Assessment of the Protein Quality of Selected Meat Products Based on Their Amino Acid Profiles and Their Myofibrillar and Connective Tissue Protein Contents†

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The amino acid profiles and levels of myosin, actin, collagen, and collagen-like proteins in extended composite meats were examined as potential indices to assess protein quality of such products. The myofibrillar and connective tissue protein levels of typical composite meat products were determined from the amounts of N^{τ}-methylhistidine and 5-hydroxylysine, respectively, found in their acid hydrolysates. When the sum of the myofibrillar and connective tissue proteins was subtracted from the total protein of these products, the difference was an accurate determination of the nonmeat proteins present. Composite meats varied in their amino acid composition and content of myofibrillar (17.4–52.3%), connective tissue (4.1–19.0%), and nonmuscle protein (2.4–67.2%), depending upon the meat cuts and nonmeat protein ingredients used to formulate them. As the content of collagen increased, three of the nonessential amino acids, glycine, proline, and 4-hydroxyproline, increased while the levels of lysine and other essential amino acids decreased. Calculated protein efficiency ratios ranged from 2.7 to 2.9 depending upon amounts of nonmuscle protein additives present.

INTRODUCTION

There has been a continuing interest in the development of reliable analytical methodology for precise assessment of the skeletal muscle, connective tissue, and nonmuscle protein contents of meat, poultry, and their products. Past efforts to assess the protein quality of these products have been based primarily on immunological assays, electrophoretic and chromatographic separation, and the determination of skeletal muscle and nonmuscle protein additives in meat products [reviewed by Pearson (1975); Olsman and Slump, 1981; Ranken, 1984; Ellis, 1987; McNeal, 1987; Berkowitz and Webert, 1987; Ashworth, 1987; Agater et al., 1986; McNeil et al., 1984]. These methods, although promising (Agater et al., 1986), have had limited success with processed meats, mainly because of the extensive denaturation and structural changes which occur in these mixtures during processing. In addition, the overall protein quality of such products differs considerably because the levels and nature of the additives and ingredients used to formulate composite meats and poultry products vary greatly. Furthermore, the presence of variable amounts of connective tissue proteins in meat blends introduces large errors in their protein determinations if the Kjeldahl conversion factor (N × 6.25) is used. Both collagen and elastin have higher nitrogen levels (≥18%) than muscle or nonmuscle proteins (≤16%). Although the Kjeldahl digesion method is satisfactory for determining total nitrogen in meats, poultry, and their products (Morries, 1983), the procedure is imprecise for determining the total protein content of such blends because a substantial quantity of nitrogen determined by the Kjeldahl procedure derives from other nonprotein nitrogenous constituents in these products (Benedict, 1987). An accurate assessment of the levels of these proteins, and their contribution to protein quality of composite meats, is therefore essential for both regulatory and scientific purposes, as well as for consumer information and international trade.

The U.S. Department of Agriculture's Food Safety and Inspection Services Expert Work Group (FSIS, 1984), and the Skylab group (Heidelbaugh et al., 1975) have recommended the use of accurate protein, amino acid, and connective tissue data of meat and poultry products as a simple and practical method for assessing their protein quality. Their recommendation is based on two major findings: a statistical correlation exists between the protein efficiency ratio (PER) values and the contents of the essential amino acids of a protein or protein mixture (Alsmeyer et al., 1974; Lee et al., 1978; Pellett and Young, 1984), and the content of collagen of meats is highly negatively correlated ($R = -0.99$) to rat PER values (Lee et al., 1978; Pellett and Young, 1984). Therefore, accurate analyses of the actual protein and the complete amino acid composition of meats and their products, including analyses of those unique amino acids found in myofibrillar and connective tissue proteins (Zarkadas et al., 1986, 1987b, 1988a,b), may be very useful predictors of protein quality in meats, poultry, and their products (Young and Pellet, 1984).

The purpose of the present study was to establish the levels and variation of all amino acids, including the methylated basic amino acids, Lys(5-OH), and related compounds in seven typical commercial composite meat products, using analytical chromatographic methods developed to quantitate these unique and other amino acids in proteins and tissues (Zarkadas, 1979; Zarkadas et al., 1986, 1987b). The aims were (1) to determine whether the levels and quantitative fluctuations of these unique basic amino acids in processed meats could be used for the determination of their myofibrillar, connective tissue, and nonmuscle proteins content; (2) to determine the protein content of meats more accurately from amino acid compositional data; and (3) to determine whether the levels of these proteins and the amino acid contents of fresh ground meat and composite meat products could be used as an accurate measure of their protein nutritional quality (Zarkadas, 1981; Zarkadas et al., 1988b,c; Karatzas and Zarkadas, 1988).

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Table I. Composition of Commercially Prepared Standard (std) and Extended (ext) Composite Wiener (W-1-4) and Hamburger (H-1-3) Products Supplied by Manufacturer I

	composite meat samples, g						
ingredients used in formulation	H_{std}-1	H_{ext}-2	H_{ext}-3	W_{std}-1	W_{ext}-2	W_{ext}-3	W_{ext}-4
lean beef chuck (10/19)[a]	70	60	49	15	12.75	10.75	12.75
beef plates (50/11.5)	30	25	21	25	21.75	17.50	21.25
lean pork trim (15/18)				20	17.00	14.00	
pork hearts				15	12.75	10.50	
pork backfat (90/2.5)				25	21.25	17.50	21.25
MD chicken (25/11)							17.00
beef tripe							12.25
total meat	100	85	70	100	85	69.25	84.50
binder (23% protein)[b]				10	10	10	10
Promate 280 (TVP; 52% protein)	0	5	10		2.5	5.0	2.5
Pro-Tn-Pea 800 (58% protein)					2.5	5.0	2.5
water	0	10	20	40	50	60	50
salt/seasoning/cure				3.58	3.62	3.62	3.62
total fresh wt	100	100	100	153.6	153.6	153.6	153.6

[a] Values in parentheses indicate approximate fat to protein levels on a fresh weight basis. [b] Values for nonmeat plant protein additives are given on a dry weight basis: Promate 280 is a textured soybean flour product; Pro-Tn-Pea 800 is a moist-heat-treated pea protein product.

Table II. Proximate Composition of Standard (std) and Extended (ext) Hamburger (H) and Wiener (W) Composite Meat Products (Grams per Kilogram of Fresh Weight)

	component					
sample description	moisture	total N	crude protein (N × 6.25)	total lipid	total ash	recovered DWB[a]
all-beef hamburger						
H_{std}-1	655.5 ± 4.5[b]	29.09 ± 0.22	181.8 ± 1.4	152.3 ± 5.0	8.55 ± 0.94	344.5
H_{ext}-2	647.5 ± 8.2	27.80 ± 0.19	173.8 ± 1.2	150.0 ± 6.8	10.11 ± 0.31	352.5
H_{ext}-3	645.5 ± 2.6	27.16 ± 0.10	169.8 ± 0.6	126.6 ± 5.5	12.02 ± 0.86	354.5
mixed-meat wieners						
W_{std}-1	502.1 ± 0.9	17.78 ± 0.08	111.1 ± 0.5	290.2 ± 2.4	34.27 ± 0.75	497.9
W_{ext}-2	524.5 ± 4.6	18.59 ± 0.09	116.2 ± 0.6	250.1 ± 2.2	36.03 ± 0.19	475.5
W_{ext}-3	555.5 ± 2.6	20.71 ± 0.15	120.4 ± 0.9	202.9 ± 14.5	37.99 ± 0.66	444.5
W_{ext}-4	526.8 ± 14.0	16.95 ± 0.12	105.9 ± 0.8	269.6 ± 7.4	35.92 ± 1.00	473.2

[b] Recovered DWB is the sum of values obtained for crude protein, total ash, and lipid. [b] Mean values ± SD for six determinations.

MATERIALS AND METHODS

Materials. Type AA-10 9.0 ± 1.0 μm spherical resin and type I standard amino acid calibration mixture were obtained from Beckman Instruments Inc., Palo Alto, CA, while types DC-6A 11.0 ± 1.0 μm, DC-4A 9.0 ± 0.5 μm, and DC-5A 6.0 ± 0.5 μm spherical resins were obtained from the Dionex Chemical Corp., Palo Alto, CA. The bovine *Ligamentum nuchae* elastin used for the preparation of desmosine and isodesmosine (Zarkadas, 1979) was purchased from Sigma Chemical Co., St. Louis, MO. The diastereoisomer mixture of 5-hydroxy-DL-lysine and *allo*-5-hydroxy-DL-lysine, N^6-methyl-L-lysine, N^6-dimethyl-L- and N^6-trimethyl-L-lysine bis(*p*-hydroxyazobenzene-*p*-sulfonate)·H_2O, N^τ-histidine hydrate, N^τ-methyl-L-histidine, D-glucosamine, *O*-phospho-L-serine, and 4-hydroxyproline were purchased from Calbiochem, La Jolla, CA. 3-Nitro-L-tyrosine was from Aldrich Chemical Co., Milwaukee, WI. All other chemicals and reagents were of the highest purity commercially available and were used without further purification.

