Processing Soybeans into Foods: Selected Aspects of Nutrition and Flavor[1]

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ABSTRACT

Since many new soy protein products are being developed which differ in enzyme activity, protein dispersibility, flavor, nutritive value, and functional properties, quality control is assuming increasing significance. The effects of dry and moist heat and hexane:ethanol azeotrope extraction upon various enzymatic activities, protein solubility, and nutritive value of defatted soy flakes differ considerably. Specifications and guidelines initially developed to establish the degree of moist heat treatment required to produce edible grade products need to be reevaluated for these processes. Flavor scores of hexane:ethanol azeotrope-extracted flakes and proteinates prepared from them are significantly higher than those prepared by current commercial practices. Because peroxidase is a much more stable enzyme than lipoxygenase, determination of peroxidase activity may be a more suitable method to define proper processing conditions which improve the flavor of soy products. A combination of hexane:ethanol extraction and steaming improves the flavor and nutritive value of defatted soy flakes. Azeotrope extraction alone does not inactivate trypsin inhibitors; nutritive value of the extracted flakes is low, and pancreatic hypertrophy occurs when they are fed to rats. Protein efficiency ratio of the processed flakes is 2.2 on a basis of a value = 2.5 for casein. Other factors to be considered to prepare soy protein isolates of good nutritional quality are: choline deficiency, variability in sulfur amino acid content, and formation of phytate complexes that affect bioavailability of essential minerals, particularly zinc.

INTRODUCTION

Problems caused by an expanding population have created a need for new sources of protein for direct consumption by man. The soybean has the best potential as a new source of protein; not only is its protein content high (39-44%), but the protein when properly processed is of good nutritional quality. Careful processing control also is required to ensure good flavor acceptability and to maintain advantageous functional properties in a wide variety of food systems. The present state of the soybean industry, governmental controls, legal barriers, markets, and technology was reviewed recently (1).

In the raw form, soybeans contain trypsin inhibitors (TI) and other substances that cause differing biological and physiological responses in various species of animals and perhaps in man (2). By live steam treatment, a process referred to as toasting, the nutritive value of fall-fat and defatted soy flours can be raised to nearly that of meat and milk. In fact, proper heat treatment is most likely an absolute requirement if the essential nutrients in soy flours, concentrates, and isolates are to be used maximally (2). Both raw and processed soy protein products also have objectionable flavors which prevent their use in bland systems. To achieve the tremendous market potential forecast for these soy protein products, the disagreeable flavors must be eliminated without sacrificing the functional and nutritional qualities of the protein (3,4). Flavor scores of hexane:ethanol azeotrope-extracted soy flakes and proteinates prepared from these extracted flakes are significantly higher than those of products prepared by present commercial practices (5).

This paper reports on the concomitant extraction of the objectionable flavors and inactivation of TI activity in raw, hexane-defatted flakes by using a combination of processes—hexane:ethanol azeotrope extraction and live steam—to improve flavor and enhance nutritive value further. Special problems associated with the preparation of soy protein isolates of good protein quality are discussed. Methods used to control processing of edible soy flours have been evaluated in relation to steaming and azeotrope-extraction processes.

PROCESSING AND ANALYTICAL TECHNIQUES

Preparation of Soy Flakes

Dehulled, defatted flakes were prepared at the Northern Regional Research Laboratory (NRRL) from certified seed-grade soybeans (Amsoy variety, 1972 crop), according to previously described procedures (6). The raw defatted flakes were extracted further with hexane:ethanol (82:18 v/v, bp 59 C) for 3 and 6 hr. The temperature in the Soxhlet thimble was 56 C. Heat treatment of hexane-defatted and azeotrope-extracted flakes was carried out in a preheated autoclave with live steam for 20 min at 100 C. The heat-treated flakes were dried in a hood for 24 hr at room temperature, then ground to a flour (100 mesh) in an air-cooled Alpine pin mill.

Analytical Procedures

Nitrogen solubility index (NSI) was determined by the

[1]One of 13 papers presented in the symposium, "Soy Protein," at the AOCS Spring Meeting, Mexico City, April 1974.

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TABLE I

Nitrogen, Protein, Lipid, and Ash Content of Dehulled Soy Flours[a]

Sample	Nitrogen	Protein (N x 6.25)	Residual lipid	Ash
Hexane-defatted	8.68	54.3	3.2	6.78
Azeotrope-extracted (3 hr)[b]	8.97	56.1	Trace	6.84
Azeotrope-extracted (6 hr)	8.99	56.2	Trace	6.86

[a]Dry basis.

