

average, the food and fiber for 56 other people.

However, we have just begun to scratch the surface in our search for ways to raise the yields of soybeans. Some researchers say that even now the actual yield potential of present varieties is between 4.75 tons per hectare and 5.45 tons per hectare.

Research is presently being conducted to raise that yield potential and also to make sure that potential is reached by a large proportion of the farmers.

One problem researchers are working to solve is that high levels of nitrogen fertilization inhibit normal symbiotic fixation by the soybean plant. They are trying to find a way to make the two systems more compatible so that the soybean plant will produce its normal 100 pounds of nitrogen per acre by symbiotic fixation while utilizing increased amounts of nitrogen fertilization to produce higher yields. Researchers are working on developing a semidwarf plant that will be high yielding and resistant to lodging.

Soybeans are inefficient users of sunlight compared to corn, sorghum, and sugar cane. Photosynthesis in soybeans is retarded by a process called photorespiration. Researchers are trying to find a way to inhibit this process and thereby make soybeans more efficient photosynthetically.

Diseases claim an average of 10 to 12% of the annual crop. Work is being done to develop new disease-resistant

varieties as well as to develop new pesticides and fungicides. In addition, crop management experts are stressing the use of clean certified seed, which has much less chance of carrying the varying diseases and consequently will cut losses.

Harvesting losses have been known to cut yields by 8 to 10%. Part of the problem stems from the fact that equipment has been adapted to soybean harvesting rather than built specifically for the job. Work is being done on producing equipment that will cut harvesting losses to 1 to 2%.

Although the American Soybean Association does not conduct research itself, it does solicit funds for and finances research toward increasing yields, reducing losses, and improving the efficiency of production practices. It works closely with other organizations, such as the National Crop Improvement Council in coordinating research work and on delineating particular research needs and setting research priorities.

Traditionally, approximately one-half of the U.S. soybean crop has been sold on the international market. ASA's efforts are therefore devoted to seeing that there is an ample supply of soybeans for that market, seeing that the soy products are top quality products, and making sure our international customers are informed of the best and most efficient ways to use these products. ●



## Nutritional Aspects of Soy Protein Products

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### ABSTRACT AND SUMMARY

This paper is concerned with the nutritional properties of the soybean and of various food products derived from it. Although primary consideration is given to the protein, cognizance is made of other nutrients such as vitamins and minerals. Much of our knowledge concerning the nutritional properties of the soybean has been derived from experiments with animals, and such knowledge is frequently directly applicable to human nutrition. Nutritional experiments with human subjects pose special problems, the most difficult of which is acceptability. People will not eat a certain food simply "because it is good for them." Thus, the most serious hurdle to be overcome in the development of products containing soybean proteins is frequently not a nutritional one but one of consumer acceptance.

### NUTRITIONAL VALUE OF THE PROTEIN OF INDIVIDUAL SOY PRODUCTS

#### General Considerations

Nutritionists generally regard the amino acid composition of a protein to give a reasonable approximation of the nutritional quality of that protein. The amino acid composition of a given protein may be compared with a suitable reference protein such as whole egg protein, which is assumed to be an "ideal" protein, or the amino acid composition may be compared with what is regarded as the amino acid requirements for the normal growth of young children (1). Such a comparison will reveal the essential amino acid which is most limiting in the test protein, and the extent to which this particular amino acid is deficient is referred to as the "chemical score." In the case of soybean protein (see Table IV for detailed tabulation of the amino

TABLE I

Biological Evaluation of the Nutritive Value of Soybean Products as Determined by Rat Feeding Experiments

Source of protein	PER <sup>a</sup>		BV <sup>b</sup>		Digestibility		Reference
	Range <sup>c</sup>	Avg <sup>d</sup>	Range <sup>c</sup>	Avg <sup>d</sup>	Range <sup>d</sup>	Avg <sup>d</sup>	
Whole soybean							
Raw, immature		1.1		49		88	Everson et al. (12)
Autoclaved, immature		2.0					Everson et al. (12)
Raw, mature	0-1.6 (25)	0.7	41-74 (14)	58	75-89 (13)	82	Kuppuswamy et al. (1958)
Autoclaved, mature	0.4-2.0 (20)	1.3	67-67 (4)	64	83-94 (4)	90	Kuppuswamy et al. (1958)
Raw, germinated		1.4					Everson et al. (12)
Autoclaved, germinated		1.9					Everson et al. (12)
Grits, toasted		2.4					Huge (53)
Meal, solvent extracted							
Uncooked	0.3-0.6 (6)	0.5	50-53 (2)	52	67-81 (2)	74	Kuppuswamy et al. (1958)
Autoclaved	1.1-2.9 (21)	1.9	61-68 (2)	65		84	Kuppuswamy et al. (1958)
Meal, expeller pressed	0.6-2.5 (22)	1.8					Kuppuswamy et al. (1958)
Flour, defatted	1.5-2.4 (13)	1.8	60-75 (5)	69	82-96 (6)	89	Kuppuswamy et al. (1958)
Full-fat		1.6					Kon and Markuze (91)
Extrusion cooked		2.0					Smith (72)
Milk <sup>e</sup>	1.6-2.3 (7)	2.0		79		91	Desikachar et al. (178); Hackler et al. (32); Harkins and Saret (30); Chang and Murray (41); Shurpalekar et al. (21)
Curd	1.7-1.9 (2)	1.8	65-69 (3)	68		96	Pian (1930); Chang and Murray (41); Kobatake et al. (1964); Standal (76); Matsuno and Tamura (81)
Tempeh	1.2-3.0 (8)	2.2					Gyorgy (73); Smith et al. (72)
steamed, 2 hr		2.1					Hackler et al. (71)
Deep fat-fried, 7 min		0.6					Hackler et al. (71)
Natto		2.6		55		72	Arimoto (79); Standal (13)
Miso				72			Matsuno and Tamura (81)
Protein concentrated							
Unheated	0.3-2.5 (5)	1.4					Meyer (50); Longnecker et al. (43)
Heated		1.7					Longnecker et al. (43)
Protein isolate	0.6-1.9 (6)	1.3					Huge (53); Longnecker et al. (43)
Textured meat analog		2.1-2.3		65		92	Bressani et al. (55); Kies and Fox (141)

<sup>a</sup>Wherever possible, protein efficiency (PER) values have been corrected on the basis of a PER of 2.5 for casein.

<sup>b</sup>BV = biological value.

<sup>c</sup>Figures in parentheses refer to the number of observations which are included in the range of values shown.

<sup>d</sup>Where more than one observation is recorded, the figure shown is the average of the range of values shown in preceding column.

<sup>e</sup>See also Table III.

acid content of various soybean products) the limiting amino acids are those which provide sulfur (cystine and methionine), and, although the exact chemical score will depend on the kind of soybean product and on which reference pattern the comparison is made, values in the range of 60-70% are obtained. The chemical score in itself, however, does not reveal one of the most valuable attributes of soybean protein, namely the fact that it has a much higher content of lysine than do most plant proteins. The importance of this feature of the amino acid composition of soy protein will become evident in a later section dealing with the use of soy protein to complement other cereal proteins.

The chemical score, based as it generally is, upon the quantity of amino acids which can be recovered in an acid hydrolysate of a protein, assumes that the animal body can utilize all of each amino acid so measured. This is an assumption which does not take into account a number of factors which can alter the physiological availability of an amino acid such as: (a) the digestibility of the protein, that is, the extent to which amino acids are released from the protein by the digestive apparatus of the animal; (b) the rate at which amino acids may be absorbed from the gastrointestinal tract; and (c) complex interactions with other nutrients which may then affect (a) and (b). It follows therefore that, although a knowledge of the amino acid

composition of a protein can provide a valuable index as to its potential nutritive value, it is the actual performance of that protein in an intact animal which must be ultimately assessed in some fashion or other.

Ideally, any answers that the nutritionist comes up with should be directly applicable to man. Since, however, experiments with humans are complicated, time-consuming, and expensive, the nutritionist must rely very heavily on extrapolated interpretations of data obtained by animal experimentation. It cannot be argued that human requirements for amino acids are identical with that of the rat, particularly if one compares the requirements of a rapidly growing animal with that of a fully mature adult human. Even a human infant does not grow on a percentage basis at rates comparable to that observed with experimental animals (2). The requirements for the growth of an animal may bear little relevance to the requirements for repleting undernourished and convalescing bodies, for human lactation, or for generating immunological defenses against infection. Despite these reservations, experience has shown that protein evaluations based upon animal studies can be of value in assessing food proteins in human nutrition (3,4).

Frequent reference in this review will be made to such terms as protein efficiency ratio (PER), biological value (BV), digestibility based largely on animal experiments; in the case of studies with human subjects, N balance data will

TABLE II

## Biological Evaluation of Soybean Food Products Based on Experiments with Human Subjects

Source of protein	Biological value		Digestibility		References
	Range <sup>a</sup>	Avg <sup>b</sup>	Range <sup>a</sup>	Avg <sup>b</sup>	
Immature bean		65			Smith and Van Duyne (9)
Whole bean	95-97 (2)	96	90-91 (2)	91	Cahill et al. (239); Smith (240)
Soybean flour, defatted	61-92 (4)	81	88-94 (5)	92	Cahill et al. (239); Smith (240) Bricker et al. (83); Murlin et al. (241); DeMaeyer and Vanderbought (24)
Soybean flour, full-fat + methionine		64 75		84 86	Parthasarathy et al. (26,121)
Soybean milk	83-95 (4)	91	80-97 (6)	89	Cahill et al. (239); Smith (240); Desikachar et al. (189); Demeyer and Vanderbought (24)
Soybean curd		64	95-97 (2)	96	Oshima (242); Cheng et al. (243)
Protein isolate	60-81 (3)	71	81-89 (2)	85	Murlin et al. (244); Supplee et al. (200); DeMaeyer and Vanderbought (24)
Textured meat analog		81		92	Bressani et al. (55)
Other animal and plant proteins					
Eggs		97		97	Block and Mitchell (3)
Milk (cow)		90		91	DeMaeyer and Vanderbought (24)
Cottonseed flour		91		87	Murlin et al. (244); DeMaeyer and Vanderbought (24)
White flour		41		97	Bricker et al. (83)
Corn		30			Block and Mitchell (3)

<sup>a</sup>Figures in parentheses refer to the number of observations which are included in the range of value shown.

<sup>b</sup>When more than one observation is recorded, the figure shown is the average of the range of values shown in preceding column.

most often be referred to. Details concerning the meaning of such terms and the various procedures employed for their evaluation may be found in publications by the National Academy of Sciences, U.S.A. (1959,1963) and by the FAO (5,6).

