2:958 (1979).
24. Holmes, W.L., G.B. Rubel and S.S. Hood, Atherosclerosis 36:379 (1980).
25. Liebermeister, H., and H. Toluipur, Dtsch. Med. Wochenschr. 105:333 (1980).
26. Gortner, W.A., Cancer Res. 35:3246 (1975).
27. Kritchevsky, D., Lipids 12:49 (1977).
28. Stern, M.P., Ann. Int. Med. 91:630 (1979).
29. Beaglehole, R., J.C. LaRosa, G. Heiss, C.E. Davis, O.D. Williams, H.A. Tyroler and B.M. Rifkind, Prevent. Med. 8:538 (1979).
30. Sacks, F.M., W.P. Castelli, A. Donner and E.H. Kass, New Engl. J. Med. 292:1148 (1975).
31. Burslem, J., G. Schonfeld, M.A. Howald, S.W. Weidman and J.P. Miller, Metabolism 27:711 (1978).
32. Sanders, T.A.B., F.R. Ellis and J.W.T. Dickerson, Am. J. Clin. Nutr. 31:805 (1978).
33. Phillips, R.L., F.R. Lemon, W.L. Beeson and J.W. Kuzma, Ibid. 31:S191 (1978).
34. "Composition of Foods, Dairy and Egg Products, Raw, Processed, Prepared," Agriculture Handbook No. 8-1, Agriculture Research Service, U.S. Dept. of Agriculture, Washington, DC, Revised, 1976.
35. Howard, A.N., and J. Marks, Lancet 2:957 (1979).
36. Antila, M., S. Ali-Yrkkö, V. Antila, P. Antila, T. Rönnemaa, H. Järveläinen and J. Viikari, Ibid. 1:602 (1980).
37. Watt, B.K., and A.L. Merrill, "Composition of Foods, Raw, Processed, Prepared," Agriculture Handbook No. 8, Agriculture Research Service, U.S. Dept. of Agriculture, Washington, DC, Revised, 1963.
38. "Provisional Food Balance Sheets, 1972-74 Average," Food and Agriculture Organization of the United Nations, Rome, 1977.

Nutritional Qualities of Soya Protein As Affected by Processing

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ABSTRACT

The nutritional qualities of soybean proteins are basically determined by amino acid patterns, amino acid availabilities (digestibility) and contents of biologically active components. Of these factors, the last two are most affected by processing conditions, whereas amino acid analysis is least affected, although it too may be modified in those cases in which soybean proteins are fractionated. In the preparation of the large variety of soya products presently available, soybeans are subjected to many different processes, all of which are discussed. Heat treatment appears to be the process which most affects protein nutritional quality; generally, quality first increases with heat treatment due to inactivation of biologically active factors, passes through a maximum and then decreases due to destruction and/or inactivation of essential amino acids such as cystine and lysine. Other processes affect protein nutritional quality to different degrees, inasmuch as they affect amino acid analysis, digestibility and content of biologically active components. When soybean proteins are used to extend animal proteins, supplement other vegetable proteins, or in vegetable protein mixtures, nutritional quality of the combined proteins appears to be affected in the same manner as that of soya proteins alone.

NUTRITIONAL QUALITY OF PROTEINS

Various means for determining and comparing protein quality have been developed, including chemical score, essential amino acid index, biological value, net protein utilization, protein efficiency ratio and nitrogen balance methods, all of which have been defined and discussed previously (1-4). Generally, these indices are classified into four groups, i.e., those based on (a) protein amino acid composition (chemical score and essential amino acid index); (b) nitrogen absorption and/or retention (biological value, net protein utilization and digestibility); (c) ability of the protein to produce growth in test animals, usually rats (protein efficiency ratio); and (d) ability of the protein to provide amino acids for the synthesis or replacement of body tissue protein (nitrogen balance methods). Of these, perhaps most limited are the chemical indices, because they depend solely on amino acid composition and not amino acid availability, and thus may give misleading information. For example, it is known that soya protein quality is significantly affected by processing under conditions that have little or no effect on its amino acid composition. The remaining indices, which all are of a biological nature, do provide a reasonable measurement of functional protein quality, and each is useful in comparing proteins from different points of view, according to the nature of each index.

Although all indices have been used in protein quality work, three have been especially useful: protein efficiency ratio (PER), nitrogen balance methods and digestibility.

The PER, first proposed by Osborne and Mendel (1), remains the most widely used technique today for biological evaluation of proteins. It is defined as the weight gain of a growing animal, usually the rat, divided by its protein intake, and when conducted under standard conditions, is capable of yielding fairly accurate and reproducible results (5). By including casein as a control and relating the observed PER of the experimental groups to casein (whose PER is usually standardized at 2.5), meaningful comparisons between laboratories can be made. Table I shows typical PER values of different proteins.

The nitrogen balance method evaluates the ability of a protein to provide amino acids for the synthesis or replacement of body tissue protein. This can be done in the intact animal by comparing the quantity of nitrogen ingested with the amount which is excreted in the urine and feces. The

TABLE I

Typical PER Values of Representative Food Proteins[a]

Protein source	Corrected PER
Animal sources	
Whole egg	3.8
Cow's milk	2.5
Beef muscle	3.2
Plant sources	
Soybeans[b]	0.7-1.8
Peanuts	1.7
Cottonseed	1.3-2.1
Rice	1.9
Corn	1.2
Wheat	1.0

[a]Corrected for casein PER = 2.5 (128,129).

[b]Range of values for raw and heat-processed.

difference between these values, referred to as "nitrogen balance," indicates whether the animal is losing, gaining or maintaining its nitrogen resources. The ability of an animal to maintain nitrogen equilibrium (as in nongrowing animals), or to retain nitrogen (as in growing animals) is dependent on the availability of a balanced assortment of essential amino acids in the diet. A diet deficient in one or more essential amino acids does not permit efficient nitrogen utilization, hence much of the dietary nitrogen would be lost in the urine and feces, a condition which would result in negative nitrogen balance. Because of its relative simplicity, the nitrogen balance technique can be applied effectively to studies with human subjects.

Finally, digestibility is defined simply as percentage of nitrogen (protein) intake which is absorbed. Strictly speaking, it is not an index of protein quality; rather, it measures efficiency of protein absorption by the animal body. Factors which determine digestibility are the ability of digestive enzymes to hydrolyze a given protein into its component amino acids, and absorption of these amino acids through the intestinal wall. Enzyme hydrolysis is usually the determining factor in digestibility. Table I also reports digestibilities of different proteins.

Further discussion of this subject is outside the scope of this work; for further information, the reader is referred to the corresponding scientific literature (1-5).

FACTORS AFFECTING PROTEIN QUALITY

It has been established that, generally, a combination of three different factors determines the nutritional quality of a given protein: (a) amino acid composition; (b) amino acid availability or digestibility; and (c) presence or absence of biologically active components.

Amino Acid Composition

Classical studies (6,7) revealed that, of the 20-odd amino acids found in nature, the human body is incapable of synthesizing eight: isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane and valine. Because of this limitation, humans must obtain these amino acids from a dietary source. These acids are therefore termed "essential." Human requirements for each of these eight essential amino acids have been determined (8). More important than the quantitative requirements for each amino acid, however, is the ratio or pattern of each amino acid requirement to another. In other words, when a protein-containing food is fed at a level which meets the total protein requirement, the overall pattern of essential amino acids is more important in determining the quality of the protein than the absolute content of each essential amino acid.

The essential amino acid pattern required by man has been a topic of much research and discussion. The first pattern originally suggested was based on that of whole hen egg protein (9). Subsequently, the FAO Committee on Protein Requirements proposed a pattern, called the "FAO Provisional Pattern," which was believed to reflect minimal essential amino acid requirements in humans (10). This pattern, however, did not distinguish between requirements for children and adults, which are now known to be different. As a result, the joint FAO/WHO expert group (11) developed and proposed separate patterns for children and adults. Both of these patterns are currently believed to reflect human minimal amino acid requirements, so that the nutritional quality of a protein will depend on how much its essential amino acid pattern resembles the corresponding reference pattern. A novel aspect of the FAO/WHO patterns is that they group together sulfur-containing (methionine and cystine) and aromatic (phenylalanine and tyrosine) amino acids, because it has been found that, although cystine and tyrosine are not, strictly speaking, essential amino acids, they may be converted by the body into methionine and phenylalanine, respectively, which are essential amino acids. Table II reports essential amino acid patterns for whole hen egg protein, the FAO provisional pattern, and the FAO/WHO patterns for adults and children.