[b]See text for details.

TABLE II

Composition of Control Diet

Ingredients[a]	Percent of diet
Casein	11.62
Corn oil	8.00
Dextrose	49.63
Salts XIV + Zn + Co	4.73
Cellulose	3.00
Vitamix	2.00
Corn starch	20.00
Water	1.02

[a]Soy ingredients were substituted for casein, dextrose, and water to maintain a 10% protein diet (N x 6.25). All diets contained 40 ppm supplemental zinc added as $ZnSO_4$ and 2 ppm Co as $CoCl_2$.

TABLE III

Property Measurement Used To Control Processing of Edible Soy Flour

Control measurement	Property affected: Flavor	Property affected: Nutritive value
Lipoxygenase	+	-
Peroxidase	+	-
NSI	-	+
PDI	-	+
Urease	-	+
TI	-	+
Available lysine		+

[a]NSI = nitrogen solubility index, PDI = protein dispersibility index, and TI = trypsin inhibitor.

TABLE IV

Processing and Nutritional Parameters of Heat-Treated Soy Flours

Heat,[a] min	NSI[b]	TI, TIU/mg[c]	PER[d]	Pancreas wt, g/100 g body wt
0	97.2	96.6	1.13	0.68
1	78.2	74.9	1.35	0.58
3	69.6	45.0	1.75	0.51
6	56.5	28.0	2.07	0.52
9	51.3	20.5	2.19	0.48
20	37.9	10.1	2.08	0.49
30	28.2	8.0	-	-

[a]Live steam at 100 C.

[b]NSI = nitrogen solubility index.

[c]TI = trypsin inhibitor and TIU = trypsin inhibitor units.

[d]Protein efficiency ratio, corrected on a basis of PER = 2.50 for casein.

NRRL procedure of Smith, et al. (7). A pH of 7.2 was specified in this procedure, because water slurries of raw, defatted flakes have pH values ranging from 6.3-6.8 for different varieties. These differences in pH affect protein extractability. Procedures for the determination of NSI and protein dispersibility index (PDI) by official AOCS Methods BA11-65 and BA10-65 (8), respectively, do not specify pH adjustment. TI activity was determined by the procedure of Kakade, et al., (9) which has been approved as an official method of the American Association of Cereal Chemists (tentatively designated as AACC Method 71-10). Nitrogen, protein, residual lipid, and ash content of soy flours are given in Table I. Residual lipid in hexane-defatted soy flours was determined by reextracting them with hexane:ethanol azeotrope mixture for 6 hr (10). The various classes of lipids comprising the residual lipid fraction are described elsewhere (5).

Lipoxygenase was determined by measuring oxygen uptake in extracts at pH 9.0 with a Gilson oxygraph (Gilson Medical Electronics, Middleton, Wis.). For quantitative determination of peroxidase, a modified procedure was used (11).

Rat Tests

A 28 day feeding trial was made with weanling male rats (5-D strain, 21 days old, initial wt 54 g) separated into groups of 5, housed in individual cages, and fed the diets ad libitum. Composition of the control diet is given in Table II. In the experimental diets, soy flour was substituted for casein and dextrose. Water content was adjusted to maintain a 10% protein diet (N x 6.25).

METHODS FOR QUALITY CONTROL OF EDIBLE SOY PROTEIN PRODUCTS

A number of official and unofficial methods has been used to evaluate the degree of heat treatment which is a primary determinant of functionality. A functional property of a protein ingredient is one that imparts desirable changes or contributes some favorable aspect to a fabricated food during processing or in the finished product. Functionality would include: nutritional value, flavor qualities, enzyme activity, protein solubility, viscosity, and other properties.

Some of the measurements used for the control of processing with respect to factors that affect flavor and nutritive value are summarized in Table III.

Lipoxygenase and peroxidase activities are two important enzymatic functions that affect organoleptic qualities of soy products (11,12).

Specifications, based upon methods used to evaluate nutritive value, have been developed to define edible soy products suitable for use in international feeding programs established by various United Nations organizations (13). Some of these same measurements also are used to prepare products that have optimum functional properties in food systems (1). Available lysine is the best method for determination of excessive heat treatment which lowers nutritive value.

TABLE V

Enzyme Activity and Nitrogen Solubility of Processed Soy Flakes

Processing conditions[a]	NSI	TI, TIU/mg[c]	Lipoxygenase, μmoles O_2/min/g	Peroxidase[d]
Hexane-defatted, raw	92.9	101.2	249	1875
Azeotrope-extracted (3 hr)	88.8	103.2	1.8	1585
Azeotrope-extracted (6 hr)	83.5	101.9	0.4	2098
Hexane-defatted, toasted	41.3	12.3	0.5	244
Azeotrope-extracted (3 hr) (toasted)	41.0	13.0	-	-
Azeotrope-extracted (6 hr) (toasted)	31.2	14.3	0.3	70

[a]See text for details.