#### Soybeans as a Vegetable

Soybeans have not been readily accepted as a fresh, frozen, or canned vegetable because of their objectionable odor and the difficulty in shelling the green bean (7). During World War II, when animal protein was in short supply, serious attempts were made to introduce certain varieties of soybean into the American diet as a fresh green vegetable. Salmon (8) estimated that a 100 g serving of fresh soybeans would supply 40% of the daily protein requirement of an adult. Smith and Van Duyne (9) and Simpson (10) have listed those varieties of soybeans which were found to be most suitable for use as a fresh vegetable or for freezing and canning.

The protein of the green immature soybean has been reported to be superior in nutritive value to the mature bean, and, when properly cooked, the BV of the protein compared favorably with that of casein and beef liver (11,12). Edamame is a popular Oriental dish prepared from green, immature soybeans which have been washed, shelled, and steamed for about 40 min. The net protein utilization (NPU) and PER of this food as measured with rats was found to be superior to that of most Oriental soybean foods (13) (see Table I for specific values). Smith and Van Duyne (9) cite an experiment involving human subjects in which cooked green soybeans had a BV of 65, a value which seems rather low compared to most soybean products (see Table II).

Mature soybeans have also been used to a limited extent in human foods (7,9). The cooking methods used in the preparation of dishes made from mature soybeans have a marked influence on the nutritive value of the protein. Preliminary overnight soaking of the bean and cooking in water rather than in the dry heat of an oven serve to enhance the nutritive properties of the protein (11,14,15). Dean (16) attempted to treat kwashiorkor in children in

Uganda by feeding them a mixture of cooked soybeans and bananas. The majority of the children were relieved of the signs of kwashiorkor and gained weight, but a few showed an aversion to this diet, and experienced digestive difficulties and a tendency to vomit.

#### Soybean Flour

One of the most common forms in which soybean protein is used in Western diets is as a flour. Soybean flour may be used in the human diet in any number of ways: (a) as a separate item of the diet, although problems of acceptability frequently limit its consumption in this fashion; (b) as an ingredient of a wide variety of common dishes such as soups, stews, beverages, and desserts; a number of recipes for preparing palatable dishes containing full-fat soybean flour have been described by Schlosser and Dawson (17); (c) in the formulation of bakery and cereal products or as a meat extender; (d) as a starting material for the preparation of infant formulas, protein concentrates, or isolates; (e) as a protein supplement to cereal grains and other foods.

Soybean flour has been used as the sole source of protein in human diets only under experimental conditions when more precise data regarding the nutritive value of the protein are desired. Representative data taken from such experiments have been included in Tables I and II based on animal and human experiments respectively. Of more practical interest is the use of soybean flour as a protein supplement to a well-accepted diet. In the early 1950s, a "Multi-Purpose Food" (MPF) in which toasted soybean flour provided the only source of protein was introduced as a dietary supplement for feeding in underdeveloped countries. The soybean flour was blended with essential vitamins and minerals in such a way that a 1-2 oz portion was estimated to provide one third of the daily requirement of these nutrients (18,19). Cooper and Bryan (20) reported that MPF at a level of 1 oz per day was an effective supplement to the diets of school children as measured by gain in weight. A formula similar to that of MPF except that it included dextrinmaltose and hydrogenated peanut oil was almost equivalent to the nutritive value of skim milk as

TABLE III

A Comparison of the Essential Amino Acid Composition of Soybean Milk with Cow and Human Milk

Essential amino acid	Source of milk			
	Soybean (traditional)	Soybean (commercial)	Cow	Human
	(grams per 16 g N)			
	Hackler and Stillings (33)	Harkins and Sarett (30)	Subrahmanyam et al. (245)	Rice (246)
Isoleucine	5.1	4.7	7.5	5.5
Leucine	8.3	8.1	11.0	9.1
Lysine	6.2	6.4	8.7	6.6
Methionine	1.4	1.2	3.2	—
Cystine	1.7	0.9	1.0	—
Total sulfur AA	3.1	2.1	4.2	4.0
Phenylalanine		5.3		
Tyrosine		—		
Total aromatics	9.0		11.5	9.5
Threonine	3.8	3.9	4.7	4.5
Tryptophan	1.3	1.1	1.5	1.6
Valine	4.9	5.0	7.0	6.2
		Nutritive value <sup>a</sup>		
BV <sup>b</sup>	80		87	100
Digestibility	95		91	90
NPU <sup>c</sup>	76		79	90

<sup>a</sup>Based on studies with African children [DeMaeyer and Vanderbought (24)].<sup>b</sup>BV = biological value<sup>c</sup>NPU = net protein utilization.

measured with rats and was recommended as a milk substitute for infants (21). Toasted full-fat soy flour (referred to in Japan as "kinako"), when fed as a main source of protein to weanling infants, was well accepted and supported good growth and N retention (22). Feeding trials on infants and children in a German orphanage conducted by Dean (23) showed that about one half of the milk in the diets of infants up to 1 yr of age could be replaced by a barley malt-soybean flour mixture and even more in the diet of older children without affecting their growth. In a study with African children, DeMaeyer and Vanderbought (24) found that soybean flour when used as protein supplement to a basal rice diet had reasonably good nutritive value although its BV was somewhat less than that of milk (see Table II). Panemangalore et al. (25), on the other hand, in similar studies with Indian children on a basal rice diet, found that defatted soybean flour when fortified with methionine was as good a protein supplement as that of skim milk. Similar results have been reported by Parthasarathy et al. (26) using full-fat soy flour. Biological evaluation with rats and chicks of full-fat soybean flour prepared by the extrusion-cooked process showed that its nutritive value was comparable to that of properly heated commercial full-fat and defatted soybean flours (27,28). This product, when used in conjunction with rice to feed infants, was not found to be appreciably different in nutritive value from whole milk powder (29).

The use of soybean flour in the formulation of bread and other baked goods and its value as a component of vegetable protein mixtures will be considered in the section dealing with soybean proteins as a protein supplement. The use of soybean flour as a starting material for the production of liquid formulas, protein isolates, and other products will be discussed in appropriate sections below.

### Soybean Milk

Soybean milk, in the traditional sense, is simply an aqueous extract of whole soybeans. Soybean milk has been of considerable interest to nutritionists as a possible substitute for cow or human milk particularly in the feeding of infants who are allergic to animal milk or where cow's milk may be either too expensive or unavailable. Soybean milk and cow's milk have approximately the same protein

content (3.5-4.0%), and a comparison of the amino acid composition of soybean and milk proteins (Table III) shows a fairly close correspondence. The main deficiency of soybean protein as compared with the protein of cow or human milk is that of the S-containing amino acids. Animal experiments in general have shown that the nutritive value of soybean milk ranges anywhere from 60% to 90% of that of cow's milk (see Table I); methionine supplementation raises its nutritive value to essentially the same level as that of cow's milk (21,30,31). Hackler et al. (32) have pointed out the sensitivity of the nutritive value of soybean milk to the time and temperature of cooking and subsequent drying of the liquid product. The rather wide variations in the biological data relating to the nutritive value of soybean milk could very well be a reflection of differences in processing conditions. Work by Hackler and associates (32-34) has shown that maximum nutritive value of the protein of soybean milk is attained within 5-10 min when the milk is heated at 121 C or in 60 min at 93 C, conditions which inactivate about 90% of the trypsin inhibitor. An impairment in nutritive value accompanies cooking at higher temperatures or for longer periods of time. Care must also be exercised in the control of heating conditions employed in the spray or roller drying of the fluid milk product to prevent heat damage to the protein.

A considerable body of data obtained with human subjects may be found in the literature pertaining to the effectiveness of soybean milk as a replacement for cow or human in the nutrition of infants and young children. Much of the earlier work has been reviewed by Jones (35) and Miller (36), and a more recent review has been provided by Swaminathan and Parpia (37). Theoretically, at least, the essential amino acids provided by the protein of soybean milk should satisfy the requirements of infants to the same extent as cow or human milk when administered at the same level of protein intake (Table IV). Criteria for measuring the ability of the protein of soybean milk to replace mammalian milk have included N-balance studies, weight gain, increase in body length, hemoglobin, and serum protein. Without going into the specific details, such studies have, in general, failed to reveal any significant margin of superiority of cow or human milk over soybean milk suitably fortified with vitamins and minerals [see, for

TABLE IV

Essential Amino Acid Requirements of Infants Compared with Intakes of Protein from Soybean Milk or Cow and Human Milk<sup>a</sup>

Amino acid	Minimum requirement (mg/kg/day)	Amino acid provided by protein fed at a level of 2 g protein/kg/day		
		Human milk	Cow milk	Soybean milk
Histidine	34	32	45	33-57
Isoleucine	119	123	128	67-117
Leucine	150	230	216	91-159
Total S-amino acid	45	73	52	31-55
Total aromatic amino acid	90	92	104	65-115
Threonine	87	89	92	51-89
Tryptophan	22	31	30	11-20
Valine	105	128	138	67-117

<sup>a</sup>Source: Holt and Snyderman (247).

example, Kay et al. (38); Fomon (39)]. These conclusions are somewhat at variance with the animal experiments cited earlier which showed soybean milk to be somewhat inferior in nutritive value to mammalian milk. This difference may perhaps be due to a less intense requirement for the sulfur amino acids by a growing child than by young rats [Block and Mitchell (3)]. Thus, although supplementation with sulfur-containing amino acids balances the protein values of soybean milk for rats, this deficiency appears to be of little consequence in practical infant feeding (40).

### Soybean Curd

From the amino acid composition of soybean curd or "tofu" shown in Table V, tofu does not appear to differ significantly from the protein found in most other soybean products. The biological evaluation of the nutritive value of soybean curd using animals or human subjects has given values (Tables I and II) which are comparable to properly heated soybean flour. Since autoclaving does not enhance the nutritive value of the curd (41), it may be concluded that tofu has received adequate heat treatment during processing for optimum nutritional value.

Tofu has been tested as a source of protein in the solid diet of weanling infants and its performance evaluated with respect to acceptability, weight gain, nitrogen balance, and serum protein level (22). On the basis of these criteria, tofu was judged to be nutritionally equivalent to the protein derived from a mixture of eggs, fish, and liver. Schroder et al. (42) have recently reported that soybean, specially prepared to reduce its beany flavor, has a PER which was ca. 85% that of casein; a deficiency of methionine was judged to be responsible for this lower value.

### Protein Concentrates

A product containing ca. 70% protein can be produced by removing much of the non-nitrogenous constituents from soybean flakes or flour by extraction with ethyl alcohol, dilute acid, or water following denaturation with heat. In addition to its high protein content, such a product is largely free of objectionable flavors and odors, and it has the functional properties which make it suitable for use in a variety of food products such as bread, cereals, and comminuted meat products.

