Effectiveness of Methods for Evaluating the Nutritional Quality of Soybean Protein

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ABSTRACT

Various approaches can be used for the nutritional evaluation of protein sources. These include human and animal bioassays and microbiological, enzymic and chemical in vitro methods. Examples of the application of each of these approaches for the evaluation of soya protein sources are presented and evaluated in this paper. The ineffectiveness of rat assays for predicting nutritive value of soya protein for humans is documented. Current procedures, based on rat bioassays, which are used for nutritional labeling of dietary protein sources in the U.S. and Canada are discussed and alternative approaches described. In particular, the use of in vitro methods for estimating amino acid composition and essential amino acid bioavailability may have significant advantages for estimating the nutritive value of soya protein sources for human consumption.

INTRODUCTION

Several methods are available for estimating nutritional values of different dietary proteins and for assessing specific factors that may influence these nutritional values (1–12). In this paper, the usefulness of some of these methods and particularly their effectiveness for assessing the nutritive value of soybean protein products for human consumption are discussed. Some approaches based on in vitro methods will be suggested as possible alternatives to animal bioassays.

METHODS AVAILABLE

Various methods for estimating overall protein nutritional value, nitrogen or amino acid digestibility, and amino acid bioavailability are listed in Tables I–IV. Selected references (13–114) to studies in which these methods have been used to evaluate soybean proteins are also listed.

In the standard procedure for assessing protein nutritional quality for human consumption, a specific protein source is fed at several nitrogen (N) intake levels (slightly above and below the level required to maintain zero balance) to subjects who have undergone an adaptation period of several days (Table I). This procedure provides two estimates of protein nutritional value. First, an estimate of the efficiency of N utilization is obtained from the slope of the line resulting from plotting N balance vs the different levels of N intake used. Second, the intake of N from a given protein source that is necessary to achieve a zero N balance can be estimated. In addition to the test proteins, a high quality reference protein (usually egg or milk protein) is fed to the same subjects. Thus, the data for the test proteins can be expressed as relative values with respect to the reference protein. In this paper, we have used the two estimates, derived from use of the standard method (including the use of reference proteins) as a basis for assessing the usefulness of the other methods.

NUTRITIVE VALUE OF SOYA PROTEIN IN HUMANS

Nutritional values, relative to milk or egg protein, of soya protein for human consumption are given in Table V. All values listed were obtained by use of the standard method (see above). In general, but with some exceptions, the estimates obtained with the adult subjects suggest that the true protein nutritional value of the products was 85 to 95% that of milk or egg protein. Data from studies with children, in which the standard procedure has been used, are limited (Table V). However, these few data suggest that soya protein is utilized as effectively by children as by adults.

Fomon and Ziegler (36) have recently reviewed studies in which infant formulas based on soya protein isolate (Edi-Pro A) were compared to formulas based on milk. They concluded that methionine-fortified soya isolate promoted N retention and growth in young infants to the same extent as milk protein. At higher protein levels, the addition of L-methionine to the isolate did not have any positive effects. Thus, at the higher level of intake, the Edi-Pro A isolate apparently was equivalent to milk.

The data presented in Table V for adults and children and the data from studies with young infants (36) indicate that properly processed soya products have a high protein value in terms of human nutrition.

RATS VS HUMAN ESTIMATES

Studies with human subjects are expensive, time consuming, and technically difficult and may involve ethical questions. Some of the more rapid methods listed in Table I partially circumvent some of these problems, but in general the shorter methods cannot be considered to be established methods. In any case, their use for routine assessments of protein nutritional value would be impractical. Assay with rats has long been recognized as a practical alternative to assay with humans. However, rat assays for estimating protein nutritional value are useful only if they provide estimates that agree with estimates obtained from human assays. This does not appear to be true for many protein sources and, in particular, for soya protein sources (15, 115, 116).

Estimates of the nutritive values of the same soya protein preparations, derived from human and rat assays are listed in Table VI. In general, the estimates from the rat assays are markedly lower than those from the human studies. These and other data (14, 115, 116) indicate that rat assays do not consistently provide accurate estimates of protein nutritive values for humans.

