Evaluating Protein Quality of Model Meat/Soybean Blends Using Amino Acid Compositional Data†

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The contents of total protein, amino acids including 5-hydroxylysine and 4-hydroxyproline, and calculated collagen of 10 experimental blends of bovine diaphragm (BD) and soybean protein concentrate (SPC) were determined using quantitative chromatographic methods. As the SPC increased from 0.5 to 21%, glutamic acid increased while the lysine and methionine contents decreased, but the percent of total essential amino acids and calculated protein efficiency ratios (PER) decreased only marginally (EAA_{10} = 51.28–50.05%; PER = 3.17–3.07). The negative linear decrease (R^2 = 0.95; $P < 0.001$) in protein-bound 5-hydroxylysine indicated that total collagen in meat blends could be accurately calculated from the amounts of 5-hydroxylysine in 96-h acid hydrolysates. However, the presence of 4-hydroxyproline in both soybean protein concentrate (1.1 g/kg of protein) and collagen suggests that the use of 4-hydroxyproline as an index of total connective tissue protein in composite meats is limited. These results indicate that amino acid composition and total collagen in composite meats can be used as useful indices for evaluating their protein quality.

INTRODUCTION

Processed meats and poultry products are important sources of proteins in human nutrition (Expert Work Group, FSIS, 1984). Such composite meats are often prepared from cuts high in connective tissue and usually include a number of protein ingredients such as milk and egg powders, gelatin, soybean, and other legume and cereal grains [reviewed by Terrell (1982) and Rust (1982)]. The levels and type of specific nonmuscle animal and plant protein ingredients used to formulate such products, however, vary greatly, resulting in wide variations in their protein quality and nutritive value. Therefore, an accurate assessment of the amounts of these proteins and their contribution to the levels of amino acids and the protein quality of composite meats is essential for both consumer information and regulatory purposes in the development of standards for labeling prepackaged meats, as well as for international trade.

The method of choice for assessing the protein quality and nutritive value of meats, poultry, and their products (AOAC, 1984) in the United States (Bodwell, 1977; U.S. Department of Agriculture, 1982) and Canada (Chapman et al., 1959; Campbell, 1963) has been the protein efficiency ratio (PER) method of Osborn et al. (1919), which measures the ability of a protein to support growth in rapidly growing rats. Other rat bioassay methods for assessing the nutritive value of foods have been proposed, including available amino acid score (Sarwar, 1984, 1987), net protein ratio (NPR) (McLaughlan et al., 1980), and relative net protein ratio (RNPR) (Happich et al., 1984). Although rat bioassays reflect the availability of essential amino acids and digestibility of the proteins in a food, they fail to take into account the quality of the various proteins present and the availability of individual amino acids. In addition, they tend to overestimate the protein quality for humans of some animal proteins while underestimating the value of some plant proteins, because rats have higher relative requirements for some essential amino acids than humans. For these reasons the USDA-sponsored Expert Work Group of the Food Safety and Inspection Services (Expert Work Group, FSIS, 1984) has recommended that a potentially more precise evaluation of the nutritional value of meat and poultry products might be obtained from an accurate knowledge of their amino acid composition and connective tissue protein contents (Pellett and Young, 1984, 1988).

The connective tissue proteins in muscle, collagen and elastin, can be determined from the amounts of 4-hydroxyproline [Pro(4-OH)] found in tissue hydrolysates [reviewed by Berg (1982)]. This approach for assessing protein quality of meat and poultry products was proposed by Alsmeyer et al. (1974), Lee et al. (1978), and Pellett and Young (1984) because, first, a statistically significant correlation exists between PER values and the contents of several or all of the essential amino acids of a protein or protein mixture and, second, the content of collagen in muscles and composite meats is highly negatively correlated (R = −0.99) to rat PER values reported by Lee et al. (1978) and Pellett and Young (1984). Therefore, accurate measurements of the amino acid composition of meats, poultry, and their products, including analyses of those unique amino acids found in collagen and elastin (Zarkadas et al., 1988a,b; Nguyen and Zarkadas, 1989; Zarkadas, 1992) would be expected to be valuable in relating amino acid composition of these products to protein quality.

The present study was undertaken (1) to quantitatively establish the amounts of total protein and amino acids, including Pro(4-OH), 5-hydroxylysine [Lys(5-OH)], and desmosine (Des) in blends consisting of adult bovine diaphragm (BD) combined with incremental additions of soybean protein concentrate (SPC) and (2) to determine whether the connective tissue protein and amino acid contents in these model meat/soybean blends could be used as an accurate measure of their nutritional quality.

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MATERIALS AND METHODS

Materials. Types DC-4A (lot 750) and DC-5A (lot 746) cation-exchange spherical resins, sized to 9 ± 0.5 and 6.0 ± 0.5 μm, respectively, were purchased from Dionex Chemical Co., Sunnydale, CA. The unusual amino acid standards were obtained as follows: N^6-lysinoalanine [N^G-(DL-2-amino-2-carboxyethyl)-L-lysine] from Miles Analytical Laboratories, Inc., Elkart, IN; the diastereoisomer mixture of 5-hydroxy-DL-lysine, D-glucosamine monohydrochloride, D-galactosamine monohydrochloride, and 4-hydroxyproline from Calbiochem-Behring Corp., La Jolla, CA; DL-ornithine (5-aminonorvaline) from Schwarz/Mann, Orangeburg, NY; norleucine and L-2-amino-3-guanidinopropionic acid from Pierce Chemical Co., Rockford, IL; and 3-nitro-L-tyrosine from Aldrich Chemical Co., Milwaukee, WI. The standard amino acid calibration mixture was purchased from Beckman Instruments, Inc., Pal Alto, CA. 2-Propanol, purchased from Caledon Laboratories, Georgetown, ON, was of HPLC grade, octanoic acid was obtained from Eastman Kodak Co., Rochester, NY, and phenol was a product of J. T. Baker Chemical Co., Phillipsburg, NJ. Bovine *Ligamentum nuchae* elastin was purchased from Sigma Chemical Co., St. Louis, MO. Desmosine (Des) and isodesmosine (iDes) were isolated by the preparative method described previously (Zarkadas, 1979). All reagents and buffers were made with high-purity laboratory water prepared according to one of the procedures as described previously by Zarkadas et al. (1987b). All other chemicals and reagents were of the highest purity commercially available and were used without further purification.

Experimental Procedures. *Sampling and Preparation of Muscle Tissues.* The bovine diaphragm samples were excised as 5 cm thick muscle sections from the left side of three commercial carcasses (Canada Grade C1) that weighed approximately 270 kg each and were randomly selected from mature (8-year-old) Holstein–Friesian cows obtained from Abattoir Soulange, Les Cedres, Quebec. All muscle tissues (approximately 200 g each) were cleaned of adhering fat, cut into small cubes, ground, frozen (−173 °C), and lyophilized. The samples were then pulverized in an electric driven end runner coffee mill (Moulinex Canada Ltd., Weston, ON) to pass through a 40-mesh screen and stored at −20 °C. Soybean concentrate samples were obtained from Griffith's Laboratories Ltd., Scarborough, ON.

Tissue Extraction Procedure. The lyophilized BD and SPC samples were extracted with a mixture of chloroform, methanol, and distilled water (1:2:0.8), essentially as described by Bligh and Dyer (1959) using a VirTis Model 45 homogenizer (VirTis, Gardiner, NY) to simplify the procedure. Since the moisture of the lyophilized samples was low, the samples were adjusted to a final moisture content of 80 ± 1% by the addition of distilled water. The volume ratio of chloroform, methanol, and water was 1:2:0.8, respectively. The defatted proteins in the methanol layer were recovered by filtration. Extraction of the insoluble protein fraction was repeated two times; the protein residue was dried overnight at room temperature, ground in a coffee mill (Moulinex), passed through a 40-mesh screen, and stored at −20 °C until needed.

Preparation of Muscle/Soybean Blends. Ten composite blends (10.0 g), prepared in triplicate, consisted of BD combined with 0.5, 1.0, 1.5, 3.0, 6.0, 9.0, 12.0, 15.0, 18.0, and 21.0% SPC (w/w, dry weight basis). Blends were homogenized in an electric coffee mill (Moulinex) and passed through a 40-mesh sieve.