On the basis of the amino acid composition of soybean protein concentrate (see Table V) one would not expect its nutritive value to differ very much from that of soybean flour. Longenecker et al. (43) found considerable variation in the PER values of a number of unheated commercial soy protein concentrates. When subjected to heat treatment (105 C for 30 min), however, values approaching that of soybean flour were obtained. The authors concluded from these observations that there must be marked differences in manufacturing processes for soybean concentrates so that optimal heat treatment is not always attained. Meyer

(44,45), on the other hand, found that soy protein concentrates have PERs equivalent to casein, and he was not able to demonstrate any improvement by heat in the nutritive value of the several commercial products examined. In general, one is forced to question the wisdom of trying to draw conclusions from feeding tests involving commercial soybean products whose processing history is not known or, if known, is not given. Furthermore, some commercial products may have deliberately received light or no heat treatment if they are intended for food applications where further heat treatment will be used.

### Protein Isolates

The nutritive value of the protein isolated from soybeans received attention as long ago as 1912 when Osborne and Mendel (46) found that the main protein of soybeans had about two thirds of the growth-promoting value of casein. Many years later De and Ganguly (47) reported that the PER of this protein was about the same as that of autoclaved soybean meal. Most of the more recent nutritional studies have been conducted with soybean protein isolated by extracting soybean flakes or flour at an alkaline pH, followed by precipitation of the protein at an acid pH, and subsequent removal of the bulk of the water. Such preparations consist almost exclusively of protein (93-95%) and represent ca. 86% of the protein from unheated soybean meal or 60% from heated meals (48). Data relating to the nutritional evaluation of soybean protein isolates with animals and human subjects may be found in Tables I and II respectively. In general, such data have shown that the nutritive value of isolated soybean protein as measured by PER or BV is ca. 65-85% that of isolated milk protein (casein), and ranks somewhat lower than the protein concentrates discussed earlier (44,45,49).

As the amino acid data indicate (Table V), soybean protein isolate is more deficient in the sulfur amino acids than are most other soybean preparations, a fact which has received biological confirmation with rats (50), pigs (51), and human subjects (52). The supplementation of protein isolates with methionine raises its nutritive value to that of casein when assayed with rats (53) and to ca. 85% of that of milk proteins when newborn pigs are used as the experimental animal (54).

Several investigators (43,48,55) have reported that the nutritive value of soybean protein isolates can be improved by heat treatment, whereas others (44,53,56) have not noted such an effect. The most obvious explanation for this discrepancy would be that the technique employed for the isolation of soybean protein may not also always lead to the elimination of heat-labile growth inhibitors. This, in turn, may be a reflection of the thoroughness with which the isolated proteins have been washed during processing.

Heating a soybean protein isolate with glucose causes a reduction in the nutritional value of the mixture which is

TABLE V  
Essential Amino Acid Composition of Various Soybean Products

Source of soybean protein	Protein <sup>b</sup> content (%)	Essential amino acids <sup>a</sup> (g/16 g N)											Trp	Val	Reference
		Ile	Leu	Lys	Met	Cys	Met + Cys	Phe	Tyr	Phe + Tyr	Thr				
Whole soybeans	34.3	4.2	7.4	6.4	1.1	—	2.3	4.5	—	3.6	1.7	4.3	Evans and Bandemer (248)		
Whole meal, defatted, dehulled	52.6	4.4	7.2	5.4	1.2	0.8	2.0	4.6	3.4	3.6	1.3	4.0	Harmon et al. (186)		
Whole meal, defatted, hulled	45.7	4.6	7.7	5.6	1.3	0.9	2.2	5.0	3.6	4.1	1.4	5.0	Harmon et al. (186)		
Whole green soybeans (Edamame)	37.5	6.6	7.1	8.5	0.8	—	—	5.0	2.8	1.9	1.0	5.6	Standal (76)		
Dehulled soybeans	47.5	4.9	7.9	6.5	1.5	1.2	2.7	5.4	3.6	3.8	0.9	5.2	Smith, A.K. et al. (72)		
Whole soybeans, defatted	44.7	4.8	7.0	6.1	1.3	2.1	3.4	4.6	3.0	4.7	1.8	5.3	Tkachuk and Irvine (249)		
Full-fat chips	—	4.8	7.7	6.2	1.4	1.7	3.1	5.2	3.8	4.2	—	4.9	Iriarte and Barnes (250)		
Whole full-fat flour	46.6	4.8	7.8	6.5	1.4	1.6	3.0	5.1	3.9	4.2	—	5.0	Iriarte and Barnes (250)		
Dehulled, full-fat flour	42.0	4.6	7.6	6.3	1.3	1.6	2.9	5.0	3.8	4.2	—	5.2	Iriarte and Barnes (250)		
Defatted flour	59.0	4.6	7.7	6.2	1.3	1.2	2.5	5.3	—	4.2	1.4	4.9	Meyer (44)		
Curd (tofu)	57.4	4.1	6.7	5.6	1.3	—	—	5.5	3.2	2.2	2.1	4.1	Standal (76)		
Curd, acid-precipitated	59.0	4.9	8.0	5.9	1.4	1.7	3.1	4.8	3.7	3.7	1.1	4.7	Hackler et al. (251)		
Tempeh, laboratory product	53.1	4.9	7.9	6.3	1.5	1.2	2.7	5.0	3.7	3.9	1.0	5.2	Smith, A.K. et al. (72)		
Tempeh	—	4.6	7.7	5.5	1.3	1.6	2.9	5.1	3.5	3.6	1.2	4.4	Stillings and Hackler (68)		
Natto	57.5	5.8	8.4	11.1	0.7	—	—	5.4	3.0	4.1	2.2	6.4	Standal (76)		
Miso	17.3	6.5	13.1	5.9	1.2	—	—	6.4	4.1	3.0	1.2	5.7	Standal (76)		
Concentrate	71.0	4.9	8.0	6.6	1.3	1.6	2.9	5.3	3.7	4.3	1.4	5.0	Huge (53); Meyer (50)		
Isolate	96.0	4.6	7.6	5.4	1.2	0.8	2.0	5.5	3.6	3.5	—	4.0	Bressani et al. (55)		
Textured meat analog	56.0	3.3	5.1	3.2	0.9	1.0	1.9	4.0	3.1	2.5	—	3.2	Bressani et al. (55)		

<sup>a</sup>The following abbreviations have been used: Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Cys, cystine; Phe, phenylalanine; Tyr, tyrosine; Thr, threonine; Trp, tryptophan; Val, valine.

<sup>b</sup>On moisture-free basis.

accompanied by an increased resistance to enzymatic hydrolysis by Pronase (57). In contrast, however, protein isolate heated in the absence of glucose caused an increase in PER and an increase in enzymatic hydrolyses. These results indicate that (a) the browning reaction may cause an adverse effect on the protein quality of soy protein when heated in the presence of carbohydrate, and (b) heating may improve the nutritive value of soy protein due to an enhancement of protein digestibility which may, to some extent, be unrelated to the presence of trypsin inhibitors.

Alkaline extraction of soybean meal under more drastic conditions may yield an inferior product not because of the failure to eliminate growth inhibitors but because of damage to certain amino acids. DeGroot and Slump (58) found that the protein isolated by acid precipitation from soybean meal which had been extracted at pH 12.2 for 4 hr at 40 C had a much lower NPU than the original meal. They attributed this decrease in nutritive value to the destruction of cystine which is accompanied by the formation of lysinoalanine (to be discussed in more detail in a subsequent section). The latter is produced by the interaction of dehydroalanine, which is a decomposition product of cystine and serine, and the  $\epsilon$ -amino groups of lysine. Supplementation of the alkaline-damaged protein with methionine partially resored its nutritive value but not fully to the level of the untreated protein source.

The use of soybean protein isolates in the formulation of infant milk formulas has received considerable attention in recent years. Using normal infants as experimental subjects, Cherry et al. (59) compared the nutritional properties of a milk formula containing 2.3% isolated soybean protein with a similar formula in which the protein was derived from skim milk at an equivalent protein concentration. Using the criteria of weight gain, levels of serum protein, amino acids, cholesterol, and other hematologic data, these authors reported that growth was somewhat better on the skim milk formula although the biochemical and hematologic data were similar. Bates et al. (60) subsequently pointed out that the inferior performance of the soybean formula may have been due to that fact that it provided only about half of the recommended level of methionine. When a milk formula containing soybean protein isolate as the sole source of protein was fortified with methionine, Bates et al. (60) were then unable to find any significant difference from formulas prepared from either cow's milk or soybean flour. Similar results were reported by Graham et al. (61) and Fomon et al. (62) who, on the basis of N-balance studies with infants could find little difference between soy protein isolate formulas fortified with methionine and fresh or processed human or cow's milk. It is significant to note that these workers, as well as others (63) have observed that the formulas containing soy protein produce a lower incidence of anal irritation than do the ones made from soybean flour, an effect which can no doubt be attributed to the absence of fiber in the purified protein.

Leake et al. (64) have reported that a soy protein isolate formula facilitated the recovery phase of infantile diarrhea produced by gastroenteritis compared to a formula based on cow's milk. The superior performance of the soy protein formula over cow's milk in this report was attributed to the fact that the infants may have had a gastrointestinal deficiency of lactose. This observation takes on added significance when it is realized that lactose intolerance among children is a very common occurrence in many parts of the world (65).

Recognizing the fact that soybean protein isolates are deficient in the sulfur amino acids, Zzulka and Calloway (66) conducted metabolic studies on men to ascertain the minimum amount of soy protein that would be needed to maintain N balance with and without supplemental methionine. In the absence of added methionine N balance was achieved with diets in which 67% to 84% of the total

protein fed was derived from soy protein. With methionine supplementation, however, the amount of soy protein required for N balance was only 33-50% of the dietary protein.

## Fermented Products

Although fermented soybean products have been used for centuries in the Orient, up to quite recently information concerning the nutritional properties of such foods has been scanty. The nutritive properties of three fermented products will be considered here—namely tempeh, miso, and natto. Miso and natto are products resulting from the fermentation of cooked soybeans by *Aspergillus oryzae* and *Bacillus subtilis* (*B. natto*), respectively, and enjoy popular consumption in Japan. Tempeh is an Indonesian dish composed of soybeans which have been cooked and then fermented by the mold *Rhizopus oryzae*.

*Tempeh*: The protein content of tempeh is ca. 20% on a wet basis and 50% when dried. It is very seldom eaten raw, but is usually roasted, cooked in soup, or fried in oil (16). The amino acid composition of the protein of tempeh (Table V) is not grossly altered by the fermentation process, although decreases in lysine and methionine have been reported to occur during fermentation (67,68). There is also a slight increase in free amino acids during fermentation (69), presumably due to the action of proteolytic enzymes elaborated by the mold. Steaming (100 C for 2 hr) of the fermented product had little effect on its amino acid composition, but as much as 20% of the cystine and lysine was destroyed during deep-fat frying (68).

