Isolation of Soybean Protein: Effect of Processing Conditions on Yields and Purity

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Abstract

The process of soybean-protein isolation, comprising extraction in dilute calcium hydroxide and precipitation with hydrochloric acid at pH 4.5, was evaluated from the viewpoint of the effect of processing conditions on yields and product purity. The course of extraction and the effect of particle size and agitation indicate that the process is quite rapid even when coarse meal is treated under mild agitation. The heat-treatment history of the meal is the main factor governing the extraction and isolation yields. The precipitation yield is unaffected by temperature. Two steps of curd washing were found to suffice for maximum product purity. The purity is also improved considerably by preliminary sifting of the meal.

Introduction

ISOLATED SOYBEAN PROTEIN has considerable advantages over soy flours from the viewpoint of human consumption. It has a nearly bland taste, white color and excellent keeping qualities. Its functional behavior may be modified to a certain extent so as to meet the required properties (dispersibility, gel formation, water- or fat absorption, whipping, foaming, etc.). One of its main shortcomings is high cost. On the basis of the same quantity of protein, the cost of edible isolated soybean protein is approximately five times that of soybean oil meal (1). This may indicate, among other things, high processing cost and low isolation yields.

The object of this study was to analyze the different steps of isolation from the viewpoints of yields and product purity.

Experimental

Soybean Oil Meal

Unless otherwise stated, the meal used was prepared as follows: Hexane-extracted soybean flakes were taken from the discharge end of the extractor in an industrial plant. The flakes were placed in a natural draught until completely desolventized. The meal contained 45.6% protein (N × 6.25) and its nitrogen dispersibility index (NDI) was approximately 84% (see definition below).

Protein Isolation Procedure

The material (meal or flour) was extracted with the appropriate amount of a 0.03 molar solution of calcium hydroxide at 55C. After a measured time interval the extract was filtered, and the filtrate acidulated to pH 4.5 with hydrochloric acid. The resulting precipitate (curd) was separated from the supernatant (whey) by decantation and washed by resuspension in water. The washed curd was pressed in cheese-cloth, resuspended in water, homogenized and spray-dried.

Processing factors, such as type and particle size of the meal (or flour), extractant-to-meal ratio, extraction time, agitation filtration and washing procedures were the variables of the study, and data regarding them are given in the appropriate places in the text. Where these data were not specifically investigated and are unspecified, they were as follows: 1) Type of meal: unheated, unground meal. 2) Extractant-to-meal ratio: 10:1. 3) Extraction time: 30 min, with agitation. 4) Filtrations: successive sieves of 1.5 mm, 20, 40, 60, and 80 mesh. 5) Number of washing steps: 2. 6) Wash water quantity: 4 weight parts of water per 1 weight part of wet curd.

Analytical Procedures

Nitrogen was determined by the macro-Kjeldahl method (6). The nitrogen dispersibility index, defined as:
NDI = % water dispersible nitrogen × 100/% total nitrogen, was determined according to Paulsen et al. (9).

Computation of Extraction and Isolation Yields

Extraction yields were computed as follows:

$$\text{Extraction yield } \% = \frac{N_1 V_1}{N_2 G_2} \times 100$$

where

N_1 = nitrogen content in extract, g per 100 ml
N_2 = nitrogen content of meal, g per 100 g
V_1 = volume of extractant added to meal, ml
G_2 = weight of meal, g

However, only part of the liquid is recovered as extract after filtration. The residue retains as much as five times its dry weight of extract. It was assumed that the amount of nitrogen retained in the absorbed liquid will not be lost in an industrial operation where countercurrent extraction could be practiced. In such a case the actual extraction yield would approach the values computed by the method described above.

To determine the over-all isolation yield a 500-ml aliquot of the filtered extract was acidulated to pH 4.5 with HCl. The curd was separated by filtration, washed twice by resuspension in 500 ml of water, filtered, pressed and weighed. A sample of the washed curd was taken for nitrogen analysis. The over-all isolation yield is defined as

$$\text{over-all isolation yield } \% = \frac{N_3 \times G_3 \times V_1}{N_2 \times G_2 \times 500} \times 100$$

where

N_3 = nitrogen content of wet curd, g per 100 g
G_3 = weight of wet curd obtained from 500 ml of extract, g
N_2, G_2, V_1 as defined above.