TABLE II

Amino Acid Reference Patterns and Patterns for Whole Egg and Soybean Proteins[a]

Amino acid	FAO provisional pattern (10)	FAO/WHO Patterns (11)		Whole egg (4)	Soybean (96)
		Children	Adults		
Isoleucine	4.2	4.0	1.8	6.3	4.2
Leucine	4.8	7.0	2.5	8.8	7.4
Lysine	4.2	5.5	2.2	7.0	6.4
Phenylalanine	2.8	2.4	1.5	5.7	4.5
Tyrosine	–	–	–	4.2	3.4
Total aromatic	–	6.0	2.5	9.9	8.0
Methionine	2.2	–	–	3.4	1.2
Cystine	–	–	–	2.4	0.9
Total sulfur	–	3.5	2.4	5.8	2.2
Threonine	2.8	4.0	1.3	5.1	3.6
Tryptophane	1.4	1.0	0.65	1.5	1.7
Valine	4.2	5.0	0.8	6.8	4.3

[a]Amino acid contents are reported as g amino acid/16 g nitrogen.

In determining the quality of a given protein, reference patterns are applied as follows. Levels of amino acids of the test protein are compared with corresponding levels in the reference pattern. Those amino acids whose levels equal or exceed reference pattern levels are termed "nonlimiting," because they are present in sufficient amounts to provide for human needs; amino acids whose levels are inferior to reference pattern levels, however, are termed "limiting," as they are not present in sufficient amounts in the protein. Limiting amino acids are classified in order of decreasing deficiency with respect to the pattern, with the most deficient being termed "first limiting amino acid." The ratio of the level of the first or most limiting amino acid in the test protein to that of the corresponding amino acid in the reference pattern, multiplied by 100, is termed the "chemical score" of the protein. Table II, which also includes the amino acid pattern for soya protein, reveals that sulfur-containing amino acids are first limiting in this protein.

The amino acid pattern of a given protein may be modified by (a) a number of processes or processing conditons such as protein fractionation, which divides a protein into separate components, each of which may or may not have an amino acid pattern different from that of the parent protein; and (b) heat treatment, which may destroy lysine, cystine and other essential amino acids (12-16).

Amino Acid Availability or Digestibility

The amino acid pattern of a protein, usually obtained by acid hydrolysis, assumes that the animal body can utilize all of each amino acid contained in the protein. This is an assumption which does not consider that a number of factors may alter the physiological availability of a given amino acid; i.e., although the amino acid may be present in the protein, it may not necessarily be available to the organism for nourishment. Thus, although the amino acid pattern may indicate a certain protein to be of potentially good quality, the unavailability of one or more essential amino acids may actually make the protein of poor quality.

As noted previously, the index which measures amino acid availability to the organism is "digestibility." Factors affecting amino acid availability or digestibility of a given protein are those which may either destroy or inactivate amino acids. "Destruction," as used here, refers to a failure to recover an amino acid upon acid or alkaline hydrolysis; "inactivation," on the other hand, refers to a situation in which there may be complete recovery of the amino acid after hydrolysis, but nonetheless, there has been a marked decrease in the biological availability of that amino acid, primarily because is has been bound into a compound or compounds which cannot be attacked by digestive enzymes.

The digestibility of a given protein is markedly impaired by excessive heat treatment. Further discussion of this subject is therefore deferred to the section on heat treatment.

Presence of Biologically Active Components

A given protein may have an adequate amino acid pattern, and the amino acids of the purified protein may be totally available to the animal organism. Purified proteins, however, do not exist in nature, and in some cases—notably legumes—are found in conjunction with certain substances which reduce their nutritive value. These substances, known as "biologically active components," are of various types, of which two are especially important: trypsin inhibitors and hemagglutinins or lectins (17-19). The most important biologically active components found in soybeans include proteinase inhibitors, phytohemagglutinins, antivitamin and mineral factors, goitrogens, allergins, saponins, sterols, phenalic compounds and flavus factors.

As the name implies, trypsin inhibitors reduce nutritional quality by decreasing or inhibiting the action of the pancreatic enzymes trypsin and chymotrypsin, thus effectively impairing digestibility (19). Hemagglutinins or lectins have the ability of agglutinating blood cells of many animals, including humans (19,20). It is known that they inhibit growth in a number of animals fed diets containing adequate quantities and qualities of proteins (21,22); this growth inhibition appears to be correlated to their toxicity and hemagglutinating activity (23,24).

It has been amply demonstrated that antinutritional factors such as trypsin inhibitors and hemagglutinins are destroyed by mild heat treatment, which is considerably less than that required to impair protein nutritional quality by destruction or inactivation of essential amino acids (25). Application of heat treatment, therefore, to proteins which contain biologically active components markedly increases their nutritive quality as a result of destruction of these substances.

EFFECT OF PROCESSING ON NUTRITIONAL QUALITY OF SOYA PROTEINS

Heat Treatment

By far, the most important process encountered with soya products is heat treatment. Defatted soya flour and grits, full-fat soya flour and grits, soyamilk, soyamilk curd and soya protein concentrate, among others, include a heat treatment step in their production.

It has generally been established by many investigators that the nutritional quality of soy proteins first increases, passes through a maximum and then decreases, all with increasing degree of heat treatment, as shown in Figures 1 and 2.

The beneficial effect of heat treatment on the nutritive value of soya proteins has been the subject of numerous studies, and the extensive literature dealing with this subject has been reviewed elsewhere (26,27). Generally, it is believed that the improvement in nutritive value of soya proteins probably results from inactivation or destruction of antinutritional factors; e.g., it has been found that nutritive value improvement closely follows trypsin inhibitor inactivation (Fig. 2; ref. 19). Figure 1 shows that moist heat is essential for obtaining the marked improvement in nutritive value, and that steaming under pressure produces this effect at a faster rate than steaming under atmospheric conditions (28). Factors which affect the efficacy of heat treatment in improving the nutritive value of soya proteins are summarized as follows.

Steaming temperature. Maximal nutritive value is obtained after steaming whole, initially dry soybeans for 20-30 min at atmospheric pressure, or for 15-20 min at 15 psi (29).

Moisture content. Atmospheric steaming inactivates most of the trypsin inhibitor in whole soybeans in 15-20 min if the initial moisture content is 20%. If the beans are soaked in water overnight to 60% moisture, 5 min in boiling water is sufficient to inactivate the inhibitors (30). Also, maximal PER obtained upon heating increases with increasing soybean moisture content (17).

Particle thickness. Atmospheric (100 C) steaming inactivates more than 95% of the trypsin inhibitor activity of raw, defatted soybean flakes in 15 min. In contrast, steaming whole soybeans, chips or cotyledons for 20 min only partially inactivates trypsin inhibitor, apparently because of the large particle size, which retards heat penetration (31).

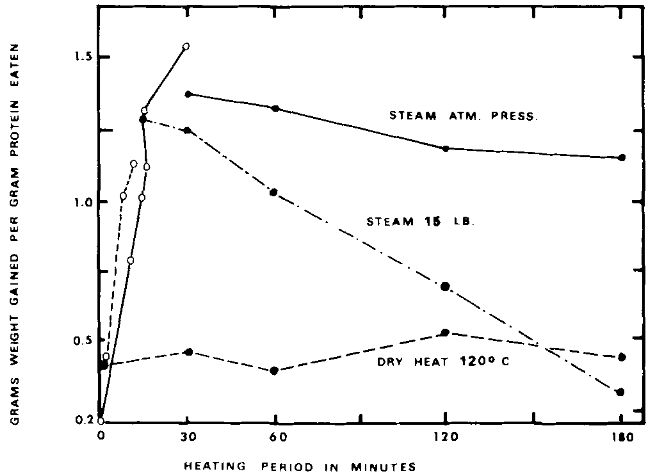

FIG. 1. Effect of type and extent of heat treatment on nutritional value of soybean protein (ground whole soybeans) (37).