For nutritional labeling of protein in the U.S. and for making claims about the nutritional value of protein foods in Canada, the official evaluation procedures involve the use of rat bioassays. In the U.S., the protein efficiency ratio (PER) assay is used (Table II). In Canada the relative net protein ratio (R-NPR) assay (Table II) was recently proposed as a replacement for the PER assay, which is now used. Due to differences in the amino acid requirements of rats and humans (14, 115), it is doubtful that any rat growth assay will provide a useful approach for regulatory purposes. The limited amount of data in Table VI along with values from different rat assays, as well as other data (14, 115, 116), support this observation.

ALTERNATIVES TO RAT BIOASSAYS

For the reasons noted above, routine assays with humans are impractical and undesirable. If rat bioassays are excluded, are there useful alternatives? For the most part, the methods listed in Tables III and IV have not been validated by evaluation of the same protein preparations in studies with humans. The amino acid scores and other indexes based on amino acid compositions are exceptions. The amino acid compositions of protein sources tested in human assays are often determined and reported or the compositions of similar proteins are known.

A generally recognized problem in the use of amino acid composition data is that all of the amino acids (as chemically determined to be present in a protein source) may not be nutritionally available. However, an approach for estimating

TABLE I

Human Bioassays Used for Evaluating Nutritional Value of Soya Protein

Method	Parameters measured	Information acquired	Use of method to evaluate soya protein (selected references)
Nitrogen balance: multiple intake levels of nitrogen (conventional, standard method)	Nitrogen intake and excretion during 8–12 day periods for each protein intake level	Estimates of the efficiency of the utilization of a protein source and of the minimal N required from a protein source for maintaining zero N balance; when a reference protein is included, relative values are obtained	13–24
Nitrogen balance: multiple intake levels of nitrogen (short-term method)	Nitrogen intake and excretion during 1–2 day periods for each protein intake level	Same parameters estimated as the conventional long-term, standard method (see above)	25–26
Nitrogen balance: single intake level of nitrogen	Nitrogen intake and excretion	Ranking of proteins when different sources are fed in the same study; values are dose-dependent, which complicates comparisons from study to study	27–33
Nitrogen balance and/or growth in infants or children	Nitrogen intake and excretion or weight gain/day, weight gain/100 kcal or length gain/day	Ability of proteins to support growth as fed; usually not used for ranking	34–48
Net protein utilization (NPU)	N balance at 1-protein intake level; % digestibility; NPU = biological value × % digestibility	Ranking of proteins within a single study; values are linear but dose-dependent	4, 15, 21
Urea nitrogen measurement	(1) Post prandial or fasting levels of plasma or serum urea N; (2) urinary urea N excretion	(1) Ranking of similar proteins; (not an established method); (2) general approximation of nutritive value (little data available)	34, 49, 50

TABLE II

Rat Bioassay Methods Used for Estimating Nutritional Value of Soya Protein

Method	Parameters measured	Information acquired	Use of method to evaluate soya protein (selected references)
Relative protein value (RPV)	Growth compared to growth obtained for reference protein (usually lactalbumin); slope-ratio assay based on multiple intake levels of protein	Ranking of proteins; values obtained are linear	15, 51–53
Protein efficiency ratio (PER)	Growth (1 point assay); casein usually fed as reference with values corrected (casein = 2.50)	Ranking of proteins; values obtained are not linear	15, 20, 51, 52, 54–68
Net protein ratio (NPR)	Growth corrected for weight loss of group fed nonprotein diet; (2 point assay)	Ranking of proteins; includes estimate of needs for maintenance; values obtained are linear	15, 53, 59, 61, 64, 68–70
Relative-NPR (R-NPR)	Same as NPR except values are expressed as a % of value for reference protein		51, 68
Relative nitrogen utilization (RNU)	Growth; allowance for maintenance calculated as a fraction of observed growth (1 point assay)	Values obtained, expressed as % of values obtained for reference protein, are similar to R-NPR values	15, 51, 52, 58
Net protein utilization (NPU)	Nitrogen balance or carcass nitrogen retention (1 point assay)	Ranking of proteins; values are linear but dose-dependent; nitrogen balance method based on determination of biological value and N digestibility	20, 51, 57, 61, 69, 71
Urea nitrogen measurements	Blood, serum or plasma urea nitrogen	Ranking of proteins; estimation of biological value; (not an established method)	72, 73

TABLE III

Chemical, Enzymatic and Microbiological Methods Used to Evaluate Nutritive Value or Digestibility of Soya Protein