Procedures for Amino Acid Analyses. Amino acid analyses were carried out on either a conventional (Beckman Spinco Model 120C) or a fully automated amino acid analyzer (equivalent to Beckman Spinco Model 121 MB). The automated instrument was interfaced with a Varian Vista 402 (Varian, Walnut Creek, CA) chromatographic data reduction system (Zarkadas et al., 1986, 1987b) to enable both rapid quantitation of amino acids at the picomole range and accurate peak area analysis.

Complete amino acid analyses were carried out both in the BD and in the SPC and in each of the model meat/soybean blends. Duplicate 24-, 48-, 72-, and 96-hydrolysate samples (0.1 g) were prepared from BD, SPC, and model blends and were then hydrolyzed under vacuum (below 10 mmHg) with 20 mL of triple-glass distilled constant-boiling HCl (6 M) at 110 °C, with the precautions described previously (Nguyen et al., 1986; Nguyen and Zarkadas, 1989; Ozols, 1990). Each hydrolysate was then analyzed in duplicate (total 48 determinations). The data reported for serine, threonine, and tyrosine represent the average of values extrapolated to zero time of hydrolysis by linear regression analysis. The values for valine, isoleucine, leucine, and phenylalanine are averages of data from 48, 72, and 96 h of hydrolysis (36 determinations). All others are reported as average values of 48 determinations from 24, 48, 72, and 96 h of hydrolysis.

The Pro(4-OH) content of muscle tissues was determined separately from six 24-h concentrated hydrolysate samples (equivalent to 0.1 mg of protein/analysis) as described previously (Berg, 1982; Zarkadas et al., 1986). Each hydrolysate containing norleucine as an internal standard was analyzed in duplicate (12 determinations), and recoveries of Pro(4-OH) were calculated relative to alanine, valine, isoleucine, and leucine present in the sample.

Methionine and cyst(e)ine were determined separately (0.1-g samples) according to the performic acid oxidation procedure of Moore (1963). Triplicate 24-h hydrolysates were prepared, and each was analyzed in duplicate (12 determinations). Norleucine was added in the hydrolysates as an internal standard, and the recoveries of cyst(e)ine as cysteic acid and methionine as the dioxide were calculated in proportion to the yields obtained by the performic acid treatment of standard solutions of these amino acids and relative to alanine and leucine present in the sample. Similarly, tryptophan in composite meat blends (0.1 g) was determined separately after alkaline hydrolysis according to the procedure of Hugli and Moore (1972) as described previously

Table I. Amino Acid Composition of Midcostal Region of Bovine Diaphragm and Soybean Protein Concentrate after Solvent Extraction (Grams of Amino Acid per Kilogram of Total Protein)

	diaphragm		soybean protein concentrate	
amino acid	mean ± SEM[a]	CV[a]	mean ± SEM[a]	CV[a]
aspartic acid	95.26 ± 0.01	0.01	113.14 ± 0.63	0.63
threonine[b]	45.21 ± 1.18	3.70	39.67 ± 0.15	0.15
serine[b]	39.45 ± 0.84	3.00	52.13 ± 1.84	1.88
glutamic acid	158.31 ± 1.38	1.23	186.43 ± 0.37	0.38
proline	39.94 ± 0.38	1.35	50.48 ± 2.70	1.35
glycine	37.62 ± 0.93	3.48	36.49 ± 0.06	0.23
alanine	54.39 ± 0.89	2.31	41.17 ± 0.20	0.67
cysteine	8.52 ± 0.03	0.34	11.86 ± 0.01	0.08
valine	53.38 ± 0.05	0.12	52.74 ± 0.42	1.13
methionine	29.11 ± 0.44	1.84	12.15 ± 0.24	2.41
isoleucine	50.97 ± 0.33	0.93	50.68 ± 1.16	1.16
leucine	87.12 ± 0.80	1.30	79.75 ± 0.55	0.98
tyrosine[b]	40.95 ± 0.38	1.31	41.72 ± 0.50	1.71
phenylalanine	45.74 ± 0.06	0.19	53.82 ± 0.28	0.74
histidine	30.34 ± 0.61	2.84	26.45 ± 0.10	0.52
lysine	93.05 ± 0.46	0.70	64.34 ± 0.14	0.30
arginine	66.22 ± 0.74	0.61	72.60 ± 0.11	0.21
tryptophan	14.62 ± 0.80	7.71	14.04 ± 0.09	1.00
ammonia[b]	12.95 ± 0.43	4.61	16.84 ± 2.71	22.76
total AA N,[c] g/kg of protein	171.53		171.34	
protein content,[d] g/kg of DM	884.38		573.20	
WE,[d] μg/nmol	0.111784		0.112231	
CF,[d] μg/nmol	0.113834		0.114578	
CF′,[d] μg/nmol	0.120446		0.121978	
Kjedahl conversion factors[e]	5.84		5.83	

[a] Mean values and standard errors of measurements (SEM) for 3 replicates and 48 determinations. CV, coefficient of variation. [b] Mean values and standard error of estimates. [c] Calculated according to the method of Heidelbaugh et al. (1975). [d] The WE and CF constants were calculated according to the method of Horstmann (1979) using eq 1. The conversion factor CF′ was also calculated according to the method of Zarkadas et al. (1988a,b) for determining protein mass in the absence of tryptophan, cyst(e)ine, proline, and 4-hydroxyproline. [e] The Kjeldahl conversion factors were calculated from the mean amino acid nitrogen contents of BD and SPC according to the method of Khanizadeh et al. (1992).

Table II. Amino Acid Composition of Experimental Blends Containing Bovine Diaphragm and Varying Amounts of Soybean Protein Concentrate after Solvent Extraction (Grams of Amino Acid per Kilogram of Total Protein in the Blends)

	soybean protein concentrate added to skeletal muscle (diaphragm) on a dry weight (w/w) basis									
	0.5%		1.0%		1.5%		3.0%		6.0%	
amino acid	mean ± SEM[a]	CV[a]	mean ± SEM[a]	CV[a]	mean ± SEM[a]	CV[a]	mean ± SEM[a]	CV[a]	mean ± SEM[a]	CV[a]
aspartic acid	95.43 ± 0.30	0.04	96.43 ± 0.34	0.05	94.64 ± 0.34	0.52	95.98 ± 0.09	0.15	97.13 ± 0.04	0.06
threonine	46.21 ± 0.49	1.51	45.95 ± 0.25	0.78	45.92 ± 0.19	0.59	45.30 ± 0.90	2.78	46.18 ± 0.66	2.05
serine	40.06 ± 5.50	5.50	41.90 ± 0.04	0.14	41.33 ± 0.16	0.55	39.37 ± 1.90	6.85	40.53 ± 1.01	3.53
glutamic acid	160.58 ± 0.26	0.22	162.16 ± 0.46	0.40	161.13 ± 1.14	1.01	162.86 ± 0.17	0.15	164.19 ± 0.27	0.23
proline	41.84 ± 0.66	2.23	40.61 ± 1.44	5.03	45.85 ± 1.40	4.31	41.28 ± 0.03	0.10	41.49 ± 0.64	2.21
glycine	37.68 ± 0.03	0.12	36.60 ± 0.11	0.40	36.92 ± 0.15	0.57	37.37 ± 0.15	0.58	37.55 ± 0.03	0.12
alanine	54.38 ± 0.18	0.46	53.56 ± 1.44	1.44	52.68 ± 0.23	0.61	53.17 ± 0.15	0.38	53.47 ± 0.30	0.87
cyst(e)ine	9.06 ± 0.01	0.03	8.77 ± 0.19	2.96	10.05 ± 0.70	9.86	10.03 ± 0.03	0.23	10.63 ± 0.06	0.74
valine	53.10 ± 0.37	0.98	52.56 ± 0.09	0.23	51.23 ± 0.36	1.02	52.23 ± 0.05	0.13	52.38 ± 0.33	0.39
methionine	28.97 ± 0.16	0.80	28.07 ± 0.03	0.16	27.85 ± 0.21	1.05	27.75 ± 0.21	1.05	27.75 ± 0.05	0.27
isoleucine	51.10 ± 0.22	0.60	50.61 ± 0.28	1.32	49.68 ± 0.23	0.65	50.21 ± 0.31	0.87	49.89 ± 0.15	0.41
leucine	87.76 ± 0.42	0.70	88.70 ± 0.54	0.86	88.28 ± 0.08	0.13	87.72 ± 0.09	0.14	86.95 ± 0.19	0.31
tyrosine	40.85 ± 0.66	2.28	40.49 ± 0.58	2.00	40.77 ± 0.86	2.98	42.24 ± 1.58	5.27	38.16 ± 0.92	3.43
phenylalanine	44.68 ± 0.06	0.19	46.71 ± 0.05	0.14	44.04 ± 0.96	3.06	45.36 ± 0.19	0.60	44.45 ± 0.11	0.35
histidine	29.62 ± 0.01	0.02	29.20 ± 0.21	1.05	29.22 ± 0.04	0.21	29.34 ± 0.17	0.80	29.02 ± 0.03	0.15
lysine	93.52 ± 0.21	0.31	94.24 ± 0.64	0.97	94.03 ± 0.04	0.05	93.17 ± 0.01	0.01	92.90 ± 0.10	0.15
arginine	67.78 ± 0.05	0.10	67.70 ± 0.71	1.48	67.66 ± 0.35	0.71	67.36 ± 0.42	0.86	67.39 ± 0.04	0.08
tryptophan	10.04 ± 0.11	1.51	10.00 ± 0.17	2.29	12.15 ± 0.03	0.03	13.33 ± 1.01	10.54	13.06 ± 0.52	5.60
ammonia	10.24 ± 0.97	0.97	12.96 ± 0.69	7.53	12.53 ± 0.52	5.92	22.46 ± 2.54	16.01	17.13 ± 1.46	12.05
total protein,[b] g/kg of dry mass	882.45 ± 3.67		879.24 ± 8.80		880.23 ± 6.78		870.45 ± 9.88		868.34 ± 14.45	
total AAN[c]	169.55		171.50		171.30		179.22		175.02	
WE,[d] μg/nmol	0.111521		0.111700		0.111689		0.111888		0.111621	
CF,[d] μg/nmol	0.113313		0.113459		0.113756		0.114043		0.113822	
CF′,[d] μg/nmol	0.119847		0.119776		0.120877		0.120437		0.120378	

