NITROGEN SOLUBILITY INDEX, ISOLATED PROTEIN YIELD, AND WHEY NITROGEN CONTENT OF SEVERAL SOYBEAN STRAINS[1]

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ABSTRACT

Two modified procedures were developed to determine nitrogen solubility index (NSI). Both methods used a double extraction of dehulled, defatted soybean flakes. The first, on a 40-g. sample, had water-to-meal ratios of 20:1 and 10:1, with slow paddle stirring at 30°C. and pH 7.2; the second, on a 2-g. sample, ratios of 60:1 and 50:1, with magnetic stirring at room temperature. Flaking cracked beans tempered to 14% moisture facilitated rapid extraction of oil and eliminated the need for grinding defatted flakes to obtain maximum and reproducible NSI values. Lower water-to-meal ratios, pH, and other type of grinding reduced NSI values somewhat. Average NSI values of 93.6 (range 91–97) were obtained with both methods on 26 soybean strains. Their yield of isolated protein varied from 39 to 56 g./100 g. dehulled, defatted meal (moisture-free basis). The nitrogen content of isolated protein varied between 13.4 and 15.2%. The small differences between strains in NSI would not aid in genetic selection of soybeans suitable for food and industrial use; however, the large differences in yield of isolated protein correlate directly with the protein content of the meal.

Knowledge of the degree of solubility in water of protein in soybean meal is useful for several reasons. Protein or nitrogen solubility measures the amount of undenatured protein and acceptability of meal for protein isolation; it indicates the heat-treatment received by the meal, and such measurements are used as control tests to maintain uniform processing operations for optimum nutritive value; also, it is useful in preparing specifications for purchase of unheated meals, soy flour, and other specialty products. Urease activity (1,2) is also used as a control in soybean meal processing.

In making water-solubility tests, both protein and nonprotein nitrogen compounds are extracted together (3). About 3–10% of the nitrogen of soybean meal is nonprotein nitrogen (4,5), which includes free amino acids, peptides, phospholipids, nucleic acids, and other nitrogenous constituents. In most instances the term nitrogen solubility index (NSI) has been adopted; however, such terms as protein dispersi-

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bility index (PDI) or protein-solubility index (PSI) are sometimes preferred (6). NSI is defined as the ratio of water-soluble nitrogen to total nitrogen × 100, it varies widely with moist heat-treatment of the meal (7), and severely heated meal may read as low as 4.0.

Several procedures have been proposed for determining changes in protein solubility during meal processing (8,9). Tentative procedures for the determination of PDI and NSI of raw and toasted soy products have been published (10). Most of the proposed methods follow the general procedure of grinding (equipment varies) and weighing a sample, extracting it with a given ratio of water for a specified time with constant stirring, recovering the extract in a centrifuge, and measuring the nitrogen (Kjeldahl) extracted in relation to the total nitrogen in the sample. Some proposed methods control the extraction pH. Soybean hulls do not have a significant effect on nitrogen solubility. With full-fat soybean meal, extractions must be made at temperatures of about 80°C. to obtain maximum nitrogen solubility (11).

Reportedly, temperature, grinding, water-to-meal ratio, time of extraction, rate of stirring, and age and variety affect NSI values (3,12).

There has been some difference of opinion as to the relative merits of using slow-speed and high-speed stirring procedures (6). High speeds have the advantage of grinding the sample coincidently with nitrogen extraction; however, for some purposes such speeds give unrealistically high values.

The present investigation was undertaken to determine whether NSI of undenatured defatted meal is a characteristic that might be used in a plant-breeding program to select improved soybean varieties for food and industrial uses. For such an investigation it was necessary to establish both a meal-processing procedure and an NSI method that gave maximum reproducible values. Yields of isolated protein and whey nitrogen content of several soybean strains were also determined.

Experimental

Undenatured Soybean Meal. Soybeans (1962 crop) were supplied by the U.S. Regional Soybean Laboratory (USRSL) and were flaked at the Northern Regional Research Laboratory (NRRL). Chemical analyses were made at each institution. Dehulled, full-fat flakes were prepared by cracking whole beans between corrugated rolls into six to eight parts and removing the hulls in a Eureka seed cleaner. The cracked beans, referred to as chips, were tempered to 11 and 14% moisture, pressed into thin flakes of about 0.005 to 0.008 in. thickness, and air-dried at room temperature to about 5–8% moisture.