The earliest comment bearing directly on the nutritive value of tempeh is that of Van Veen and Schaeffer (70) who claimed that tempeh is more easily digested than the unfermented bean and that the protein is excellent in quality. Specific data relating to the biological evaluation of the protein value of tempeh are to be found in Table I. Most investigators have found tempeh to offer little, if any, nutritional advantage over unfermented soybean products (67,71,72). On the other hand Gyorgy (73) noted considerable variation in the PER values of a number of tempeh preparations which had been processed under varying conditions, and some of those did have PER values which were higher than an unfermented control. Smith et al. (72) saw no evidence of pancreatic hypertrophy in animals fed tempeh, which would indicate that the trypsin inhibitor had been destroyed, probably during the preliminary cooking of the soybeans prior to inoculation with the mold. This perhaps explains why mild heating of tempeh (steaming for 2 hr) produces no further improvement in nutritive value (71). Deep-fat frying (7 min), on the other hand, significantly reduced its PER and digestibility. These biological effects are in accord with the amino acid data discussed in the previous paragraph.

Bai et al. (74) have prepared a tempeh-like product from a mixture of soybeans and peanuts which had a higher PER than did tempeh made from soybeans alone.

In view of the fact that tempeh is a product intended solely for human consumption, it is indeed surprising that so little work appears to have been done with human subjects. In this connection, the following quote from Grant (75) is of interest: "... many attempts were made (by prisoners in Japanese prison camps during World War II) to make soybeans palatable and digestible, the only satisfactory method proving to be one common in Indonesia, involving inoculation with a fungus. Otherwise these beans were likely to give much digestive disturbances when used in any quantity, even if first reduced to a fine meal." This statement would suggest that the principal virtue of tempeh lies in the fact that some of the oligo-saccharides believed to be responsible for flatulence have been eliminated, this loss most likely occurring when the water used for cooking is discarded.

TABLE VI

Examples of the Supplemental Relationship between Soybean and other Plant Proteins

Source of test protein	Level of protein in diet (%)	(% of total protein in diet)		PER <sup>a</sup>	Reference
		Test protein	Soybean protein		
Corn meal	9	100	0	1.43	Bressani and Elias (125)
	9	80	20	2.15	
	9	60	40	2.53	
	9	40	60	2.71	
	9	20	80	2.61	
Wheat flour	9	100	0	0.77	Sure (252)
	9	91	9	1.03	Jones and Divine (92)
	9	65	35	2.16	
Polished rice	6	100	0	1.76	Sure (95)
	6	92	8	1.84	
	7	79	21	2.08	
	8	68	32	2.15	
Rye flour	6	100	0	1.29	Sure (253)
	8	38	62	2.43	Kon and Markuze (91)
Sesame	10	100	0	1.73	Tasker et al. (254)
	10	35	65	2.17	Chang and Murray (41)

<sup>a</sup>PER = protein efficiency ratio.

*Natto*: Natto is used as a side dish or in combination with cooked rice. The protein content of natto is approximately the same as that of tempeh, and the essential amino acid composition of the protein (Table V) does not differ appreciably from most other soybean products except for somewhat higher values for tryptophan and lysine (76). Experiments with rats (Table I) have shown that the nutritive value of natto is somewhat less than that of unfermented cooked beans (13,77-79). The product obtained from 8 hr of fermentation was superior in nutritive value to that which had been fermented for only 4 hr (79). Supplementation with methionine and/or lysine had little effect on the protein quality of natto (78).

There is some indication that a more judicious choice of organisms may enhance the nutritive value of natto. For example, Chah et al. (80) have reported that natto prepared with ten out of eleven strains of *Aspergillus* produced a significant improvement in the weight gain and feed efficiency of chicks. Amino acid analyses indicated that this improvement may have been due to an increased supply of essential amino acids.

Only limited studies have been conducted on natto with human subjects. Muto et al. (22) tested natto as a source of protein in the diet of infants and concluded that it could substitute, at least in part, for animal protein with no adverse effects on growth, digestibility, and N retention. Natto in the form of a powder has also been used for making biscuits which were well accepted by children without adverse effects (79).

*Miso*: Miso differs from natto and tempeh in that it is prepared from a mixture of soybeans and rice in varying ratios with added salt. The rice is first inoculated with *A. oryzae* and, after the rice is mixed with soybeans, the fermentation is allowed to proceed for periods of up to 1 yr. The final protein content ranges from 10% to 17%. The largest single use for miso is in soups. It is also spread on bread and on raw vegetables to add flavor and is used, in combination with sugar and oil, for cooking fish, meat, and vegetables.

The amino acid composition of a preparation of miso made from a 1:1 mixture of soybeans and rice is shown in Table V. The growth rate of rats fed this preparation of miso was comparable to that of those fed natto and heated soybeans (78). Matsuno and Tamura (81) reported the biological values of miso to be 71-73 compared to a value of 69 and 80 for tofu and casein, respectively.

Diamant and Laxer (82) have described the preparation of a miso-type product prepared from defatted soybean flakes and one of the following cereals: corn, wheat, barley, sorghum, or potatoes. Such products, however, were very poor in nutritive value, and amino acid analysis showed an absence of methionine and inadequate levels of tryptophan and arginine. Supplementation with these three amino acids improved growth somewhat, but growth was still far less than that of the unfermented mixture of soybeans and cereal. The authors presented evidence to indicate that the high salt concentration (8%) of the miso-type product may have been partly responsible for its poor growth-promoting quality.

The paucity of reports dealing with the nutritive evaluation of miso with human subjects may, perhaps, be due to the fact that its high salt content precludes its testing at a protein level needed to produce meaningful results. It may also be that miso has been for so long such an important part of the Japanese diet with apparent impunity to health that its nutritive merits have gone unchallenged.

### BLENDING SOY PRODUCTS

The deficiency of sulfur-containing amino acids which characterizes the protein of most soybean products can sometimes be minimized by combining such products with other proteins which are not deficient in these amino acids. But more important, perhaps, is the fact that any amino acid which may be limiting in the other protein may be present in excess amounts in the soybean protein. In other words, it is possible to combine soybean protein with other proteins in such a way as to provide a mixture which is nutritionally superior to each one alone. Since soybean protein is a rich source of lysine, the soybean provides an excellent means of correcting the lysine deficiency of most plant proteins. Examples of the complementary effect of soybean protein on cereal proteins are shown in Table VI. It is evident that marked improvements in PER values are obtained when soybean protein is used to complement protein derived from various cereals. This general conclusion, based largely on experiments with rats, has been confirmed in the case of wheat protein in N-balance studies with human subjects; Brickler et al. (83) reported that the biological value of white flour, which was 41, could be increased to 55 if 19% of the wheat flour were replaced by soybean flour.



## Blend with Wheat Protein

*Bread:* Because of the varied complementary effect which soybean protein exerts on lysine-deficient wheat protein (84), much attention has been directed to use of soybean flour or soybean protein concentrates in bread formulations [see reviews by Bailey et al. (85); Horvath (86); Burnett (87); Diser (88); Hayward and Diser (89)]. As early as 1921, experiments conducted by the USDA showed that bread made with a mixture of 25 parts soybean flour and 75 parts wheat flour was adequate for the growth of rats (90). In 1931, Kon and Markuze (91) reported that the PER of a bread formula could be markedly increased when 11-25% of the wheat flour was replaced by soybean flour. This improvement in the nutritive value of bread by adding soybean flour has been subsequently confirmed in many laboratories (35,92-97). The effect of substituting soybean flour for dried skim milk solids in bread formulas has also been studied. Carlson et al. (98) reported that bread which contained 3% soybean flour was equal in nutritive value to bread containing 3% milk solids and significantly better than bread made from unsupplemented formulas. Harris et al. (99), Volz et al. (94), and Henry and Kon (100) likewise showed that soy flour could not only completely replace the milk solids of bread without affecting its nutritive properties, but actually improved the nutritional value of bread already containing skim milk solids. Although the protein quality of bread continues to increase as the proportion of soybean flour in the formula is increased, there is a practical upper limit to the amount of soybean flour which can be used in this fashion. When more than 6% of the wheat flour is replaced by soybean flour there is some deterioration in bread character, and alterations in dough handling techniques become necessary (87,101,102).

Only limited human studies on the nutritive value of breads containing soybean flour have been reported in the literature. Horvath in his review of 1938 (86) refers to N-balance experiments which were conducted on human subjects in Italy and Germany in the 1920s. It was concluded from these studies that white or rye bread containing as much as 20% soybean flour was well tolerated, and utilization of the protein was better than that of breads containing wheat or rye flours alone.

Although soybean flour has been the most common vehicle for incorporating soybean protein into bread, the possibility of using more concentrated sources of soybean protein has not been overlooked. Wilding et al. (103) have studied the nutritional consequences of supplementing the wheat protein of bread with a soybean concentrate containing 70% protein. Increasing the percentage of soy protein in relation to the wheat protein produced significant increases in PER with a maximum value being obtained with 75% soybean protein and 25% white bread protein. The supplementation of wheat flour with soybean protein isolates has likewise proved to be a very effective way of enhancing the nutritive value of bread (104-107). The improvement in protein quality produced by supplementation with soybean protein isolate is proportional to the amount of lysine provided by the soybean protein (105,107).

*Other Wheat-containing Goods:* Experimental data are also available to demonstrate that soybean flour can be used to enhance the nutritional quality of other food products containing wheat protein. Reynolds and Hall (108) reported that various cakes and pastries to which soybean flour had been added were nutritionally superior to the nonsupplemented baked goods in terms of growth, PER, and N retention in experiments with rats. The addition of soybean protein in the form of grits to commercial formulas for cookies (89) or graham crackers (109) markedly improved the nutritive quality of the protein. The nutritional property of pasta (durum wheat flour) is

markedly enhanced by the addition of soy flour (110).

## Blend with Corn Protein

It is evident from the data presented in Table VI that soybean protein has a marked supplemental effect on the nutritive value of corn protein, particularly in a ratio of 40% corn protein to 60% soybean protein. This supplementary relationship is also evident from the data of Bressani and Marengo (111) who found that the PER of lime-treated corn flour was increased from 1.0 to 2.5 by adding 8% soy protein isolate or 10% soybean flour. Along related lines, Cravioto et al. (112) reported that the PER of tortillas made from lime-treated corn plus 10% soybean flour was 1.80 compared to only 1.0 when only lime-treated corn was used. Bressani (84) also reported the nutritional advantage obtained by adding soy flour (at a level of 8%) or whole soybean (at a level of 15%) to 85% corn for making tortillas. Advantage has been taken of the supplementary relationship of corn and soybean proteins in the formulation of special mixtures of plant proteins for use in developing countries, as is described below in the section dealing with vegetable protein mixtures.

Akinrele and Edwards (113) have described the preparation of a mixture of 70 parts soured corn, which is a native dish in Nigeria, and 30 parts full-fat soybean flour. This mixture, referred to as "soy-ogi," was successfully used as a weaning food for the restoration of normal health to children suffering from kwashiorkor.