[a] Mean values ± standard error of measurements (SEM) for 3 replicates, 24 determinations; CV, coefficient of variation. [b] Protein mass determined according to the method of Horstmann (1979) and dry mass as reported previously (Zarkadas et al., 1987a). [c] Calculated according to the method of Heidelbaugh et al. (1975). [d] The WE and CF constants were calculated according to the method of Horstmann (1979) using eq 1. The conversion factor CF′ was calculated according to the method of Zarkadas et al. (1988a,b).

(Zarkadas et al., 1986). Triplicate 24-h alkaline hydrolysates were prepared, and each sample was analyzed in duplicate (six determinations).

Determination of the diastereoisomer of Lys(5-OH) and related compounds were carried out with six concentrated 96-h hydrolysates (equivalent to 1–2 mg of protein) by the accelerated single-microcolumn (17.5 ± 0.28 cm) system as described previously (Zarkadas et al., 1986). Each hydrolysate was analyzed in duplicate (12 determinations).

Accuracy and Precision of Methods. Tests for accuracy and precision of these methods were carried out in a series of 48 nonconsecutive quantitative analyses of synthetic calibration mixtures containing the 24 amino acids most commonly encountered in protein hydrolysates. Norleucine was added in the hydrolysates prior to acid hydrolysis as the internal standard. The results of the overall mean recoveries (x) for the common amino acids, which were expressed as percentage error (% error = SD/x), showed little apparent difference between replicates. Using an automated amino acid analyzer, recoveries of 100 ± 2.0% (ranging from 100 ± 0.1 to 100 ± 2.1%; n = 48) were obtained for the majority of the amino acids, except for proline which gave a reproducibility of 100 ± 3.9%. This methodology also gave high precision (100 ± 2.5%) and reproducible recoveries for all of the unique amino acids analyzed, even though 100–400-fold increases in protein, equivalent to 100–400 μg of protein/analysis, were used. Recoveries of 100 ± 2.6% for Lys(5-OH), 100 ± 1.9% for aLys(5-OH), and 100 ± 4.3% for desmosine (Des) were obtained. These results indicate that these analytical methods (Zarkadas et al., 1986, 1987a,b) can accommodate a wide range of sample concentrations and still maintain an overall reproducibility of 100 ± 2.77% and an accuracy of 100 ± 3.32%. The accuracy was tested by the one-sample t test [$t = (x - \zeta)/\mathrm{SD}$], where ζ is the known concentration for each of the 50 amino acids in the standard calibration mixtures, i.e., ζ = 1000 pmol/amino acid. For the 24 common amino acids analyzed, the accuracy was very high and ranged from 100 ± 0.5 to 100 ± 4.1% (n = 48), except for proline which was 100 ± 5.4% (n = 48).

Determination of Total Protein Mass. Recoveries of amino acids were calculated on the basis of the protein content of individual hydrolysates determined according to the procedure described by Horstmann (1979) by the following equation:

$$\mathrm{WE} = \sum_{i=1}^{18} (a_i b_i) \tag{1}$$

According to this method, a mean residue weight (WE, in micrograms per nanomole) is calculated for the amino acids constituting the proteins in the composite meat products; a_i is the mole fraction of a specific amino acid i found in the analyzed aliquot, and b_i is the molecular weight of amino acid residue i. The conversion factor CF, which represents the apparent average residue molecular weight (in micrograms per nanomole) of the proteins in the mixtures, but in the absence of tryptophan and cyst(e)ine, and protein concentration of each hydrolysate were then calculated as described previously (Zarkadas et al., 1988a,b; Nguyen and Zarkadas, 1989).

Determination of Connective Tissue Proteins in Meats. A method for calculating the amount of a specific protein j in processed meats has been described previously (Zarkadas et al., 1988a) and is

$$P_{j=1} = C_1 \frac{[1000]}{n_i} \frac{\mathrm{WE}_{Pj}}{M_r(i)} \tag{2a}$$

where WE_{Pj} is the weight equivalent of a specific connective tissue protein j, determined from eq 1 according to the method of Horstmann (1979), n_i is the number of residues of a unique amino acid per 1000 amino acid residues, and $M_r(i)$ is the anhydrous molecular weight of the unique amino acid i.

The following analytical conventions derived from eq 2a as described previously (Zarkadas et al., 1988a,b) can therefore be used for calculating collagen as grams per kilogram of total protein

$$\text{amt of collagen } [\mathrm{P_c}] = \text{amt of Lys(5-OH)} \times 63.3 \tag{2b}$$

and for computing total connective tissue proteins (grams per kilogram of total protein)

$$\text{amt of connective tissue } [\mathrm{P_{CT}}] = \text{amt of Pro(4-OH)} \times 8.03 \tag{2c}$$

This value (8.03) is in close agreement with that reported by Etherington and Sims (1981).

Table II (Continued)