Preparation of Composite Meat Products. The commercially blended all-beef wiener (W) emulsions with condiments (+C) or without (–C) used in these studies were obtained immediately after mixing from ordinary commercial sources (manufacturer II), although information concerning their composition was not disclosed by the manufacturer. Five additional unknown composite meat products (four wiener samples, labeled W_{ext}-1-4, and three hamburger samples, labeled H_{ext}-1-3) were obtained from a major manufacturer (I) in eastern Canada. In this case, however, information concerning their method of preparation and composition with regard to the muscle type, the meat yielding species, and the extenders employed for their preparation, as presented in Table I, was not disclosed until after the results of these analyses were compiled (Table II–V). Each of the commercially blended samples was homogenized in a Lourdes stainless steel blender (Lourdes Instrument, Corp., Brooklyn, NY) operated at top speed (3 min; 5 °C), dried to constant weight in vacuo (95–100 °C) or by lyophilization, pulverized in an electrically driven end-runner mill, passed through a 152-μm mesh sieve, and stored at –20 °C until needed.

Proximate Composition. Standard methods from AOAC (1984) were followed for the determination of moisture (Sections 7.003, 24.002), petroleum-ether-extractable lipids (Sections 10.132, 24.005), and total ash (Sections 24.009, 31.012) as described previously (Zarkadas et al., 1987a). Total nitrogen of the composite meat products was determined according to the official Kjeldahl method (Section 2.057) using the automated Technicon II system (Technicon Instruments Co., Tarrytown, NY) to analyze the digests (Section 24.028; AOAC, 1984).

Extraction Procedures for Composite Meats. To effectively remove all traces of soluble histidine dipeptides known to be present in skeletal muscle tissues (Carnegie et al., 1982, 1984; Harris and Milne, 1987), samples (10 g) of the pulverized composite meats were suspended in 200 mL of 75% ethyl alcohol in 0.1 M HCl (Rangeley and Lawrie, 1977) and homogenized for 3 min in a VirTis Model 45 (VirTis, Gardiner, NY) homogenizer (speed set at 30/100). The homogenates were centrifuged at 50000*g* (SS-34 Sorvall rotor) for 30 min at 2 °C, and the pellets were extracted a further two times. The extracted composite meat samples were finally ground in an electrically driven end-runner coffee mill (Moulinex Canada Ltd., Weston, ON) to pass through a No. 40 mesh screen and stored at –20 °C until needed.

Preparation of Tissue Hydrolysates. Triplicate samples (0.5 g) of all meat blends were hydrolyzed in Pyrex (No. 9860) test tubes (18 × 150 mm) under vacuum (below 25 μm of Hg) with triple-glass-distilled constant-boiling HCl (6.0 M) containing 0.2% (v/v) phenol at 110 ± 1.0 °C for periods of 24, 48, 72, and 96 h as described previously (Nguyen et al., 1986; Nguyen and Zarkadas, 1989). Analyses of individual acid hydrolysates were performed on the clear filtrate in duplicate according to methods described previously (Zarkadas, 1975, 1978, 1979; Zarkadas et al., 1986, 1987b; 1988a,c).

Procedures for Amino Acid Analyses. Amino acid analyses were carried out either on a Model 120C conventional or on a fully automated Beckman Spinco Model 121 MB amino acid analyzer using single-column methodology (Zarkadas, 1979; Zarkadas et al., 1986, 1987b). The standard instrument was equipped with a module control (Autolab Spectra-Physics GmbH, Darm-

Table III. Comparison of the Total Protein Content of Commercially Prepared Composite Meat Products Calculated by Three Methods

meat product	N content, g of N/100 g DWB: Kjeldahl A[a]	N content, g of N/100 g DWB: sum of amino acid B[b]	N content, g of N/100 g DWB: % difference, $[(A-B)/A] \times 100$	conversion factors calcd from amino acid composition	total protein content, g of protein/100 g DWB, calculated from Kjeldahl N: conversion factor 6.25	total protein content, g of protein/100 g DWB, calculated from Kjeldahl N: individual conversion factors	total protein content, g of protein/100 g DWB, calculated from: sum of amino acid composition[b,d]
all-beef hamburger							
H_{std}-1[c]	8.44[c]	7.95	5.90	5.76	52.78	48.61	45.81 ± 0.48
H_{ext}-2	7.89	7.65	2.88	5.69	49.29	44.87	43.56 ± 0.37
H_{ext}-3	7.66	7.20	6.00	5.66	47.88	43.36	40.78 ± 0.45
mixed-meat wieners							
W_{std}-1[c]	3.57	3.26	8.79	5.97	21.94	21.32	19.42 ± 0.29
W_{ext}-2	3.91	3.81	2.63	5.58	24.44	21.82	21.23 ± 0.36
W_{ext}-3	4.66	4.28	8.11	5.48	29.12	25.53	23.49 ± 0.40
W_{ext}-4	3.58	3.24	9.51	5.67	22.39	20.31	18.38 ± 0.11
Skylab meats[e]							
filet mignon	11.75	10.54	10.30	6.35	73.44	73.44	74.56
prime rib	12.20	9.44	22.62	5.45	76.25	76.25	66.47

[a] Analyzed in sextuplet by the Kjeldahl method (AOAC, 1984). [b] Mean values expressed on a dry weight basis (DWB) and standard error of measurements (SEM) for 12 determinations; calculated according to the method of Heidelbaugh et al. (1975). [c] Data taken from Karatzas and Zarkadas (1988). [d] Data calculated according to the method of Horstmann (1979) using eqs 1–3. [e] Data taken from Heidelbaugh et al. (1975).

Table IV. Comparison of the Amino Acid Composition (Grams of Amino Acid per Kilogram of Total Protein) of Extended (ext) and Composite Hamburger Samples (H-2 and H-3) before and after Solvent Extraction with 0.1 M HCl in 75% Ethyl Alcohol[a] (Manufacturer I)

amino acid (AA)	extended hamburger samples: H-2 untreated[b]	H-2 extracted[b]	H-3 untreated[b]	H-3 extracted[b]
aspartic acid	90.81 ± 1.37	93.79 ± 0.35	96.36 ± 0.69	96.16 ± 3.34
threonine	39.81 ± 0.22	40.38 ± 0.15	40.49 ± 0.21	41.38 ± 2.66
serine	39.72 ± 0.38	39.34 ± 0.13	41.97 ± 0.44	41.21 ± 1.25
glutamic acid	158.02 ± 2.38	149.00 ± 2.63	164.42 ± 1.83	157.96 ± 5.29
proline	50.11 ± 1.56	48.23 ± 1.70	48.22 ± 1.59	44.29 ± 1.49
glycine	51.89 ± 1.05	58.87 ± 0.75	47.40 ± 0.64	47.40 ± 1.43
alanine	54.89 ± 0.76	57.13 ± 0.65	52.22 ± 0.53	49.57 ± 1.52
cysteine	23.98 ± 0.09	24.99 ± 0.10	27.99 ± 0.12	26.57 ± 0.13
valine	49.74 ± 0.22	51.78 ± 0.39	52.72 ± 0.49	51.52 ± 0.92
methionine	25.25 ± 0.54	30.36 ± 2.66	21.43 ± 0.57	31.69 ± 2.15
isoleucine	51.31 ± 1.94	49.06 ± 1.99	50.56 ± 0.51	51.45 ± 1.49
leucine	81.48 ± 1.01	78.46 ± 0.57	79.46 ± 0.24	80.25 ± 1.24
tyrosine	34.18 ± 0.25	34.03 ± 0.03	33.27 ± 0.51	36.49 ± 1.39
phenylalanine	42.07 ± 0.45	42.11 ± 0.52	43.30 ± 0.44	45.81 ± 0.55
histidine	32.69 ± 0.36	28.87 ± 1.31	32.11 ± 0.27	29.24 ± 1.13
lysine	83.41 ± 1.15	80.33 ± 0.54	81.04 ± 0.62	81.67 ± 2.78
arginine	65.22 ± 0.79	67.28 ± 0.83	66.43 ± 0.64	67.67 ± 2.16
tryptophan	11.16 ± 0.39	14.72 ± 0.51	10.36 ± 0.43	10.16 ± 0.39
4-hydroxyproline	11.43 ± 0.85	12.12 ± 0.97	8.55 ± 0.74	8.05 ± 0.09
N^{τ}-methylhistidine	0.983 ± 0.02	0.131 ± 0.00	0.715 ± 0.03	0.094 ± 0.01
unknown 17, nmol/mg of protein	29.35 ± 0.58	13.49 ± 1.53	nd	nd
ammonia	16.07 ± 2.12	17.35 ± 0.79	18.03 ± 0.95	17.83 ± 1.07
total protein, g/kg of dry weight	435.61 ± 3.71	886.68 ± 20.49	407.82 ± 4.45	878.43 ± 17.89
total AA-N[c]	175.82	177.36	176.58	175.61
total EAA,[d] mg/g of N	2887.2	2887.5	2871.45	2987.65
EAA index[d]	78.61	78.00	77.99	79.70
protein score[d]	69.40	71.83	65.67	62.29
WE,[e] μg/nmol	0.109397	0.108448	0.109869	0.110361
F,[e] μg/nmol	0.113013	0.112163	0.113964	0.114300
F',[f] μg/nmol	0.121483	0.120310	0.121913	0.121631

[a] Method of Rangeley and Lawrie (1976, 1977). [b] Mean values and standard error of measurements (SEM) for 12 determinations. nd, not determined. [c] Calculated according to the method of Heidelbaugh et al. (1975). [d] From Oser (1951) and Block and Mitchell (1946). [e] The weight equivalent (WE) and conversion factor F were calculated according to the method of Horstmann (1979). [f] The conversion factor F' (μg/nmol) was also calculated according to the method of Horstmann (1979) using eq 2 but in the absence of tryptophan, cyst(e)ine, proline, and 4-hydroxyproline.

stadt, West Germany) and a companion Autolab System AA (Beckman Methodology Bulletins AA-TB-001–TB-014) for computing peak concentrations (Zarkadas, 1978, 1979). The automated instrument was equipped with a Varian Vista 402 chromatographic data reduction system (Varian Instruments Group, Walnut Creek, CA) to increase the sensitivity of the analysis and to enable quantitation of amino acids at the picomole level as described previously (Zarkadas et al., 1987b).