## Blend with Rice

The supplementary effect of soybean flour on rice protein as demonstrated with rats (see Table VI) has been corroborated in studies with infants and children. Mixtures of soybean flour and rice have been fed to infants and found to support growth comparable to that resulting from cow's milk although the digestibility of this mixture is somewhat less than that of milk (29,114,115). Panemangalore et al. (116) fed a group of children in India a basal diet consisting mainly of rice supplemented with suitably processed soybean flour or skim milk powder. Both supplements resulted in a significant increase in N retention, biological value, and NPU, with little difference between the soybean flour and milk. Experiments along similar lines were conducted by DeMaeyer and Vanderbought (24) on African children. In this case, however, the soybean flour proved to be somewhat less satisfactory than the skim milk, which the authors attributed to improperly processed soybean flour.

Siegel et al. (117) have described the preparation of noodles containing 18% protein of which 10% was derived from full-fat soybean flour and 8% from rice. The PER of soy-rice noodles was 2.56 compared to a value of 2.17 for noodles made from rice alone and a value of 2.5 for casein as a control. Along similar lines a mixture of 82.5 parts rice and 15.4 parts tempeh gave a PER equivalent to casein (118).

## Formulation of Vegetable Protein Mixture for Use in Underdeveloped Countries

Recognizing the many advantages to be derived from the use of properly processed soybean flour as a protein supplement to other plant proteins, considerable effort has gone into the formulation of blends of soybean protein with other plant proteins. These mixtures, when suitably fortified with vitamins and minerals, have great potential for the feeding of infants, children, and adolescents in developing areas of the world. Whether used as a sole source of food for the weaning of infants or as a protein supplement, the nutritional quality of these mixtures should be of the highest order because it must supply the nutrients missing in the diets being consumed by populations of low economic resources.

TABLE VII

## Nutritive Value of Protein-rich Supplements Containing Soybean Protein

Source of proteins	Proportions <sup>a</sup>	Protein content (%)	PER <sup>b</sup>	Reference
Corn/soy flour (Incaparina 14)	58/38	26	2.6	Bressani (124)
Corn/soy flour + 0.2% methionine	58/38	26	2.9	Bressani (124)
Corn/soy flour + 0.2% methionine + 0.2% threonine + 0.2% lysine	58/38	26	3.4	Bressani (124)
Corn/extrusion cooked full-fat soy flour	43/40	21	2.5	Smith, O.B. (131)
Wheat/soy flour (WSB)	73/20	23	2.1	Senti (127)
Wheat/soy flour	40/60		2.6	Parpia (255)
Wheat/soy flour + methionine	40/60		3.0	Parpia (255)
Peanut protein isolate/full-fat soy flour	50/50	26	2.3	Korula et al. (256); Shurpalekar et al. (257-259)
Same as above + 0.4% methionine	50/50	26	2.9	
Peanut flour/full-fat soy flour + 1% methionine	50/50	50	2.5	Shurpalekar et al. (258)
Peanut flour/full-fat soy flour + 1% methionine + 1% lysine	50/50	49	2.8	Narayana Rao et al. (119,260)
Skim milk powder/soy flour	70/30	57	2.2	Prasanna et al. (123)
Peanut flour/sesame flour/soy flour	48/20/30	16	2.3	Krishnamurthy et al. (15)
Peanut flour/sesame flour/soy flour	40/30/30	44	2.4	Panemangalore et al. (261)
Peanut flour/sesame flour/soy flour	40/20/40	54	2.4	Prasanna et al. (123)
Corn meal/skim milk/soy flour (CSM)	64/5/24	19	2.5	Senti (127)
Corn/cottonseed flour/soy flour (Incaparina 15)	58/19/19	26	2.2	Bressani (124)
Corn/cottonseed flour/soy flour + 0.2% methionine + 0.1% lysine	58/19/19	26	2.7	Bressani (124)
Peanut flour/wheat/soy flour	30/60/10		2.4	Parpia (255)
Peanut flour/wheat/soy flour + 1% methionine	30/60/10		2.5	Parpia (255)
Wheat/sesame/soy flour	60/15/25		2.6	Parpia (255)
Peanut flour/coconut meal/soy flour	30/30/40	42	2.3	Tasker et al. (254)
Peanut flour/Bengal gram/soy flour	48/25/25	16	1.7	Krishnamurthy et al. (262)
Sesame flour/chick pea flour/soy flour	35/47/18	38	2.9	Guggenheim and Szmelcman (133)
Peanut flour/sesame flour/Bengal gram/soy flour	38/20/28/20	16	2.1	Krishnamurthy et al. (262)
Peanut flour/sesame flour/chick pea flour/soy flour	30/10/30/30	26	1.8	Joseph et al. (263)

<sup>a</sup>Proportions shown in same sequence as presented in column 1.

<sup>b</sup>Wherever possible, protein efficiency ratio (PER) values have been corrected on the basis that the PER of casein or skim milk powder = 2.5.

The formulation of vegetable protein mixtures has generally involved the blending of soybean protein with the proteins derived from plant sources which may already be a traditional part of the diet in a particular country. Scientists at the Central Food Technological Institute (CFTI) in India, for example, have been particularly active in the development of mixtures in which soybean protein is used to supplement the protein of peanuts and indigenous legumes. At the Institute of Nutrition of Central America and Panama (INCAP) in Guatemala, corn and cottonseed meal have been used in conjunction with soybean protein. Such mixtures are fortified with essential vitamins and minerals, and, in some cases, essential amino acids, in order to insure an adequate intake of these nutrients. The biological evaluation of some of these plant protein mixtures, in terms of their PER as determined with rats, is presented in Table VII. It will be noted that with some of these mixtures, particularly those which have been fortified with methionine, PER values closely approaching those of milk protein have been obtained.

A number of these protein blends have been evaluated in clinical trials with children. A blend composed of an equal mixture of full-fat soybean flour and peanut flour, and fortified with 1% L-lysine and 1% DL-methionine as well as vitamins and minerals, when fed as a supplement to children on basal diet of corn and tapioca, produced an increase in height, weight, and hemoglobin content of the blood comparable to skim milk powder (25,119). This same blend of soybean and peanut flours markedly increased the growth and N retention of school children on a basal diet of rice (120,121). Blends containing sesame flour or coconut meal in addition to soybean and peanut flours have also been tested on children. A blend of soybean flour, peanut flour, and coconut meal (4:3:3), when used to supplement a basal wheat diet of young school children, brought about a significant increase in the retention of nitrogen (122). A microatomized blend of soybean flour, peanut flour, and sesame flour (2:2:1), administered as a liquid emulsion to children suffering from moderate to severe kwashiorkor,

proved to be an effective cure (123).

Incaparina is the generic name given to a series of vegetable protein mixtures developed by INCAP, so formulated as to provide 25% or more of protein comparable in quality to that of animal origin. Originally the Incaparinas were mixtures of corn and cottonseed flour, but, in some of the more recent formulations, the cottonseed flour has been replaced wholly or partially by soybean flour. The formulation of Incaparina was based on the observation previously noted that maximum complementation between corn protein and soybean protein is obtained when 20-40% of the protein in the diet is derived from corn and 60-80% from soybean. Incaparina 14 consists of 59% corn, 38% toasted soybean flour, 3% torula yeast, 1% CaCO<sub>3</sub>, and 4500 IU vitamin A per 100 g (124). These mixtures may be used directly to prepare a beverage by simply adding water or they may be incorporated into foods such as soups, puddings, cookies, precooked baby foods, etc.

PER values for Incaparina with and without amino acid supplementation, may be found in Table VII. The nutritive value of the unsupplemented formulas was somewhat inferior to casein. When supplemented with a combination of methionine, threonine, and lysine, however, their PER values exceeded that of casein. Incaparina had a BV value of 73% and a digestibility coefficient of 80% when tested with dogs, values close to what was obtained with milk protein under the same conditions (125). The following data, taken from studies with children (125) likewise show that the nutritive value of Incaparina was quite comparable to that of skim milk.

	BV	Digestibility	Mg N required for N equilibrium
Skim milk	80.6	92.0	79
Incaparina 14	78.6	91.8	92

A food supplement containing 19% protein derived from

a blend of 63.8% processed corn meal, 24.2% toasted defatted soybean flour, and 5% nonfat dried milk was developed by the American Corn Millers Federation in cooperation with USDA (126,127). This mixture, referred to as CSM, also contains 5% soybean oil and is fortified with 2% of a vitamin-mineral premix. CSM was designed to provide a level of all the essential nutrients sufficient to render adequate the total diet of a preschool child if consumed at a level to supply ca. 25% of the energy needs. CSM may be served in the form of a gruel or porridge for school children and infants or used in baked goods, soups, and other recipes. Feeding experiments with rats have shown CSM to have a PER essentially equivalent to that of casein (127). CSM has also been tested in a number of feeding trials with infants and children (128-130). The results of these trials have uniformly showed CSM to be well accepted and capable of supporting satisfactory weight and N equilibrium.

Smith (131) has described the preparation of an extrusion cooked mixture of full-fat soy flour and degerminated corn meal (40:43) as well as a variety of blends with other cereal flours and oilseed proteins. Only PER values with rats were reported, but these data indicated that the protein value of such mixtures is in many cases equivalent to that of casein.

Because of the world-wide popularity of wheat, a formulation (WSB) involving a blend of soybean flour with wheat protein in the form of straight-grade or bulgar flour or a concentrate has been recently developed by USDA (127). The formula consists of 20% defatted soybean flour, 73.4% wheat fraction, 4% soybean oil, and 2.6% minerals and vitamins, and the final protein content is about 20%. The PER as determined in rat feeding tests is 2.1. Preliminary results of child feeding tests in Peru show that WSB maintains children in N balance when fed as a primary source of protein (130). Beghim et al. (132) studied the nutritive value of a noodle preparation formulated with corn, soy, and wheat (60:30:10, w/w) with a protein content of 20.5%. This food was found to be a very effective supplement for rehabilitating malnourished Brazilian children.

Guggenheim and Szmelcman (133) were interested in formulating a protein-rich mixture of cereal proteins which are commonly available in the Middle East including sesame flour and chick peas (*Cicer arietinum*). It was found that a mixture containing 37.3% protein derived from 47% autoclaved chick peas, 35% defatted sesame flour, and 18% defatted soybean flour had a higher nutritive value than any of its components alone when assayed with growth tests on rats. The authors refer to preliminary trials in which this protein mixture was administered to infants with good acceptance and tolerance. Sesame flour and chick pea (also known as Bengal gram) have also been used in combination with soybean flour by workers in India, to produce a protein mixture of good quality (134).