soybean protein concentrate added to skeletal muscle (diaphragm) on a dry weight (w/w) basis									
9.0%		12.0%		15.0%		18.0%		21.0%	
mean ± SEM[a]	CV[a]	mean ± SEM[a]	CV[a]	mean ± SEM[a]	CV[a]	mean ± SEM[a]	CV[a]	mean ± SEM[a]	CV[a]
100.87 ± 0.42	0.59	100.61 ± 0.76	1.07	101.23 ± 0.21	0.29	102.18 ± 0.29	0.40	102.52 ± 0.04	0.05
44.55 ± 0.66	2.10	44.15 ± 0.28	0.91	44.98 ± 0.31	0.98	45.19 ± 0.27	0.83	44.71 ± 0.68	2.16
36.83 ± 6.62	3.64	39.13 ± 1.01	3.64	41.01 ± 0.35	1.18	41.42 ± 0.04	0.14	40.55 ± 1.22	4.25
163.56 ± 3.54	3.07	168.14 ± 0.68	0.58	169.49 ± 0.68	0.58	171.08 ± 0.08	0.08	171.04 ± 0.45	0.37
39.97 ± 1.19	1.19	39.02 ± 0.95	3.46	40.22 ± 0.31	3.46	37.56 ± 0.22	0.82	38.61 ± 1.66	6.08
39.10 ± 1.58	5.71	37.06 ± 0.09	0.37	37.01 ± 0.25	1.00	36.76 ± 0.03	0.10	36.95 ± 0.02	0.06
55.53 ± 0.02	0.05	52.54 ± 0.01	0.01	52.54 ± 0.23	0.61	52.75 ± 0.11	0.29	51.84 ± 0.03	0.09
10.36 ± 0.63	8.64	9.95 ± 0.28	3.98	7.94 ± 0.72	12.77	9.72 ± 0.21	3.04	10.01 ± 1.22	17.24
53.34 ± 0.38	1.00	53.55 ± 0.26	0.69	53.28 ± 0.12	0.33	53.40 ± 0.12	0.32	53.40 ± 0.01	0.04
26.68 ± 0.28	1.48	25.74 ± 0.02	0.09	25.16 ± 0.21	1.15	25.02 ± 0.04	0.32	24.01 ± 0.38	2.26
50.79 ± 0.62	1.74	50.54 ± 0.07	0.18	51.05 ± 0.24	0.66	51.18 ± 0.08	0.22	51.06 ± 0.05	0.14
86.45 ± 0.87	1.43	85.66 ± 0.14	0.23	86.32 ± 0.40	0.66	86.14 ± 0.08	0.13	86.23 ± 0.20	0.33
39.23 ± 1.11	4.00	39.88 ± 0.72	2.55	38.92 ± 0.83	3.03	38.43 ± 0.23	0.85	41.29 ± 1.00	3.26
46.24 ± 0.16	0.49	45.24 ± 0.06	0.19	45.57 ± 0.33	1.01	46.60 ± 1.26	3.85	46.45 ± 0.03	0.10
30.26 ± 0.32	1.50	28.42 ± 0.07	0.36	28.87 ± 0.15	0.71	28.53 ± 0.06	0.27	28.32 ± 0.06	0.29
89.96 ± 0.85	1.34	90.29 ± 0.23	0.36	89.61 ± 0.06	0.10	88.57 ± 0.05	0.08	87.66 ± 0.27	0.43
67.51 ± 0.04	0.09	66.94 ± 0.04	0.84	67.32 ± 0.01	0.03	67.16 ± 0.05	0.11	68.24 ± 0.91	1.89
12.59 ± 0.11	0.23	16.38 ± 1.30	11.21	13.47 ± 1.05	11.06	12.08 ± 0.01	0.14	10.38 ± 0.21	1.89
19.10 ± 0.08	0.51	13.74 ± 0.85	8.77	15.81 ± 2.01	17.96	12.79 ± 1.14	12.79	16.36 ± 1.26	2.23
856.03 ± 12.34		846.70 ± 23.56		837.38 ± 6.67		828.06 ± 15.67		818.73 ± 2.78	
176.73		171.53		173.25		170.41		173.34	
0.111515		0.111970		0.111844		0.111854		0.111892	
0.113645		0.114333		0.113744		0.113881		0.113840	
0.119836		0.120409		0.119970		0.119836		0.119988	

Table III. Unique Basic Amino Acid Contents of Experimental Blends Containing Bovine Diaphragm and Varying Amounts of Soybean Protein Concentrate after Solvent Extraction (Grams of Amino Acid per Kilogram of Total Protein)

% soybean protein concentrate in mixes[a]	5-hydroxylysine		4-hydroxyproline		desmosine	
	mean ± SEM[b]	CV[b]	mean ± SEM[b]	CV[b]	mean ± SEM[b]	CV[b]
0	0.5484 ± 0.021	0.90	8.01 ± 0.87	18.90	0.1490 ± 0.020	21.0
0.5	0.5419 ± 0.002	0.62	5.69 ± 0.31	9.42	0.0214 ± 0.002	12.0
1.0	0.5348 ± 0.004	1.38	5.19 ± 0.52	17.38	0.0254 ± 0.005	31.0
1.5	0.5377 ± 0.004	1.41	5.16 ± 0.31	10.56	0.0222 ± 0.003	22.0
3.0	0.5330 ± 0.001	0.21	5.47 ± 0.04	1.48	0.0165 ± 0.002	20.0
6.0	0.5166 ± 0.001	0.49	5.79 ± 0.23	6.76	0.0232 ± 0.001	4.0
9.0	0.5070 ± 0.002	0.57	4.86 ± 0.18	6.54	0.0177 ± 0.017	12.0
12.0	0.4832 ± 0.003	1.00	4.47 ± 0.31	11.90	0.0179 ± 0.001	13.0
15.0	0.4661 ± 0.005	1.97	4.77 ± 0.38	13.64	0.0177 ± 0.002	20.0
18.0	0.4502 ± 0.010	5.51	5.60 ± 0.47	14.43	0.0191 ± 0.002	21.0
21.0	0.4344 ± 0.008	3.19	5.94 ± 0.03	0.92	0.0203 ± 0.004	32.0
100.0			1.10 ± 0.04	5.83		

[a] Dry weight (w/w) basis. [b] Mean values ± standard error of measurements (SEM) of 12 determinations; CV, coefficient of variation.

Statistical Analysis. Data processing and statistical analysis of the results were carried out by a FORTRAN computer program developed for this purpose. Analysis of variance conducted on the amino acid data for a completely randomized block design (factorial) was carried out by the Statistical Analysis System (SAS, 1982) general linear model procedure.

Orthogonal comparisons of means from each treatment with that of the control samples were carried out on all of the amino acid values obtained (Steel and Torrie, 1980). These orthogonal polynomials were used since the independent variables were not equally spaced, especially at the high concentrations. Tests of significance of the intensity of association between amino acids and the BD/SPC blends were performed using the Pearson simple correlation method (Robson, 1959).

RESULTS AND DISCUSSION

Table I contains the amino acid composition of the BD and SPC, as well as the calculated values for total amino acid nitrogen (AA N) determined according to the procedure described by Heidelbaugh et al. (1975). These authors found that the best estimate of the protein content of a food is the summation of the amino acid nitrogen content and recommended that whenever accurate data on the protein content of individual foods are required, conversion factors based on the actual amino acid composition should be used. In the present study the protein Kjeldahl conversion factors of 5.84 and 5.83 for the bovine diaphragm and soybean protein concentrate, respectively, were calculated from their mean amino acid nitrogen contents (Table I) as described previously (Khanizadeh et al., 1992).

The overall amino acid composition of the midcostal region of the BD and SPC after extraction is summarized in Table I. The mean residue weight (WE) and conversion factors CF and CF′ (all in micrograms per nanomole) of these samples determined by the summation of the weights of the amino acids present, as described by Hortsmann (1979), are also given in Table I. The data for BD are typical of a skeletal muscle tissue (Young and Pellett, 1984; Zarkadas et al., 1988a). The amino acid profile of SPC appeared to be high in acidic amino acids, which together accounted for almost 30% of all residues, while the total basic amino acids accounted for about 16% of the total amino acid residues. The total content of hydroxylated amino acids accounted for almost 14% compared to 22%

Table IV. Polynomial Regression Equations Obtained from Orthogonal Comparisons of All Amino Acids Present in Experimental Blends Containing Bovine Diaphragm and Varying Amounts of Soybean Protein Concentrate (from 0.0 to 21.0%, Dry Weight Basis) after Solvent Extraction (Grams of Amino Acids per Kilogram of Total Protein)

	coefficient				
amino acid[a,c]	β_0[b]	linear β_1[b]	quadratic β_2[b]	cubic β_3[b]	R^2100[d]
threonine	45.78 ± 0.25	−0.06 ■ 0.002*			40.1
glutamic acid	160.40 ± 0.54	0.56 ± 0.05***			93.3
proline	41.12 ± 0.76	−0.19 ± 0.07*			44.9
cyst(e)ine	8.52 ± 0.37	0.8490 ± 0.2209**	−0.1000 ± 0.0268**	0.0030 ± 0.0008**	67.9
methionine	28.65 ± 0.15	−0.22 ± 0.1***			96.3
isoleucine	50.93 ± 0.25	−0.3903 ± 0.1481*	0.0478 ± 0.0180*	−0.0014 ± 0.0006*	62.5
leucine	87.91 ± 0.27	−0.106 ■ 0.0250			65.7
phenylalanine	44.96 ± 0.31	0.066 ± 0.029			37.2
histidine	29.69 ± 0.23	−0.062 ± 0.022*			46.8
lysine	93.98 ± 0.29	−0.303 ± 0.027***			93.5
4-hydroxyproline	6.23 ± 0.30	−0.300 ± 0.089**	0.0140 ± 0.0044**		27.8
5-hydroxylysine	0.547 ± 0.002	−0.005 ± 0.0002***			95.0
desmosine	0.052 ± 0.02	−0.002 ± 0.001*			14.5