Complete amino acid analyses were carried out on each of the composite meat samples according to the standard procedures described previously (Zarkadas, 1979; Zarkadas et al., 1986, 1987b).

The data reported for serine and threonine represent the average values extrapolated to zero time of hydrolysis. The values for valine, isoleucine, leucine, and phenylalanine are averages of data from 48, 72, and 96 h of hydrolysis. All others are reported as the average values from 24, 48, 72, and 96 h of hydrolysis.

4-Hydroxyproline was determined separately from a concen-

Table V. Amino Acid Composition (Grams of Amino Acid per Kilogram of Total Protein) of Mixed-Meat Extended (ext) Wiener Sausage Samples (W-2–4) before and after Solvent Extraction with 0.1 M HCl in 75% Ethyl Alcohol[a] (Manufacturer I)

	composite meat samples					
	W_{ext}-2		W_{ext}-3		W_{ext}-4	
amino acid (AA)	untreated[b]	extracted[b]	untreated[b]	extracted[b]	untreated[b]	extracted[b]
aspartic acid	85.47 ± 1.08	95.43 ± 0.47	97.35 ± 0.61	99.11 ± 0.44	85.48 ± 0.41	95.20 ± 0.30
threonine	41.12 ± 0.29	40.25 ± 0.19	34.83 ± 0.07	40.83 ± 0.12	38.11 ± 0.14	39.35 ± 0.18
serine	42.77 ± 0.65	42.11 ± 0.16	29.85 ± 0.02	44.44 ± 0.10	43.26 ± 0.43	43.19 ± 0.36
glutamic acid	170.48 ± 2.55	164.76 ± 0.44	182.66 ± 0.44	167.44 ± 1.36	168.15 ± 1.81	163.07 ± 1.12
proline	58.17 ± 1.91	49.78 ± 0.50	64.18 ± 0.17	49.13 ± 0.97	61.94 ± 1.16	55.99 ± 1.33
glycine	48.16 ± 0.53	47.64 ± 0.20	49.81 ± 0.31	42.36 ± 0.27	58.31 ± 0.38	55.90 ± 0.61
alanine	50.53 ± 0.55	51.10 ± 0.38	51.89 ± 0.17	48.76 ± 0.49	52.45 ± 0.29	52.43 ± 0.48
cysteine	18.35 ± 1.81	18.56 ± 1.82	16.14 ± 0.24	15.16 ± 0.24	18.31 ± 1.34	18.29 ± 1.63
valine	55.96 ± 0.19	55.08 ± 0.31	58.91 ± 0.12	56.02 ± 0.20	55.16 ± 0.33	53.20 ± 0.32
methionine	32.30 ± 3.20	27.45 ± 0.56	25.19 ± 0.38	25.69 ± 0.25	29.46 ± 2.15	26.58 ± 0.29
isoleucine	50.07 ± 0.24	50.80 ± 0.16	50.65 ± 0.13	50.44 ± 0.12	49.35 ± 0.28	48.86 ± 0.21
leucine	82.37 ± 0.51	83.80 ± 0.09	83.77 ± 0.13	85.66 ± 0.04	78.54 ± 0.21	79.41 ± 0.31
tyrosine	31.80 ± 0.76	35.21 ± 0.14	18.65 ± 0.03	37.74 ± 0.14	29.75 ± 0.51	34.19 ± 0.34
phenylalanine	44.28 ± 0.22	46.22 ± 0.18	47.65 ± 0.07	48.50 ± 0.07	42.98 ± 0.11	44.33 ± 0.23
histidine	32.17 ± 0.51	29.27 ± 0.26	32.70 ± 0.07	28.70 ± 0.19	29.72 ± 0.23	27.96 ± 0.46
lysine	76.50 ± 0.34	80.39 ± 0.21	76.55 ± 0.21	77.93 ± 0.50	70.78 ± 0.63	74.59 ± 0.66
arginine	58.71 ± 2.00	64.60 ± 0.29	59.10 ± 0.37	67.24 ± 0.18	58.28 ± 1.94	65.93 ± 0.44
tryptophan	10.79 ± 2.02	10.90 ± 1.63	11.07 ± 0.01	10.40 ± 0.34	12.09 ± 1.30	11.16 ± 0.86
4-hydroxyproline	8.41 ± 0.58	5.39 ± 0.84	7.55 ± 0.11	3.68 ± 0.79	14.11 ± 0.89	8.63 ± 1.01
N^{π}-methylhistidine	0.402 ± 0.004	0.02 ± 0.00	0.324 ± 0.003	0.177 ± 0.01	1.311 ± 0.04	0.00 ± 0.00
unknown 17, nmol/mg of protein	2.50 ± 0.01	1.15 ± 0.08	2.55 ± 0.001	1.24 ± 0.21	3.91 ± 0.002	1.34 ± 0.25
ammonia	19.50 ± 0.43	18.63 ± 0.62	27.26 ± 0.73	21.98 ± 0.33	19.66 ± 0.76	15.78 ± 1.02
total protein, g/kg of dry weight	212.31 ± 3.60	718.95 ± 14.75	234.94 ± 4.32	823.38 ± 10.80	183.83 ± 1.05	716.29 ± 5.59
total AA-N[c]	179.33	175.62	182.35	177.62	176.31	174.16
total EAA, mg/g of N	2553.3	2922.59	2646.12	2902.76	2738.44	2847.41
EAA index[d]	76.87	78.52	74.35	76.99	75.95	76.89
protein score	75.66	68.26	73.77	64.87	75.60	72.37
WE,[e] μg/nmol	0.109933	0.110069	0.109533	0.110888	0.108282	0.108877
F,[e] μg/nmol	0.112864	0.113037	0.112187	0.113441	0.111201	0.111766
F',[f] μg/nmol	0.122148	0.120681	0.122162	0.120822	0.121505	0.120563

[a] Method of Rangeley and Lawrie (1977). [b] Mean values and standard error of measurements (SEM) for 18 determinations. [c] Calculated according to the method of Heidelbaugh et al. (1975). [d] From Oser (1951) and Block and Mitchell (1946). [e] The weight equivalent (WE) and conversion factor (F) were calculated according to the method of Horstmann (1979). [f] The conversion factor F' was also calculated according to the method of Horstmann (1979) using eq 2 but in the absence of tryptophan, cysteine, proline, and 4-hydroxyproline.

trated 24-h hydrolysate (equivalent to 0.1 mg of protein/analysis) using a single column (21 × 0.6 cm) packed with Dionex DC-6A resin (Zarkadas et al., 1986). Recoveries of Pro(4-OH) were calculated relative to alanine, isoleucine, and leucine. Determination of tryptophan in meat samples (0.1 g) was carried out separately after alkaline hydrolysis (Hugli and Moore, 1972) on the same column as described previously (Zarkadas et al., 1986, 1987b).

Methionine and cyst(e)ine were determined separately (0.2-g samples) according to the performic acid procedure of Moore (1963). Norleucine was added in the hydrolysates as an internal standard, and the recoveries of cyst(e)ine as cysteic acid and methionine as methionine S,S-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, valine, leucine, and isoleucine present in the sample.

Determination of the methylated basic amino acids, the diastereoisomers of Lys(5-OH), and related compounds were carried out with concentrated hydrolysates (equivalent to 1–2 mg of protein) by the accelerated single-microcolumn (50 × 0.28 cm) system described previously (Zarkadas et al., 1987a) so that peaks adequate for these compounds would be obtained.