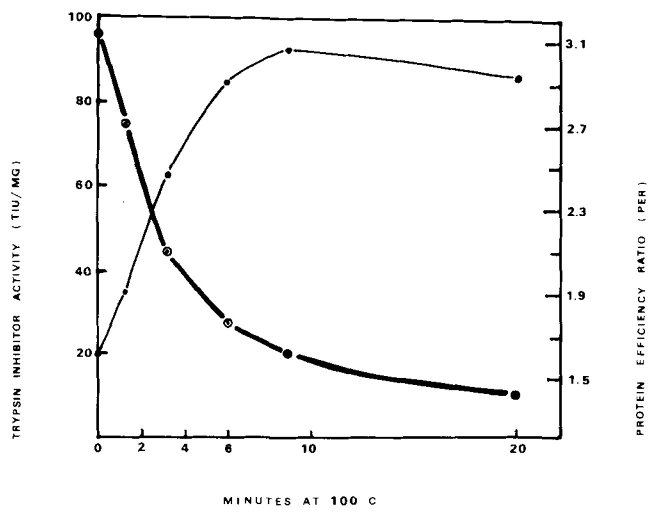

FIG. 2. Effect of atmospheric steaming on trypsin inhibitor activity and protein efficiency ratios of soybean-meal-fed rats (19).

Adverse effects of heat treatment on protein quality previously were explained as being due to the destruction or inactivation of certain essential amino acids. Cystine is particularly sensitive to heat, and as much as one-half to one-third of the cystine content of soybean protein may be destroyed by excessive heat (12-14). Lysine, besides undergoing destruction when proteins are overheated (15,16), is also rendered unavailable by the Maillard or browning reaction, in which its epsilon-amino groups react with the carbonyl groups of reducing sugars. In the case of soybeans subjected to heat treatment, the hydrolysis of sucrose may give rise to appreciable levels of reducing sugars which interact with lysine. Lysine so modified is no longer physiologically available, because the peptide bond containing the modified lysine is not susceptible to cleavage by trypsin; thus, the digestibility of proteins by pancreatic enzymes, whether in vitro or in vivo, is considerably reduced (32,33). A direct consequence of impaired digestion is retardation of the rate at which amino acids are released from the protein during digestion (33-36). Although not all workers have been able to demonstrate that lysine becomes limiting in overheated soybean products (13), it is important to remember that one of the principle assets of soybean protein is its high lysine content, which can be used to supplement lysine-deficient cereal products. Thus, the lysine deficiency of overheated soybean protein may not become apparent unless such products are used in combination with other proteins which are limiting in lysine. With regard to cystine, it has been noted that its destruction further intensifies the deficiency of methionine in overheated soybeans (37). In any case, it has definitely been established that heat damage to soya protein may be overcome by supplementation with lysine and sulfur-containing amino acids (37).

In addition to cystine and lysine, a number of other amino acids, including arginine, tryptophane, histidine and serine have been found to be either partially destroyed or inactivated as a result of excessive heating (26). On the other hand, because these amino acids are not limiting in soya proteins, their destruction or inactivation does not appear to greatly affect protein quality (26).

Finally, it has been found that nutritive quality loss in soybeans by heat proceeds at a much faster rate when steaming is done under pressure than when it is done atmospherically, and also that dry heat appears to have no effect (29).

Solvent Extraction

Extraction of soybean flakes for removal of oil with aliphatic hydrocarbons such as hexane, heptane and cyclohexane is used in the industrial production of defatted grits and flour (38). As far as we know, solvent extraction per se has no effect on soybean protein quality; rather, this appears to be affected by the degree of heat treatment applied in the desolventizing step (20). Different types of desolventizers exist which apply different degrees of heat treatment for the production of defatted products of different types (39). Generally, heat treatment denatures proteins, decreasing their solubility so that, in these products, degree of heat treatment is determined indirectly by measuring protein solubility. Table III compares the nutritive value of defatted soy flakes which were extracted with hexane under identical conditions, but were desolventized at different temperatures (40). As degree of heat treatment increased, protein solubility (PDI) decreased and PER increased. However, the factor which determined protein quality was heat treatment and not solvent extraction.

A few words on why extraction of soybeans with aliphatic hydrocarbons has no effect on protein quality might be of interest. First, extraction is usually done at moderate temperatures. Second, because extraction solvents used are hydrophobic or nonpolar, they are capable of dissolving soybean oil, but have little or no effect on soybean proteins. Also, because of the total incompatibility of the extraction solvent with water, except for the small amount of moisture present in soybean flakes, extraction is essentially done under anhydrous or "dry" conditions, and it has been established that dry heat has no effect on protein quality (Fig. 1). It is known, on the other hand, that use of more polar solvents for extraction (ethanol, isopropanol or trichloroethylene) does affect soya protein

TABLE III

Effect of Heat Treatment on Solubility and Nutritive Value of Soy Proteins (40)

Heat treatment	PDI[a]	Corrected PER
Untoasted	85 +	1.31
Lightly toasted	60-75	1.59
Fully toasted	20-40	2.19

[a] PDI = Protein dispersibility index, a measure of solubility of protein in water under standard conditions, AOCS-MOCS Method 10-65.

solubility and hence may affect protein quality as well (41,42).

Leaching of Low Molecular Weight Substances (Soya Protein Concentrate)

Defatted soya flour and grits have ca. 50% protein. To prepare products of higher protein content, it is necessary to remove low molecular weight components such as water-soluble sugars, ash and other minor constituents by leaching. Products in which the protein content has been increased by this method are known as "soy protein concentrates" (20).

Three processes are used commercially to prepare concentrates. They differ from each other mainly in the method used to insolubilize the major proteins while the low molecular weight components are removed (43,44). In the first process, the soluble constituents are leached out with aqueous alcohol leaving the proteins and polysaccharides, which are desolventized and dried. In the second process, the major proteins are insolubilized by extracting with dilute acid at the isoelectric point (pH 4.5), after which the insoluble protein-carbohydrate mixture is neutralized and dried. Because some of the minor proteins are soluble at pH 4.5, there is some loss and fractionation of proteins in this process. The third process takes advantage of the heat sensitivity of soy proteins: the flakes or flours are heated with moisture to denature the proteins and insolubilize them; the soluble constituents are then leached out with hot water (20).

Despite the different processes used, overall composition of the concentrates are very similar, and their protein content on a dry basis lies within a relatively narrow range, as shown in Table IV (45). Protein solubility, on the other hand, is very low for the alcohol and moist, heat-treated products, and relatively high for the acid-extracted product.

The effect of leaching on the nutritive value may be determined by analyzing its effect on the three factors which are known to affect protein quality, i.e., amino acid composition, amino acid availability and biologically active factors.

No appreciable difference exists between amino acid composition of defatted soyaflour and soya protein concentrates obtained therefrom. Leaching by the different methods, therefore, apparently does not substantially alter amino acid compositions. It may be concluded that either a negligible amount of protein is removed by leaching (as is probably the case with the alcohol and hot water processes) or else whatever protein is removed has approximately the same amino acid pattern as that which remains (which is probably the case with the acid-leach).

Regarding amino acid availability and biologically active components, both have been shown to depend primarily on degree of heat treatment. Unfortunately, not much information is available on heating conditions employed industrially for making soya protein concentrates. However, some data available in the literature might shed light on this point. Longenecker (46) found considerable variation in the PER values of a number of unheated commercial soya protein concentrates which, when heated at 105 C for 30 min, gave values approaching those of defatted soya flour. On the other hand, Meyer (45) was unable to demonstrate any improvement by heat treatment in the nutritive value of several commercial products examined. Generally, PER values reported in the literature for concentrates (Table V) shows them to vary little from each other, and to approximately equal known values for defatted soya flour.

The preceding observations, and the general effect of leaching on protein nutritive value in the preparation of concentrates, may be summarized as follows. If the defatted soya flour or flakes from which the concentrate is made has received adequate heat treatment, concentrate nutritive value probably cannot be further improved by heat treatment, and will probably equal that of the flour. On the other hand, if the defatted flour has not received sufficient heat treatment, concentrate nutritive value will either equal or exceed that of the flour. It will equal that of the flour if it receives further heat treatment during its preparation or thereafter; it will be greater than that of the flour if heat treatment is applied in the leaching step or thereafter. Because it does not appear to appreciably alter amino acid pattern, leaching per se (independently of heat treatment) probably has no effect on protein nutritonal quality.