[a] Mean values ± standard error of measurements (SEM), $N = 3$ (number of replicates), $N \times 16$ = number of determinations. [b] β_0 is the amount of a given amino acid (g/kg of protein), β_1, β_2, and β_3 are the coefficients of the linear, quadratic, and cubic terms of X, respectively, calculated according to eq 3. [c] Aspartic acid, serine, glycine, alanine, valine, tyrosine, arginine, tryptophan, and ammonia were not significantly correlated and were not included in this table. [d] Significance: R^2100, values of the coefficient of determination from multiple regression analysis; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

Table V. Protein Quality of Muscle (Diaphragm) and Plant Protein Mixes Based on Their Amino Acid Composition

% soybean protein concentrate added to bovine diaphragm (w/w, dry weight basis)	essential amino acids (EAA)					PER predicted by[d]		collagen content,[e] % total protein	connective tissue content,[f] % total protein
	total EAA,[a] mg/g N	EAA index[b]	chemical score[b]	EAA_7,[c] % total protein	EAA_{10},[c] % total protein	eq 4 (PER_7)	eq 5 (PER_{10})		
0.0	3118.1	83.8	65.4	40.45	51.58	3.16	3.11	3.47	3.63
0.5	3144.0	81.8	66.3	40.53	51.28	3.17	3.09	3.43	3.58
1.0	3106.6	80.6	64.3	40.68	51.37	3.18	3.09	3.39	3.54
1.5	3103.6	81.3	66.3	40.10	51.00	3.13	3.07	3.40	3.52
3.0	2998.5	79.1	65.7	40.17	51.17	3.14	3.08	3.37	3.51
6.0	3025.1	80.7	66.8	40.00	50.97	3.12	3.07	3.27	3.39
9.0	2986.1	79.7	65.3	39.80	51.63	3.11	3.11	3.21	3.37
12.0	3080.0	83.1	62.7	39.52	50.69	3.08	3.05	3.06	3.17
15.0	3022.2	80.5	58.7	39.60	50.56	3.09	3.04	2.95	3.05
18.0	3072.0	81.4	61.7	39.61	50.38	3.09	3.03	2.85	2.96
21.0	3017.7	78.5	60.5	39.35	50.05	3.07	3.01	2.75	2.86
100.0	2871.9	75.4	44.8	35.32	46.63	2.74	2.79		

[a] Computed from reference protein standards (FAO/WHO, 1965, 1973). [b] Computed according to the methods of Block and Mitchell (1946) and Oser (1951). [c] Calculated according to the method of Lee et al. (1978). EAA_7: threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine. EAA_{10}: EAA_7 plus histidine, arginine, and tryptophan. [d] PER were calculated according to the method of Lee et al. (1978) from eq 4 (PER = 0.08084 (EAA_7) − 0.1094) and eq 5 (PER = 0.06320 (EAA_{10}) − 0.1539). [e] Total collagen was calculated from the amounts of Lys(5-OH) found using eq 2b. [f] Total connective tissue proteins were calculated from amounts of Pro(4-OH) present using eq 2c.

for the total hydrophobic amino acids. This composition is characteristic of the amino acid profiles of the two principal storage proteins, i.e., glycinin (350-kDa protein) and β-conglycinin (175-kDa protein), which together account for 70% of the total proteins in the soybeans (Hughes and Murphy, 1983; Wilson, 1987; de Lumen, 1990). There was good agreement between the mean amino acid values obtained for SPC in the present study and those values reported by Wolf (1982) and Zarkadas et al. (1988c).

The presence of small amounts of the unique amino acid Pro(4-OH) in the acid hydrolysate of soybean protein concentrate (1.1 g/kg of total protein) is very important. These results are in accord with those reported by Zarkadas et al. (1988c) for soybean flours, protein concentrates, and isolates and by Cassab et al. (1985, 1988), Cassab and Varner (1987), Averyart-Fullard et al. (1988), and Ye and Varner (1991), who have demonstrated the presence of Pro(4-OH) in soybean seed coats. These authors have shown that Pro(4-OH) makes up 45.5% of the polypeptide backbone, corresponding to 455 Pro(4-OH) residues/1000 amino acid residues. Thus, the content of 4-hydroxyproline-rich glycoproteins of soybean protein concentrate was calculated by multiplying the amounts of Pro(4-OH) found in their acid hydrolysates (Table III) by 2.128 as described previously (Khanizadeh et al., 1989). Although Pro(4-OH) has been used as the basis for determining the connective tissue fibrous proteins collagen and elastin in animal tissues (Eastoe, 1967), the use of Pro(4-OH) as an index for determining the connective tissue content of composite meats containing such plant protein additives is limited.

Tables II and III summarize the results obtained on the amino acid contents of each of the 10 BD/SPC blends after extraction. The average weight equivalent and conversion factors CF and CF′ obtained are listed in Table II. Each of these blends has a characteristic amino acid profile depending upon the amounts of specific muscle and nonmuscle ingredients used to formulate each product. Specifically, when compared with the midcostal region of the diaphragm (Table I), the aspartic acid, glutamic acid, lysine, proline, and threonine contents in the experimental mixes are increased, while serine, phenylalanine, valine, and leucine contents decreased. Although the values presented in Tables II and III reflect the composition of the extracted blends, these results are in close agreement with those reported by Noda et al. (1977) for all-meat and soybean-containing wieners.

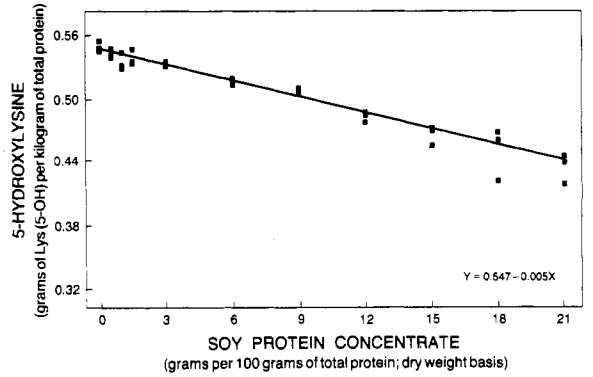

Figure 1. Relation between soybean protein concentrate (grams per 100 g of protein) and 5-hydroxylysine content (grams per kilogram of total protein) for composite meats containing bovine diaphragm and varying amounts of soybean protein concentrate after solvent extraction (Bligh and Dyer, 1959).

The values obtained for protein-bound Lys(5-OH), Pro(4-OH), and Des of all blends show high reproducibility and low coefficient of variation, and within the precision of the analytical methods used (100 ± 3.0%), recoveries were found to be quantitative (Table III). In the case of Des, however, the higher coefficients of variation observed may be related to its quantification being carried out near the lower limits of detection by the ninhydrin procedure.

Table IV shows the polynomial regression equations obtained from orthogonal comparisons of means from each treatment with that of the reference materials used as controls for all amino acid values reported in Tables I–III. Tests of significance (Robson, 1959) and the sums of squares attributable to the various powers of X were computed as

$$Y = \beta_0 + (\beta_1 X) + (\beta_2 X^2) + (\beta_3 X^3) \quad (3)$$

where Y is the content of any amino acid found in the mixes, X is the predicted soybean protein content of a given composite meat blend (expressed as a percentage on a dry weight basis), β_0 is the amount of a given amino acid in grams per kilogram of protein, and β_1, β_2, and β_3 are the coefficients of the linear, quadratic, and cubic terms of X, respectively. Thus, β_0 is the zero degree or mean effect, β_1 is the first degree or linear effect, and so on (Steel and Torrie, 1980).