Method	Parameters measured	Information acquired	Use of method to evaluate soya protein (selected references)
Nutritive Value			
Chemical			
Amino acid analysis	Amino acid composition of hydrolyzed test protein	1) Prediction of limiting amino acid and estimates of nutritive value from amino acid scores;	74–76
		2) values used to predict PER	77, 78
Chemical and enzymatic			
Computed protein efficiency ratio (C-PER)	Amino acid composition and in vitro enzyme digestibility (pH change)	Values used to predict PER	79
Pepsin digest residue (PDR)	Amino acid composition of test protein and of the enzymatic digest of test protein	Calculated PDR index (based on measured parameters) roughly estimates BV or NPU for a series of proteins	80
Pepsin pancreatin digest dialyzate (PPDD)	Amino acid composition of test protein and of the dialyzate of the enzymatic digest of test protein	Calculated PPDD index (based on measured parameters) roughly estimates BV or NPU for a series of proteins	81
Microbiological			
Tetrahymena	Organism growth by cell count or tetrahymenol produced	Relative utilization of a test protein compared to a standard protein or standard test media	82–84
Streptococcus zymogenes	Organism growth by cell count	Relative utilization of a test protein compared to a standard protein or standard test media	69, 85
Clostridium perfringens	Organism growth by manometry	Relative utilization of a test protein compared to a standard protein or standard test media	86
Digestibility			
Chemical			
Discriminant computed digestibility (apparent)	Amino acid composition	Values used to calculate nitrogen digestibility	77, 87
Enzymatic	1) Specific amino acids released after enzymatic digestion; 2) pH change during enzymatic digestion	1) Relative in vitro enzymatic release of specific amino acids (e.g., as affected by processing;	64, 70, 80, 81, 88, 89
		2) values are used to estimate total nitrogen digestibility	90–92

protein nutritional values based on amino acid composition data and upon some indication of the nutritional availability of the amino acids present, would appear to be potentially useful.

One such approach has been developed by Satterlee and co-workers (87, 91) and others (77, 79), who attempted to estimate PER values determined in rats, from empirical equations based on amino acid composition data and on either of two in vitro estimates of apparent nitrogen digestibility. The calculated values are designated as the C-PER (computed protein efficiency ratio), based on amino acid data and apparent nitrogen digestibility estimated by enzymic digestion, and the DC-PER (discriminant computed protein efficiency ratio), based on amino acid data and apparent nitrogen digestibility estimated from amino acid composition data.

When a large number of samples of widely varying protein nutritional values were studied, the correlations were quite high between the observed PER values and the C-PER or DC-PER values (87, 91). For specific protein sources, however, the agreement among PER, C-PER and DC-PER values is unsatisfactory (Table VII). This is unfortunate because if the empirical approach successfully predicted rat PER values of individual protein sources, a similar approach might successfully predict protein nutritive values for humans. The approach might be useful for quality control monitoring in which the same or similar protein sources are routinely evaluated, and the data can be compared to a reference data base for the same sources.

A possible problem with this approach is the use of nitrogen digestibility as an indirect indication of possible differences in amino acid availability. Although some protein sources (e.g., sorghum) may have a low nitrogen digestibility, in general, as noted by Hopkins (117), this is not so. Properly processed soya protein sources have high nitrogen digestibility levels (Table VIII). For 42 protein sources, estimates of protein nutritional values obtained in human studies were compared with estimates obtained from the use of various amino acid scores (74). The correction of the amino acid composition data used in calculating the scores, by nitrogen digestibilities of 12 of the 42 protein sources (for which various estimates of digestibility were available), did not improve the relationships between the protein nutritive values determined in the human studies and those estimated by use of the amino acid scores.