Results and Discussion

Extraction Time

The course of nitrogen extraction from soybean oil meal, under the conditions of the experiments, is illustrated in Fig. 1. The changes in pH and refractive index in the course of extraction are also recorded. The results refer to pilot plant runs where the batch size was 300 liters.

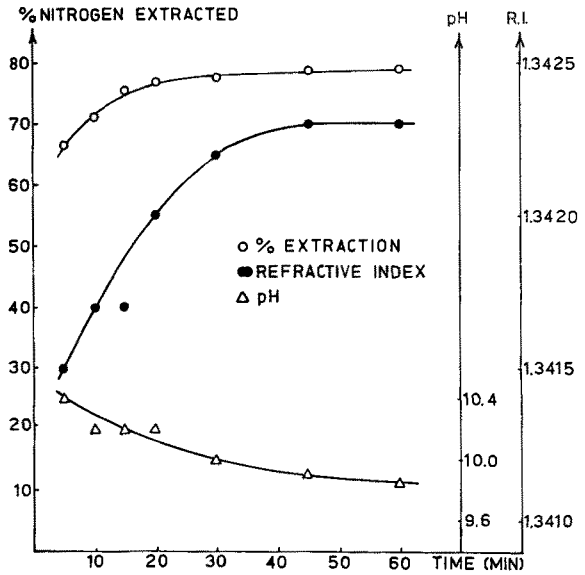

FIG. 1. Percent nitrogen extraction, refractive index and pH, vs. time.

The amount of nitrogen extracted increased steadily in the first 30 min and reached a nearly constant level after 45 min. This is generally in accordance with results reported elsewhere (2,3,8). However, Smith et al. (11) reported a steady increase in nitrogen extraction in water during 60 min. In their investigation even extraction periods of 120 min failed to yield maximum peptization with meal obtained from older beans. These discrepancies are attributable to differences in type of extractant and raw material.

The change in refractive index parallels exactly the extent of protein peptization, while the pH continues to drop slightly even after completion of extraction. This may serve as an indication of the possible use of refractometry in following the course of protein extraction in industry. The use of refractive index as a rapid method for the evaluation of the heat-treatment history of soybean oil meal has been advocated by Pomeranz and Lindner (10).

Meal/Extractant Ratio

A ratio of 20 parts extractant to 1 part meal is generally recommended as optimal (2,3). Our results, summarized in Table I, show that a 10-to-1 ratio may be used without considerable detriment to extraction yields. The batch size was 300 liters.

The advantages of working with more concentrated suspensions are obvious. Less material has to be treated for an equal output. Furthermore, a more concentrated "whey" is obtained, so that utilization of this fraction becomes more economical.

Agitation

Table II shows the effect of agitation on extraction yields after 5 and 30 min of contact. "No agitation" refers to extraction without constant mixing. The suspension was only stirred with a glass rod, once every 5 min. "Strong agitation" was secured by means of a "Turrax" colloid blender, an extremely

TABLE I
Effect of Extractant/Meal Ratio on Protein Extraction Yield

Extractant/meal ratio	Percentage of total N extracted after	
	30 min	60 min
30:1	80.5	82.0
20:1	78.5	80.2
10:1	77.3	78.0

TABLE II
Effect of Agitation on Extraction

Agitation	Percentage of total N extracted after	
	5 min	30 min
No agitation	53	71
100 rpm, propeller	48	64
180 rpm, propeller	58	71
280 rpm, propeller	60	76
600 rpm, propeller	69	74
"Turrax"	....	76

effective mixing device producing a high degree of shear and disintegration; when this blender was used, the liquid had to be cooled to keep the temperature at the 55C level. In the intermediate range, agitation is expressed as the speed of a stirrer working under constant conditions specified as: vessel diameter, 128 mm; stirrer type, three-blade propeller; stirrer pitch, standard; stirrer diameter, 52 mm; stirrer position, 45 mm below surface and 45 mm above bottom; batch size, 1 liter.