Protein Determination. The content of total protein in each of these meat products was determined according to three methods: first, the conventional Kjeldahl method (AOAC, 1984; Morries, 1983) and the multiplication of nitrogen by 6.25; second, the multiplication of Kjeldahl nitrogen by the new conversion factors calculated from the amino acid composition of a given product as described by Heidelbaugh et al. (1975) for Skylab Foods; and third, the protein mass of individual samples calculated by summation of the 18 standard amino acid residues plus Pro(4-OH), Lys(5-OH), and His(τ-Me) of which each sample is composed according to the procedure described by Horstmann (1979) and Zarkadas et al. (1988a) as follows:

$$\mathrm{WE} = \sum_{i=1}^{21} (a_i b_i) \tag{1}$$

The mean residue weight (WE in micrograms per nanomole) and conversion factors F (in micrograms per nanomole) for determining the protein mass in each sample analyzed in the absence of tryptophan and cyst(e)ine were calculated as described previously (Horstmann, 1979; Nguyen et al., 1986). A conversion factor F' (in micrograms per nanomole) was also calculated according to the method of Horstmann (1979), but for determining protein mass in the absence of tryptophan, cyst(e)ine, proline, and/or Pro(4-OH) the expression

$$F' = \sum_{i=1}^{15} (a_i b_i)/[1 - (a_{\mathrm{Trp}} + a_{\mathrm{Cys}} + a_{\mathrm{Pro}} + a_{\mathrm{Pro(4\text{-}OH)}} + a_{\mathrm{Lys(5\text{-}OH)}} + a_{\mathrm{His}(\tau\text{-}\mathrm{Me})})] \tag{2}$$

was used, where a_i is the nanomole fraction of an amino acid i found in the analyzed aliquot and b_i is the molecular mass of amino acid residue i (in micrograms) as described by Horstmann (1979). Both of these factors, F and F', can be used in all subsequent quantitations of a given sample. The protein content of each sample was calculated by multiplying F or F' by the nanomoles of total amino acids found in each acid hydrolysate as follows:

$$P = F' \sum_{i=1}^{15} \chi_i \tag{3}$$

Statistical Analysis. Data processing and linear regression 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 general linear model procedure (SAS, 1982).

RESULTS AND DISCUSSION

Accurate and detailed amino acid determinations were carried out in five selected meat products, to ascertain whether the amino acid profiles and levels of myosin, actin, collagen, and collagen-like proteins in extended composite meats could be used as indices for assessing their protein quality (FSIS, 1984; Young and Pellett, 1984; Lee et al., 1978; Zarkadas, 1981; Zarkadas et al., 1988a–c). Samples of typical all-beef and mixed-meat wieners and hamburger, prepared commercially by varying both the amounts and the type of meat cuts and nonmuscle plant or animal additives used to formulate them (Table I), selected from two major Canadian manufacturers, were subjected to proximate and complete amino acid analyses. All determinations were carried out according to the single-column chromatographic methods developed for this purpose (Zarkadas, 1979; Zarkadas et al., 1986, 1987b). In this study the amino acid compositions of two all-beef wiener emulsions with and without condiments and fresh hamburger were also included for comparison.

Proximate Composition. The average proximate composition of standard and extended hamburger and wiener composite meat products, prepared by manufacturer I (Table I), are summarized in Tables II and III. The data on Kjeldahl nitrogen, crude protein, moisture, fat, and ash (Table II) are given on a wet weight basis (WWB). The mixed-meat wiener blends had a high lipid content (20.3–29.0%) and lower crude protein content (10.5–12.0%), compared to the high crude protein content (17.0–18.2%) and lower lipid content (12.7–15.2%) found in the all-beef hamburger blends (Table II). These results are comparable to those reported previously for other composite meat products (Zarkadas et al., 1987b). Variation was also found to be highly significant in the ash contents on a dry weight basis among the all-beef hamburger and the mixed-meat wieners.

Protein Determination. Table III compares the total nitrogen content determined according to the Kjeldahl procedure and the total nitrogen content determined by the sum of the amino acids present in each of these products. The total protein contents of the selected composite meat blends used for this survey were calculated from their amino acid composition (Tables IV–VI) as described by Heidelbaugh et al. (1975) and Horstmann (1979) and were compared with crude protein values calculated using the Kjeldahl protein conversion factor of 6.25. Variation in protein content as a function of method of calculation ranged from 2.6 to 9.5% (Table III). The apparent differences in protein or nitrogen content calculated by the Kjeldahl method were higher than calculations based on amino acid composition. Similar variability has been reported previously by Heidelbaugh et al. (1975) for Skylab foods, which included bovine filet mignon and prime rib (Table III). These authors recommended that whenever accurate data on the protein content of meats are required, conversion factors based on the actual amino acid nitrogen content should be used.

To correct for this variation, I have calculated new Kjeldahl protein conversion factors based on the actual amino acid nitrogen content of meats. These protein conversion factors are characteristic for each product and can be used in all subsequent quantitations of these products to convert Kjeldahl nitrogen into total protein. The protein contents of the seven meat blends evaluated by the conventional Kjeldahl nitrogen proceudre (AOAC, 1984) and by quantitative amino acid analysis (Heidelbaugh et al., 1975; Horstmann, 1979) differ considerably (Table III). It appears that a substantial quantity of Kjeldahl nitrogen is derived from nonprotein nitrogenous constituents present in these products. From these results it is also apparent that a more accurate method for calculating total protein is the summation of the weights of each amino acid present in foods and that less than 30 μg of protein can be quantitated.

Table VI. Amino Acid Composition (Grams of Amino Acid per Kilogram of Protein) of Lyophilized All-Beef Wiener Emulsions with Condiments[a] (+C) or without (–C) following Extraction with 0.1 M HCl in 75% Ethyl Alcohol (Manufacturer II)

	all-beef wieners	
amino acid (AA)	–C[a]	+C[a]
aspartic acid	87.35 ± 1.29	84.01 ± 0.62
threonine	40.33 ± 0.46	41.28 ± 0.31
serine	42.06 ± 0.38	43.33 ± 0.48
glutamic acid	128.59 ± 2.66	147.60 ± 2.05
proline	66.50 ± 1.70	60.69 ± 1.28
glycine	84.25 ± 3.13	66.61 ± 1.99
alanine	64.40 ± 1.36	56.89 ± 1.19
cysteine	12.21 ± 0.07	10.78 ± 0.05
valine	60.46 ± 1.36	60.05 ± 0.47
methionine	27.28 ± 2.42	36.67 ± 1.11
isoleucine	39.55 ± 1.16	49.33 ± 0.28
leucine	73.68 ± 1.31	79.19 ± 0.89
tyrosine	32.12 ± 0.47	34.03 ± 0.15
phenylalanine	38.79 ± 0.67	41.85 ± 0.38
histidine	27.73 ± 0.52	29.44 ± 0.34
lysine	72.69 ± 1.40	73.53 ± 1.17
arginine	64.50 ± 1.04	59.51 ± 0.30
tryptophan	8.96 ± 0.09	7.91 ± 0.10
4-hydroxyproline	25.23 ± 2.32	15.55 ± 0.93
N^{π}-methylhistidine	0.149 ± 0.003	0.174 ± 0.015
ammonia	14.01 ± 0.1737	12.46 ± 0.22
total protein, g/kg of dry wt	604.51 ± 10.76	766.07 ± 13.73
total AA-N[b]	177.62	173.29
total EAA, mg/g of N	2649.28	2849.68
EAA index[c]	70.50	75.43
protein score[c]	77.99	88.74
WE,[d] μg/nmol	0.104433	0.106959
F,[d] μg/nmol	0.106280	0.108668
F',[d] μg/nmol	0.117629	0.118484

[a] Mean values and standard error of measurements (SEM) for 18 determinations. It should be noted that information concerning the composition of the added condiments was not disclosed by the manufacturer. [b] Calculated according to the method of Heidelbaugh et al. (1975). [c] From Oser (1951) and Block and Mitchell (1946). [d] The WE and F constants were calculated according to the method of Horstmann (1979). The F' value was calculated in the absence of tryptophan, cysteine, proline, and 4-hydroxyproline.

Amino Acid Composition. Results of the amino acid analyses carried out in this study are summarized in Tables IV–VI. A comparison of different methods of expressing results (Eastoe, 1967) indicated that, within any given product, the least variability occurred when the data are expressed on a moisture-, fat-, and ash-free basis. Results have therefore been calculated as grams of anhydrous amino acids per kilogram of total protein. The advantages of this method are that calculations of percentage amino acid recovery and essential amino acid contents can be carried out by simple summation, which allows comparisons to be made between the present data (Tables IV–VI) and those reported in food compositional tables (Tristram and Smith, 1963; Richardson et al., 1980). Values for all determinations show a reproducibility of 100 ± 3% for all amino acids. The mean residue weight (WE, μg/nmol) and conversion factors F and F' (μg/nmol) calculated from the amino acid composition of these products are given in Tables IV–VI.

The data presented in Tables IV–VI indicate that each of these composite meat blends has a characteristic amino acid profile, reflecting the amounts of meat and nonmeat plant or animal additives used to formulate them. The amino acid profiles of the two extended hamburger products evaluated in this study, i.e., H-2 and H-3 (Table IV), appeared to be very similar in composition. The acidic amino acids in the extended hamburgers were present in substantially high quantities and when taken together accounted for almost 25% of all amino acid residues. The total basic amino acids, including arginine, lysine, small amounts of Lys(5-OH), and histidine, comprised approximately 20% of the total amino acids, which is a slightly lower percentage than that of the acidic amino acids. The proteins in the extended hamburger mixtures were overall acidic proteins, which reflects the higher content of plant protein ingredients used to formulate them. Methionine and cysteine accounted for only 4–5% of the total amino acid residues. Although these two amino acids still represent the minor components of these products, it appears that their concentration in meats might also be used to predict their protein quality. To meet minimum FAO recommendations for the essential amino acids in meats, the concentration of methionine should be 3.0% of the total amino acid content. These results are in close agreement with those obtained recently (Karatzas and Zarkadas, 1988).