[b]NSI = nitrogen solubility index.

[c]TI = trypsin inhibitor and TIU = trypsin inhibitor units.

[d]Absorption units/g, at pH 5.0.

TABLE VI

Biological Evaluation of Hexane:Ethanol Azeotrope-Extracted Soy Flakes (Untoasted)

Diet	Trypsin inhibitor content, mg/100 g diet	Body wt, g	PER[a]	Nitrogen digestibility, %	Pancreas wt, g/100 g body wt
Hexane-defatted, raw	1025	97[b]	1.23[b]	75	0.68[b]
Azeotrope-extracted (3 hr)	1053	86[b]	1.11[b]	72	0.71[b]
Azeotrope-extracted (6 hr)	1047	99[b]	1.32[b]	77	0.63[b]
Hexane-defatted, toasted	128	151	2.10	84	0.51

[a]Protein efficiency ratio (PER) corrected to casein control diet of 2.50.
[b]Statistical significance, $P<0.01$.

TABLE VII

Biological Evaluation of Toasted Hexane:Ethanol Azeotrope-Extracted Soy Flakes[a]

Diet	Trypsin inhibitor content, mg/100 g diet	Body wt, g	PER[b]	Nitrogen digestibility, %	Pancreas wt, g/100 g body wt
Toasted, defatted	128	151	2.10	84	0.51
Toasted, azeotrope (3 hr)	147	171	2.17	86	0.48
Toasted, azeotrope (6 hr)	133	176	2.20	83	0.49
Casein	0	178	2.50	93	0.53

[a]Toasting: live steam, 20 min, 100 C.
[b]Protein efficiency ratio (PER) corrected to casein diet of 2.50.

PROCESS PROCEDURES FOR PRODUCTION OF SOY FLOUR PRODUCTS

Precise control of heat treatment is critical in that the degree of inactivation of enzymes, improvement of nutritive value, and change in protein solubility and dispersibility depend upon temperature, time, and moisture conditions (13). However, it may be necessary to reevaluate many of the processing guidelines. For example, Pour-El and Peck (14) found no parallelism between lipoxygenase, peroxidase, and urease activity or between these and protein solubility and TI activity when dry heat (130 C) and wet heat (steaming at 100 C) treatments were evaluated.

Live Steam Process

The adverse nutritional effects of raw flour disappear at different levels of TI activity in the diet (15). Effect of live steam treatment at 100 C upon TI activity, NSI, and nutritive value of hexane-defatted soy flour is shown in Table IV. TI activity decreases more rapidly than nitrogen solubility when hexane-defatted flakes are treated with live steam. Normal pancreas wt were obtained with rats fed soy flour in which 53% of the original TI activity was destroyed (45 trypsin inhibitor units [TIU]/mg sample). Maximum protein efficiency ratios (PER) were obtained with rats fed soy flours containing 20.5 TIU/mg which corresponds to nearly 80% inactivation of the TI activity in raw soy flour.

NSI and PDI values in the range of 12-25% are specified for edible, toasted soy flours to indicate adequate heat treatment for good protein nutritional value (13). This study shows that, when minimum heat treatment (9 min at 100 C) was applied, maximum PERs were obtained and the heated soy flours have an NSI value of 51%. Soy flours treated for 30 min at 100 C have NSI values of 28%. The additional heat treatment does not lower nutritive value significantly and provides for a margin of safety to ensure destruction of antinutritional factors. To guard against excessive heat treatment which destroys essential amino acids, available lysine determinations should be made (13).

Hexane:Ethanol Azeotrope Process

Effect of azeotrope extraction upon NSI, TI, lipoxygenase, and peroxidase activity of hexane-defatted soy flour is given in Table V. Over 99% of the lipoxygenase activity was destroyed by azeotrope extraction, whereas there was no effect upon peroxidase activity. Soybean peroxidase is more resistant to dry heat treatment than lipoxygenase (14). Lipoxygenase is very sensitive to moist heat and acid treatment (16). Because peroxidase is a more stable enzyme, measurement of peroxidase activity, rather than lipoxygenase activity, may have greater significance in defining conditions for production of bland soy products. In addition, peroxidase is a very active enzyme capable of utilizing lipohydroperoxides (11). Hydroperoxides can be produced even in the absence of lipoxygenase activity. There appears to be no correlation between changes in nitrogen solubility, TI activity, and enzyme activity in respect to processing soy flakes with hexane:ethanol azeotrope solvents.