TABLE IV

Compositions of Soy Protein Concentrates Obtained by Different Processes (45)

Component	Alcohol leach	Acid leach	Moist heat, water leach
Protein (%)	66	67	70
Moisture (%)	6.7	5.2	3.1
Fat (%)	0.3	0.3	1.2
Crude fiber (%)	3.5	3.4	4.4
Ash (%)	5.6	4.8	3.7
Nitrogen Solubility Index	5.0	69.0	3.0
pH of 1:10 water dispersion	6.9	6.6	6.9

TABLE V

PER Values for Defatted Soybean Meal, Defatted Flour and Soy Protein Concentrates[a]

Product	PER Range	PER Average	Reference
Solvent extracted meal, uncooked	0.3-0.6	0.5	130
Solvent extracted meal, autoclaved	1.1-2.9	1.9	130
Defatted flour	1.5-2.4	1.8	130
Protein concentrate	0.3-2.5	1.4	45,46

[a] Wherever possible, PER values have been corrected on the basis of a PER of 2.5 for casein.

Protein Solubilization and Precipitation (Soya Protein Isolate)

Another process to which soya proteins are subjected is solubilization in dilute alkali, followed by precipitation at the isoelectric point, in the production of isolates. Processing conditions are usually pH at 7-8, temperature 50-55 C for solubilization, and pH of 4-5 and ambient temperature for precipitation (20,47,48). After separating the protein curd by filtration or centrifugation, it is washed with water and either directly dried to produce the isoelectric protein, or neutralized and spray-dried to produce the sodium proteinate form. Isolates generally contain above 90% of highly soluble protein because the starting material is raw, unheated flakes and the temperature is kept relatively low throughout the process.

Processing of soya proteins in the production of isolates affects their nutritive values. In the first place, because not all protein is solubilized in the alkaline extraction step, and not all solubilized protein is precipitated in the isoelectric precipitation step, a certain fractionation of protein occurs. This fractionation reduces the sulfur-containing amino acids, a fact which has received biological confirmation with rats (19), pigs (49) and human subjects (50). The supplementation of soy protein isolates with methionine, on the other hand, raises their nutritive value to that of casein when assayed with rats (51), and to 85% of milk proteins when assayed with pigs (52).

Second, because isolates are prepared from raw flakes, isolated proteins may not be totally free from trypsin inhibitor and other biologically active components. It has been found that elimination of these components depends, to a large extent, on the thoroughness of the washing step (28). Thus, several investigators have found that nutritive value of soya isolates can be improved by heat treatment (45,51,53).

Finally, alkaline extraction of soybean meal under more drastic conditions may also damage certain amino acids. De Groot and Slump (54) 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, which in turn is produced by the interaction of dehydroalanine—a decomposition product of cystine and serine—and the epsilon amino group of lysine. Supplementation of the alkali-damaged protein with methionine partially, but not totally, restored its nutritive value.

Typical PER values for soya isolates reported in Table VI show that all are inferior to values reported for soya flour. Interestingly, however, in studies conducted with human subjects using nitrogen balance techniques, Young and his associates (55) found that for young children and adults, under conditions of normal usage of soya proteins, methionine supplementation of good quality soya protein isolates was unnecessary, and was required only for newborn infant formulas.

Spun Soya Fiber

An important and interesting application of isolates is the production of spun fiber for use in textured foods and meat analogs (56-58). In this process, soya protein is dissolved in alkali and then extruded through a die or forced through spinnerets into an acid or acid-salt bath to form fibers, which can be manipulated to simulate a wide variety of foodstuffs.

The amino acid compositions of some textured foods made from spun soya fiber (50) indicate that cystine has not been destroyed, probably because the protein isolate was treated at pH 12 for only 10 min at room temperature (59). Bressani et al. (59) made a comprehensive study of the protein quality of soybean protein textured foods, and concluded that their nutritive value was equivalent to that of beef protein, and ca. 80% that of casein. PER values reported for textured meat analogs made from spun soy fiber are of the order of 2.3 (46,51).

Because of considerably milder processing conditions, and probably better washing, spun soya fiber, although a form of isolate, apparently has a higher nutritive value (PER) than normal forms of isolates.

TABLE VI

PER Values for Defatted Soybean Meal, Defatted Flour, Soy Protein Isolate and Textured Meat Analog (spun fiber)[a]

Product	PER		Reference
	Range	Average	
Solvent extracted meal, uncooked	0.3-0.6	0.5	130
Solvent extracted meal, autoclaved	1.1-2.9	1.9	130
Soy protein isolate	0.6-1.9	1.3	46,51
Textured meat analog		2.3	59

[a]Wherever possible, PER values have been corrected on the basis of a PER of 2.5 for casein.

Extrusion

In extrusion cooking, soybean products are subjected to temperatures, pressures and shear rates of different intensities for varying periods of time. Several types of industrial extruders exist for use in the food industry, each with its own peculiar characteristics (60).

Generally, three types of soya products are made by extrusion processes: textured soy protein, full-fat soy flour and extrusion-cooked mixtures of soybeans with cereals and/or other legumes.

Textured protein is produced by extrusion cooking of defatted soya flour or grits. By proper control of processing characteristics (temperature, pressure, and moisture content) in the extruder, as well as raw material characteristics (fat content and protein solubility), a product with texture similar to that of meat may be obtained (61). Adequate control of temperature, pressure and moisture during extrusion inactivates antinutritional factors (62). On the other hand, comparison of amino acid data for textured soya protein and defatted soy meal reveal the textured protein to have suffered some reduction in methionine and lysine, suggesting excessive heat treatment during extrusion (63,64). Generally, however, PER values for textured soy protein tend to resemble closely those for defatted soy meal, showing inactivation of antinutritional factors during extrusion to be adequate, and heat treatment to not be over-excessive (65,66).

Full-fat soya flour is produced by extrusion cooking of whole, raw soybeans which have usually been dehulled; the product leaving the extruder is then finely ground to the required mesh size. (It should be noted that extrusion cooking is not the only process used for making full-fat soy flour; other processes employ cooking, toasting or dry roasting of soybeans to apply proper heat treatment, followed by fine grinding [67-69]). Available data reveal that this process (extrusion cooking) affects the nutritive value of full-fat soy flour in much the same way that it affects that of textured soy protein, i.e., inactivation of antinutritional factors with the possibility of some heat destruction or inactivation of amino acids (70). Indeed, Table VII shows that the nutritive value of full-fat soy flour extruded from dehulled soybeans reflects the typical effect of heat treatment, i.e., it first increases with increasing extrusion temperature (revealing increased inactivation of antinutritional factors), passes through a maximum (at ca. 143 C) and then decreases with increasing temperature (probably because of inactivation or destruction of some

TABLE VII

Effect of Extrusion Temperature on the Nutritive Value of Full-Fat Soy Flour (70)

Extrusion temperature (C)	Corrected PER
Raw, dehulled soybeans	1.01
121	1.35
127	1.42
132	1.41
138	1.55
143	1.94
149	1.78

amino acids as a result of excessive heat treatment).

Protein nutritive values of mixtures of soybeans and other legumes behave in essentially the same way, and are affected by the same factors as those which affect textured soy protein and extrusion-cooked, full-fat soy flour.

Fractionation of Proteins (Soyamilk and Soyamilk Curd)

In the preparation of soyamilk as well as soya curd by the traditional method, soybean proteins are fractionated. In the first case, water soluble or dispersible proteins are separated from insoluble or nondispersible proteins, which are left in the residue. In the second case, proteins which are precipitated by acid, calcium or magnesium ions are separated from those which are not so precipitated. This fractionation of proteins can, and does, also result in a fractionation of amino acids. This change necessarily modifies the nutritional value of the fractions.

To prepare soyamilk by the traditional method (71-73), whole soybeans are thoroughly washed and soaked in water 3 hr or more, depending on the temperature of the water, and ground wet with addition of enough water to give the final desired solids content. The ratio of water to beans is ca. 10:1. The slurry is heated at near its boiling point for 15-20 min to inactivate toxic factors and reduce microbial load. The suspension is finally filtered, yielding soyamilk (filtrate) and an insoluble residue.

