Hydroxylysine, Elastin Crosslinks, Other Amino Acids and the Amino Sugars in Proteins and Tissues". *Anal. Biochem.* **1987b**, *160*, 251–266.

Zarkadas, C. G.; Meighen, E. A.; Zarkadas, G. C.; Karatzas, C. N.; Rochemont, J. A.; Berthelet, M. "The Determination of the Myofibrillar and Connective Tissue Proteins in the Bovine Diaphragm". *J. Agric. Food Chem.* **1988a**, preceding paper in this issue.

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Comparison of the Total Protein, Nitrogen, and Amino Acid Composition of Selected Additives and Ingredients Used in Composite Meat Products[1]

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To validate the possible use of N^{τ}-methylhistidine, desmosine, and 5-hydroxylysine as markers for assessing, respectively, the myofibrillar and connective tissue contents of composite meats, 16 typical nonmeat protein additives and ingredients used to formulate such products were subjected to detailed amino acid analyses. It was found that these products contained no N^{τ}-methylhistidine, 5-hydroxylysine, or desmosine, suggesting that their quantitation in meat hydrolysates could be used to evaluate protein quality in composite meats. By contrast, the presence of 4-hydroxyproline in these products suggests that the use of 4-hydroxyproline as an index of total connective tissue proteins in composite meats is limited. The least variability in tissue amino acid content was found when the data were expressed on a protein-, fat-, and ash-free basis. A comparison between the Kjeldahl vs amino acid methods for protein quantitation showed that by far the most accurate, sensitive, and least variable method is the summation of the weights of individual amino acid residues present in each product, as determined by detailed amino acid analysis.

Previous work from this laboratory (Zarkadas, 1981; Karatzas and Zarkadas, 1988) showed that an accurate assessment of the protein quality of composite meats can be based on the determination of their myofibrillar and connective tissue protein contents, since the contribution of these classes of proteins to the overall nutritive value of meats differs considerably. In this proposed chemical approach the myofibrillar myosin and actin contents of muscles and prepared composite meats can be determined from the amounts of N^{τ}-methylhistidine [His(τ-Me)] found in their acid hydrolysates. Collagen and collagen-like proteins (Anglister et al., 1976; Porter and Reid, 1978) of the extracellular matrix can be calculated from the amounts of 5-hydroxylsine [Lys(5-OH)] present and the elastin content from the amounts of Des found (Zardadas et al., 1986, 1987b; Nguyen et al., 1986). Therefore, when the sum of the muscle intracellular myofibrillar and other muscle soluble proteins and the extracellular matrix connective tissue proteins is subtracted from the total protein of a composite meat hydrolysate sample, the difference is an accurate assessment of the nonmuscle protein additives and ingredients used in meat products.

This quantitation is based on three concepts: first, that 1 mol of actin contains 1 mol of His(τ-Me) and that 1 mol of myosin contains 2 mol of His(τ-Me) (Elzinga et al., 1973; Vandekerckhove and Weber, 1978; 1979; Elzinga and Collins, 1977; Maita et al., 1987); second, that skeletal muscle collagens have a calculated average Lys(5-OH) content of 10.0 residues/1000 total amino acid residues (Light and Champion, 1984; Light et al., 1985) while muscle elastin contains 3.0 residues of desmosine/1000 residues (Foster, 1982)f third, that His(τ-Me), Lys(5-OH), and desmosine (Des) are absent from all other muscle and nonmuscle proteins [reviewed by Huszar (1984), Olsmand and Slump (1981), Ranken (1984), and McNeal (1987)]. Although numerous studies have described the distribution and occurrence of His(τ-Me) in vertebrate muscle tissues from several species (Haverberg et al., 1975; Hancock and Harding, 1982, 1984; Asatoor and Armstrong, 1967) and in various composite meat products (Rangeley and Lawrie, 1977; Poulter and Lawrie, 1980; Olsman and Slump, 1981; Jones et al., 1985, 1987), there are limited data on the content of these unique basic amono acids in nonmeat ingredients and additives used to formulate such products.