Furthermore, a high value for total nitrogen digestibility may not always be indicative of the availability of specific amino acids (95, 99, 111). Accordingly, in any method based on amino acid composition data, it would seem prudent to consider amino acid availability. Of the methods listed in Table IV for estimating the availability of specific amino acids, the dye-binding or the dinitrofluorobenzene in vitro chemical methods for estimating nutritionally available lysine and the *Streptococcus zymogenes* microbiological in vitro method for estimating nutritionally available methionine would appear to have considerable

TABLE IV

Methods Used to Evaluate Amino Acid Bioavailability in Soya Protein

Method	Parameters measured	Information acquired	Use of method to evaluate soya protein (selected references)
Chemical assays			
Available lysine	% of total lysine that reacts with dinitrofluorobenzene (Carpenter's method of modifications) or that binds a selected dye	Estimate of available lysine (lysine with free ε-amino groups)	66, 71, 88, 93–100
Available methionine	% of total methionine that reacts with sodium nitroprusside or dimethylsulfoxide	Estimate of available methionine (non-oxidized and/or sterically accessible methionine); may underestimate available methionine if methionine sulfoxide is utilized	101, 102
Microbiological assays			
Tetrahymena pyriformis	Organism growth by cell count or tetrahymenol produced	Relative microbiological utilization of selected essential amino acids; values used to estimate availability	71, 85, 93
Streptococcus zymogenes	Organism growth by cell count	Relative microbiological utilization of selected essential amino acids (excluding lysine); values used to estimate availability	71, 85, 88, 93, 99
Rat, pig, or chick growth assays	Relative response to multiple intake levels of lysine or methionine provided from protein source and from added lysine or methionine	Relative availability of selected amino acids	71, 98, 104–106
Peripheral or portal plasma amino acid analyses	Amino acid levels (lysine or methionine) in plasma as a function of time after meal in humans and rats	Plasma response appears in some cases to reflect relative absorbability of specific amino acids	70, 107–110
Fecal amino acid analyses	Amino acids in protein source and in feces	Recovered fecal amino acid levels used to estimate amino acid absorption or availability	67, 111–113
Ileal content amino acid analyses	Amino acids in protein source and in post prandial ileal contents	Ileal amino acids levels used to estimate amino acid absorption; presumably less affected by microbial production or consumption of amino acids than fecal amino acids level	99, 114

TABLE V

Relative Value of Soya Proteins and Milk or Egg Protein for Consumption by Adults and Children As Determined in Studies with Multiple-Intake Levels of Protein (Standard Method)

Protein source	Relative value[a]		Reference
	Based on efficiency of N utilization	Based on N required for zero N balance	
Adults			
Nonfat dried skim milk	(100)	(100)	13, 14
Soy isolate (Supro 620)	95	81	-
Soy isolate (Supro 710)	102	93	-
Milk	-	(100)	26
Soy isolate (Supro 710)	-	82, 88	-
Soy isolate (Supro 620)	-	74, 88	-
Egg (whole)	(100)	(100)	14
Soy isolate (Supro 620)	87	80	-
Egg white	(100)	(100)	15, 74
Soy isolate (Promine F)	85	77	-
Textured Soy protein (Supro 50-4)	87	90	-
Milk	(100)	(100)	26
Textured vegetable protein	76	91	-
Children			
Whole milk	(100)[b]	(100)[b]	19
Soy isolate (Supro 620)	101	98	-
Soy isolate (Supro 710)	92	142	-

[a]Values obtained for the reference protein, within each study, assigned a value of 100.
[b]Values obtained with different children (24) than those fed the soy protein.

TABLE VI

Comparisons of Estimates of Protein Nutritive Value of the Same Soya Protein Preparations Fed to Humans and Rats

Protein source	Relative value for humans		Rat bioassays					Reference
	Based on efficiency of N utilization	Based on amount of test protein needed for zero N balance	PER	R-NPR	RNU	RPV	NPU	
Adults								
Egg white	-	(100)	2.83	(100)	(100)	(100)	-	15
Soy isolate (Promine F)	-	77	1.77	70	69	47	-	-
Textured soy protein (Supro 50-4)	-	91	2.09	78	78	58	-	-
Whole egg	(100)	-	3.24	-	-	-	-	21, 55
Soy isolate (Supro 710)	86	-	1.65	-	-	-	-	-
Whole egg	(100)	-	-	-	-	-	(100)	20
Soy isolate (Supro 620)	79	-	-	-	-	-	71	-
Dried skim milk	(100)	-	-	-	-	-	(100)	20
Soy isolate (Supro 710)	87	-	-	-	-	-	66	-
Children								
Whole egg	(100)	-	-	-	-	-	-	19, 22–24, 55
Milk	110	-	-	-	-	-	-	-
Soy isolate (Supro 620)	112	-	1.63	-	-	-	-	-
Soy isolate (Supro 710)	101	-	1.78	-	-	-	-	-