The extent of fractionation of amino acids from the original soybeans into milk and residue may be appreciated from Table VIII (74-79). The most important differences between milk and residue are higher lysine and aromatic amino acid contents for the milk, but higher sulfur amino acid, threonine, tryptophane, leucine and valine contents for residue. Because soya protein is abundant in lysine, but limiting in sulfur-containing amino acids, it follows that, as a result of this fractionation, soyamilk residue should have a somewhat higher nutritive value than soya milk, and this has indeed been found to be true (4,78,80,81).

Concerning other processing parameters in soyamilk production, Hackler et al. (82) stated that the time and temperature of heat treatment of soyamilk from 1-6 hr at 93 C had no adverse effect on rat growth, PER, or available lysine.

Soyamilk curd, or tofu, is prepared from soyamilk by precipitation of protein, usually with calcium or magnesium ions, but sometimes also with acid. This process fractionates proteins between those insolubilized by the precipitating substance and those not so affected. Reference to Table VIII shows that milk and curd proteins are not much different in amino acid pattern, although milk proteins are somewhat richer in lysine, sulfur-containing amino acids, threonine, tryptophane and valine. Because this difference is not too great, PER values are not expected to be different, although soyamilk should have a higher value than curd. This is indeed true (4,78,80,81). It might be noted that acid-precipitated tofu apparently has a better amino acid pattern than either calcium- or magnesium-precipitated tofu, because the PER value of the acid-precipitated tofu exceeds that of the other (78).

Concerning effect of heat treatment, recall that tofu is produced from soyamilk which has already been heated to inactivate toxic factors. This heat treatment appears to be adequate; Chang and Murray (83) found that autoclaving did not enhance the nutritive value of tofu.

Drying

In drying soya products, all available evidence indicates that nutritional value is affected only by degree of heat treatment. Most work on drying of soya products has involved dehydration of soyamilk, usually by spray drying, but also by other methods. Spray drying has been preferred because it minimizes thermal damage by the exceedingly short contact times and high evaporation rates. Of all process variables encountered in spray drying, inlet temperature has the most marked effect on protein quality of dried soyamilk (84). Thus, Hackler et al. (82) found that temperatures below 277 C did not affect protein quality, but raising temperatures above that value produced marked decreases in available lysine and protein quality. Generally, PER values of soyamilk spray-dried under adequate conditions have been found to differ little from those of liquid soyamilk (76,84).

Hand et al. (85) and Van Buren et al. (86) made extensive investigations of the effect of vacuum roller drying, atmospheric roller drying and freeze drying on the nutritive value of soyamilk. They found essentially the same PER values for all products, when processed under adequate conditions, thus verifying the fact that in drying, degree of heat treatment alone, and not method of drying, determines nutritive value of the final product.

Grinding

Information about the effect of grinding on the nutritive value of soybean proteins is quite limited. However, evidence indicates that with grinding, as with drying, nutri-

TABLE VIII

Amino Acid[a] and Other Data for Soymilk and Soymilk Curd Fractions (74-79)

Measurement	Beans	Milk	Residue	Curd	Whey
Protein content, dry basis (%)	45	52	24	59	59
Percentage of original protein	100	83	17	74	9
Amino acids					
Isoleucine	4.8	4.8	4.5	4.9	2.9
Leucine	7.8	7.9	8.0	8.0	4.0
Lysine	6.5	6.1	5.1	5.9	8.8
Methionine	1.4	1.4	1.0	1.4	2.2
Cystine	1.6	1.6	2.3	1.7	2.7
Total sulfur	3.0	3.0	3.3	3.1	4.9
Phenylalanine	5.1	4.9	4.9	4.8	2.0
Tyrosine	3.9	3.9	3.0	3.7	3.8
Total aromatic	9.0	8.8	7.9	8.5	5.8
Threonine	4.2	3.9	4.1	3.7	5.2
Valine	5.0	4.8	5.0	4.7	3.2

[a] Amino acid content expressed as g/16 g nitrogen.

tional quality is affected only by heat treatment. On the other hand, because temperature rise in grinding—even in the case of very finely ground flours (100-300 mesh) is only 10-15 C (M. Vega, personal communication), the amount of heat treatment received is negligible, so that protein qualities of finely ground soya flours—defatted and full-fat—are expected to be essentially the same as those of grits or flakes from which they were made, and indeed, they are (87-89).

Fermentation

Numerous fermented soybean products exist in the Orient. Of these, the most important for human nutrition are those which are consumed in appreciable amounts in meals, i.e., tempeh, natto and miso. Fermented soy sauces are less important because they are used only as seasonings, so that total amounts of these products consumed per capita are rather small when compared to the others (20,38).

Tempeh, natto and miso are quite similar; all are basically soybeans which have been inoculated and fermented by different organisms (tempeh by *Rhizopus oryzae*, natto by *B. subtillis* or *B. natto*, and miso by *Aspergillus oryzae*; ref. 38). Miso differs from natto and tempeh in that it also contains rice. The procedures for preparing the three products all include a soybean cooking step before inoculation and fermentation, so that it is safe to conclude that little or no toxic factors remain in the final products (20,38).

Decreases in lysine and methionine have been reported for tempeh (90,91), increases in lysine and tryptophane for natto (92), and increases in leucine, aromatic amino acids and valine for miso (92). Nutritive values of all these products are fairly similar to those of cooked, unfermented soybeans, with the exception of deep-fat-fried natto which, probably because of excessive heat damage, has suffered a considerable drop in PER value. These results (i.e., no appreciable difference in nutritive value between fermented and unfermented soybeans) are consistent with previous observations on similarity of amino acid patterns between fermented and unfermented beans, and adequate heat treatment (by cooking) of all products to inactivate or destroy antinutritional factors.

Germination

The effect of germination on the nutritive value of soybean proteins has been studied by a number of investigators (80,81,93,94), all of whom have found an increase over ungerminated beans for rats. On the other hand, a decrease in nutritive value for chicks has been reported (95).

Comparison of amino acid patterns of germinated and ungerminated beans (92,96) shows little difference, except that germinated beans appear to contain somewhat more lysine and aromatic amino acids, but somewhat less sulfur-containing amino acids than ungerminated beans. Because lysine is abundant, and sulfur amino acids are limiting in soybeans, the difference would point to an effect opposite that observed, i.e., a decrease, and not an increase, in nutritive value (Table IX).

Desikachar and De (93) found no change in trypsin inhibitor content, but Bau and Debry (94) reported a 30% decrease upon germination.

Finally, because all nutritive value determinations reported here were conducted with unraw, unheated soybeans, the possibility of inactivation of toxic factors, or heat damage to proteins must be ruled out.

The effect of germination on soybean protein nutritive value remains unclear and is not easily explained, but appears to be real. Standal (81,92) concluded from her studies that germinated soybeans compared very favorably with other soybean products which are used as Oriental foods, their NPU being intermediate between that of tofu and natto.

Storage

Mitchell (97) and Mitchell and Beadles (98) reported a definite deterioration in digestibility and biological value of soybeans which had been stored for almost 3 years at 25.5 C. This deterioration was particularly marked with whole raw beans, but could largely be prevented by pretreatment with heat. For this reason, the decrease in digestibility and biological value were reported to be caused by enzymatic reactions. Zimmerman et al. (99) found that the NPU of defatted meal decreased with prolonged storage times at different temperatures, and was correlated with a decrease in available lysine. This loss in available lysine, which was also exhibited by isolated soya protein, was attributed to the formation of an atypical peptide bond between lysine and glutamic or aspartic acids (100).

Dry Roasting

Cowan (69) and J.M. Harper (personal communication) have studied the dry-bed roasting of soybeans and other legumes. The seeds were dropped into an inclined, rotating drum which contained sand, salt or ceramic pellets as a heat-transfer medium and held at 196-204 C for ca. 20-25 sec. They were then dropped into a separator for transfer of the salt, sand or pellets back to the rotating drum heater. Roasted beans may be ground to a flour or converted to another product.