TABLE VIII

Flavor Scores of Processed Soy Flakes

Processing conditions	Flavor scores[a]
Raw, hexane-defatted	4.0
Toasted, hexane-defatted	6.6
Azeotrope extraction	7.4
Azeotrope extraction plus live steam, 10 min, 100 C	7.9

[a]A 15 member taste panel scored for intensity on a 10 point scale where 1 is strong and 10 is bland.

A feeding trial was conducted to determine the effect of azeotrope extraction upon the protein nutritional value of defatted soy flour (Table VI). Because azeotrope extraction for 3 and 6 hr did not inactivate the TI, nutritive value was poor. Rat growth, PER, and nitrogen digestibility corresponded to that obtained with diets containing raw soy flour. Azeotrope-extracted soy flour also caused pancreatic hypertrophy.

However, a combination of hexane:ethanol azeotrope extraction and live steam treatment resulted in maximum nutritive value, as measured by wt gain, PER, and nitrogen digestibility (Table VII). Normal pancreas wt of rats indicated sufficient inactivation of TI activity. The TI content of the diets for toasted azeotrope-extracted flakes was below biological threshold levels, in agreement with previously reported studies with diets containing varying levels of TI (15).

TABLE IX

Effect of Alkaline Treatment upon Nutritive Value of Soy Beverage (20)

Sample	pH	Cystine, g/16 g N	PER[a]
Milk, raw	6.7	1.74	1.44
Milk, processed[b]	6.7	1.66	2.41
Milk, processed[b]	7.4	1.41	2.18
Milk, processed[b]	8.0	1.37	2.20
Milk, processed[b]	9.2	1.15	1.70

[a]Protein efficiency ratio (PER) corrected on a basis of PER = 2.5 for casein.

[b]Heat processed for 10 min at 121 C.

TABLE X

Biological Availability of Zinc in Foods (22)

Product	Availability, %	
	Chick assay	Rat assay
Cereals	59-62	38-57
Soybean meal	67	-
Fish meal	75	84
Nonfat milk	82	79
Casein	-	84[a]
Soy protein isolate	-	44[a]

[a]See ref. 21.

Although the differences in PER between toasted hexane-defatted and toasted azeotrope-extracted flakes were not statistically significant (17), maximum PER and the highest body wt were obtained with rats fed toasted, 6 hr, azeotrope-extracted flakes.

In 1971, the Food and Nutrition Service, USDA, U.S. Department of Agriculture, released a special specification allowing textured soy protein products to be used as acceptable meat alternates in National School Lunch Programs; this is the famous FNS Notice 219 (18). Since then, the Food and Nutrition Service requires suppliers of vegetable proteins to provide biological determination of the protein quality of their products. The minimum PER requirement is 1.8 on a basis of PER = 2.5 per casein. A list of acceptable FNS 219 products has been issued (19). As shown in Table VII, soy flours prepared by a combination of hexane:ethanol extraction and steaming had PERs of 2.17-2.20. The PER value of 2.2 for toasted 6 hr azeotrope-extracted soy flakes is 88% of that for casein (PER = 2.5), whereas the minimum PER requirement of 1.8 for protein products acceptable as meat alternates is 72% of that for casein (18). The azeotrope-extracted products, besides good nutritional value, have improved flavor characteristics. Flavor scores are given in Table VIII.

Soy Beverage Process: Alkaline Pretreatment

To prepare beverage products from whole soybeans, many investigators report flavor acceptability is improved by presoaking soybeans in alkaline solution, a process that also accelerates inactivation of TI activity during heat treatment (2). A summary of some of the effects of alkaline treatment is shown in Table IX (20). There is a sharp drop in PER of soy beverages processed at pH values above 8.0. Reduction in PER with increasing pH is related to the decrease in cystine content. Since sulfur amino acids are first limiting in soybeans, destruction of even small amounts of cystine under alkaline conditions has a large effect in decreasing the PER of soy beverages.

SOY PROTEIN ISOLATES

Nutritional Considerations

In contrast to soybean meal, a different spectrum of nutritional and biological factors affects protein quality of soy isolates (2). As the sole source of protein in the diet, soy protein isolates increase requirements for certain vitamins, decrease phosphorus utilization, and decrease bioavailability of essential trace minerals. Zinc availability is affected to the greatest extent.