Processed meats, however, have been reported to contain variable amounts of soluble histidine dipeptides (Carnegie et al., 1982, 1983, 1984; Harris and Milne, 1987; Kohen et al., 1988) including carnosine (β-alanyl-L-histidine), anserine (β-alanyl-L-N^{π}-histidine), and balenine (β-alanyl-L-N^{τ}-methylhistidine), which upon acid hydrolysis yield β-alanyl, histidine, His(π-Me), and His(τ-Me). To quantitatively establish the levels of protein-bound amino acids, including histidine, His(τ-Me), Pro(4-OH), and Lys(5-OH), these histidine dipeptides must be extracted from composite meats prior to acid hydrolysis (Zarkadas et al., 1988a,b). The composition of lyophilized hamburger samples before and after solvent extraction (Table IV) shows that the soluble amino acids extracted by 0.1 M HCl in 75% ethanol prior to acid hydrolysis ranged from 0.75 to 1.0% of the total amino acid content. The results in Tables IV and V show that approximately 9.0–12.2% of the total histidine and practically all of the His(π-Me) have been extracted at ambient temperatures by the 0.1 M HCl in 75% ethyl alcohol solvent. A sizable proportion of the total non-amino-acid nitrogen extracted from the wiener composite meats (Table V) was free ammonia.

Although the amino acid profiles of the products evaluated in this study were similar, some differences were noted. The mean values obtained for aspartic acid in the extracted meat blends ranged from 0 to 10.44% higher than those of the corresponding untreated samples (Tables V and VI). For comparison, the recalculated mean values for aspartic acid from USDA Handbook 8-7 (Richardson et al., 1980) ranged from 87.9 to 98.9 g/kg of protein for mixed-meat types of wieners. These values are considerably higher than those of the untreated wiener samples evaluated in this study (Tables IV–VI) but slightly lower than those of the corresponding reference hamburger sample. The arginine values of the hamburger and wieners ranged from 2.0 to 12.1% higher than those of the corresponding untreated samples. Extended wiener samples were high in glutamic acid (17.5%), proline (55.0%), valine (5.5%), and the basic amino acids, which accounted for a further 16.5% of all residues (Tables V and VI).

Table VII. N^{τ}-Methylhistidine and 5-Hydroxylysine Contents (Grams of Amino Acid per Kilogram of Total Protein) of Standard (std) and Extended (ext) Hamburgers (H), All-Beef Wieners with Condiments (+C) and without (–C), and Mixed-Meat Wiener (W) Products following Extraction with 0.1 M HCl in 75% Ethyl Alcohol

	protein-bound			
	N^{τ}-methylhistidine		5-hydroxylysine	
meat product	mean ± SEM[a]	CV	mean ± SEM[a]	CV
all-beef				
hamburger (N = 6; R = 3)				
H_{std}–1[b]	0.533 ± 0.027	10.17	1.755 ± 0.035	3.97
H_{ext}–2	0.485 ± 0.013	3.75	1.625 ± 0.010	0.87
H_{ext}–3	0.358 ± 0.008	3.00	1.085 ± 0.057	7.49
wiener (–C)	0.366 ± 0.015	8.26	3.003 ± 0.110	7.30
wiener (+C)	0.293 ± 0.003	3.10	1.743 ± 0.010	1.16
mixed-meat (N = 4; R = 2)				
W_{ext}–2	0.228 ± 0.011	7.10	1.071 ± 0.026	3.38
W_{ext}–3	0.177 ± 0.017	13.35	0.647 ± 0.055	11.94
W_{ext}–4	0.216 ± 0.007	4.39	1.576 ± 0.042	3.80

[a] Mean values and standard error of measurements (SEM): R, replicates; N, determinations. [b] Data taken from Karatzas and Zarkadas (1988).

These results are in good agreement with those reported by Karatzas and Zarkadas (1988) for similar meat products.

From the results presented in Tables IV–VI, it is apparent that the Pro(4-OH) content of composite meats varies considerably, possibly reflecting the connective tissue protein contents of these meat blends. Until recently Pro(4-OH) was thought to be confined almost exclusively to the connective tissue fibrous proteins, i.e., collagen and elastin (Eastoe, 1967; Bentley and Hanson, 1969), and has been used as the basis for determining the connective tissue protein content of meats primarily with the methods of either Woessner (1961), Kivirikko (1963), Laurent et al. (1978), or Berg (1982). Recent studies indicate that this hydroxylated unique amino acid occurs in the extracellular matrices of primary cell wall glycoproteins, i.e., extensins, arabinogalactan proteins and salt-extractable glycoproteins, lectins, and agglutinins (Lamport, 1977; Fincher et al., 1983; Stuart and Varner, 1980; Smith et al., 1986; Cooper et al., 1987; Cassab and Varner, 1988). It has also been shown that Pro(4-OH) makes up 45.5% of the polypeptide backbone of some of these glycoproteins. In addition, Pro(4-OH) was found to be present in oilseed and cereal-derived nomeat protein additives as well as in sensory enhancers, potato protein isolate, and alfalfa meal proteins (Zarkadas et al., 1988b). Therefore, the use of this unique amino acid as an index for determining collagen and elastin in composite meats is limited. Although in the present study Pro(4-OH) was used as the basis for determining the total connective tissue contents of extended wieners, the results presented in Table VIII indicate that such calculations gave overestimated connective tissue values for most of these products.

Unique Basic Amino Acid Content of Composite Meats. The results obtained for the protein-bound His(τ-Me) and Lys(5-OH) contents of the all-beef standard and extended hamburger samples (H_{std}-1, H_{ext}-2, and H_{ext}-3) and mixed-meat wiener blends (W_{ext}-2–4) after extraction are presented in Table VII and represent the average values of sextuplet determinations. From these results it is evident that each of these meat products has a typical His(τ-Me) and Lys(5-OH) profile, reflecting the amounts of specific meat cuts and nonmuscle protein ingredients used to formulate them. The data reported in Table VII show high reproducibility and low coefficients of variation, and within the precision of the chromatographic procedure (100 ± 2.5%), recoveries were found to be quantitative (Table I). Table VII lists the His(τ-Me) and Lys(5-OH)

Table VIII. Myofibrillar and Connective Tissue Protein Contents (Grams of Protein per Kilogram of Total Protein) of Standard (std) and Extended (ext) Hamburger (H) and Wiener (W) Composite Meats and of Wieners with Condiments (+C) and without (–C)

	composite meat products[a]								skeletal muscle psoas major (Yates and Greaser, 1983), % of total muscle protein
	all-beef								
	hamburger			wieners		mixed-meat wieners			
protein class	H_{std}–1[f]	H_{ext}–2	H_{ext}–3	–C	+C	W_{ext}–2	W_{ext}–3	W_{ext}–4	
Skeletal Muscle Proteins									
i, intracellular (ia + ib)[b]	851.63	774.66	571.81	584.59	467.98	364.17	282.71	345.00	
ia, myofibrillar[b]	523.12	475.79 ± 12.75	351.20 ± 7.85	359.05 ± 14.71	287.43 ± 2.94	223.67 ± 10.79	173.64 ± 16.68	211.90 ± 6.87	57.71
actin[b]	110.48	100.40 ± 2.69	74.11 ± 1.66	75.76 ± 0.11	60.65 ± 0.62	47.20 ± 2.28	36.64 ± 3.52	44.71 ± 1.45	12.69
myosin[b]	229.72	209.04 ± 5.60	154.30 ± 3.45	157.75 ± 6.47	126.28 ± 1.29	98.27 ± 4.74	76.29 ± 7.33	93.10 ± 3.01	24.82
actomyosin[b]	340.20	309.43 ± 8.29	228.40 ± 5.10	233.51 ± 9.57	186.93 ± 1.91	145.46 ± 7.02	112.93 ± 10.85	137.81 ± 4.47	37.52
ib, other soluble proteins[b]	328.51	298.87 ± 8.01	220.61 ± 4.93	225.54 ± 9.24	180.55 ± 1.85	140.50 ± 6.77	109.07 ± 10.48	133.10 ± 4.31	
ii, extracellular matrix[c]	46.38	42.20 ● 1.13	31.15 ± 0.77	31.84 ± 1.31	25.49 ± 0.26	20.10 ± 0.87	15.40 ± 1.50	18.79 ± 0.61	
iia, collagen[c]	32.52	29.54 ± 0.79	21.80 ± 0.49	22.30 ± 0.90	17.84 ± 0.18	13.89 ± 0.61	10.78 ± 1.04	13.15 ± 0.43	
total (i + ii)	898.01	816.86	602.96	616.43	493.47	384.27	298.11	363.79	
Nonmuscle Additives and Ingredients									
iii, connective tissue[d]	88.89	91.78 ± 6.83	68.66 ± 5.94	202.60 ± 18.60	124.87 ± 7.47	67.53 ± 4.66 (43.28 ± 6.75)	60.63 ± 0.88 (29.55 ± 6.34)	113.30 ± 7.15 (69.30 ± 8.11)	
iv, total collagen and collagen-like proteins[e]	110.13	102.86 ± 0.63	68.68 ± 3.61	190.09 ± 6.39	110.33 ± 2.85	63.30 ± 1.65	40.96 ± 3.48	99.76 ± 2.66	
v, added collagen (iv – iia)	77.61	73.32	46.88	167.79	92.49	49.41	30.18	86.61	
vi, $\sum_{i=1}^{3}$ (i + ii + v)	975.62	890.18	649.84	784.22	585.96	433.68	328.29	450.40	
vii, added nonmuscle proteins	24.38	109.82	350.16	215.78	414.04	566.32	671.71	549.60	
Essential Amino Acids									
EAA_7,[g] %	38.2	37.3	36.9	35.3	38.2	38.3	36.5	36.4	
EAA_{10},[g] %	48.9	48.2	44.9	45.4	47.9	48.4	46.5	46.4	
Protein Efficiency Ratio (PER)									
PER[g] predicted by									
eq 8 (PER_7)	2.98	2.90	2.87	2.85	2.98	2.98	2.84	2.83	
eq 9 (PER_{10})	2.94	2.89	2.68	2.71	2.87	2.90	2.75	2.78	
eq 10 ($PER_{collagen}$)	2.90	2.91	2.99	2.72	2.94	3.03	3.05	2.92	