The rate of extraction is obviously increased by stronger agitation. However, protein extraction seems to be quite rapid even under "no agitation" conditions. The low rate obtained at the stirrer speed of 100 rpm is instructive. At this speed the stirrer caused considerable turbulence of the suspension above the propeller but failed to lift the meal from the bottom of the vessel; the result was even poorer than that obtained by occasional striring with a rod.

The following conclusion may be reached with respect to agitation: Whenever batch-wise extraction with contact times of 10 to 30 min is considered it is important to keep the meal in suspension, but it is apparently unnecessary to maintain a state of high turbulence by vigorous agitation.

These findings disagree with results reported elsewhere (11), but the conditions of extraction used by these authors were different. The extractant was water at pH 6.6, and the fact that the amount of nitrogen extracted under "vigorous agitation" conditions continued to increase considerably after 30 and 60 min indicates an apparent "bottle-neck"—not in the liquid film but within the meal particle (low solubility of the protein).

Particle Size

Three samples of meal were prepared, as follows: Sample 1: meal flakes, unground. Sample 2: meal, medium-ground. Sample 3: meal, finely ground. The particle-size distribution of the samples is given in Table III.

The results seem to indicate that, under the given extraction conditions, the effect of mean particle size on the rate of protein extraction is insignificant. There is no effect on the "equilibrium" extraction yields (i.e., yields after prolonged extraction). This is in accordance with the conclusion that, under the conditions in question, diffusional resistance to material transfer in the liquid film was rather small and that any decrease in extraction yields should be at-

TABLE III

Mesh size		Weight percentage of fractions		
		Flakes	Medium ground	Finely ground
	+ 18	70.7	15.2	0
− 18	+ 20	8.3	17.2	3.3
− 20	+ 25	6.3	9.3	6.7
− 25	+ 40	4.4	31.8	11.0
− 40	+ 60	3.5	13.7	16.8
− 60	+100	3.5	6.3	38.3
−100	+140	2.1	3.9	15.4
−140	+200	0.7	1.9	6.8
	−200	0.5	1.0	2.0

The meals were extracted in 1-liter batches (100 g meal per batch).

TABLE IV
Effect of Particle Size on Nitrogen Extraction

Type of meal	Percentage of total N extracted after			
	5 min	15 min	30 min	60 min
Flakes	60	68	76	77
Medium-ground	62	71	76	77
Finely ground	62	69	77	77

tributed to the state of the proteins (heat-treatment history) rather than to kinetic factors.

It should be emphasized that this statement applies only to the conditions described. The original material was soybean meal in the form of flakes, i.e., particles with only one dimension already reduced considerably. It may be that in other cases, such as when comparing grits and flour, the effect of particle size on extraction rate is more significant.

Particle size may also affect the rate of nitrogen dispersion in cases where extraction is more difficult than under the conditions described. Thus, Loska and Melnick (7), extracting soy flour with water, found that 73.5% of the protein could be extracted from the flour fraction coarser than 100 mesh, while the flour fraction passing through 100 mesh gave an extraction percentage of 84.7.

Heat Treatment of the Meal

Commercial practice for the production of soybean oil meal comprises several steps of heat treatment. These operations are known to cause a given degree of protein "denaturation" (5) and may be expected to impair protein extraction yields to some extent.

One of the largest oil extraction plants in Israel was chosen as a model for these investigations. This plant uses a horizontal De Smet extractor. Desolventizing and toasting are carried out as two separate operations. Samples were drawn from seven different points along the production line in a single shift (8 hr) at 1-hr intervals and their nitrogen dispersibility indices determined. Some of the samples were used for protein isolation runs, according to the usual procedure. Results are given in Table V.

The most significant decrease in NDI occurred after extraction, i.e., during desolventizing and toasting; alkali extraction and precipitation yields of protein are less affected. The decrease in the extractability of protein is almost quantitatively reflected on the over-all isolation yield. This seems to indicate that "denaturation" bears mainly on the "curd" proteins, causing a larger quantity of these to remain in the residue. The percentage of total nitrogen lost in the whey remains fairly constant (9–11%, Fig. 2).