As shown in Figure 1, as the amount of SPC in the experimental mixes increased, there was a significant linear decrease ($P < 0.01$) in the amount of protein-bound Lys(5-OH). The negative linear effect was due to the decreased amounts of connective tissue proteins in these mixes. The orthogonal models developed for Lys(5-OH) ($R^2 = 0.95$) and several other amino acids are highly reliable, except for Des ($R^2 = 0.15$), which is found at very low concentration (<20–50 pmol) in these blends. There was also evidence of highly significant ($P < 0.001$) linear effects for lysine and methionine by comparison to the less pronounced ($P < 0.05$) linear relationships that exist among threonine, proline, and histidine and the amounts of muscle and nonmuscle proteins in the experimental mixes. These results are in accord with those reported by Pellett and Young (1984). These authors indicated that since the lysine content of muscle proteins does not vary greatly but is present in much lower amounts in collagen and cereal proteins, it would appear to be useful to monitor the content of lysine as well as the levels of Pro(4-OH) and nitrogen, with a view to establishing a minimum value for muscle and nonmuscle proteins in meat and poultry products.

As the amount of SPC in the experimental mixes increased, the levels of glutamic acid increased significantly ($P < 0.001$) in a linear fashion. There was also evidence of significant ($P < 0.01$) in the linear, quadratic, and cubic components of the blends for the amount of cyst(e)ine and possibly isoleucine as well as Pro(4-OH) in these experimental mixes. The significant ($P < 0.01$) quadratic effect observed for Pro(4-OH) was probably due to the small amounts of this unique amino acid found in soybean protein (Table I). For the above reasons, the authors do not recommend the use of Pro(4-OH) as an index for determining connective tissue proteins in composite meats.

The experimental blends contained all of the essential amino acids (EAA) required for human nutrition (Tables V and VI). Mean values for total EAA ranged from 3018 to 3118 mg/g of N (Table V), which are similar to those of cow's milk (3200 g/g of N) and hen's whole egg (3215 mg/g of N) (FAO/WHO, 1965, 1973) and higher than that of soybean protein concentrate (2872 mg/g of N). Similarly, the EAA indices and chemical score for these meat mixes, as determined by the methods of Block and Mitchell (1946) and Oser (1951), were high.

Until recently various bioassay methods have been widely used for assessing the nutritive value and protein quality of meats and poultry products, using the growth of rats as indirect indices of amino acid availability. These methods include protein efficiency ratio (PER), available amino acid score, net protein ratio (NPR), and relative NPR (RNPR) (Sarwar, 1984, 1987; Happich et al., 1975, 1984; McLaughlan et al., 1980). Bioassay values, however, tend to underestimate the protein quality of composite meats since rats have higher relative requirements for the sulfur-containing and other essential amino acids than humans. Thus, Lee et al. (1978), Pellett and Young (1984, 1988), Young and Pellett (1984), and Young et al. (1989) recommended that the complete amino acid composition and total collagen content of meat and poultry products would be a more accurate assessment of protein quality in these foods.

FAO/WHO/UNU (1985) developed reference amino acids patterns for four different age groups (infants, 2–5-year-old children, 10–12-year-old children, and adults). They recommended that, in conjunction with in vivo protein digestibility data (primarily from rat studies), the most appropriate approach would be to use amino acid values for the 2–5-year-old child as the reference pattern (Table VI) in the evaluation of mixed diets for all persons. Since this scoring procedure is based on the essential amino acid content of foods, the protein efficiency ratio (PER) of these mixes was calculated by the equations (eqs 4 and 5 listed in Table V) developed by Lee et al. (1978). Both prediction equations (EAA_7 and EAA_{10}) show that the calculated mean PER values for the experimental meat mixes varied (3.07–3.17) with the amounts of SPC present. Replacing the BD with 21.0% (dry weight basis) SPC decreased the calculated PER values only marginally (0.1 PER unit). These results are consistent with those reported by Happich et al. (1975) for lean beef, collagen, soybean protein concentrate, and various combinations of these products. The higher PER values calculated for these experimental meat blends are because of the complementation effect of the plant and animal proteins.

The essential amino acid composition of experimental meat mixtures containing BD and varying amounts of SPC (from 0.5 to 21.0%) is compared with that of the selected reference pattern (FAO/WHO/UNU, 1985) for a 2–5-year-old child, and the results are shown in Table VI. These

Table VI. Reference Amino Acid Pattern and Amino Acid Compositions of Experimental Blends Containing Bovine Diaphragm and Varying Amounts of Soybean Protein Concentrate (Milligrams of Amino Acid per Gram of Protein)

essential amino acid	reference pattern[a]	diaphragm	soybean protein concentrate added to bovine diaphragm (dry weight basis)										
			0.5%	1.0%	1.5%	3.0%	6.0%	9.0%	12.0%	15.0%	18.0%	21.0%	100.0%
histidine	19	30	30	29	29	29	29	30	28	29	28	28	26
isoleucine	28	51	51	49	50	50	50	51	51	51	51	51	51
leucine	66	87	89	89	88	87	87	86	86	86	86	86	80
lysine	58	93	94	94	94	93	93	90	90	89	88	87	64
methionine + cyst(e)ine	25	39	38	37	38	38	38	37	35	33	35	34	24
phenylalanine + tyrosine	63	86	85	87	85	87	83	85	85	84	85	87	95
threonine	34	45	46	46	46	45	46	45	44	45	45	45	40
tryptophan	11	15	10	10	12	13	13	12	16	13	12	10	14
valine	35	53	53	53	51	52	52	53	52	53	53	53	53
total	339	499	499	493	493	494	501	489	488	483	483	481	447

[a] FAO/WHO/UNU (1985) and FAO/WHO (1990) reference pattern for 2–5-year-old child.

blends provide an excess of all of the essential amino acids ranging from 44.1 to 49.9% (Table VI) of the total amino acids, which is considerably higher than the value of 33.9% in the reference pattern (FAO/WHO, 1990). These results correspond closely with the mean essential amino acid values listed in Table V (EAA = 46.6–51.6% of the total amino acids), which were calculated according to the method of Lee et al. (1978) and Pellett and Young (1984) using eqs 4 and 5, and are in close agreement with those values reported by Bodwell (1987) for skeletal muscle for beef, pork, lamb, and chicken. It should be noted that there is little tryptophan in excess of that recommended in either of the components or the experimental blends investigated. A problem might arise if skeletal muscle and SPC are replaced with ingredients high in connective tissue proteins, i.e., skin (rind) (Lindberg et al., 1985; Laser-Reutersward et al., 1985a,b) in a meat mixture, since collagen contains little if any tryptophan. Because of this, Expert Work Group, FSIS (1984), recommended that the connective tissue protein content of such products should be limited to 35% of the total protein.

In the present study, the amount of collagen in the experimental blends was estimated from the amount of Lys(5-OH) found in the acid hydrolysates. The amount of collagen in the experimental meat mixtures ranged from 2.75 to 3.43% of the total composite meat proteins compared to 3.47% found in the bovine diaphragm (Table V). These results are similar to those presented by Bendall (1967), Dransfield (1977), Light and Champion (1984), Light et al. (1985), and Light (1987) for the distribution of collagen (average 4.35%; spread 2.22–15.1%) in 34 bovine skeletal muscles and are in reasonable agreement with the collagen content of 2.62% reported for the costal region of the bovine diaphragm by Zarkadas et al. (1988a). The small difference noted in the collagen contents of the costal region of the diaphragm between the present and the previously reported values may be attributed to the anatomical arrangement of the connective tissue proteins in each level of muscle organization of this tissue. The bovine diaphragm is a dome-shaped sheet of skeletal muscle tissue, separating the thoracic and abdominal cavities. The muscle fibers are large and project radially from its central dome near the sternum to the periphery, thus giving rise to a costal region and a largely tendinous central dome. A study showed that the connective tissue proteins in the costal and dome regions of the diaphragm accounted for 2.6 and 18.01%, respectively, of the total protein (Zarkadas et al., 1988a).

The mean total connective tissue protein values for the experimental meat mixes, calculated from the amounts of Pro(4-OH) found, ranged from 2.86 to 3.58%, which are higher than the values found from the Lys(5-OH) content of these meat mixtures. Pro(4-OH) has been widely used as an index for determining the connective tissue proteins of meats and poultry products. However, this amino acid has been found in numerous plant tissues, including soybean protein concentrate and other oilseed and cereal-derived nonmeat protein additives as well as in sensory enhancers, potato protein isolate, and alfalfa proteins (Zarkadas et al., 1988c) and in all three classes of extracellular matrices of plant cell wall glycoproteins, i.e., extensins (Cooper et al., 1987; Cassab and Varner, 1988; Varner and Lin, 1989). For this reason the use of this unique amino acid as an index for determining the connective tissue proteins for this purpose is limited.