[a] Mean values and standard error of measurements (SEM) for 18 determinations following extraction with a mixture of 75% ethyl alcohol in 0.1 M HCl. [b] Calculated using His(τMe) data from Table VII and eq 4 [amount of myosin and actin = $638C_T$, where C_T is the amount of His(τ-Me); Zarkadas et al., 1988c]. The SDS-soluble muscle protein fraction in meat products could also be computed by using His(τ-Me) data from Table VII and eq 4a [amount of other muscle SDS-soluble proteins = $616.2C_T$; Karatzas and Zarkadas, 1988]. [c] Calculated by using His(τ-Me) data from Table VII and eq 5 [amount of extracellular matrix = $87C_T$; Karatzas and Zarkadas, 1988]. [d] Calculated by using Pro(4-OH) data from Tables IV–VI and eq 6 [amount of connective tissue (P_{CT}) = amount of Pro(4-OH) × 8.03; Nguyen and Zarkadas, 1989]. The values in parentheses are those obtained from the extracted products (Table V). [e] Calculated by using Lys(5-OH) data from Tables IV–VI and eq 7 [amount of collagen (P_C) = amount of Lys(5-OH) × 63.3; Nguyen and Zarkadas, 1989]. [f] Data quoted from Karatzas and Zarkadas (1988). [g] Calculated according to the method of Lee et al. (1978). EAA_7 include the amino acids isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine; EAA_{10} include the preceding seven amino acids plus tryptophan, histidine, and arginine. PER were also calculated from eq 8 [PER = 0.08084(EAA_7) – 0.1094], eq 9 [PER = 0.06320(EAA_{10}) – 0.1539], and eq 10 [PER = –0.02290(collagen) = 3.1528].

contents of all-beef wiener emulsions with and without condiments and a standard all-beef hamburger (H_{std}-1) analyzed previously (Karatzas and Zarkadas, 1988). Both the all-beef hamburger and wiener emulsion with and without condiments were found to contain considerably higher concentrations of His(τ-Me) and Lys(5-OH) compared to the all-beef extended hamburger and mixed-meat wiener blends. The extended products contained lower myofibrillar and connective tissue proteins. These results are in accord with those reported by other authors (Rangeley and Lawrie, 1977; Poulter and Lawrie, 1980; Olsman and Slump, 1981), although some differences were noted. These differences may have arisen because other methods were employed for these determinations.

Essential Amino Acid Content of Meats. A comparison of the essential amino acid (EAA) profiles (milligrams of EAA per gram of dietary nitrogen) of the selected composite meat products examined in this study, as recommended by Block and Mitchell (1946), Oser (1951), and FAO/WHO (1965, 1973), indicates that these composite meat products contain significant amounts of all EAA required for human nutrition. Mean values for total EAA profiles of composite meat samples before and after extraction ranged from 2553 to 2922 mg/g of N (Tables IV–VI), compared to the total EAA of cow's milk (3200 mg/g of N) or hen's egg (3215 mg/g of N; FAO/WHO, 1965, 1973). Similar results were obtained from the EAA indices and chemical scores.

From the data presented in Tables IV–VI, it may be concluded that a potentially more complete assessment of the protein quality of meats, poultry, and their products might be obtained from a knowledge of their complete amino acid composition. This concept of evaluating the protein quality of meat from its amino acid composition was first introduced by Block and Mitchell (1946) and was improved by Alsmeyer et al. (1974) and Happich et al. (1975). As these predictive tests fail to take into account differences in digestibility, the quality of the various proteins present, and the availability of individual amino acids, more reliable nutritional methods (Sarvar, 1984), including rat bioassays, have been developed to assess their nutritive value and protein quality. However, because such assays are both expensive and time-consuming (Lee et al., 1978; Pellett and Young, 1984; Young and Pellett, 1984), both the complete amino acid composition and the collagen content of meats, poultry, and their products have been used as a basis for assessing their potential nutritive value. The contents of tryptophan, lysine, Pro(4-OH), and His(τ-Me) have also been used as indicators of protein quality in meats.

Lee et al. (1978) identified total EAA in two ways, either as 7 or as 10 amino acids, the 7 (EAA_7) being isoleucine,

leucine, lysine, methionine, phenylalanine, threonine, and valine and the 10 (EAA_{10}) being these 7 plus tryptophan, histidine, and arginine. Mean values for total EAA_7 ranged from 35.3 to 38.3% and for EAA_{10} from 45.4 to 48.9% in the composite meat products evaluated (Table VIII). These results are consistent with those listed in the paper by Pellett and Young (1984) for similar meat products but are considerably above the minimum value of 33% required for the seven (EAA_7) by USDA regulations (FSIS, 1984). Because this scoring procedure is limited to the essential amino acids, Lee et al. (1978) developed equations (eqs 8–10 listed in Table VIII) for predicting the protein efficiency ratios (PER) of meats from amino acid data. In using the prediction, eqs 8 (EAA_7), 9 (EAA_{10}), and 10 ($PER_{collagen}$) all show that the calculated mean PER values for composite meats, which for skeletal muscle protein averages 3.2, also varied (2.7–2.9) with the amounts of nonmuscle plant and animal proteins present. These values are also considerably above the minimum PER value of 2.5 required for such products by USDA regulations (FSIS, 1984); in fact, the higher PER values observed for these products are indicative of the complementation of the plant and animal proteins present. It should also be noted that as the content of collagen increased (Table VIII) three of the nonessential amino acids, glycine, proline, and 4-hydroxyproline, increased while the levels of lysine and other essential amino acids decreased (Tables IV–VI).

Contents of Intracellular Muscle Proteins in Meats. (*i*) *Actin and Myosin Components.* The data in Table VII show that the quantitation of protein-bound His(τ-Me), known to occur exclusively in actin and myosin (Elzinga et al., 1973; Maita et al., 1987), can be used as an index for determining these two principal myofibrillar proteins in composite meats, as described previously (Zarkadas et al., 1988b,c; Karatzas and Zarkadas, 1988), and the results are summarized in Table VIII. Data for a typical skeletal muscle, psoas major (Yates and Greaser, 1983), and for a standard all-beef hamburger sample (Karatzas and Zarkadas, 1988) are also included for comparison. From the data presented in Table VIII, it is apparent that the extended hamburger samples, H_{ext}-2 and H_{ext}-3, contained 10.0 and 7.4% actin and 20.9 and 15.4% myosin, respectively, of the total muscle proteins. This corresponds to 47.6 and 35.1% myofibrillar proteins for H_{ext}-2 and H_{ext}-3, respectively. In the case of standard all-beef hamburger (H_{std}-1), myosin accounted for an estimated 22.9% of the total muscle protein, which corresponds to 43.9% of the myofibrillar proteins. Actin accounted for an estimated 11.0% of the total muscle proteins, which corresponds to 21.1% of the myofibrillar proteins (52.3% of the total protein). Although hamburger is usually prepared from tougher meat cuts, which are higher in connective tissue proteins, the levels of the myofibrillar proteins present were in close agreement with those reported by Yates and Greaser (1983), who have shown that skeletal muscle, i.e., psoas major, contains 57.71% myofibrillar protein of the total muscle mass and that the myofibrils contain 22% actin and 43% myosin by weight. Therefore, the observed differences between the myofibrillar protein contents of standard and extended hamburger samples represent an accurate assessment of the nonmuscle plant or animal protein additives present.

The data of Table VIII also show that the myofibrillar proteins in mixed-meat wiener blends, which ranged from 17.36 to 22.37% compared to 28.7 and 35.9%, respectively, in all-beef wieners with condiments and without, indicate a substantial amount of nonmuscle proteins present in these products. These results are in good agreement with the myofibrillar content of 14.7–32.2% reported for other mixed-meat products (Karatzas and Zarkadas, 1988).