Filtration of the Extract

The alkaline extract of soybean meal contains a considerable amount of fine particles in suspension, whose elimination, prior to precipitation, is necessary in order to obtain a curd of acceptable color, taste and nitrogen content. These particles may be partly removed by sifting the meal before extraction, by filtration of the extract, or by a combination of both methods.

TABLE V
NDI and Protein Isolation Data for Samples Drawn from Industrial Line

Sample no.	Description	NDI %	Protein extraction yield %	Protein isolation yield %
1	Raw beans after cleaner	91.6	.....	.....
2	Grits after breaker	90.9	.....	.....
3	Grits after pre-heater	86.7	.....	.....
4	Flakes after rolls	86.0	.....	.....
5	Meal after extractor	84.6	86.1	75.0
6	Meal after desolventizer	64.4	76.5	65.0
7	Toasted meal	22.0	60.0	51.8

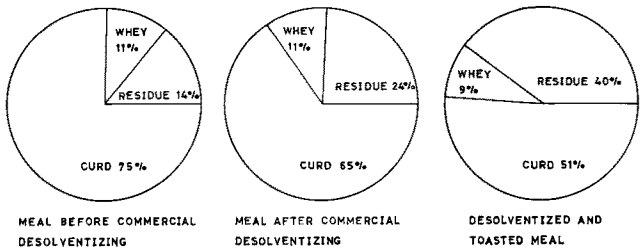

FIG. 2. Distribution of nitrogen in the three products of soybean protein isolation.

Commercial flakes with particle size distribution similar to that described in sec. 4 were sifted on a 60-mesh screen to remove the fines, which were discarded. Extracts were then prepared from sifted and unsifted flakes. Portions of each extract were filtered through screens of different fineness or completely clarified by centrifugation. The filtered or clarified extracts were acidified and the curds obtained were analyzed for nitrogen (Table VI).

The purity of the curd increases when clarification of the extract is more complete (4). A considerable increase in purity was obtained when the 140-mesh filtrate was further filtered through 200 mesh. It is interesting to note that in dry sifting the particles passing through 140 mesh represented only 1.2% of the meal. Since particles undergo swelling in the extraction medium, one can conclude that considerable particle distintegration takes place during extraction. On the other hand, dry sifting of the meal through 60 mesh improved purity considerably.

Precipitation of Curd

Early investigations (12) indicated that in the isoelectric precipitation of soy protein the type of acid used to lower the pH of extract does not affect precipitation yields. This was confirmed by our results. We also found that precipitation of the protein with calcium chloride (0.33 g per 100 ml of extract) at boiling point gives essentially identical yields. Eighty-two to 84% of the nitrogen of the extract was precipitated.

The temperature of the extract at the moment of acidification does not affect precipitation yields; nor does "tempering" of the extract (i.e., heating to 90C for 1 hr and cooling prior to precipitation). Tempering is reported to be an important factor in the production of soy curd in the Orient (13), but its effect seems to be limited to firmness and volume of the curd.

Washing the Curd

Table VII shows the effect of repeated washing on the purity of isolated soy protein.

The results indicate considerable increase in purity as a result of the two first washing steps. Further

TABLE VI
Effect of Meal Sifting and Extract Filtration on Purity of Isolated Protein

Sieve mesh no.	Percentage protein in dry curd (N × 6.25)	
	From unsifted meal	From sifted meal
20	83.2	.....
40	83.9	87.9
60	.....	91.8
80	85.8	.....
100	88.7	92.4
140	89.5	92.4
200	91.2	.....
Centrifuge	93.3	94.2

TABLE VII

Effect of Washing on Purity of Curd

No. of successive washing steps	% Protein (N × 6.25) in curd (dry basis)	
	Experiment 1	Experiment 2
0	88.9	89.0
1	95.3	94.8
2	96.0	95.1
3	96.3	94.9
4	96.3	94.9
5	96.3	....

washing had no significant effect on purity. When continous washing is envisaged, a process equivalent to two steps of leaching, using a sludge-to-water ratio of 1:4, will suffice.

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