Formulations usually include a number of nonmuscle animal and plant protein additives to enhance texture and reduce cost (Terrell, 1982; Rust, 1982), such as milk and egg powders, gelatin, soybean, and other types of oilseed protein products, wheat gluten and other cereal grain binders and fillers, etc., and cheaper meat cuts, which are frequently high in connective tissue. The actual levels of meat binders or fillers being used in such processed meats vary, depending upon the cost and nature of such nonmuscle protein form modifiers available to the processor.

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As a result, the protein quality and nutritive composition of such composite meat products are highly variable and this has become the subject of major interest and concern to both the consumer and manufacturer as well as the regulatory agencies concerned with the development of standards for labeling prepackaged meats (Benedict, 1987; Ellis, 1987). An accurate assessment of the levels of these protein form modifiers and nutritional composition of composite meats is therefore essential.

The purpose of the present study was to show whether or not these unique amino acids, reported to occur exclusively in the myofibrillar myosin and actin, and connective tissue collagen and elastin, are in fact absent from all nonmeat animal and plant-derived protein additives and ingredients for use in meat products. The amino acid composition of two typical sensory enhancers normally used industrially in the preparation of composite meat products, as well as other protein supplements for use in experimental diets, were also included in this study. The content of total protein in each of these nonmeat protein additives and ingredients was determined from their detailed amino acid compositions.

EXPERIMENTAL SECTION

Materials. Types DC-4A (Lot No. 750) and DC-5A (Lot No. 746) cation-exchange spherical resins, sized to 9.0 ± 0.5 and 6.0 ± 0.5 μm, respectively, were purchased from Dionex Chemical Co., Sunnyvale, CA. The unusual amino acid standards were obtained as follows: diastereoisomer mixture of 5-hydroxy-DL-lysine, N^6-methyl-L-lysine, N^6,N^6-dimethyl-L- and N^6,N^6,N^6-trimethyl-L-lysine bis(*p*-hydroxyazobenzenesulfonate) hydrate, N^τ-methyl-L-histidine, N^π-methyl-L-histidine hydrate, D-glucosamine hydrochloride, D-galactosamine hydrochloride, and 4-hydroxyproline from Calbiochem-Behring Corp., La Jolla, CA; DL-ornithine (5-aminonorvaline) from Schwartz/Mann, Orangeburg, NY; norleucine from Pierce Chemical Co., Rockford, IL; 3-nitro-L-tyrosine from Aldrich Chemical Co., Milwaukee, WI. The standard amino acid calibration mixture was purchased from Beckman Instruments, Inc., Palo Alto, CA. Octanoic acid was obtained from Eastman Kodak Co., Rochester, NY, and phenol was a product of J. T. Baker Chemical Co., Phillipsburg, NJ. All other chemicals and reagents were of the highest purity commercially available and were used without further purification.

Methods. *Sampling and Preparation of Nonmeat Additives and Ingredients for Analysis.* The four commercially available oilseed products used in this study, which include textured soybean flour Promate made by thermoplastic extrusion (Mounts, 1982), soybean protein concentrates produced by aqueous ethanol solvent extraction, and soybean protein isolates made from undenatured defatted flakes by dilute alkali extraction, were obtained from manufacturer I located in eastern Canada (Toronto, Ontario) while mustard seed full-fat flour was supplied by manufacturer II also located in eastern Canada (Hull, Quebec). Two of the animal-derived form modifiers used in this survey include milk solid nonfat powder (Brink and Lofgren, 1982) obtained from manufacturer II and egg white solids in powder form supplied by manufacturer III (Western Ontario). The cereal grain derived protein additives included two typical Hygrade flour binders, H-82 and H-93, and were provided by Hygrade-LaBelle Fermiere, Montreal, Quebec. The same manufacturer also generously provided two typical sensory enhancers for use in sausage products and include mixed nonmeat binders and flavorings and spices and spice extractive flavorings. Vital wheat gluten and biscrum flour were purchases from manufacturer II. Also included in this survey are commercially available protein supplements normally used as feed ingredients for experimental diets. These were originated from Athens, Greece, and were contributed by N.J.D. and include wheat gluten bran and gluten feed, potato protein, and alfalfa meal.