TABLE VII

PER Values for Soya Protein Products Determined by Rat Assay and Estimated from In Vitro Assays[a]

Soya protein	PER (rat)	PER predicted from in vitro analyses		% Difference	
		C-PER	DC-PER	PER minus C-PER	PER minus DC-PER
Concentrate	2.0	2.5	2.1	−25	−5
Flour	1.6	2.2	2.1	−38	−31
Flour	1.6	1.4	1.5	+12	+6
Isolate	1.3	1.3	1.5	0	−15
Isolate "#1"	1.7	2.5	2.1	−47	−24
Isolate "#2"	1.9	2.3	1.9	−21	0
Isolate "#3" (cooked)	1.4	2.1	1.8	−50	−29
Textured "#1"	2.1	2.3	2.1	−10	0
Textured "#2"	1.9	2.1	1.9	−11	0

[a] From Jewell et al. (77). C-PER = PER calculated from amino acid composition data and nitrogen digestibility estimated by a 4-enzyme in vitro digestion; DC-PER = PER calculated from amino acid composition data and nitrogen digestibility estimated from amino acid composition data.

potential for rapidly monitoring the bioavailability of these amino acids.

SUGGESTED APPROACHES

Reasonable agreement between estimates of protein nutritive value obtained in human studies and those predicted by several different amino acid scores can be demonstrated (74). Agreement, however, is best when the reference amino acid pattern (which is used for scoring) is based on estimates of human amino acid requirements. Comparisons of relative protein nutritional values of soya products, estimated by studies with adults and by two amino acid scores calculated by use of the FAO/WHO (4) reference amino acid pattern (which is based on estimates of human requirements for essential amino acids), are shown in Table IX.

Based on these and similar data (74), some possible approaches for estimating protein nutritive value are given in Table X. In approach A, the composition would be determined, including all of the usual essential and non-essential amino acids. The values for each of the essential amino acids would be corrected for availability (as estimated by in vitro methods for one or two key amino acids) as needed. The corrected values would be used to calculate a score based on all the essential amino acids (4). In approach B, similar procedures would be used but analyses and scoring would be based on only three or four key amino acids. In this approach, a complete amino acid analysis would not be required and simpler methods of analysis than ion-exchange chromatography might be adequate. Approach

TABLE VIII

True and Apparent Nitrogen Digestibilities of Soya Protein Estimated in Adults and Children[a]

	Adults			Children		
Soya protein	True	Apparent	Reference	True	Apparent	Reference
Flour	75	-	118	88	-	124
Flour	92	70	119	88	-	124
Flour	90	79	120	84	65	125
Flour	84	66	29	-	-	-
Flour	88	76	74	-	-	-
Isolate	93	82	74	93	85	126
Isolate	97	-	13	95	-	124
Isolate	95	81	121	92	-	127
Spun	101	83	122	-	-	-
Spun	107	88	123	-	-	-

[a] From Hopkins (115). Apparent digestibility = (nitrogen intake minus fecal nitrogen) divided by nitrogen intake; true digestibility = (nitrogen intake minus fecal nitrogen [corrected for obligatory fecal nitrogen]) divided by nitrogen intake; obligatory fecal nitrogen is the fecal nitrogen excreted on a nonprotein diet (4).

TABLE IX

Comparisons of Relative Protein Nutritional Values of Soya Proteins Estimated from Studies with Adults and from Two Amino Acid Scores[a]

	Relative value in humans				
Protein source	Based on efficiency of N utilization	Based on amount of N required from test protein to obtain zero N balance	Reference	Chemical score based on values for all essential amino acids	Chemical score based on 4 amino acids[b]
Egg white	(100)	(100)	15, 74	(100)	(100)
Textured soya protein (Supro 50-4)	87	90	-	88	94
Soya isolate (Promine F)	85	77	-	79	79
Nonfat dried milk	(100)	(100)	13, 14	(100)	(100)
Soya isolate (Supro 710)	102	93	-	88	94
Soya isolate (Supro 620)	95	81	-	76	76
Nonfat dried milk	(100)	(100)	13, 14	(100)	(100)
Beef + soya isolate (Supro 620) bologna	69	100	-	85	85
Beef bologna	93	95	-	85	100
Milk	(100)	(100)	26	(100)	(100)
Textured vegetable protein (TVP)	76	91	-	88	94
TVP + beef	97	95	-	99	100
Beef	97	103	-	100	100

[a] From Bodwell (74). Scores calculated by use of amino acid compositions not corrected for nitrogen digestibility; FAO/WHO (4) amino acid pattern (based on estimates of human requirements for essential amino acids) used as reference pattern for scoring; reference protein, in each study, assigned a value of 100.