Defatted meal was prepared from full-fat flakes by repeated extrac-

tion of oil with hexane at room temperature. Residual hexane in the meal was removed in a laboratory hood. Samples of both full-fat flakes and of defatted soybean meal, which were used by both institutions for nitrogen solubility determinations, were blended and quartered; opposite parts were then combined so that variation in the samples due to flaking and residual oil should be minimized.

NSI and Yield of Isolated Protein. Different NSI procedures were developed at both NRRL and USRSL to determine on the same samples the factors that affect nitrogen solubility. Figure 1 is a schematic diagram of conditions used at NRRL for determining NSI and preparing isolated soybean protein.

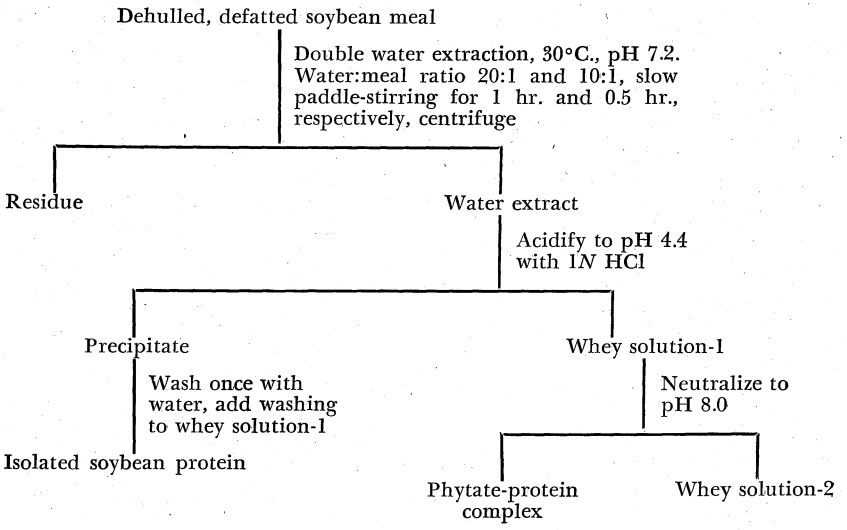

Fig. 1. Procedure for the determination of nitrogen solubility index and yield of isolated protein in soybean meal.

Meal was ground in a hammermill to pass a 100-mesh screen to minimize sampling errors because 40-g. samples were used in the extractions at NRRL. Grinding in a hammermill had little effect, if any, on NSI. In pilot-plant operations, defatted flakes could be used directly.

Slow paddle-stirring was employed. Distilled water was used in all extractions. The pH of water extracts varied between 6.52 and 6.85 depending on strain, whereas that of water extracts from the USRSL procedure (described later) was slightly higher. NSI experiments were carried out at pH 7.2 and were based on the work of Smith and Circle (13), who earlier reported that higher nitrogen solubility occurs at this pH. However, the amount of alkali required to raise the pH to 7.2

varied widely between meals and had no relation to the initial pH of the water extract. Washing the acid-precipitated protein was done in one centrifuge cup, to minimize weight losses during recovery. The curd was sufficiently compacted during centrifugation that it could be lifted out of the cup with a spatula almost intact.

Centrifugation for 10 min., about 2,000 × *g*, was employed in all operations. All nitrogen analyses are reported as Kjeldahl nitrogen. Distribution of nitrogen in various meal fractions made according to Fig. 1 are reported in percentage of the total nitrogen in dehulled, defatted soybean meal on a moisture-free basis.

NSI measurements at USRSL were made according to the following procedures: a 2-g. sample of dehulled, defatted flakes was placed in a 250-ml. centrifuge bottle marked at 100-ml. and 125-ml. levels, and distilled water was added to the 125-ml. level. The sample was stirred on a magnetic stirrer at about 1,000 r.p.m. for 1 hr. by means of a 1-in. Teflon-covered magnet. After centrifugation at 1,900 × *g* for 15 min., the extract was decanted into a 250-ml. volumetric flask, and the residue was re-extracted with 100 ml. of water for 1 hr. with stirring. The combined extracts were made up to volume. Macro-Kjeldahl analyses were made on 50-ml. aliquots of the extraction and on 0.8-g. samples of the defatted flakes: NSI = 200 × titration from 50 ml. of extract/Kjeldahl titration from 0.8-g. flake sample. High water-to-meal ratios, without pH adjustment, were used in the USRSL procedure.