#### Soy Protein as Meat Extender

Soy flour and soy protein preparations have been widely used as a means of extending or replacing meat protein with favorable results (135,136). For example, Meyer (50) reported that the protein quality (in terms of PER) of a meat protein mixture remained the same even when meat protein was replaced up to 25% by a soybean isolate. Although the nutritional quality of the textured protein will be discussed more fully below, it is pertinent to mention here the studies reported by Wilding (136) on the use of textured soy protein as a meat replacement for beef and chicken patties, meat loaf, meatballs, and chili. With the exception of chili, it was found that the replacement of as much as 30% of the meat protein with the textured protein gave PERs which exceeded that of casein. Skurry and Osborne (137) have recently reported that the replacement of 60% of the meat protein of cooked sausages with

isolated soybean protein lowered the PER from 2.0 to 1.7. The authors attributed this depression in protein value to a further accentuation of the deficiency of the sulfur amino acids produced by a substitution of the meat protein with soy protein. Unlike the supplementary effect obtained between cereal proteins and soy proteins, meat protein and soy protein do not mutually supplement each other since both proteins are limiting in sulfur amino acids. As will be discussed below, similar results are obtained by Kies and Fox (138,139) who, in N-balance studies with human subject, found that measuring the proportion of textured soy protein with respect to beef protein caused a progressive decrease in N retention.

#### TEXTURED MEAT ANALOGS

One of the most dramatic and promising uses of soybean protein is in the formulation of textured foods or meat analogs. In this process, the protein isolate is dissolved or suspended in alkali and then extruded through a die or force through spinnerets into an acid or an acid-salt bath to form fibers. Alternatively, defatted soy flour processed by extrusion cooking can be used to formulate texturized products. These can be manipulated to give products simulating the texture and flavor of a wide variety of meat foodstuffs. Such products contain, on the average, about 25% protein, or on a dry basis about 50% protein (140).

Bressani et al. (55) appear to be the first group of workers to make a comprehensive study of the protein quality of soybean protein textured foods using rats, dogs, and children as experimental subjects. From these studies, the authors concluded that the nutritive value of the textured foods was equivalent to that of beef protein and ca. 80% of that of casein. It was readily accepted by children and did not produce any adverse physiological effects.

Since the earlier report by Bressani et al. (55), a number of research projects have been reported dealing with various aspects of the nutritional quality of textured soy products for the human being. Most of these have used the traditional philosophical approach—that is, that the nutritional standard for the textured protein product should be that of the nutritional value of the traditional product it resembles or which the textured protein product is partially or totally predicated to replace in the food pattern. In the United States, meat is a highly popular food. It contributes high quality protein, vitamins, and minerals to the typical American diet. It is not surprising therefore that most of these studies have involved a comparison between the protein quality of meat and textured protein products. Chemical evaluation of a textured protein product can be used to predict the first limiting amino acid as an index of protein quality. Such analyses (Table V) indicate that the amino acid composition of the textured protein is not grossly different from the soybean protein from which it was derived; hence the sulfur-containing amino acids are the most limiting. This conclusion has been verified for the extruded defatted soymeal product in human bioassay evaluations (141,142). The protein nutritional value of the extruded defatted soymeal product (processed to resemble ground beef, referred to as TVP, texturized vegetable protein), methionine-enriched TVP and beef were compared in controlled human feeding studies at two levels of nitrogen intake. At the 8.0 nitrogen intake level all three sources equalled or exceeded the protein requirements of the adult subjects. The 4.0 nitrogen intake level was inadequate to meet protein needs of the subjects; hence, it constituted a better test level. At this level of nitrogen intake, beef was found to be superior to the TVP on the basis of nitrogen balance data. DL-methionine supplementation of the TVP product was effective in improving the nitrogen balance. In a replication of the 4.0 g nitrogen intake section of this study with adolescent boys, beef again was found to give

superior nitrogen retention in comparison to the unsupplemented TVP product. However, 1% DL-methionine addition to the TVP product resulted in an improvement in nitrogen balances of subjects to a degree to equal those achieved with beef.

In another adult human bioassay study TVP and beef were mixed to provide TVP nitrogen to beef nitrogen ratios of 4:0, 3:1, 2:2, 1:3, and 0:4 (138,139). These mixtures were fed to human subjects in controlled nitrogen balance studies at a 4 g nitrogen intake level. Mean nitrogen retention of subjects progressively improved as the proportion of beef in the mixture was increased in a straight line arithmetic relationship. Along similar lines, N balance studies with human adults showed that N equilibrium could be achieved with a mixed diet in which 30-40% of the daily protein was provided by texturized soybean protein or by an equivalent level of beef protein (143,144).

Textured protein foods of the soy protein isolate type, processed to resemble familiar animal protein foods, were successfully fed to a group of human subjects by Koury and coworkers (140,145). All subjects consuming the diet remained in good health. Clinical and laboratory determinations confirmed that subjects were maintaining good health. The study suggested that this type of textured protein product was nutritionally acceptable as a major source of protein in human diets.

Adequacy of a spun-soy protein product containing egg albumin to meet human protein requirements was studied by Turk et al. (146). Results of two studies in which either increasing or decreasing increments of product nitrogen were fed indicated that protein needs for subjects were met by feeding between 0.4 and 0.5 g nitrogen from this product per day. Morse et al. (147) compared spun-soy protein with a mixture of casein and lactalbumin (5:1) in human subjects at an intake of 0.4 to 0.45 g protein per kg body weight per day. Using as criteria of utilization nitrogen balance, urea nitrogen, and ammonia excretion, they found no difference when both sources of protein were supplemented with amino acids to give the same amino acid pattern.

### NUTRITIONAL VALUE OF NONPROTEIN CONSTITUENTS

Although the principal contribution which the soybean can make to the nutrition of man lies in the quantity and quality of protein which it contains, consideration should, nevertheless, be given to other soybean constituents which could have nutritional significance under special circumstances. Situations might arise, for example, in which the oil and carbohydrate components of soybeans might be called upon to serve to satisfy the caloric requirements of an undernourished population. The vitamin and mineral constituents of soybeans might also prove to be decisive nutritional factors under conditions where the availability of these nutrients might be limiting or marginal.

#### Available Energy of Caloric Considerations

The energy available for metabolism (metabolic energy) from the soybean may be calculated from its content of carbohydrate, fat, and protein, taking into account the digestibility of each one of these components as well as the heat of combustion. Except under conditions of extreme caloric deprivation, the protein would not be expected to be utilized to any significant extent as a source of energy. The amount of energy theoretically available from soybean protein may be calculated by multiplying the protein content by the factor 3.47 Cal per g (148). Little need be said here regarding the energy available from soybean oil except to indicate that soybean oil is highly digestible and has a caloric value of 8.37 Cal per g of fat (148). Most soybean products intended for human consumption, however, have

been defatted so that, unless indicated otherwise, the caloric contribution of soybean oil can generally be neglected. On the other hand, since full-fat soy flour usually contains about 20% oil, the use of soy flour as a supplement to other proteins provides an additional source of calories where needed (149). In addition, full-fat soy flour can make a valuable nutritional contribution because of the high proportion of essential fatty acids in the oil, containing about 5% linoleic acid and 9% linolenic acid (150). The carbohydrate content of soybean products will vary considerably depending on the extent to which carbohydrate material may have been removed during processing and will range from 22-29% for whole soybeans (151) to only 0.3-4.2% for the isolated protein (152). Only a portion of this material is actually metabolizable for energy purposes. Such substances as galactans, pentosans, and hemicelluloses, which are utilized poorly, if at all, by the body, represent a substantial proportion of the total (153). The actual percentage of the total carbohydrate which is available depends on the experimental animal and ranges from a figure of 14% determined with chicks (154) to 40% with rats (155). In the absence of conclusive data on human beings, the latter figure has been taken by the FAO (156) to be the digestibility of carbohydrate from soybeans and soybean products, and, on this basis, the calorie factor becomes 1.68 Cal per g of carbohydrate. The USDA Table of Composition (148), on the other hand, assumes the carbohydrate of soybeans to be almost completely digestible (97%) and bases its caloric calculations for soybeans and soybean products on a factor of 4.07 Cal per g. In the case of soybean milk, soybean curd, and protein isolates, this factor may be applicable since the preparation of these products involves the removal of most of the insoluble and indigestible carbohydrates.

#### Crude Fiber

The significance of the crude fiber content of the diet has received considerable attention of late because of its possible role in reducing the incidence of colon tumors. The fraction of total carbohydrate which may be classified as "crude fiber" will, of course, depend on the type of soybean product. Crude fiber values range from 0.1% or less for soybean curd and soybean milk to ca. 5% for the whole seed (148,156). Since about one half of the crude fiber in the latter case is attributable to the seed coat, the crude fiber content of soybean products is much reduced by removal of its hull. Although the presence of crude fiber may have a beneficial effect in terms of the risk of colon cancer, there is some indication that the crude fiber may interfere with the digestibility of other nutrients, particularly in monogastric animals such as man. Their presence in the cell wall may prevent the access of digestive juices to the protein within the cells. To what extent this may account for the poor digestibility of inadequately processed soybean products is not known. It is known, however, that the digestibility of plant protein concentrates, which are relatively low in crude fiber, is much higher than the crude plant materials which have a higher content of crude fiber (157).

#### Vitamins

When soybean products are consumed as part of a mixed diet, they can hardly be considered to be an important source of vitamins. However, when consumed in the form of a protein-rich supplement to a basal diet that may be deficient in vitamins as well as protein, the vitamin contribution of the soybean may assume a very decisive role in the maintenance of health. In the usual determination of PER the basal diet already contains an adequate supply of vitamins, so that any additional contribution of vitamins made by the soybean is obscured. In those few studies where the vitamins contributed by soybeans have been

TABLE VIII

Soybean product	Vitamin Content of Soybean Food Products <sup>a</sup>										
	$\beta$ -Carotene ( $\mu$ g/g)	Thiamine <sup>b</sup> ( $\mu$ g/g)	Riboflavin <sup>b</sup> ( $\mu$ g/g)	Niacin <sup>b</sup> ( $\mu$ g/g)	Pantothenic acid <sup>b</sup> ( $\mu$ g/g)	Pyridoxine <sup>b</sup> ( $\mu$ g/g)	Biotin <sup>b</sup> ( $\mu$ g/g)	Folic acid <sup>b</sup> ( $\mu$ g/g)	Inositol <sup>b</sup> (mg/g)	Choline <sup>b</sup> (mg/g)	Ascorbic acid (mg/g)
Immature bean	2-7	6.4	3.5	20.0-25.9	12	3.5	0.5	1.3		3.0-3.3	0.2
Mature bean	0.2-2.4	11.0-17.5	2.3	29.9-48.0	12	6.4	0.6	2.3	1.9-2.6	3.4	0.2
Sprouts		11.9-21.9	4.8-7.0	19.0-40.0	18.8-34.4	14.1-17.7	1.1-1.7	3.7	2.5-3.9		0.4
Meal		12.0-44.1	2.7-3.3	20.3-29.1	13.3-16.0	8.8	0.2	4.0-4.9	1.8-2.1	3.5-3.8	
Flour		11.0-15.0	4.0-4.4		47.0-50.6			0.8-0.9			
Curd (tofu)		3.9	3.7	5.5							
Milk <sup>c</sup>	7.50	0.8	1.1	2.5							21.6
Miso		1.3	1.4								
Infant formula		0.4-0.5	0.6-1.1	6.0-7.4	2.6-5.0	0.4					40-53
Textured soy protein		3.0	3.0-6.0	23-160	25	4.0-14.0					<10

<sup>a</sup>Source: Data compiled from the following: Burkholder (264); Burkholder and McVeigh (265); Chang and Murray (41); Engel (266); Flynn (267); Guggenheim and Szmelcman (133); Hamdy (268); Hoff-Jorgensen et al. (269); Kondo et al. (270); Miller (169); Mustakas et al. (27); Sherman and Salmon (162); Shurpalekar et al. (21); Sugawara (271); Van Duyne et al. (272); Weisberg (273).