PER and digestibility values reported for roasted soybeans are superior to those for autoclaved beans, and trypsin inhibitor activity is apparently reduced 75% to 4×10^{-3} units/g, and hemagglutinin units to 0.2 (69).

Hydrolysis

Hydrolyzed soybean protein is used as a seasoning (soya sauces), flavoring agents for foods and, to a lesser extent, for its functional properties. On an industrial scale, mainly two types of hydrolytic processes are used: acid and enzyme hydrolysis. Hydrochloric is the most commonly used acid, which easily leads to a 100% completion of the hydrolysis. Hydrolysis by isolated proteolytic enzymes or under alkaline conditions proceeds substantially slower, and results only in partial hydrolysis, making the resulting products useful for their functional properties. Generally, hydrolyzed vegetable proteins are defined as mixtures, composed primarily of amino acids, other substances such as peptides obtained also by hydrolysis, and salt (101).

Because hydrolyzed soya protein is used in relatively small amounts in foods, little or no information exists in the literature regarding its nutritive value. However, much about the chemistry of its production is known, so that

TABLE IX

PER Values for Ungerminated and Germinated Soybeans

Product	PER[a]	Reference
Ungerminated soybean, raw, immature	1.1	80
Ungerminated soybean, raw, vine-ripened	0.5	66
Ungerminated soybean, raw, mature	0.7	130
Germinated soybeans, raw	1.36	81
	1.4	80

[a] Corrected PER values have been reported whenever possible.

from this information some conclusions concerning its nutritive value may be drawn.

First, because antitrypsins and hemagglutinins are proteins, they are also probably attacked by the hydrolysis process, and hence are either destroyed or inactivated. That this happens may be inferred from the fact that vegetable protein hydrolysates, including soya protein hydrolysates, are considered generally recognized as safe (GRAS) in the U.S. (101) in their present level of use in foods.

Concerning amino acids resulting from proteins by hydrolysis, it is known that, under conditions of hydrolyzed vegetable protein production, or during the concentration or drying steps, part of these react with sugars or sugar degradation products in nonenzymatic browning reactions, Strecker degradations or oxidation and condensation reactions into meat-like flavor compounds (101). This would point to the loss of appreciable amounts of essential amino acids, including lysine and possibly cystine, with a consequent loss in nutritive value.

Homogenization

In a number of processes, liquid soyamilk is homogenized in order to increase stability to settling of dispersed solid particles (73,102,103). Although homogenization is usually done at high pressures (2,000-4,000 psi), in the author's experience, the resulting temperature rise of soyamilk in passing through the homogenizer is very low, on the order of 2-5 C. This would indicate, excluding unexpected high pressure effects, that because of the insignificant amount of heat treatment applied, homogenization per se would probably have negligible effect on soybean protein quality. That this is, indeed, the case may be inferred from the fact that PER values of properly heat-processed homogenized soyamilks are essentially the same as those of properly heat-processed raw materials from which the milks were made (85,104).

Combinations of Soybean Proteins with Other Proteins

A number of important applications exist in which soybean proteins are combined with other proteins. Three cases are especially important: extension, supplementation and vegetable protein mixtures.

Extension involves addition of soya to animal proteins for the purpose of increasing yields and decreasing finished product cost, all at essentially constant nutritional content (105-107). Its most important application is in the meat products field, especially processed meats (108-111)—extension of, e.g., hamburger patties, meatballs, frankfurters and salami—although dairy and egg products have also been extended (112). Supplementation refers to addition of soy proteins to other vegetables, mostly cereal proteins, with the purpose of improving their nutritional values, usually by supplying lysine, which is abundant in soy but limiting in cereal proteins. In this case, soya proteins are usually the minor component. Examples of supplementation include enrichment of bread and other baked goods, tortillas and other corn products, and rice-based products with soya proteins (113-122). Finally, as their name implies, vegetable protein mixtures refer to blends of different vegetable proteins whose combined amino acid pattern, because of amino acid complementation, is superior to those of the individual components (123-126). Examples of vegetable protein mixtures include the Incaparinas developed at the Institute of Nutrition of Central America and Panama (125) and the soy-oats infant formula developed at the Chihuahua Institute for Nutritional Research and Development (126).

All evidence available in the literature indicates that the effect of processing on the nutritional value of mixtures of soya with other proteins is essentially the same as that for soya proteins alone and that heat treatment is the most important single process variable which determines nutritive values.

A few examples of the effect of processing on nutritive value of mixtures of soy with other proteins may be mentioned. Tribelhorn et al. (127) and Cummings et al. (70), using a low-cost Brady extruder, found that PER of corn-soy, cassava-soy and potato-soy mixtures first increased, passed through a maximum and then decreased, all with increasing extrusion temperature in exactly the same manner as that encountered for full-fat soya flour. Del Valle and Pérez-Villaseñor (121) and Del Valle and Montemayor (122) found that properly heat-processed corn-soy tortillas had PER values comparable to those of properly processed soybeans alone, and considerably greater than those of tortillas made for corn alone. Young et al. (55) found that adequately heat-processed beef-soy frankfurters (using soy protein isolate) had a nutritive value, by nitrogen balance studies with humans, similar to that of 100% beef frankfurters. And Bressani et al. (124) found that properly heat-processed mixtures containing corn, defatted soy flour and defatted cottonseed meal had PER values superior to those of the individual components. More information is available to the interested reader (131-149).

REFERENCES

1. Osborne, T.B., and L.B. Mendell, J. Biol. Chem. 32:369 (1917).
2. Munro, H.N., and J.B. Allison, "Mammalian Protein Metabolism," Academic Press, New York, 1944.
3. Miller, D.S., "A Procedure for Determination of NPU Using Rats Body Techniques: Evaluation of Protein Quality," Publication no. 1100, Natl. Acad. of Sciences, Washington, DC, 1963.
4. "Amino Acid Content of Foods and Biological Data on Proteins," FAO, Food Policy and Food Science Service, Nutrition Division, Rome, 1970.
5. Derse, P.H., J. Assoc. Offic. Agric. Chem. 41:192 (1962).
6. Rose, W.C., Fed. Proc. 8:546 (1949).
7. Rose, W.C., Nutr. Abstr. Rev. 27:631 (1957).
8. "Evaluation of Protein Nutrition," Natl. Acad. Sci. Natl. Res. Council Publ. 711, 1959.
9. Mitchell, H.H., and R.J. Block, J. Biol. Chem. 163:599 (1946).
10. "Protein Requirements," Vol. 16, FAO UN Studies, 1957.
11. "Energy and Protein Requirements," FAO/WHO, Ad Hoc Expert Committee, World Health Organization Tech. Report, Series no. 522, Rome, 1973.
12. Evans, R.J., A.C. Groschke and H.A. Butts, Arch. Biochem. 30:414 (1951).
13. Iriarte, B.J.R., and R.H. Barnes, J. Food Technol. 20:131 (1966).
14. Taira, H., T. Koyanagi, T. Takanohashi and K. Oikawa, Agric. Biol. Chem. (Tokyo) 33:1387 (1969).
15. Evans, R.J., and H.A. Butts, J. Biol. Chem. 175:15 (1948).
16. Taira, H., H. Taira, K. Sugimura and Y. Sakurai, Agric. Biol. Chem. (Tokyo) 29:1074,1080 (1965).
17. Rackis, J.J., Fed. Proc. 24:1488 (1965).
18. Liener, I.E., and M.L. Kakade, in "Toxic Constituents of Plant Foodstuffs," edited by I.E. Liener, Academic Press, New York, 1969.
19. Rackis, J.J., JAOCS 51:161A (1974).
20. Wolf, W.J., and J.C. Cowan, "Soybeans as a Food Source," Butterworth's, London, 1971.
21. Liener, I.E., Ibid. 49:527 (1953).
22. Birk, Y., and A. Gertler, Ibid. 75:379 (1961).
23. Demuelenaere, H.J.H., Nature 206:827 (1965).
24. Stead, R.H., H.J.H. Demuelnaere and G.V. Quicke, Arch. Biochem. Biophys. 113:703 (1966).
25. Liener, I.E., and E.G. Hill, J. Nutr. 49:609 (1953).
26. Liener, I.E., in "Processed Plant Protein Foodstuffs," edited by A.M. Altschul, Academic Press, 1958.
27. Liener, I.E., in "Toxic Constituents of Plant Foodstuffs," Academic Press, New York, 1969.
28. Smith, A.K., J.J. Rackis, L.L. McKinney, D.J. Robbins and A.N. Booth, Feedstuffs 36:46 (1964).