Several investigators have found considerable variability in the PER values of commercial isolates; with some of the commercial isolates PERs can be improved to some extent by additional moist heat treatment. However, it is evident that the formation of protein-phytic acid-mineral complexes during manufacture is one of the primary factors responsible for the lower nutritive value of isolates compared to that of full-fat and defatted soy products (2). At the present time, vitamin-mineral supplementation is required to prepare soy protein formulas of acceptable nutritive value.

TABLE XI

Zinc Requirement and Carrier Capacity of Soy Protein Products (24)

Product	Supplementation, zinc, ppm	Solubility of ^{65}Zn,[a] %
Soybean meal	None	28
Soy protein isolate-A	15	7
Soy protein isolate-B	30	2

[a]Solubilization of ^{65}Zn from an insoluble ^{65}Zn-labeled phytate complex at intestinal pH of 6.8.

Availability of Zinc

As shown in Table X, zinc availability in soy protein isolates is very low compared to that for soybean meal, casein, and other animal products. Availability for isolates is in the midrange for cereals (21). Availability of zinc in defatted soybean meal is somewhat higher than in cereals and not as high as in animal products (22).

Although some of the variability in PER values of commercial soy isolates can be accounted for by differences in amino acid composition (23), the need for supplemental zinc is most likely the primary factor that affects nutritive value (24). As shown in Table XI, optimal growth of chicks fed soybean meal did not require addition of zinc to the diet. On the other hand, 15-30 ppm supplemental zinc was required to increase growth rate of chicks fed two commercial soy protein isolates.

The need for supplemental zinc in different soy protein isolate diets is due to the unavailability of the protein's zinc rather than the total zinc content of the ration. Chicks fed cottonseed meal and soy isolates without added zinc showed severe signs of zinc deficiency. Those fed safflower meal showed moderately severe zinc deficiency; those given soybean meal did not exhibit signs of zinc deficiency. The type of phytate-protein-mineral complexes, rather than phytate content, was responsible for these differences in zinc-binding capacity of these vegetable protein diets (24). Zinc in soybean meal digests is present as a water-soluble, dialyzable complex at pH 6.8 in the digestive tract of chicks. In this form, zinc is assimilated readily by the animal. The binding agent in soybean meal digests was termed a "carrier," and, as shown in Table XI, the digest solubilized 28% of the labeled ^{65}Zn in an insoluble Ca-Mg-^{65}Zn-phytate complex. Similar digests prepared from soy protein isolates A and B were able to solubilize only 7 and 2% of the zinc, respectively. On the basis of these results, protein isolates A and B contained low or no carrier properties. These differences in their ability to solubilize zinc at intestinal pH most likely must be related to the processing conditions used to manufacture these two isolates.

Research is needed to determine processing conditions that will prevent the formation of phytate complexes that

TABLE XII

Growth Response of Chicks to Supplements Added to Different Preparations of Soy Protein Isolates (25)

Supplement to basal diet	Mean body wt, g[a]			
	Defatted soy meal	Isolate A	Isolate B	Isolate C
None	138	70	51	58
Choline 0.2%	143	112	55	65
Methionine 0.4%	164	90	90	89
Choline + methionine	160	148	125	139

[a]14 Day trial; initial wt, 30-32 g.

reduce in vivo availability of zinc and other minerals. Probably the best solution to the problem is to prepare phytate-free isolates. As yet, no economical process has been developed.

Choline and Methionine Supplementation

Other factors that also need to be considered to improve the nutritive value of soy protein isolates are shown in Table XII. Of the three isolates evaluated in chick assays, only isolate A was found to have a biological value close to that of soybean meal, after supplementation with a mixture containing choline and methionine. When either choline or methionine or combinations of the two were added to diets containing the three different isolates, growth response of the chicks differed widely (25).

Nutritional quality of soy protein isolates can be rated as fair to good depending upon the conditions of manufacture. The four main factors to be considered during processing are: (A) choline deficiency; (B) variability in amino acid content, especially sulfur amino acids; (C) bioavailability of some vitamins and essential minerals; and (D) formation of phytate complexes, particularly those with zinc.

Studies now are underway to evaluate the nutritional properties of soy protein isolates prepared from hexane:ethanol azeotrope-extracted flakes. Normally, soy protein isolates contain appreciable amounts of choline containing phospholipids and other minor constituents. These components are readily extracted with aqueous alcohol (26). Since azeotrope extraction effectively removes residual lipids from hexane-defatted soy flakes, isolates prepared from azeotrope-extracted flakes would contain much lower amounts of choline derivatives.

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