From the foregoing results, it may be concluded that addition of significant amounts of soybean protein concentrate (from 0.5 to 21.0%, dry w/w basis) to meat and poultry products could be made without greatly reducing their nutritive value in terms of meeting essential amino acid requirements for humans. These results also indicate that amino acid composition and connective tissue protein contents of composite meats can be used as useful indices for evaluating their protein quality, as recommended by the Expert Work Group, FSIS (1984), and Pellett and Young (1984, 1988).

LITERATURE CITED

Alsmeyer, R. H.; Cunningham, A. E.; Happich, M. L. Equations Predict PER from Amino Acid Analysis. *Food Technol.* **1974**, *28*, 34–40.

AOAC. *Official Methods of Analysis*, 14th ed.; Association of Official Analytical Chemists: Washington, DC, 1984.

Averyart-Fullard, V.; Datta, K.; Marcus, A. A Hydroxyproline-Rich Protein in the Soybean Cell Wall. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 1082–1085.

Bendall, J. R. The Elastin Content of Various Muscles of Beef Animals. *J. Sci. Food Agric.* **1967**, *18*, 553–558.

Berg, R. A. Determination of 3- and 4-Hydroxyproline. *Methods Enzymol.* **1982**, *82*, 372–398.

Bligh, E. G.; Dyer, W. J. A Rapid Method for Total Lipid Extraction and Purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911–917.

Block, R. J.; Mitchell, H. H. The Correlation of the Amino Acid Composition of Proteins with their Nutritive Value. *Nutr. Abstr. Rev.* **1946**, *16* (2), 249–398.

Bodwell, C. E. Problems in the development and Application of Rapid Methods of Assessing Protein Quality. *Food Technol.* **1977**, *31*, 730–737.

Bodwell, C. E. *Effect of Collagen on the Nutritional Value of Foods*; Pearson, A. M., Dutson, T. R., Bailey, A. J., Eds.; Advances in Meat Research 4; AVI, Van Nostrand Reinold: New York, 1987; pp 333–350.

Campbell, J. A. *Methodology of Protein Evaluation. A Critical Appraisal of Methods for Evaluating of Protein in Foods*; Publication 21; American University of Beirut: Beirut, Lebanon, June 1963; pp 1–104.

Cassab, G. I.; Varner, J. E. Immunocytolocalization of Extensin in Developing Soybean Seed Coats by Immunogold-Silver Staining and by Tissue Printing on Nitrocellulose Paper. *J. Cell Biol.* **1987**, *105*, 2581–2588.

Cassab, G. I.; Varner, J. E. Cell Wall Proteins. *Annu. Rev. Plant Physiol. Mol. Biol.* **1988**, *39*, 321–353.

Cassab, G. I.; Nieto-Sotelo, J.; Cooper, J. B.; Van Holst, G. J.; Varner, J. E. A Developmentally Regulated Hydroxyproline-Rich Glycoprotein from the Cell Walls of Soybean Seed Coats. *Plant Physiol.* **1985**, *77*, 532–535.

Chapmann, D. G.; Castillo, R.; Campbell, J. A. Evaluation of Proteins in Food. I. A Method for the Determination of Protein Efficiency Ratios. *Can. J. Biochem. Physiol.* **1959**, *37*, 679–760.

Cooper, J. B.; Chen, J. A.; Van Holst, G. J.; Varner, J. E. 4-Hydroxyproline-rich Glycoproteins of Plant Cell Walls. *Trends Biochem. Sci.* **1987**, *12*, 24–27.

de Lumen, B. O. Molecular Approaches to Improving the Nutritional and Functional Properties of Plant Seeds as Food Sources: Developments and Comments. *J. Agric. Food Chem.* **1990**, *38*, 1779–1788.

Dransfield, E. J. Intramuscular Composition and Texture of Beef Muscles. *J. Sci. Food Agric.* **1977**, *28*, 833–842.

Eastoe, J. E. Composition of Collagen and Allied Proteins. In *Treatise on Collagen*; Ramachandran, G. N., Ed.; Academic Press: New York, 1967; pp 1–72.

Etherington, D. J.; Sims, T. J. Detection and Estimation of Collagen. *J. Sci. Food Agric.* **1981**, *32*, 539–546.

Expert Work Group, FSIS. Final Report and Recommendations: Food Safety and Inspection Service (FSIS), USDA. *Am. J. Clin. Nutr.* **1984**, *40*, 675–684.

FAO/WHO. *Protein Requirements*; FAO/WHO Nutrition Meeting, Report Series 37; Food and Agriculture Organization/World Health Organization: Rome, 1965.

FAO/WHO. *Energy and Protein Requirements*; FAO/WHO Nutrition Meetings, Report Series 522; Food and Agriculture Organization/World Health Organization: Rome, 1973.

FAO/WHO Expert Consultation. *Protein Quality Evaluation*; Report Series 51; Food and Agriculture Organization/World Health Organization: Rome, 1990.

FAO/WHO/UNU Consultation. *Energy and Protein Requirements*; FAO/WHO Nutrition Meetings, Report Series 724; Food and Agriculture Organization/World Health Organization: Geneva, 1985.

Happich, M. L.; Swift, C. E.; Naghski, J. Equations for Predicting PER from Amino Acid Analyses: A Review and Current Scope of Application. In *Protein Nutritional Quality of Food and Feeds. Part I. Assay Methods—Biological, Biochemical and Chemical*; Friedman, M., Ed.; Dekker: New York, 1975; pp 125–135.

Happich, M. L.; Bodwell, C. E.; Hackler, L. R.; Phillips, J. G.; Derse, P. H.; Elliot, J. G.; Hartnagel, R. E., Jr.; Hopkins, D. T.; Kapiszka, E. L.; Mitchell, G. V.; Parsons, G. F.; Frescher, E. E.; Robaidek, E. S.; Womak, M. Net Protein Ratio Data: AACCC-ASTM Collaborative Study. *J. Assoc. Off. Anal. Chem.* **1984**, 255–262.

Heidelbaugh, N. D.; Huber, C. S.; Bernarczyk, J. F.; Smith, M. Methods for Calculating Protein Content of Foods. *J. Agric. Food Chem.* **1975**, *23*, 611–613.

Horstmann, J. H. A Precise Method for the Quantitation of Proteins Taking Into Account Their Amino Acid Composition. *Anal. Biochem.* **1979**, *96*, 130–138.

Hughes, S. A.; Murphy, P. A. Varietal Influence on the Quality of Glycinin in Soybeans. *J. Agric. Food Chem.* **1983**, *31*, 376–379.

Hugli, T. E.; Moore, S. Determination of the Tryptophan Content of Proteins by Ion Exchange Chromatography of Alkaline Hydrolysates. *J. Biol. Chem.* **1972**, *247*, 2828–2834.

Khanizadeh, S.; Buszard, D.; Zarkadas, C. G. Seasonal Variation of Proteins and Amino Acids in Apple Flower Buds (*Malus pumila* Mill. cv. McIndoch/M7). *J. Agric. Food Chem.* **1989**, *37*, 1246–1252.

Khanizadeh, S.; Buszard, D.; Zarkadas, C. G. Comparison of Three Methods for Calculating Protein Content in Developing Apple Flower Buds. *J. Assoc. Off. Anal. Chem.* **1992**, *75*, 734–737.

Laser-Reutersward, A.; Asp, N.-G.; Bjorck, I. Effect of Collagen and Heat Treatment on Protein Digestibility and Biological Value of Meat Products. *J. Food Technol.* **1985a**, *20*, 745–751.

Laser-Reutersward, A.; Andersson, H.; Asp, N.-G. Digestibility of Collagenous Fermented Sausage in Man. *Meat Sci.* **1985b**, *14*, 105–112.

Lee, X. B.; Elliot, J. G.; Rickansrud, D. A.; Hagberg, E. C. Predicting Protein Efficiency Ratio by the Chemical Determination of Connective Tissue Content in Meat. *J. Food Sci.* **1978**, *43*, 1359–1362.

Light, N. D. *The Role of Collagen in Determining the Texture of Meat*; Pearson, A. M., Dutson, T. R., Bailey, A. J., Eds.; Advances in Meat Research 4; AVI, Van Nostrand Reinhold: New York, 1987; pp 87–107.