[b] The four amino acids are lysine, methionine, cystine and tryptophan.

C differs from approach B in that the results would be expressed not as an amino acid score but as a percentage of RDA for each amino acid.

By definition, amino acid chemical scores are based on the level of the most limiting essential amino acid. Thus, information is not given about the usefulness of the protein when it is consumed with a protein that may be limiting in the same amino acid or in a different amino acid. In approach C, the benefits that can be expected by mixing specific amounts of two or more different protein sources with differing limiting essential amino acids can be readily derived. In nutritional labeling, this would be particularly valuable, as shown by the examples of labels for two proteins given in Table XI.

Variations in the above approaches could also be used. In particular, a minimal level of each of the essential amino acids could be required, regardless of whether levels of total essential amino acids were listed on the label.

For any of the approaches, a determination of total nitrogen (or of alpha amino acid nitrogen) would be needed. Values could be expressed on the label as a percentage of an RDA for nitrogen or in terms of protein with the factor used for converting nitrogen to protein specified; this would not be necessary if the conversion factors to be used were established by the appropriate regulatory agency.

As noted previously (115), an ideal assay for protein nutritive value would: (a) provide quantitative information about the nutritive value of a protein as a single source of protein for humans and its potential value when consumed with other proteins; (b) provide quantitative information about the amount and nutritional availability of the limiting amino acid and also about the total content and distribution of the nutritionally available nonlimiting essential amino acids in the protein; (c) provide a basis for accurately

TABLE X

Possible Approaches for Estimating Protein Nutritive Value

Approach A

1. Determine amino acid composition
2. As needed, correct values for low amino acid availability, e.g., lysine, methionine (estimated by in vitro methods)
3. Calculate amino acid scores by using reference pattern based on estimates of essential amino acid requirements for humans (e.g., FAO/WHO [4])

Approach B

1. Determine levels of selected essential amino acids (lysine, methionine, cystine ± tryptophan)
2. As needed, correct values for low amino acid availability, e.g., lysine and methionine (estimated by in vitro methods)
3. Calculate scores based on the amino acids selected by use of a reference pattern based on estimates of essential amino acid requirements for humans (e.g., FAO/WHO [4])

Approach C

1. Determine levels of selected essential amino acids (lysine, methionine, cystine ± tryptophan)
2. Estimate availabilities of amino acids measured; if low, make appropriate corrections
3. Evaluate protein in terms of the % of a standard level (e.g., a U.S. RDA for each of the 3 or 4 amino acids; see text and Table II)

TABLE XI

Amounts and Percent of a Hypothetical U.S. RDA for Specific Amino Acids Provided by 20 g Protein from Two Sources[a]

Amino acids	Soya isolate		Whole wheat	
	g	% U.S. RDA	g	% U.S. RDA
Lysine	1.27	35	0.55	15
Tryptophan	0.27	42	0.25	39
Methionine plus cystine	0.59	26	0.87	38
Total essential amino acids	8.08	33	7.10	29

[a] Hypothetical U.S. RDA for lysine, tryptophan, methionine + cystine, and total essential amino acids of 3.48, 0.65, 2.28, and 24.5 g, respectively (based on 1973 FAO/WHO [4] values). Levels of total essential amino acids could be omitted from the label. However, a minimal level of each essential amino could be required regardless of whether levels of total essential amino acids were listed.

predicting the nutritive value of a mixture of two or more protein sources that have been assayed separately; and (d) for nutritional labeling purposes, be simple in application and accurately reflect (as differences in protein quality) only those differences that are real under practical conditions.

None of the methods used (including human assays) fulfill all the requirements for this "ideal" assay. However, particularly if the uncertainties (precision, reproducibility, accuracy) associated with even the best estimates of protein nutritional quality are recognized (20, 128), the use of amino acid scores (with monitoring and corrections for possible low levels of availability), together with a consideration of nitrogen content, may provide a useful, practical approach for the evaluation and documentation of protein nutritional value.