(*ii*) *Other Soluble Muscle Protein Components.* In addition to myofibrillar proteins, skeletal muscles contain a large quantity of other soluble intracellular muscle proteins (McCollester, 1962; Laurent et al., 1981) which on the average represent 42.5% of the total muscle protein among the bovine and porcine skeletal muscles investigated (Zarkadas et al., 1988b,c), which is in accord with the findings of Szent-Gyorgi et al. (1955). The latter, using two different extraction procedures, found that the soluble muscle protein fraction accounted for an estimated 41.6% of the total muscle proteins. The work of Hanson and Huxley (1957), however, reported that the salt-soluble (ca. 0.15 M) sarcoplasmic protein fraction represented only 28.0% of the total muscle proteins. In the present study, the quantity of intracellular SDS-soluble muscle protein fraction, which could also be estimated from His(τ-Me) data and eq 4a given in Table VII (Zarkadas et al., 1988c; Karatzas and Zarkadas, 1988), ranged from 10.9 to 32.9% of the total protein found in the various meat products investigated (Table VIII). These data indicate that the sum of the myofibrillar and other intracellular soluble muscle proteins in composite meats ranged from 28.27 to 77.47% on a total protein basis, compared to 85.16% found in the reference all-beef hamburger sample (Karatzas and Zarkadas, 1988).

(*iii*) *Determination of the Connective Tissue Proteins.* In the present study, an attempt was also made to relate the amounts of protein-bound Lys(5-OH), which occurs exclusively in collagen and collagen-like proteins (Porter and Reid, 1978; Anglister et al., 1976), to the contents of total connective tissue proteins in composite meats. In this chemical approach the distribution of collagenous fibers in composite meats could be calculated from the amounts of Lys(5-OH) found in the acid hydrolysates of these products. Since types I and III skeletal muscle collagens accounted, respectively, for 61 and 35% of the recovered collagen in the muscle connective tissue in epimysium, perimysium, and endomysium, while type IV collagen accounted for the remaining 5% (Light and Champion, 1984; Light et al., 1985; Light, 1985), a mean for the diastereoisomers of Lys(5-OH) content of n'_i = 10.0 residues per 1000 total amino acid residues in muscle collagen could be computed from the relative distribution of collagen types; their respective Lys(5-OH) contents are presented in Table IX, using eq 7 (Table VIII). The average residue weight (WE) for collagen is 91.1, and each of the diastereoisomers of Lys(5-OH) has an anhydrous M_r of 145.18.

Significant variations in collagen content were found in the extended meats and all-beef wiener types of cured sausages and among the standard and extended hamburger samples evaluated. The results, summarized in Table VIII, show that the amount of collagen in the extended hamburger samples ranged from 6.9 to 10.2% and in the wiener samples from 4.9 to 19.0% of the total protein. High collagen values were found in wiener samples both with condiments (11.0%) and without 19.0%), compared to the reference hamburger sample (11.0%). Thus, the higher collagen content of these products (Table VIII), compared to the average collagen value of 4.2% calculated for skeletal muscles (Bendall, 1967; Dransfield, 1977), may be attributed to the inclusion of tougher meat cuts, which are high in connective tissue proteins. These results correspond closely to those reported by Terrell (1982) for beef plate and cow meat but are much higher than the

Table IX. Average Values for 5-Hydroxylysine and 4-Hydroxyproline Computed from the Known Distribution of Types I, III, and IV Collagens in Skeletal Muscle[a]

collagen type standard	chain assoc	av no. of residues/1000 total residues[a] Pro(4-OH)	Lys(5-OH)	collagen type distrib as % of total muscle collagen[b]	av no. of residues contrib by collagen type/1000 residues Pro(4-OH)	Lys(5-OH)
I	$[\alpha 1(I)]_2\alpha 2(I)$	98	10	61.5	60.3	6.2
III	$[\alpha 1(III)]_3$	118	5	33.5	39.5	1.7
IV	$[\alpha 1(IV)]_2\alpha 2(IV)$	120	42	5.0	6.0	2.1
$\sum_{i=1}^{3} n'_i$					105.8	10.0

[a] Average values computed from the data of Miller and Gay (1982), Laurent et al. (1981), and Light (1985). [b] Data taken from Light and Champion (1984) and Light et al. (1985). The average mean residue weight for muscle collagen and connective tissue was calculated according to the method of Horstmann (1979), as described previously (see also Table V; Zarkadas et al., 1988c).

$$\sum_{i=1}^{3} \text{WE muscle collagen} = \frac{[(0.615 \times 91.14) + (0.335 \times 90.90) + (0.05 \times 95.94)]}{100} = 91.30 \text{ g/mol}$$

$$\sum_{i=1}^{3} \text{WE muscle connective tissue} = \frac{[(0.0466 \times 85.06) + (0.9534 \times 91.10)]}{100} = 90.8 \text{ g/mol}$$

calculated average collagen value of 4.2% (which ranged from 1.2 to 15.1%) reported for 34 different bovine skeletal muscles (Bendall, 1967; Dransfield, 1977; Light et al., 1985).

For purposes of comparison of the products in this study, the average value of 4.2% collagen has been used to calculate the added collagen in the products. This figure was used until an accepted upper limit for collagen in meats, poultry, and their products has been agreed upon. Mean values for total collagen ranged from 4.1 to 19.0% in the meat products evaluated. If the amount of collagen normally associated with skeletal muscle tissues is subtracted from the total collagen found in composite meats, the difference is an accurate assessment of the nonmuscle collagen being added to these products. For example, all-beef wieners with and without condiments contained 19.0% total collagen, as estimated from the amount of Lys(5-OH) found (Table VII) in its acid hydrolysate, of which 16.7% was added as nonmuscle collagen to this product. Values for collagen being added to all-beef mixed hamburger and wieners with and without condiments were high and ranged from 46.9 to 167.8 g/kg of total protein. The mixed-meat wiener samples contained the lowest levels of collagen of all of the products analyzed (Table VIII).

(*iv*) *Contents of Nonmuscle Proteins.* The data presented in Table VIII show that when the sum of the intracellular and extracellular muscle protein content is subtracted from the total protein of a given meat product, the difference represents an accurate assessment of the nonmuscle protein additives and ingredients present. All-beef extended hamburger samples, H_{ext}-2 and H_{ext}-3, contained 11.0 and 35.0% nonmuscle protein additives of the total proteins, respectively, compared to only 2.4% found in the reference hamburger sample (H_{std}-1). Similarly, the nonmuscle proteins in mixed-meat wiener blends ranged from 54.9 to 67.2% compared to 21.6 and 41.4% found in all-beef wieners with and without condiments, respectively. Sample W_{ext}-4 was also found to contain 8.7% additional connective tissue collagen, as estimated from the amount of Lys(5-OH) compared to sample W_{ext}-2 or W_{ext}-3. The largest amount of added collagen was found in the all-beef wiener samples W(+C) and W(−C), which represented 9.3 and 16.8% collagen, respectively.

From the foregoing results, it may be concluded that the direct method of analysis described in this paper for determining the content of myosin, actin, collagen, and total connective tissue proteins in muscle tissues and composite meats from the amounts of His(τ-Me) and Lys(5-OH) found, respectively, should be especially valuable for assessing the overall protein quality of these products. The data presented in this paper show that the commercially prepared composite meats selected for this survey varied in their myofibrillar (17.4–52.3%), connective tissue (4.1–19%), and nonmuscle protein contents (2.4–67.2%). Each of these composite meat blends has a characteristic amino acid profile, reflecting the amounts of meat cuts and nonmeat plant or animal additives used to formulate them.

LITERATURE CITED

Agater, I. B.; Briant, K. J.; Llewellyn, J. W.; Sawyer, R.; Bailey, F. J.; Hitchcock, C. H. The Determination of Soy and Meat Protein in Raw and Processed Meat Products by Specific Peptide Analysis. An Evaluation. *J. Sci. Food Agric.* **1986**, *37*, 317–331.

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

Anglister, L.; Rogozinski, S.; Silman, I. Detection of 4-Hydroxyproline in Preparations of Acetylcholinesterase from the Electric Organ of the Electric Eel. *FEBS Lett.* **1976**, *69*, 129–132.

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

Ashworth, R. B. Amino Acid Analysis for Meat Protein Evaluation. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 81–85.

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

Benedict, R. C. Determination of Nitrogen and Protein Content of Meat and Meat Products. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 69–74.

Bentley, J. P.; Hanson, A. N. The Hydroxyproline of Elastin. *Biochim. Biophys. Acta* **1969**, *175*, 339–344.

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

Berkowitz, D. B.; Webert, D. W. Determination of Soy in Meat. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 85–90.

Block, R. J.; Mitchell, H. H. The Correlation of the Amino Acid Composition of Proteins with their Nutritive Value. *Nutr. Abstr. Rev.* **1946**, *16*, 249–278.

Carnegie, P. R.; Hee, K. P.; Bell, A. W. Ophidine (β-analyl-L-N^{τ}-methylhistidine, "Balenine") and Other Histidine Dipeptides in Pig Muscles and Tinned Hams. *J. Sci. Food Agric.* **1982**, *33*, 795–801.

Carnegie, P. R.; Ilic, M. Z.; Etheridge, M. O.; Collins, M. G. Improved High-performance Liquid Chromatographic Method for Analysis of Histidine Dipeptides Anserine, Carnosine and Balenine Present in Fresh Meat. *J. Chromatogr.* **1983**, *261*, 153–157.

Carnegie, P. R.; Collins, M. G.; Ilic, M. Z. Use of Histidine Dipeptides to Estimate the Proportion of Pig Meat in Processed Meats. *Meat Sci.* **1984**, *10*, 145–154.