Moisture and Oil Analyses. Moisture content was determined by drying weighed samples in an oven for 3 hr. at 120°C. Hold-up water was first removed in a vacuum oven at 60°C. from the isolated protein, which was then ground and equilibrated to the atmosphere before moisture analysis. Residual oil in defatted meal was analyzed by official AOCS methods (10).

Results

Effect of Water-to-Meal Ratio and pH on NSI. In these experiments, dehulled, defatted meal was doubly extracted with water at 30°C., at two levels of dilution, using water-to-meal ratios of 20:1 followed by 10:1 at the first level, or water-to-meal ratios of 10:1 followed by 5:1 at the second (see Table I). Both levels of dilution were conducted at two conditions of pH, 7.2 and 6.5. Regardless of strain, the highest NSI values were obtained with the higher dilution and higher pH. NSI values were reduced 0.2 to 2.8 percentage units when either the lower water-to-meal ratio was used at pH 7.2 or when extractions were made at the normal pH of a water extract with no change in the water-to-meal ratio of 20:1 and 10:1. When extractions were made

TABLE I

EFFECT OF WATER-TO-MEAL RATIO AND pH ON NITROGEN SOLUBILITY INDEX (NSI) OF DEHULLED, DEFATTED SOYBEAN FLAKES

STRAIN	NSI [a]			
	20:1, 10:1 pH 7.2	10:1, 5:1 pH 7.2	20:1, 10:1 pH 6.5	10:1, 5:1 pH 6.5 [b]
Lindarin	94.6	92.8	93.5	90.1
Hawkeye	95.2	94.2	93.6	90.7
Harosoy	94.4	91.7	93.2	90.0
M 316 G	93.2	92.6	90.8	88.5
Bethel	94.5	91.7	93.9	90.5
Lee	95.5	94.5	94.3	91.0
D60–9647 [c]	94.3	94.1	92.6	89.9

[a] All extractions were made at 30°C.
[b] Normal pH of the water extracts for these strains ranged from pH 6.4 to 6.6.
[c] Represents an experimental strain.

with a water-to-meal ratio of only 10:1 and 5:1 without pH adjustment, NSI values were decreased nearly 5 units. On the basis of these experiments, further nitrogen solubility measurements at NRRL were carried out according to the conditions illustrated in Fig. 1. Comparable values could, however, be obtained if water-to-meal ratios of 60:1, 50:1 were used at about pH 6.8 (USRSL method).

Effect of Processing Conditions on NSI. Two series of experiments were made to determine the effect of tempering and flaking on nitrogen solubility. The beans were cracked and dehulled as usual. Different lots were then tempered to 11 and 14% moisture before flaking was done. The smooth rolls were preset to the same position in all operations.

After four hexane extractions with fresh solvent for each decantation of the oil miscella, defatted meal prepared from beans tempered at 11% moisture contained as much as 3–5% residual oil. Several more hexane extractions were required to reduce the oil content of the meal to less than 0.1%. Defatted meal prepared from beans tempered to 14% moisture contained less than 0.05% residual oil after four hexane extractions. Uniformly thin flakes, without the production of fines, were obtained with beans tempered to 14% moisture. When flaked under the conditions described in this report, Clark and Shelby soybeans contained the largest amounts of residual oil in defatted meal.

As shown in Table II, reproducibility in NSI values of defatted meals prepared from beans tempered to 14% moisture was within 0.8 to 2.1 NSI units for the three flaking runs. NSI values of the same meals analyzed at USRSL did not vary by more than 1.5 units. NSI values of meal prepared from full-fat flakes tempered to 11% moisture were 0.8 to 5 percentage units lower, depending on variety.

The effect of grinding on NSI of dehulled, defatted flakes is shown in Table III. NSI values of unground flakes analyzed at USRSL and

TABLE II

EFFECT OF PROCESSING ON NITROGEN SOLUBILITY INDEX (NSI) OF DEHULLED, DEFATTED SOYBEAN FLAKES[a]

STRAIN	NSI IN FLAKING RUNS[b]			
	I	II	III	Range in NSI
Adams	94.0	92.6	93.8	1.4
Clark 63	93.6	95.7	93.8	2.1
Harosoy 63	93.4	92.8	93.6	0.8
Shelby	92.5	92.9	92.8	0.9

[a] Cracked beans tempered to 14% moisture before flaking.
[b] Northern Laboratory procedure.