Proximate Composition. Standard methods from the AOAC (1980) were followed for the determination of moisture (sections 7.003 and 24.002) and total ash (sections 24.009 and 31.012). Petroleum ether extractable lipids were determined by the Goldfish method (sections 10.132 and 24.005) essentially as described by Crampton (1956). The total nitrogen content of nonmeat animal and plant additives and ingredients for use in meat products was determined by 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, 1980).

Procedures for Amino Acid Analyses. Amino acid analyses were carried out on either a conventional (Beckman model 120C) or on an updated and fully automated amino acid analyzer (equivalent to Beckman Model 121MB). The automated instrument was equipped with a Varian Vista 402 chromatographic data reduction system (Varian Instruments Group, Walnut Creek, CA) to amplify its sensitivity and to automate quantitation of amino acids at the picomole level.

Dried samples and/or lyophilized powders (0.1 g) were hydrolyzed in Pyrex test tubes (18 × 150 mm) under vacuum (below 10 μmHg) with 10 mL of triple-glass-distilled constant-boiling HCl (6.0 M) at 110 °C in duplicate for 24, 48, 72, and 96 h, respectively, with the usual precautions described by Moore and Stein (1963) and Blackburn (1978). The small amounts of insoluble materials formed during acid hydrolysis were removed by filtration (0.22-μm Millipore microfilters; Millipore Corp., Bedford, MA) and washed with the same acid (6.0 M HCl). Foaming of hydrolysates was suppressed during evacuation and filtration by the addition of 5–10 μL of octanoic acid. The clear filtrate and washings were combined, evaporated to dryness in a Rotary Evapo Mix (Buchler Instruments, Fort Lee, NJ) at 45 °C, and brought to volume (usually 5.0 mL) with 0.2 M sodium citrate buffer, pH 2.2. Norleucine or Tyr(NO_2), selected as the internal standards, were included in this step or prior to hydrolysis. The data reported for serine and threonine represent the average of values extrapolated to zero time of hydrolysis (Rees, 1946). Addition of phenol (10–15 μL) to the hydrolysates usually prevented chlorination of tyrosine (Sanger and Thompson, 1963). The values for valine, isoleucine, leucine, and phenylalanine are averages of data from 48, 72, and 96 h of hydrolysis (Blackburn, 1978).

4-Hydroxyproline [Pro(4-OH)] was determined separately from a concentrated 24-h hydrolysate (equivalent to 0.1 mg of protein/analysis) as described previously (Zarkadas et al., 1986). Recoveries of Pro(4-OH) were calculated relative to alanine. Tryptophan in nonmeat protein samples (0.1 g) was also determined separately after alkaline hydrolysis (Hugli and Moore, 1972) by an improved chromatographic procedure (Zarkadas et al., 1986) using Tyr(NO_2) as an internal standard. Methionine and cysteine were determined in separate samples (0.1 g) as their oxidation products by the performic acid procedure of Moore (1963) as described previously (Zarkadas et al., 1987b) using norleucine as the internal standard. Recoveries were calculated relative to alanine and leucine.

Analyses for the methylated basic amino acids, the diastereoisomers of Lys(5-OH), and related compounds were

carried out with concentrated 96-h hydrolysates (equivalent to 100–250 μg of protein/analysis) by the single-microcolumn (50 × 0.28 cm) system using Dionex DC-4A resin (Zarkadas et al., 1987b) so that peaks adequate for these components could be obtained. The unusual amino acid calibration standards employed for peak identification and standardization of the instrument were prepared essentially as described previously (Zarkadas, 1975, 1979), using Tyr(NO_2) as the internal standard (Zarkadas et al., 1987b). Recoveries of these unique basic amino acids were calculated on the basis of protein content of individual hydrolysates determined according to Horstmann (1979) as described previously (Zarkadas et al., 1988).