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Determination of Soya Protein in Processed Foods

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ABSTRACT

Many qualitative and quantitative analytical procedures for determining vegetable proteins in processed foods have been studied by researchers throughout the world, but each technique seems to have limitations. Several analytical procedures that have potential for both qualitative and quantitative determination of soya protein in foods are reviewed.

INTRODUCTION

Processed foods may contain added vegetable protein for a number of reasons. Most commonly the supplements act as binders; they are added for improvement of texture and nutrition or for retention of water and/or fat. Soya protein can be incorporated into foods as defatted flour, a concentrate, of an isolate. Complications arise in the analysis of food products that contain the soya protein, because commercially available products can be obtained in texturized forms that may be artificially colored and fortified with vitamins and minerals. The food technologist procures these products, mixes them with other ingredients, and manufactures a product. During processing, proteins interact both chemically and physically with other components to form intricate composites. This mass is then given to the analyst to determine the amounts of additives introduced into the food.

Since most food products in the United States and other countries must meet standards of identity, it has been necessary to develop methods that will detect and quantitate vegetable protein products in foods. Two excellent reviews on the determination of vegetable proteins have recently been published (1,2).

MICROSCOPY AND HISTOLOGICAL METHODS

Probably the oldest, best known microscopy method is inspection for characteristic hourglass and/or palisade cells in the residue that remains after extracting with potassium hydroxide (3). Determining the presence of calcium oxalate crystals in the soybean cotyledon cells (4) has also been used as a qualitative test to detect soya meal or a textured soya meal in meat products (5).

Pomeranz and Miller (6) developed a method that enables one to detect soya flour in wheat flour by observing the canary-yellow fluorescence of soybean particles viewed under ultraviolet light (360 mμ) with low magnification. The smallest quantity of soya flour determined was 0.01%.

If histological stains are used, more elaborate methods exist that enable the measurement not only of carbohydrates but also of proteins. Specifically, detection and even quantitative approximation can be made of textured soya flour (TSF). Smith (7) suggests four useful stains: toluidine blue, iodine, periodic acid/Schiff reagent, and acridine orange. Coomaraswamy and Flint and Meech (8,9) quantitated TSF added to meat products by using a toluidine blue stain; they measured TSF with a standard deviation of 1.85% at the 45% level of addition. They reported that an experienced person can analyze one or two samples per day, a rate too slow for routine screening. Concentrates or isolates cannot be determined because the amount of carbohydrate present in these products is variable. That is, a defatted soya flour has 29% carbohydrate, a soya concentrate has 16%, and an isolate may contain only 2% carbohydrate (10). Consequently, it is necessary to know what type of product is present, and the techniques are not applicable when more than one type is added to the food item. However, Parisi et al. (11) in Italy claim that they can detect soya flours, concentrates, and isolates in commercial meat products by the periodic acid-Schiff base reaction, which is dependent on the presence of carbohydrate.

Bergeron and Durand (12), using several protein stains, developed a histological technique that is reported to be rapid and capable of detecting as little as 1% soybean protein in meat products. They report satisfactory results with fresh, heated or putrefied meat containing soy flour, concentrates or isolates.

IMMUNOCHEMICAL ANALYSIS

Immunological techniques should be the best procedures for the determination of nonmeat proteins in meat products because of the high specificity of antibodies and the sensitivity of the antigen-antibody reaction. By having several different antibodies available, i.e., for casein, wheat, corn, and so on, a researcher or analyst should be able to determine which substances have been added to food products. An excellent review of the literature in this field has recently been published by Olsman and Hitchcock (2). Since much of the immunological research has been done in Europe, they have done an outstanding job of providing a review of the European journals, which may not be available to everyone.

Poli et al. (13) recently reported a unique crossover electrophoresis technique that uses antisera. In the procedure, the unknown protein sample is solubilized in buffer containing sodium dodecylsulphate and mercaptoethanol, and the migrated against a soy-specific rabbit antisera. The resultant precipitin band may be enhanced with a sheep anti-rabbit gamma globulin that has been coupled to a fluorescent compound. The arcs of precipitation are observed as fluorescent bands.