29. Klose, A.A., B. Hill and H.L. Fevold, Food Technol. 2:201 (1948).
30. Albrecht, W.J., G.C. Mustakas and J.E. McGhee, Cereal Chem. 43:400 (1966).
31. Rackis, J.J., Food Technol. 20:1482 (1966).
32. Evans, R.J., and J. McGinnis, J. Nutr. 31:449 (1964).
33. Evans, R.J., J. McGinnis and J.L. St. John, Ibid. 33:661 (1947).
34. Riesen, W.H., D.R. Clandinin, C.A. Elvehjem and W.W. Cravens, J. Biol. Chem. 167:143 (1947).
35. Evans, R.J., and H.A. Butts, J. Biol. Chem. 178:543 (1949).
36. Clandinin, D.R., and A.R. Robblee, J. Nutr. 46:525 (1952).
37. Almquist, H.J., in "Amino Acids and Proteins," edited by D.M. Greenberg, Charles W. Thomas, Springfield, IL, 1951.
38. Smith, A.K., and S.J. Circle, "Soybeans: Chemistry and Technology"; Vol. I, Proteins, AVI Publ. Co., Inc., Westport, CT, 1978.
39. Kingsbaker, C.L., JAOCS 47:458A (1970).
40. Kellor, R.L., Ibid. 51:77A (1974).
41. Okotomo, S., Contemp. Manchuria. 1(3):83 (1937).
42. Eldridge, A.C., JAOCS 46:458A, 460A, 462A, 464A, 496A, 498A, 500A, 502A (1969).
43. O'Hara, J.B., and A.E. Schoepfer, U.S. Patent 3,207,774 (1965).
44. Mustakas, G.C., L.D. Kirk and E.L. Griffin, Jr., JAOCS 39:222 (1962).
45. Meyer, E.W., Proc. Int. Conf. on Soybean Protein Foods, U.S. Dept. Agr., ARS-71-35, 1967, p. 142.
46. Longenecker, J.E., W.H. Martin and H.P. Sarett, J. Agric. Food Chem. 12:411 (1964).
47. Wolf, W.J., Baker's Dig. 43(5):30 (1969).
48. Circle, S.J., P.L. Julian and R.W. Whitney, U.S. Patent 2,881,159 (1959).
49. Berry, T.H., G.E. Combs, H.D. Wallace and R.C. Robbins, J. Anim. Sci. 25:722 (1966).
50. Longenecker, J.B., in "Newer Methods of Nutritional Biochemistry," edited by A.A. Albanese, Academic Press, New York, 1963.
51. Huge, W.E., "Present and Potential Uses of Soybean Flour, Grits, and Protein Concentrates in Foods," Proc. Conf. Soybean Products for Protein in Human Foods, USDA, Peoria, IL, 1961.
52. Schneider, D.L., and H.P. Sarett, J. Nutr. 98:279 (1969).
53. Booth, A.N., "Physiological Effects of Feeding Soybean Meal and Its Fractions," Proc. Conf. Soybean Products for Protein in Human Foods, USDA, Peoria, IL, 1961.
54. DeGroot, A.P., and P. Slump, J. Nutr. 98:45 (1969).
55. Young, V.R., and N.S. Scrimshaw, JAOCS 56:110 (1979).
56. Irmiter, T.F., Nutr. Rev. 22:33 (1964).
57. Thulin, W.W., and S. Kuramoto, Food Technol. 21:168 (1967).
58. Kiratsous, A.S., Cereal Sci. Today 14:147 (1969).
59. Bressani, R., et al., Ibid. 93:349 (1967).
60. Harper, J.M., "Food Extrusion," CRC Critical Reviews in Food Sci. Nutr. (Feb. 1979).
61. Ziemba, J.V., Food Eng. 41(11):72 (1969).
62. Mustakas, G.C., W.J. Albrecht, G.N. Bookwalter, J.E. McGhee, W.F. Kwolek and E.J. Griffin, Jr., Food Technol. 24:1290.
63. Harmon, B.G., D.E. Becker, A.H. Jensen and D.H. Baker, J. Anim. Sci. 28:459 (1969).
64. Hamdy, M.M., JAOCS 51:85A (1974).
65. Hamdy, M.M., W.H. Case, M.S. Cole and F.E. Horan, "Economical Textured Vegetable Protein Foods from Defatted Soy Flakes," Protein Advisory Group, FAO/WHO/UNICEF, Geneva, Document 2.24/1, Sept. 1969.
66. Everson, G., H. Steenbock, D.C. Cederquist and H.T. Parsons, J. Nutr. 27:225 (1944).
67. Huang, P.C., T.C. Tung, H.C. Lue and H.Y. Wei, J. Formosan Med. Assoc. 64:591 (1965).
68. Pringle, W., JAOCS 51:74A (1974).
69. Cowan, J.C., Ibid. 56:168A (1979).
70. Cummings, D.A., G.R. Jansen, J.D. Kellerby and R.E. Tribelhorn, Annual Report: "*Evaluation of Low-Cost Extrusion Cookers for Use in LDC's*," Department of Agricultural and Chemical Engineering and Food Science and Nutrition, Colorado State University, Fort Collins, CO, 1979, p. 28.
71. Miller, H.W., "Traditional Method of Processing and Use of Soy Liquid and Powdered Milk," Proc. Conf. Soybean Products for Protein in Human Foods, Peoria, IL, USDA-ARS, June 1962, p. 149.
72. Smith, A.K., and A.C. Beckel, Chem. Eng. News 24:54 (1946).
73. Banzon, J.A., and E.E. Escueta, Paper presented at Fifth International Congress of Food Science and Technology, Kyoto, Japan, 1978.
74. Rackis, J.J., et al., J. Agric. Food Chem. 9:409 (1961).
75. Rackis, J.J., et al., Cereal Chem. 40:531 (1963).
76. Van Etten, C.H., et al., J. Agric. Food Chem. 7:129 (1959).
77. Smith, A.K., et al., Feedstuffs 36:46 (1964).
78. Hackler, L.R., D.B. Hand, K.A. Steinkraus and J.B. Van Buren, J. Nutr. 80:205 (1963).
79. Hackler, L.R., B.R. Stillings and R.J. Polimeni, Cereal Chem. 44:638 (1967).
80. Everson, G., H. Steenbock, D.C. Cederquist and H.T. Parsons, Ibid. 27:225 (1944).
81. Standal, B.R., J. Nutr. 81:279 (1963).
82. Hackler, L.R., et al., J. Food Sci. 30:723 (1965).
83. Chang, I.C.L., and H.C. Murray, Chin. J. Physiol. 16:83 (1949).
84. Ang, H.G., W.L. Kwik, S.F. Tan and O.Y. Theng, in "Proceedings of the International Soya Protein Food Conference," Republic of Singapore, Jan. 1978.
85. Hand, D.B., et al., Food Technol. 18:139 (1964).
86. Van Buren, J.P., et al., J. Agric. Food Chem. 12:524 (1964).
87. Kuppuswamy, S., M. Srinivasan and V. Subrahmanyan, "Proteins in Foods," Indian Council Med. Res., New Dehli, India, 1958.
88. Smith, C.R., in "Protein-Enriched Cereal Foods for World Needs," edited by M. Milner, Am. Assoc. Cereal Chemists, St. Paul, 1969.
89. Kon, S.K., and Z. Markuze, Biochem. J. 25:1476 (1931).
90. Stillings, B.R., and L.R. Hackler, J. Food Sci. 30:1043 (1965).
91. Steinkraus, K.H., D.B. Hand, J.P. Van Buren and L.R. Hackler, "Proc. Conf. Soybean Products for Proteins in Human Foods," USDA, Peoria, IL, 1961.
92. Standal, B.R., J. Am. Diet. Assoc. 50:397 (1967).
93. Desikachar, H.S.R., and S.S. De, Science 106:421 (1947).
94. Bau, H.M., and G. Debry, JAOCS 56:160 (1979).
95. Mattingly, J.P., and H.R. Bird, Poult. Sci. 24:344 (1945).
96. Evans, R.J., and S.L. Bendemer, J. Agric. Food Chem. 15:439 (1967).
97. Mitchell, H.H., Ind. Eng. Chem. Anal. Edn. 16:696 (1944).
98. Mitchell, H.H., and J.R. Beadles, J. Nutr. 39:463 (1949).
99. Zimmerman, G., I. Ben-Gara, S. Weismann and S. Yanal, Deut. Ges. Chem. Apparatewesen. Monograph 63:347 (1969).
100. Ben-Gara, I., and G. Zimmerman, Nature 202:1007 (1964).
101. Olsman, H., Ibid. 56:375 (1979).
102. Nelson, A.I., M.P. Steinberg and L.S. Wei, J. Food Sci. 41:57 (1976).
103. Mustakas, G.C., et al., Food Technol. 46:623 (1970).
104. Suberbie, F., F. Tello, L. Guzmán, R. Rocha and J. Pérez-Villaseñor, Paper presented at the Third Congress of *Asociación Nacional de Técnicos en Alimentos de México*, Mexico City, Mexico, 1972.
105. Rosenfield, D., and W.E. Hartman, JAOCS 51:91A (1974).
106. Wolford, K.M., Ibid. 51:131A (1974).
107. Karpati, G., Ibid. 51:159A (1974).
108. Senti, F.R., Ibid. 51:138A (1974).
109. Hutton, G.H.W., Ibid. 51:146A (1974).
110. Graham, G.G., and J.M. Baertl, Ibid. 51:152A (1974).
111. Balla, F., Ibid. 51:156A (1974).
112. Del Valle, F.R., et al., Paper presented at the XXXIX Annual Convention of the Institute of Food Technologists, St. Louis, MO, 1979.
113. Jones, D.B., Fed. Proc. 3:116 (1944).
114. Jones, D.B., and J.P. Divine, J. Nutr. 41:41 (1944).
115. Sure, B., "Nutritional Improvement of Cereal Grains with Small Amounts of Foods of High Protein Content, Univ. Arkansas Coll. Agric. Expt. Sta. Bull., 1950, p. 493.
116. Bornstein, S., and B. Lipstein, Isr. J. Agric. Res. 12:63 (1962).
117. Carlson, S.C., E.C. Hermann, R.M. Bnn and J.W. Hayward, Cereal Chem. 24:215 (1947).
118. Hayward, J.W., and C.M. Diser, Soybean Dig. 21(10):14 (1961).
119. Bressani, R., and E. Marenco, J. Agric. Food Chem. 11:517 (1963).
120. Cravioto, D.Y., et al., Ciencia (Mex) 10:145 (1950).
121. Del Valle, F.R., and J. Pérez-Villaseñor, J. Food Sci. 39:244 (1974).
122. Del Valle, F.R., and E. Montemayor, Ibid. 41:249 (1976).
123. Orr, E., and D. Adair, "The Production of Protein Foods and *Concentrates from Oilseeds*," Trop. Prod. Inst. Rept. Gel, London, 1967.
124. Bressani, R., and L.G. Elias, J. Food Sci. 31:626 (1966).
125. Bressani, R., and L.G. Elias, Adv. Food Res. 16:1 (1967).
126. Del Valle, F.R., et al., J. Food Sci. (in press).
127. Tribelhorn, R.E., J.M. Harper, M.L. Stone, G.R. Jansen, J.A. Maga and K.J. Lorenz, Paper presented at 1976 Winter