Determination of Total Protein. The content of total protein in each of these nonmeat protein products was determined by three different methods: first, the conventional Kjeldahl method (AOAC, 1980) and the multiplication of the nitrogen by 6.25; second, the multiplication of the 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; third, the protein mass of individual samples calculated by summation of the weights of individual amino acid residues of which each sample is composed as described by Horstmann (1979). For the quantitation of the protein contents in biological materials by Horstmann's (1979) method, the mean residue weight (WE, μg/nmol) and conversion factor F (μg/nmol) for determining the protein mass in each sample analyzed in the absence of tryptophan and cyst(e)ine were calculated as described previously (Nguyen et al., 1986; Karatzas and Zarkadas, 1988), by complete amino acid analysis. A conversion factor, F' (μg), was also calculated according to eq 1 but for determining protein mass in the absence of tryptophan, cyst(e)ine, proline, and/or Pro(4-OH) from

$$F' = \frac{\sum_{i=1}^{16}(a_i b_i)}{1 - [a_{Trp} + a_{Cys} + a_{Pro} + a_{Pro(4\text{-}OH)}]} \quad (1)$$

where a_i is the nmol fraction of an amino acid i found in the analyzed aliquot and b_i is the molecular weight of amino acid residue i (μg) as described by Horstmann (1979). These factors, F and F', can be used in all subsequent quantitation of a given biological sample and by the procedures described below:

Step 1: Hydrolysis of Proteins or Tissues. In order to hydrolyze dry materials for protein determination, the same procedure described above was used, except that a smaller sample (from 0.3 to 2 mg) was weighed into a small Pyrex test tube (10 × 70 mm), 500 μL of triple-glass-distilled constant boiling HCl (6.0 M) was added, and the tube was pulled, evacuated to below 10 μmHg, sealed, and hydrolyzed for 24 h at 110 °C as before.

Step 2: Removal of Ammonia. After hydrolysis, the tube was opened and cooled in liquid nitrogen (−170 °C); about 1.5 times of 10 M NaOH was added to the hydrolysate and mixed. The final pH should be about 10. The tube was positioned in a desiccator containing 1 M H_2SO_4 and evacuated to 100 mmHg. The diffusion of ammonia was allowed to proceed at room temperature for at least 5 h, or overnight. The hydrolysate was then acidified with a calculated volume of 6.0 M HCl, and the contents were dried under vacuum in a desiccator containing P_2O_5 and pellets of KOH.

Step 3: Determination of Ninhydrin Color. The dried hydrolysate from step 2 was dissolved in an appropriate volume of 0.2 M sodium citrate dilution buffer, pH 2.2, and made to volume (i.e., from 500 to 1.00 mL). The convenient final concentraton of total amino acids is about 1 nmol/μL, corresponding to about 0.1 μg of protein/μL.

The total amino acids are quantitated without fractionation by either the manual spectrophotometric ninhydrin procedure described by Hirs et al. (1956) or using the continuous flow system of a standard amino acid analyzers (e.g., Beckman Model 120C) or a fully automated amino acid analyzer (equivalent to Beckman Model 121MB) adopted from the procedure described by Horstman (1979). The instruments are quickly modified by connecting the sample injector valve between the outlet of the resin column and the ninhydrin line and detector through a Y mixer. The time-delay reaction coil was maintained at 100 °C in a boiling water bath or at 129 ± 0.1 °C in a heating oil bath as described previously (Zarkadas et al., 1987b). In the case of the Beckman Model 120C amino acid analyzer, using a 0.6 × 25 cm column at 65 °C, the flow of the starting buffer (pH 3.25) was adjusted to 35 μL/h. The ninhydrin flow rate was maintained at 25 mL/h. By this means, the buffer pressure waves caused by the Accu Flo Beckman pumps are minimized to zero before they arrive at the Y mixer.

When the Beckman Model 121MB amino acid analyzer was used, the previously described chromatographic conditions were employed (Zarkadas et al., 1987b). Aliquots from 20 to 100 μL of each hydrolysate containing from 50 pmol to 15 nmol of amino acids were injected into the running buffer stream through the Y mixer, using the sample loops of the sample injector valve. Injections were completed within 10–50 s and were repeated every 7 min.