TABLE III

EFFECT OF GRINDING ON NITROGEN SOLUBILITY INDEX (NSI) OF DEHULLED, DEFATTED SOYBEAN FLAKES

STRAIN	NSI			FLAKES — BAUER MILL NSI BY DIFFERENCE
	Unground Flakes[a]	Hammermill-Ground Flakes[b]	Bauer Mill-Ground Meal[a]	
Clark 63	93.7	94.0	86.3	+ 7.4
Harosoy 63	94.6	93.7	87.6	+ 7.0
Kanrich	96.1	96.1	86.0	+10.1
Ottawa Mandarin	94.0	93.0	90.4	+ 3.6
Perry	93.6	94.1	86.5	+ 6.9
Shelby	93.6	92.7	83.0	+10.7

[a] U.S. Regional Soybean Laboratory data.
[b] Northern Laboratory data.

hammermill-ground flakes analyzed at NRRL were within one NSI unit of each other. NSI of meals prepared from whole beans ground in a Bauer mill and then defatted with hexane were 3.6 to 10.7 units lower compared with values obtained from flakes. NSI values remained unchanged even when the samples were ground in a cold Bauer mill. Regrinding the Bauer mill samples in a mortar and pestle increased NSI values only 2 to 3 units. Protein denaturation and incomplete break-up of cell membranes are most likely responsible for the large decrease in NSI of meals ground in a Bauer mill. The data of Tables II and III indicate that flaking has a great effect on nitrogen solubility and oil extractability, and that cellular membranes surrounding oil and protein bodies must be ruptured for efficient extraction.

Soybean Strains Classified as to Protein Content, NSI, and Isolated Protein Yield. In Table IV, 23 strains are arranged in decreasing order according to the protein content of dehulled, defatted meal ranging from 60.3 to 44.5%. The meals were also arbitrarily classified into high, intermediate, and low protein. When so classified, the yields of isolated protein also can be separated into three categories as indicated in Table IV. There appears to be a direct relationship between the

TABLE IV

CLASSIFICATION OF SOYBEAN STRAINS IN RELATION TO PROTEIN CONTENT, NSI, YIELD OF ISOLATED PROTEIN, AND WHEY NITROGEN CONTENT OF DEHULLED, DEFATTED MEAL

STRAIN	PROTEIN CONTENT [a]	NSI	ISOLATED SOYBEAN PROTEIN		WHEY NITROGEN
			Yield	Nitrogen Content	
	%		*g./100 g. meal* [a]	%	*% of total*
D60–9647 [b]	60.3	94.3	56.0	14.80	9.0
D60–8107	59.8	92.3	50.4	14.90	13.0
D59–9048	58.7	93.7	49.5	14.96	13.0
Lindarin	58.1	94.6	49.6	14.90	12.6
Ottawa Mandarin	57.3	91.7	49.2	15.10	11.0
Lee	56.9	95.5	49.7	14.53	13.6
Delmar	56.8	92.2	50.1	14.60	10.5
Hawkeye	54.7	95.4	49.9	14.63	10.2
High range	54.7–60.3		48.5–56.0		
Clark	54.5	92.4	46.7	14.70	12.1
D60–8335	54.5	94.9	47.0	14.45	14.8
Chippewa	54.3	91.8	46.8	14.67	12.0
Harosoy	54.1	94.5	48.0	14.30	12.4
Comet	54.0	92.5	46.9	14.60	12.1
D58–1894	53.9	95.0	47.2	14.40	14.4
Shelby	53.9	91.9	46.9	14.51	12.0
Adams	53.3	94.8	47.8	14.70	12.7
D60–12,327	52.4	91.4	46.6	14.01	13.3
Intermediate range	52.4–54.5		46.6–48.0		
Kanrich	51.0	94.1	44.5	14.56	12.5
D60–11,215	50.2	93.4	44.3	14.55	13.8
Bethel	49.0	94.5	44.2	14.42	12.9
Hampton	48.9	93.8	45.8	13.57	14.4
Jackson	46.6	95.2	45.9	13.50	12.6
M316 G	44.5	93.8	39.9	14.00	15.3
Low range	44.5–51.0		39.9–45.8		
Average	53.7 [c]	93.7 [d]			

[a] N × 6.25, dry basis.
[b] Entries listed by number represent experimental strains.
[c] An average for all strains.
[d] Average value from USRSL data was 93.5.

protein content in meal and yield of isolated protein. Although NSI values for all strains differed within a narrow range (91.4–95.5%), differences in NSI and whey nitrogen content can influence the yield of isolated protein. For example, dehulled, defatted meal prepared from Hawkeye soybeans had the lowest protein content of the strains placed in the high-protein category. Because of a combination of a high NSI and a low whey nitrogen content, yields of isolated protein for Hawkeye were comparable to the other strains except for D60–9647. Average nitrogen content of the isolated proteins was 14.5 ± 0.5%. Nitrogen content of isolated protein from Jackson and Hampton soybeans was quite low.