Meeting, American Society of Agricultural Engineers, Chicago, IL.

128. Altschul, A.M., "Proteins: Their Chemistry and Politics," Basic Books, New York, 1965.
129. "Protein Requirements," Food Agric. Org., UN Nutr. Meeting, Rept. Ser. 37, 1965.
130. Kuppuswamy, S., M. Srinivasan and V. Subrahmanyan, "Proteins in Foods," Indian Council Med. Res., New Dehli, India, 1958.
131. Desikachar, H.S.R., S.S. De and V. Subrahmanyan, Ann. Biochem. Exptl. Med. (Calcutta) 6:49 (1946).
132. Harkins, R.W., and H.P. Sarett, J. Nutr. 91:213 (1967).
133. Shurpalekar, S.R., S. Korula, M.R. Chandrasekhara and M. Swaminathan, J. Sci. Food Agric. 16:90 (1965).
134. Plan, J.H.C., Chin. J. Physiol. 4:431 (1930).
135. Smith, A.K., et al., Cereal Chem. 41:173 (1964).
136. Murata, K., H. Ikehata and T. Miyamoto, J. Food Sci. 32:580 (1967).
137. Györgi, P., in "Meeting the Protein Needs of Infants and Children," Natl. Acad. Sci.—Natl. Res. Council Publ., 1961, p. 843.
138. Matsuno, N., and E. Tamura, Ann. Rept. Natl. Inst. Nutr. (Tokyo) 8:9 (1964).
139. Hackler, L.R., K.H. *Steinkraus*, J.P. Van Buren and D.B. Hand, J. Nutr. 82:452 (1964).
140. Wodicka, V.C., JAOCS 51:101A (1974).
141. Mussman, H.C., Ibid. 51:104A (1974).
142. Ward, A.G., Ibid. 51:104A (1974).
143. Rakosky, J., Jr., Ibid. 51:125A (1974).
144. Wilding, M.D., Ibid. 51:128A (1974).
145. Arimoto, K., in "Meeting Protein Needs of Infants and Children," Natl. Acad. Sci.—Natl. Res. Council Publ. 843, 1961.
146. "Promosoy, Soy Protein Concentrate," Central Soya Co., Inc., Chicago, IL, Technical Service Bulletin.
147. "Promine, an Isolated Soy Protein," Central Soya, Inc., Chicago, IL, Technical Service Bulletin.
148. Poullain, B., D. Guisard and G. Debry, Nutr. Metabol. 14:298 (1972).
149. Kies, C., and H. Fox, J. Food Sci. 36:841 (1971).

Considerations in Development of Regulations for New Protein Sources

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ABSTRACT

In the development of regulations for new protein food sources, considerations are given to such factors as product definition, product safety, nutritional quality, consumer perceptions and fair marketing practices.

In the U.S. the food additive status of any new protein source would have to be considered. A determination would be necessary, depending on the type and use of the product, as to whether a new protein product has GRAS (generally recognized as safe) status or whether a food additive petition would be necessary before marketing. A new product would be considered GRAS if there is general agreement that a product is safe for human consumption and no concerns exist that the product could cause harm if consumed by any segment of the population. Although a manufacturer can make this determination on his own, generally, it would be best for the manufacturer to review his findings with the Food and Drug Administration (FDA) prior to marketing the new protein product to be sure that the agency agrees with his determination. Should the manufacturer or the FDA find unanswered questions about a product's safety, then a food additive petition would be required.

PRODUCT DEFINITION

The first consideration in regulation of a new protein source is the same as for any other food additive—that of defining the product. In general, two approaches have been used to describe a new product source. The first and most preferred is to describe the physical and chemical properties of the end item. Such a description should be as complete as possible to distinguish the product from other products in the marketplace. The description should contain information about the complete chemical profile of the product, including levels of all nutrients and contaminants found.

A second method for defining a new protein product is to detail the way in which the product was produced. This approach is generally less desirable because it requires records on production and disclosure of processing techniques. A manufacturer who wishes to describe a product in this manner may elect to file a food additive petition, even if he thinks the product is, in reality, GRAS. Many new products are described on the basis of a combination of these approaches. In such cases, the manufacturer elects to provide information on both production techniques and composition of the end product. For example, a general outline of the production process is provided, in which the starting products are specified, the processing steps are listed and the acceptance criteria used for the end product are stated. It is obvious that approval to market a new protein source or any other food additive requires a reasonable basis on which the product can be identified in the marketplace.

PRODUCT SAFETY

After a new product has been adequately defined, the next consideration is an assessment of its safety. Traditionally, the agency has required a so-called 100-fold safety factor for approval of new food additives. This strategy permits the use of 1% of the highest level shown to have no adverse biochemical or physiological effect in man or