<sup>b</sup>Where a range of values is shown, average value is very closely given by taking the average of the two extreme values.

<sup>c</sup>Expressed as mg/liter with the exception of  $\beta$ -carotene which is expressed in terms of IU of vitamin A per liter.

evaluated by employing a basal diet lacking vitamins of the B complex, it has been observed that if at least 10% of the protein is derived from soybean flour then the latter provides enough of the B vitamins to satisfy the animals' (rat) requirement (158,159). Westerman et al. (160) reported that soy flour at a level of 3% could replace wheat germ as a vitamin B supplement to nonenriched wheat flour. Zucker and Zucker (158) have estimated that if approximately one half of the daily protein requirement of an adult male was derived from soy flour, then one third to one half of his requirement for thiamine, riboflavin, and nicotinic acid would be met.

The vitamin content of various soybean products is summarized in Table VIII. Rather wide variations in analytical values may be found in the literature. These are most likely a reflection of the analytical techniques employed as well as the influence of processing conditions.

*Fat-soluble Vitamins:*  $\beta$ -Carotene, the biological precursor of vitamin A, is present in green, immature soybeans to the extent of 2-7  $\mu$ g per g, while the mature bean contains a significantly lesser amount (161,162). The  $\beta$ -carotene content of soybean milk is about half that of cow's milk (21). Soybean and soybean products may be considered to be essentially devoid of vitamin D. Anderson (163) has pointed out that if soy milk is to be used as a replacement for cow's milk it should be supplemented with vitamin D. Any vitamin E present in soybean products is contained in the oil to the extent of 1.4  $\mu$ g per g of oil (164). During the course of the fermentation of soybeans, as in the preparation of tempeh, an antioxidant is produced which retards the development of rancidity and prevents a vitamin E deficiency in rats (73). Murata et al. (165) reported higher levels of  $\alpha$ -tocopherol in the serum of rats fed tempeh than in rats fed unfermented beans. Gyorgy et al. (166) succeeded in isolating from tempeh three active antioxidants which were identified as daidzein (7,1'-dihydroxyisoflavone), genistein (5,7,4'-trihydroxyisoflavone), and "factor 2" (6,7,4'-trihydroxyisoflavone). Factor 2 was later reported to be ineffective in preventing the hemolysis of red blood cells from vitamin E deficient rats, nor did it prevent the autoxidation of soybean oil or full-fat soybean flour (167). Since factor 2 apparently does not occur in soybeans, it would appear that some of the pre-existing isoflavones of soybeans are converted to factor 2 as a consequence of the fermentative process.

*Water-soluble vitamins:* In comparison with cereal grains, soybean products are fairly good sources of the vitamins of the B complex. Because of its thermolability, however, thiamine is apt to be present in somewhat lower amounts in heat-processed soybean products. Thus, the toasting of solvent-extracted soybean flakes destroys about half of this vitamin (168). Thiamine losses ranging from 10% to 75% have been reported for soybeans which had been cooked under varying conditions (9, 169-173). The B vitamins of extrusion-cooked full-fat soybean flour, however, seem to suffer little damage as a result of the cooking process (27). In a study designed to establish the effect of vitamin enrichment in textured soy protein on N balance on human adults, Kies and Fox (138,139) noted that supplementation with niacin improved the N balance by 16% to 23%. Appreciable losses in ascorbic acid also accompany the cooking of soybeans so that cooked soybean products should be regarded as a relatively poor source of vitamin C (171,172,174,175).

As will be noted in Table VIII, a significant increase in the concentration of most of the B vitamins and ascorbic acid accompanies the germination process. Soybean sprouts may be considered to be a particularly good source of vitamin C (176). Miller et al. (177) have estimated that soybean milk may retain 50-90% of the thiamine, 90% of the riboflavin, and 60-80% of the niacin found in the soybeans from which the milk was made. Desikachar et al.

TABLE IX  
Mineral Content of Soybeans and Soybean Food Products<sup>a</sup>

Soybean product	Calcium (%)	Phosphorus (%)	Magnesium (%)	Zinc (mg/kg)	Iron (mg/kg)	Manganese (mg/kg)	Copper (mg/kg)
Immature bean	0.10	0.26			21.3		
Mature bean	0.16-0.47	0.42-0.82	0.22-0.24	37	90-150	32	12
Sprouts	0.40				100		
Meal	0.24-0.31	0.60	0.24-0.30	55-77	140	24-29	14-24
Flour	0.42-0.64	0.60			110-160		
Curd (tofu)	0.80	0.80-1.0			105		
Milk, traditional	0.76	0.15			68		
Milk, powdered proprietary	0.7-1.0	1.1			30-170		
Miso	0.11				35		
Natto	0.18	0.42			62		
Textured soy protein	0.25	0.74	0.30	56	96	36	16

<sup>a</sup>Source: Data compiled from the following: Adolph and Chen (190); Bailey et al. (85); Cartter and Hopper (1942); Chang and Murray (41); Dewar (1967); FAO (1954); Guggenheim and Szmelcman (133); Hamdy (268); Harmon et al. (186); Miller and Robbins (174); Mitchell (216); Morse (217); Pant and Kapur (1963); Shurpalekar et al. (21); Watt and Merrill (148). All values corrected for moisture content. Where range of values is shown, average value is very closely given by taking the average of the two extreme values.

(178) found soy milk to be ca. 80% as potent as cow's milk with regard to its vitamin B-complex content. When the curd is separated from the milk only about half of the thiamine and one fourth of the nicotinic acid is retained in the curd, while the riboflavin content is equivalent to the original bean (41). The fermentation of soybeans, such as is involved in the preparation of natto and tempeh, has been observed to cause an increase in most of the B vitamins except thiamine which undergoes a significant decrease (67,69,179,180).

Since soybeans are devoid of vitamin B<sub>12</sub> it is not surprising that diets containing soybean protein require supplementation with this vitamin in order to produce maximum growth. Vitamin B<sub>12</sub> supplementation, however, improves the growth of animals receiving raw soybeans to a greater extent than similar supplementation of diets containing heated soybeans (181-183). Using the urinary excretion of methyl malonic acid as an index of vitamin B<sub>12</sub> deficiency, Edelstein and Guggenheim (184) found that the excretion of this metabolite was reduced to a greater extent when vitamin B<sub>12</sub> was given to rats on a raw soybean diet compared to rats administered this vitamin on a diet containing heated soybeans. These observations suggest not only that is raw soybean deficient in vitamin B<sub>12</sub> but also that it contains a heat-labile substance which increases the requirement for this vitamin.

### Minerals

Table IX shows the mineral composition of soybeans and soybean products. Reference will be made to this table in the following paragraphs that deal with each mineral individually. It is questionable whether too much nutritional significance should be attached to these analytical values since there is ample evidence to indicate that the availability of most minerals from soybeans is quite low (185,186). Also there appear to be constituents in soybeans which interfere with the availability or utilization of certain minerals, particularly calcium and phosphorus (187).

**Calcium:** Most of the nutritional interest in the calcium content of soybeans has involved a comparison of soybean milk with cow's milk. Analytically, the calcium content of soybean milk prepared in the traditional manner (0.08%) compares quite favorably with cow's milk (0.11%) (21). Schroeder et al. (188) compared the availability of calcium from soybean milk with that from evaporated cow's milk for human subjects and concluded that 22.6% of the calcium of a proprietary preparation of soybean milk was available compared to 29.1% from cow's milk. These values agree fairly well with earlier experiments with human subjects which indicated that the utilization of the calcium of soybean milk prepared in the traditional manner is about 90% that of cow's milk (189). The calcium content of soy-

bean curd is about four times higher than that of soybean milk because of the use of calcium salts for precipitating the protein. Adolph and Chen (190) found little difference in the availability of the calcium of soybean curd and cow's milk for human subjects.

In contrast to the generally high availability of calcium from soybean milk and curd, the availability of calcium from the whole bean is quite low. Schroeder et al. (188) reported that only 10% of the calcium of cooked soybeans could be effectively utilized by man. Experiments with animals strongly suggest that phytic acid contained in soybeans interferes with the availability of calcium (191) which, in turn, may account in part for the rachitogenic properties of isolated soybean protein (192).

**Phosphorus:** Although soybeans contain almost twice as much phosphorus as most cereals, approximately one half to one third of it is present as phytic acid (193). The availability of phosphorus when present in the form of phytic acid depends on the species and age of the experimental animal. Thus, the phosphorus of soybeans appears to be well utilized by the rat (194,195) but not by the chick (191). Whether or not an animal can utilize phytate phosphorus seems to depend on the level of phytase activity in the intestinal tract (193). Even soybean protein which has been isolated by isoelectric precipitation seems to contain enough phytic acid, 0.5% (196), to interfere with the availability of phosphorus for bone mineralization in the pig (197). Although no experiments with humans have been conducted to directly determine the availability of phosphorus from soybeans, it has been estimated that anywhere from 40 to 80% of the phytate phosphorus in cereals is available to man (198).

**Zinc:** Although soybeans contain an appreciable amount of zinc, from rat experiments it has been estimated that only 44% of it is actually available (199). A number of investigators have reported that the dietary requirement of chicks and turkey poults for zinc is significantly increased when soybean protein is the main source of protein in the diet (200-205). Autoclaving the protein or adding ethylenediaminetetraacetate (EDTA) to the diet eliminated this interference with the availability of zinc. In vitro experiments have provided direct evidence that isolated soybean protein can bind zinc, an effect which can be partially eliminated by autoclaving the protein or by adding EDTA (206). Phytic acid appears to be the specific component responsible for the zinc-binding properties of soybean protein (190,196,207), although other lines of evidence suggest that a glycoprotein might be involved (208). The availability of zinc from soybean protein isolates varies from one source to another (208,209).