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

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.

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*; Ramachadran, G. N., Ed.; Academic Press: New York, 1967; Vol. I, pp 1–72.

Ellis, R. L. Chemical Analysis of Meat Products. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 77–80.

Elzinga, M.; Collins, J. H.; Kuehl, W. M.; Adelstein, R. S. Complete Amino Acid Sequence of Actin of Rabbit Skeletal Muscle. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 2687–2691.

FAO/WHO. *Protein Requirements*; Technical Report Series FAO 37 and WHO 301; Food and Agricultural Organization/World Health Organization: Rome, 1965.

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

Fincher, G. B.; Stone, B. A.; Clarke, A. E. Arabinogalactan Proteins: Structure, Biosynthesis, and Function. *Annu. Rev. Plant Physiol.* **1983**, *34*, 47–70.

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

Hanson, J.; Huxley, H. E. Quantitative Studies on the Structure of Cross-striated Myofibrils. II. Investigations by Biochemical Techniques. *Biochim. Biophys. Acta* **1957**, *23*, 250–260.

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

Harris, C. I.; Milne, G. The Identification of the N^{τ}-Methylhistidine-Containing Dipeptide, Balenine, in Muscle Extracts from Various Mammals and the Chicken. *Comp. Biochem. Physiol.* **1987**, *86B*, 273–279.

Heidelbaugh, N. D.; Huber, C. S.; Bednarczyk, J. F.; Smith, M. C.; Rambaut, P. C.; Wheeler, H. O. Comparison of Three Methods for Calculating Protein Content of Foods. *J. Agric. Food Chem.* **1975**, *23*, 611–613.

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

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.

Karatzas, C. N.; Zarkadas, C. G. Determination of the Myofibrillar and Connective Tissue Protein Contents and Amino Acid Composition of Selected Composite Meat Products. *J. Agric. Food Chem.* **1988**, *36*, 1109–1121.

Kivirikko, K. I. Hydroxyproline-Containing Fractions in Normal and Cortisone-Treated Chick Embryos. *Acta Physiol. Scand.* **1963**, *60* (*Suppl. 219*), 1–92.

Kohen, R.; Yamamoto, Y.; Cundy, K. C.; Ames, B. N. Antioxidant Activity of Carnosine, Homocarnosines and Anserine Present in Muscle and Brain. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 3175–3179.

Lamport, D. T. A. Structure, Biosynthesis and Significance of Cell Wall Glycoproteins. In *Recent Advances in Phytochemistry*; Loewus, F. A., Runeckless, V. C., Eds.; Plenum Press: New York, 1977; Vol. II, pp 79–115.

Laurent, G. J.; Sparrow, M. P.; Bates, P. C.; Millward, D. J. Turnover of Muscle Protein in the Fowl. Collagen Content and Turnover in Cardiac and Skeletal Muscles of the Adult Fowl and the Changes During Stretch-Induced Growth. *Biochem. J.* **1978**, *176*, 419–427.

Laurent, G. J.; Cockerell, P.; McAnulty, R. J.; Hastings, J. R. B. A Simplified Method for Quantitation of the Relative Amounts of Type I and Type III Collagen in Small Tissue Samples. *Anal. Biochem.* **1981**, *113*, 301–312.

Lee, Y. B.; Elliott, 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. *Collagen in Skin: Preparation and Analysis*; Skerow, D., Skerow, C. J., Eds.; Methods in Skin Research; Wiley: New York, 1985.

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

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

Maita, T.; Hayashida, M.; Tanioka, Y.; Komine, Y.; Matsuda, G. The Primary Structure of Myosin Head. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 416–420.

McCollester, D. L. A Method for Isolating Skeletal Muscle Cell-Membrane Components. *Biochim. Biophys. Acta* **1962**, *57*, 427–437.

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

McNeal, J. E. Rapid Methods for Determination of Meat Composition. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 95–100.

McNeil, M.; Darville, A. G.; Fry, S. C.; Albersheim, P. Structure and Function of the Primary Cell Walls of Plants. *Annu. Rev. Biochem.* **1984**, *53*, 625–663.

Miller, E. J.; Gay, S. Collagen: An Overview. *Methods Enzymol.* **1982**, *82*, 3–32.

Moore, S. On the Determination of Cystine as Cysteic Acid. *J. Biol. Chem.* **1963**, *238*, 235–237.

Morries, P. A Century of Kjeldahl (1883–1983). *J. Assoc. Public Anal.* **1983**, *21*, 53–58.

Murakami, U.; Uchida, K. Contents of Myofibrillar Proteins in Cardiac, Skeletal, and Smooth Muscles. *J. Biochem.* **1985**, *98*, 187–197.

Nguyen, Q.; Zarkadas, C. G. A 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. A.; 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*, 565–572.

Olsman, W. J.; Slump, P. *Methods of Determination of Connective Tissue Free Muscle Protein in Meat Products*; Lawrie, R. L., Ed.; Developments in Meat Science; Elsevier: New York, 1981; Vol. 2, pp 192–240.

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

Pearson, D. The Examination of Meat Products with Special Reference to the Assessment of the Meat Content. A Review. *Analyst* **1975**, *100*, 73–81.

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.

Porter, P. R.; Reid, K. B. M. The Biochemistry of Complement. *Nature* **1978**, *275*, 699–704.

Poulter, N. H.; Lawrie, R. A. The Practical Application of 3-Methylhistidine in Determining the Lean Meat Content of Food Products. *Meat Sci.* **1980**, *4*, 21–31.

Rangeley, W. R. D.; Lawrie, R. A. II. Methylamino Acids as Indices in Meat Products. Further Examination of Protein Sources and the Practical Application of Methylamino Acid Titres in Predicting Meat Content. *J. Food Technol.* **1977**, *12*, 9–26.

Ranken, M. D. *Composition of Meat: Some Structural and Analytical Implications*; Hudson, B. J. F., Ed.; Developments

in Food Proteins; Elsevier Applied Science Publishers: New York, 1984; Vol. 3, pp 1–32.

Richardson, M.; Posati, L. P.; Anderson, B. A. *Composition of Foods*; USDA Handbook 8-7; U.S. Department of Agriculture: Washington, DC, 1980.

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

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

Smith, J. J.; Muldoon, E. P.; Willard, J. J.; Lamport, D. T. A. Tomato Extensin Precursors P1 and P2 are Highly Periodic Structures. *Phytochemistry* **1986**, *25*, 1021–1030.

Stuart, D. A.; Varner, J. E. Purification and Characterization of a Salt Extractable Hydroxyproline-Rich Glycoprotein from Aerated Carrot Discs. *Plant Physiol.* **1980**, *66*, 787–792.

Szent-Gyorgyi, A. G.; Mazia, D.; Szent-Gyorgyi, A. On the Nature of the Cross-Striation of Body Muscle. *Biochim. Biophys. Acta* **1955**, *16*, 339–342.

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

Tristram, G. R.; Smith, R. H. *The Amino Acid Composition of Some Purified Proteins*; Anfinsen, C. B., Anson, M. L., Edsall, J. T., Eds.; Advances in Protein Chemistry 18; Academic Press: New York, 1963; pp 227–318.

Woessner, J. F. The Determination of Hydroxyproline in Tissue and Protein Samples Containing Small Proportions of this Amino Acid. *Arch. Biochem. Biophys.* **1961**, *93*, 440–447.

Yates, L. D.; Greaser, M. L. Quantitative Determination of Myosin and Actin in Rabbit Skeletal Muscle. *J. Mol. Biol.* **1983**, *168*, 123–141.

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.

Zarkadas, C. G. A Simple Chromatographic Method for the Determination of the Methylated Basic Amino Acids in Proteins. *Can. J. Biochem.* **1975**, *53*, 96–101.

Zarkadas, C. G. A Rapid Chromatographic Method for the Determination of ω-N-Methylarginines in Protein and Muscle Tissues. *Can. J. Biochem.* **1978**, *56*, 952–957.

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

Zarkadas, C. G. *The Determination of the Myofibrillar and Connective Tissue Contents of Meats and Composite Meat Products*; Proceedings of the 27th European Fleischforscher-Kongress II, Vienna, Austria, 1981; Vol. 27, pp 512–575.

Zarkadas, C. G.; Zarkadas, G. C.; 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. N.; Kanizadeh, 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 Proteins and Tissues. *Anal. Biochem.* **1987b**, *160*, 251–266.

Zarkadas, C. G.; Drouliscos, N. J.; Karatzas, C. J. Comparison of the Total Protein, Nitrogen and Amino Acid Composition of Selected Additives and Ingredients Used in Composite Meat Products. *J. Agric. Food Chem.* **1988a**, *36*, 1131–1146.

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 Muscle. *J. Agric. Food Chem.* **1988b**, *36*, 1131–1146.

Zarkadas, C. G.; Meighen, E. A.; Zarkadas, G. C.; 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.* **1988c**, *36*, 1095–1109.

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Registry No. His(τ-Me), 332-80-9; Lys(5-OH), 6000-08-4; Pro(4-OH), 51-35-4; Pro, 147-85-3; Gly, 56-40-6; ammonia, 7664-41-7.