For 20 of the strains given in Table IV, NSI values determined by

USRSL procedure were within 2 percentage units of the values obtained at NRRL and reported in Table IV. Differences in NSI values for the other three strains were between 2.4 and 3.3 units. Ten of the NSI values determined at NRRL were higher and 13 were lower. These data represent good agreement between the two institutions. Total whey nitrogen was calculated by determining the Kjeldahl nitrogen of whey solution-2 (Fig. 1) and adding a correction factor of 0.3% nitrogen to account for the nitrogen represented by the phytate-protein complex. There is continuous precipitation of nitrogen in whey solution-1 with time.

Residue Fraction Analyses and Nitrogen Balance Studies. Composition and yield of the water-insoluble residue of several strains were determined as part of the nitrogen recovery experiments (Table V).

TABLE V

COMPOSITION AND YIELD OF SOYBEAN RESIDUE FRACTION AND NITROGEN RECOVERY VALUES

STRAIN	YIELD	NITROGEN CONTENT	TOTAL NITROGEN OF MEAL	NSI	NITROGEN RECOVERY [b]
	g./100 g. meal [a]	%	%		%
D59–9048 [c]	20.0	3.01	6.6	92.5	99.1
Hawkeye	20.1	2.47	5.6	94.5	100.5
D60–8335	20.1	2.39	5.7	94.0	99.7
Harosoy	21.0	2.73	6.2	94.0	100.2
Jackson	20.8	2.07	6.0	94.5	100.5
Ottawa Mandarin	20.0	2.36	7.5	91.3	98.8

[a] Dehulled, defatted meal, dry basis.
[b] (Residue nitrogen plus soluble nitrogen)/(total nitrogen in the meal).
[c] Entries listed by number represent experimental strains.

Although protein content ranged from 46.6 to 58.7% and yield of isolated protein varied from 45.9 to 49.9 g./100 g. of meal, yield of residue was nearly the same for all strains. Residue nitrogen for these accounted for 5.6 to 7.5% of the total nitrogen of the meal. Insoluble residue nitrogen plus soluble nitrogen accounted for 99 to 101% of the total nitrogen of the meal. Other nitrogen recovery experiments showed that isolated soybean protein nitrogen and whey nitrogen accounted for at least 97% of the soluble nitrogen. These nitrogen recovery experiments constituted a good check on the accuracy of the Kjeldahl nitrogen determinations. Protein content of all the defatted, dehulled meals, when analyzed at both institutions, was well within the range of experimental error.

Discussion

Proper moisture-tempering and flaking of soybeans appear to be essential processing factors. They facilitate the efficient and rapid

extraction of oil and protein and eliminate the need for grinding. Maximum NSI values and better reproducibility were obtained with flaked samples. Water-to-meal ratios, as well as pH, are other important factors that affect NSI values. Flaking soybeans before heat-treatment increases their nutritive value and markedly improves utilization of full-fat soybean meal by the chick (14). These beneficial effects from flaking suggest that physical barriers, such as cell membranes, are completely ruptured by flaking but not by grinding. Electron-microscopic studies indicate that soybeans and other oilseeds contain subcellular particles composed of protein bodies and oil globules, presumably surrounded by membrane (15).

Reduction in nitrogen solubility and large amounts of residual oil in dehulled, defatted soybean meal prepared from poorly flaked soybeans plus the difficulties encountered in flaking some varieties indicate that meal-processing factors may have been responsible for the varietal differences in NSI as previously reported (3). Average NSI values of 93.5% (range 91–97%) for 26 soybean strains reported here also indicate that the individual strain has a minor effect on nitrogen solubility. As a result, NSI is not a good criterion for making selections in a plant-breeding program. However, soybean strains can be classified according to the protein content of dehulled, defatted meal and the yield of isolated protein; yet the low nitrogen content of the isolated protein of certain strains shows that some of the isolates may contain appreciable amounts of nonprotein substances. Protein content ($N \times 6.25$) of the protein isolates prepared from Jackson and Hampton soybeans was particularly low.

Because of their high protein content and yield of isolated proteins, some of the strains may be of economic importance in the manufacture of shoyu (soy sauce) (unpublished data), tofu (11), and other food and industrial products.

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