**Other minerals:** The ability of soybean protein to interfere with the availability of minerals can be extended to

TABLE X

Antinutritional Factors Present in Soybeans	
Trypsin inhibitor <sup>a</sup>	Phytate <sup>a</sup>
Hemagglutinins <sup>a</sup>	Goitrogens <sup>a</sup>
Saponins	Anti-vitamin factors <sup>a</sup>
Estrogens	Flatulence factors
Lysinoalanine	

<sup>a</sup>Destroyed by heat

include manganese, copper, and molybdenum (203,210). Here again this interference can usually be effectively counteracted by supplementing the diet with EDTA. Reports regarding the availability of iron from soybeans have been quite variable, and values for percentage of availability of iron have ranged from 28.5% to 80% (211-213). Experiments dealing with the effect of soybean protein on the availability of iron have likewise been inconclusive. In experiments with chicks, Davis et al. (203,214) found that isolated soybean protein did not interfere with the availability of iron for growth and hemoglobin synthesis. On the other hand, Fitch et al. (215) observed reduced gastrointestinal absorption of iron and anemia due to an iron deficiency in monkeys fed a diet containing isolated soybean protein. Not only is the soybean lower than most cereals in chlorine and iodine content (216,217) but, in addition, one must take into consideration its goiterogenic properties which exacerbate an iodine deficiency (187). Anderson (163) has emphasized the nutritional importance of supplementing infant soybean milk formulas with iodine.

Measures are frequently taken to increase the iron content of such soy protein products as infant milk formulas. In this case it appears that the source of iron used for this purpose becomes important (218). Although heat processing has no effect on the availability of iron from ferrous sulfate added to liquid soybean isolate infant formulas, processing increased the availability of iron from ferric pyrophosphate from 39% to 93%, and of iron from sodium ferric pyrophosphate from 15% to 66%.

### ANTINUTRITIONAL FACTORS

Soybeans in their native state are known to contain a number of factors which are known to cause an adverse physiological response in rats (187). A listing of these is given in Table X. Much of the beneficial effect which heat treatment is known to exert on the nutritional properties of soybean protein may be attributed to the destruction of most of these factors. At the same time, it is well recognized that excessive heat treatment can induce serious damage to the nutritional quality of the protein. The real question thus becomes—what level of these antinutritional factors can still be tolerated in soybean products without posing a risk to the consumer? This of course becomes a very difficult question to answer since most of the evidence dealing with this point must come from experiments with animals, and these results must be extrapolated to the human subject. Nevertheless an attempt will be made to evaluate some of the experimental evidence which might shed some light on the possible physiological significance of some of these antinutritional substances with respect to human nutrition.

#### Heat-labile Factors

**Trypsin inhibitor:** This component of soybeans has been the object of considerable study since an inhibitor of trypsin was first isolated from soybeans by Kunitz in 1944, and no attempt will be made to review this literature here [see review by Liener and Kakade, (219)]. Most soybean products which have been properly processed have undergone at least a 90% destruction of the trypsin inhibitor.

Rackis et al. (220) have shown, however, that only 80% of the trypsin inhibitor need be destroyed in order to obtain maximum PER and only 50% destruction eliminates the pancreatic hypertrophy associated with the feeding of raw soybeans. It would appear, therefore, that most soy products which have been properly processed should be well within the tolerance level as judged by experiments with rats. Churella et al. (221) have recently reported that although a number of soy-based commercial infant formulas had low but measurable levels of trypsin inhibitor activity, they did not produce pancreatic hypertrophy in rats. Low levels of antitryptic activity are also found in some textured soy products, ca. 10% of that found in unheated soy flours (Liener, unpublished data), but again such levels are of questionable physiological significance, particularly since such products are subjected to further heat treatment during household cooking.

Aside from the fact that the levels of trypsin inhibitor in most soy products are probably too low to give much concern, there is the real question as to whether these inhibitors are really capable of playing any significant role in human nutrition at all. It is important to note that practically all of the *in vitro* studies with trypsin inhibitors have involved the use of bovine trypsin because of its ready commercial availability as a crystalline preparation. It has been demonstrated (222,223) that human trypsin is much less inhibited by the soybean inhibitor and by ovomucoid, the egg white trypsin inhibitor, than is bovine trypsin. The inability of ovomucoid to inhibit human trypsin may explain perhaps why this inhibitor has virtually no effect on the nitrogen balance of human subjects (224). From these various lines of evidence, therefore, one is tempted to conclude that, despite the considerable body of evidence which implicates the trypsin inhibitors as a factor contributing to the poor nutritive value of improperly processed legumes in animals, their relevance to human nutrition remains uncertain.

**Hemagglutinins:** Although there is ample evidence to indicate that the phytohemagglutinins are responsible for much of the growth inhibition and toxic effects which one sees with many unheated legumes (225), there have, until recently, been very few studies dealing with the possible nutritional significance of the soybean hemagglutinins. Using affinity chromatography to selectively remove the hemagglutinins from crude soybean extracts, Turner and Liener (226) found that this treatment did not serve to increase the PER of the protein contained in such extracts. Birk and Gerlter (227) had concluded earlier, on the basis of more indirect evidence, that the soybean hemagglutinin probably made a very minor contribution to the poor nutritive value of raw soybeans.

**Other heat-labile factors:** The ability of phytate to interfere with the availability of such minerals as zinc, manganese, copper, molybdenum, calcium, magnesium, and iron is largely eliminated by heat treatment. For added safety, however, it would appear to be desirable to supplement soy protein containing diets with an adequate supply of minerals. Although the precise factors in soybeans responsible for an interference with the availability of vitamins D and E is not known, heat processing seems to be an effective means of eliminating this effect. The goiterogenic effect of soybeans can also be effectively abolished by heat treatment or by the addition of small amounts of iodine.

#### Heat Stable Factors

**Saponins:** Although saponins from some plants have antinutritional properties, saponins isolated from soybeans are relatively innocuous to chicks, rats, and mice even when fed at a level three times greater than the level in diets containing soy flour (228). It would appear that the saponins of soybeans should be removed from the list of

antinutritional factors in soybeans.

*Estrogens:* Phenolic compounds capable of eliciting an estrogenic response in animals have been isolated from soybeans and products prepared therefrom (184). These substances, primarily genistein and daidzein, although stable to autoclaving, are present in such low levels in the soybean that they are believed to be physiologically insignificant.

*Flatulence factors:* As with most leguminous plants, the ingestion of soybeans leads to the formation in many individuals of gastrointestinal gas which in turn can cause nausea, cramps, diarrhea, and general social discomfort when the gas is egested rectally. The gas-producing factor resides mainly in the low molecular weight carbohydrate fraction consisting mainly of raffinose and stachyose (184). Stachyose, in particular, has high flatus-producing activity. Consequently flatus activity has been noted primarily with soybean products from which the carbohydrate has not been removed such as soybean flours and, to a lesser extent, soy protein concentrates. Protein isolates, textured soy protein products, and fermented soy foods such as tempeh, are essentially devoid of flatus activity.

It should be emphasized that there is considerable variability in the response of individuals to the flatus-producing effects of soybeans. Many individuals are completely unaffected by the ingestion of soybeans and other legumes, but the exact reason for this variable response is not known. Since the flatus-producing factors in soybeans are heat-stable, attempts have been made to eliminate these factors by enzymatic hydrolysis (229,230). Although treatment with soybeans with a mold enzyme virtually eliminated stachyose and raffinose, there was no significant reduction in flatus activity in human subjects (230). The treatment of soy milk by an enzyme purified from *Aspergillus saitoi* also resulted in the complete hydrolysis of its constituent oligosaccharides (231), but, in this case, the effectiveness of this treatment on reducing flatulence was not evaluated. A significant reduction in the oligosaccharide content of soybeans can also be achieved by a combination of soaking, germination, and re-soaking (232), although in this case it is difficult to assess how much of this reduction is due to a leaching out of the oligosaccharides or to autolysis by endogenous enzymes.

*Lysinoalanine:* Reference has already been made to the fact that alkaline treatment of soybean protein results in the formation of lysinoalanine (58). It was subsequently shown that alkali-treated soy protein produced kidney lesions in the rat (233). Although this effect could be reproduced by the administration of low levels of free lysinoalanine (234), no evidence of toxicity was apparent when the lysinoalanine was peptide-linked in the alkaline treated protein (235). This difference was probably due to the fact that free lysinoalanine was absorbed and excreted in the urine, whereas the lysinoalanine of the alkali-treated protein was excreted primarily in the feces. Apparently lysinoalanine provides a peptide bond which is resistant to attack by intestinal enzymes and hence cannot be released for absorption which is necessary for the toxic effect. It is significant to note that a commercial type of spun soy isolate, such as that employed in the fabrication of textured meat analogs, did not induce any renal lesions when fed to rats (236). These latter authors stressed the importance of using a nutritionally well-balanced diet in interpreting the results of toxicological experiments. It also appears that the strain of rat employed for such studies may influence the results, since Struthers et al. (237) found that some strains of rats were more susceptible to the effects of lysinoalanine than others. In addition, the rat kidney lesions may be peculiar to the rat since several other species of animals, including the dog, mouse, hamster, quail, and monkey, do not develop such lesions even with free lysinoalanine.

Steinberg et al. (238) have recently shown lysinoalanine

to be widely distributed in cooked foods, commercial food preparations, and food ingredients, many of which had never been subjected to alkaline treatment. Many of these foods had levels of lysinoalanine which were considerably higher than those found in commercial samples of soy protein isolate. The wide distribution of lysinoalanine among commonly cooked foods would tend to indicate that this is neither a novel or serious problem, since some humans have long been exposed to proteins containing lysinoalanine with apparent impunity. Its presence in soy protein can hardly be considered a serious problem for man.

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## Falb to direct ASA soy oil effort

Richard A. Falb has been named director of the American Soybean Association's new soy oil market development program, the first ASA market development program to be conducted in the United States.

Falb has been communications director for the ASA's market development program the past four years. ASA has conducted programs promoting U.S. soybean products in approximately 55 nations during the past 21 years.

"Even though soy oil is the most widely used edible oil in the United States, research already done shows most American consumers are unfamiliar with it," an ASA spokesman said. "Consequently, inroads are being made by imported palm oil, an edible but highly saturated oil."

Palm oil imports may reach one billion pounds by 1980, the spokesman said.

Falb's initial efforts in the new program will be to gather and distribute information, building a rapport within the industry and doing market research. Soy oil is classified in the high polyunsaturate oils along with corn oil, sunflowerseed oil, and safflowerseed oil, Falb said, but this is not well known among consumers.

Falb will be seeking information on the basic composition of soybean oil, what factors in production, extraction, and processing affect its properties. Persons interested in the project may contact him at ASA headquarters in Hudson, Iowa, 50643 (tele: 319-988-3295).

ASA decided to launch the program after a market research study showed that a successful program could mean the difference between having or not having a market for 2.4 billion pounds of soy oil in the United States.

Falb has spent more than 25 years in marketing, advertising, promotion, and public relations. He has a master's degree from the University of Minnesota and is a member of the AOCs and the Institute of Food Technologists.

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