Light, N. D.; Champion, A. E. Characterization of Muscle Epimysium, Perimysium and Endomycium Collagens. *Biochem. J.* **1984**, *219*, 1017–1026.

Light, N. D.; Champion, A. E.; Voyle, A. J.; Bailey, A. J. The Role of Epimysial, Perimysial and Endomysial Collagen in Determining Texture in Six Bovine Muscles. *Meat Sci.* **1985**, *13*, 137–149.

Lindberg, W.; Ohman, J.; Wold, S. Determination of the Proteins in Mixtures of Meat, Soybean and Rind from their Chromatographic Amino Acid Pattern by the Partial Least Squares Method. *Anal. Chim. Acta* **1985**, *171*, 1–11.

McLaughlan, J. M.; Anderson, G. H.; Hackler, L. R.; Hill, D. C.; Jansen, G. R.; Keith, M. O.; Sarvar, G.; Sosulski, F. W. Assessment of Rat Growth Methods from Estimating Protein Quality: Interlaboratory Study. *J. Assoc. Off. Anal. Chem.* **1980**, *63*, 462–467.

Moore, S. On the Determination of Cystine and Cysteine as Cysteic Acid. *J. Biol. Chem.* **1963**, *243*, 235–237.

Nguyen, Q.; Zarkadas, C. G. Comparison of the Amino Acid Composition and Connective Tissue Protein Contents of Selected Bovine Skeletal Muscles. *J. Agric. Food Chem.* **1989**, *37*, 1279–1286.

Nguyen, Q.; Fanous, M.; Kamm, L. H.; Khalili, A. D.; Schuepp, P. H.; Zarkadas, C. G. Comparison of the Amino Acid Composition of Two Commercial Porcine Skins (Rind). *J. Agric. Food Chem.* **1986**, *34*, 566–572.

Noda, I.; Sofos, J. N.; Allen, C. E. Nutritional Evaluation of All-meat and Meat-soy Wieners. *J. Food Sci.* **1977**, *42*, 567–569.

Osborn, T. B.; Mendel, L. B.; Ferry, E. L. A Method of Expressing Numerically the Growth-promoting Value of Proteins. *J. Biol. Chem.* **1919**, *37*, 223–231.

Oser, B. L. Method for Integration Essential Amino Acid Content in the Nutritional Evaluation of Protein. *J. Am. Diet. Assoc.* **1951**, *27*, 396–402.

Ozols, J. Amino Acid Analysis. *Methods Enzymol.* **1990**, *182*, 587–601.

Pellett, P. L.; Young, V. R. Background Paper 4: Evaluation of the Use of Amino Acid Composition Data in Assessing the Protein Quality of Meat and Poultry Products. *Am. J. Clin. Nutr.* **1984**, *40*, 718–736.

Pellett, P. L.; Young, V. R. Protein and Amino Acid Needs for Adults. *Ecol. Food Nutr.* **1988**, *21*, 312–330.

Robson, D. S. A Simple Method for Constructing Orthogonal Polynomials When the Independent Variable is Unequally Spaced. *Biometrics* **1959**, *49*, 444–449.

Rust, R. E. *Cured and Processed Meat and Meat Products*; Wolff, I. A., Ed.; CRC Handbook of Processing and Utilization in Agriculture 1; CRC: Boca Raton, FL, 1982; pp 83–105.

Sarwar, G. Available Amino Acid Score for Evaluating Protein Quality of Foods. *J. Assoc. Off. Anal. Chem.* **1984**, *67*, 623–626.

Sarwar, G. Digestibility of Protein and Bioavailability of Amino Acids in Foods. *World Rev. Nutr. Diet.* **1987**, *54*, 26–70.

Statistical Analysis System (SAS). *User's Guide: Basics*; SAS Institute: Cary, NC, 1982.

Steel, R. G. D.; Torrie, J. H. *Principles and Procedures of Statistics. A Biometrical Approach*, 2nd ed.; McGraw-Hill: New York, 1980.

Terrell, R. N. *Sausage Products*; Wolff, I. A., Ed.; CRC Handbook of Processing and Utilization in Agriculture; CRC: Boca Raton, FL, 1982; pp 107–155.

U.S. Department of Agriculture. Standards and Labelling Requirements for Mechanically Separated (species) and Products in which it is Used. Final Rule. *Federal Register*; 9CFR Parts 317, 318, 319, U.S. GPO: Washington, DC, June 29, 1982; Part 2, 47, 28214-58.

Varner, J. E.; Lin, L.-S. Plant Cell Wall Architecture. *Cell* **1989**, *56*, 231–239.

Wilson, R. F. Seed Metabolism. In *Soybean: Improvements, Production and Uses*, 2nd ed.; Wilcox, J. R., Ed.; Agronomy Monograph 16; American Society of Agronomy: Madison, WI, 1987; pp 643–686.

Wolf, W. J. *Edible Soybean Protein Products*; Wolff, I. A., Ed.; CRC Handbook of Processing and Utilization in Agriculture II; CRC: Boca Raton, FL, 1982; pp 23–55.

Ye, Z.-H.; Varner, J. E. Tissue-Specific Expression of Cell Wall Proteins in Developing Soybean Tissues. *Plant Cell* **1991**, *3*, 23–37.

Young, V. R.; Pellett, P. L. Background Paper 5: Amino Acid Composition in Relation to Protein Nutritional Quality of Meat and Poultry Products. *Am. J. Clin. Nutr.* **1984**, *40*, 737–742.

Young, V. R.; Bier, D. M.; Pellett, P. L. A Theoretical Basis for Increasing Current Estimates of the Amino Acid Requirements in Adult Man, with Experimental Support. *Am. J. Clin. Nutr.* **1989**, *50*, 80–92.

Zarkadas, C. G. Accelerated Methods for Determining Methylated Basic Amino Acids in Proteins and Muscle Tissues and Stable Crosslinks in Elastin and Collagen. *J. Appl. Biochem.* **1979**, *1*, 148–163.

Zarkadas, C. G. An Assessment of the Protein Quality of Selected Meat Products Based on Their Amino Acid Profiles and Their Myofibrillar and Connective Tissue Protein Contents. *J. Agric. Food Chem.* **1992**, *40*, 790–800.

Zarkadas, C. G.; Zarkadas, C. G.; Karatzas, C. N.; Khalili, A. D.; Nguyen, Q. Rapid Method for Determining Desmosine, Isodesmosine, 5-Hydroxylysine, Tryptophan, Lysinoalanine and the Amino Sugars in Proteins and Tissues. *J. Chromatogr.* **1986**, *378*, 67–76.

Zarkadas, C. G.; Marshall, W. D.; Khalili, A. D.; Nguyen, Q.; Zarkadas, G. C.; Karatzas, C. H.; Khanizadeh, S. Mineral Composition of Selected Bovine, Porcine and Avian Muscles, and Meat Products. *J. Food Sci.* **1987a**, *52*, 520–525.

Zarkadas, C. G.; Rochemont, J. A.; Zarkadas, G. C.; Karatzas, C. N.; Khalili, A. D. Determination of Methylated Basic, 5-Hydroxylysine, Elastin Crosslinks, Other Amino Acids and the Amino-sugars in Protein and Tissues. *Anal. Biochem.* **1987b**, *160*, 251–266.

Zarkadas, C. G.; Meighen, E. A.; Zarkadas, C. G.; Karatzas, C. N.; Khalili, A. D.; Rochemont, J. A.; Berthelet, M. The Determination of the Myofibrillar and Connective Tissue Proteins in the Bovine Diaphragm. *J. Agric. Food Chem.* **1988a**, *36*, 1095–1109.

Zarkadas, C. G.; Karatzas, C. N.; Khalili, A. D.; Khanizadeh, S.; Morin, G. Quantitative Determination of the Myofibrillar Proteins and Connective Tissue Content in Selected Porcine Skeletal Muscles. *J. Agric. Food Chem.* **1988b**, *36*, 1131–1146.

Zarkadas, C. G.; Drouliscos, N. J.; Karatzas, C. N. Comparison of the Total Protein, Nitrogen, and Amino Acid Composition of Selected Additives and Ingredients Used in Composite Meat Products. *J. Agric. Food Chem.* **1988c**, *36*, 1121–1131.

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