The standard amino acid calibration mixtures employed for peak area calibration and standardization of the instrument were the same as those in the amino acid analyzers except that ammonia was removed as described in step 2 prior to analysis. The absorbance was measured at 570 nm. Standard curves were established by plotting the areas under the peaks versus the nanomoles of standard amino acids injected. Linear regression analysis of the results was carried out, and the nanomoles of amino acids of the sample was then computed directly from the observed areas.

Step 4: Calculation of the Protein Concentration. Horstmann (1979) has presented the considerations, procedures, and calculations for this method of protein quantitation. The amount of protein (P, μg) in each hydrolysate can then be calculated as

$$P = F' \sum_{i=1}^{16} X_i \quad (2)$$

where X_i are the nanomoles of each amino acid i found in the analyzed aliquot according to steps 1–3. Peterson (1983) introduced the reaction with *O*-phthalaldehyde, which is more sensitive than the ninhydrin reaction for the determination of absolute protein concentration in biological samples, but it has not yet been applied as a routine general laboratory procedure.

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 (Statistical Analysis System, 1982), using the computing center (VAX) at Datacrown, Inc., IBM, Toronto, Ontario.

RESULTS AND DISCUSSION

To ascertain whether the four unique basic amino acids proposed as markers for determining specific myofibrillar and connective tissue proteins in processed meats are ab-

Table I. Proximate Composition (Grams per Kilogram, Dry-Weight Basis) of Selected Nonmeat Additives and Ingredients and Protein Supplements Supplied by Five Different Manufacturers

	component				
		Kjeldahl			
nonmeat protein form modifiers	moisture	N	crude protein (N × 6.25)	lipid	ash
		Oilseed Derived			
mustard seed full-fat flour[c]	56.40 ± 0.37	55.20 ± 0.07	345.00 ± 0.47	271.10 ± 0.86	44.40 ± 0.04
textured soybean flour, Promate[b]	53.20 ± 0.13	83.30 ± 0.95	520.63 ± 5.99	3.80 ± 0.11	79.10 ± 0.38
soybean protein[b] concentrate	59.80 ± 0.70	107.60 ± 0.67	672.50 ± 4.19	3.70 ± 0.04	39.50 ± 0.08
soybean protein[b] isolate	55.20 ± 1.31	140.00 ± 0.28	875.00 ± 1.77	3.70 ± 0.15	45.60 ± 0.54
		Cereal Grain Derived			
biscrum flour[b]	86.40 ± 0.92	24.00 ± 0.03	150.00 ± 0.18	6.03 ± 0.05	5.20 ± 0.14
vital wheat gluten[b]	57.15 ± 0.20	133.4 ± 0.55	833.75 ± 3.43	16.90 ± 0.72	3.10 ± 0.04
		Animal Derived			
milk solid nonfat powder[b]	29.00 ± 0.26	58.00 ± 0.02	362.50 ± 0.18	3.30 ± 0.06	83.00 ± 0.09
egg white solids[d]	65.19 ± 0.26	136.90 ± 0.16	855.63 ± 1.01	143.80 ± 1.08	56.70 ± 0.68

[a] Mean values and standard error of measurements for 12 determinations. [b] Manufacturer I. [c] Manufacturer II. [d] Manufacturer III.

Table II. Comparison of Total Protein, Nitrogen, and Conversion Factors for Calculating Protein Content from Kjeldahl Nitrogen of Selected Nonmeat Additives and Ingredients

	nitrogen content,[a] g N/100 g DWB			conversion[a] factor	total protein content, g protein/100 g DWB		total
nonmeat protein product	Kjeldahl (A)	sum of amino acid (B)	% diff, (A − B)/ A × 100	calcd from amino acid content	Kjeldahl N[a]	amino acid compn[b]	essential amino acids, mg/g N
			Oilseed Derived				
soybean							
textured flour, Promate	8.33	8.78	5.40	5.56	46.31	48.81 ± 1.92	2771.8
concentrate	10.76	10.56	1.86	5.64	59.83	59.57 ± 2.74	2821.8
isolate	14.00	13.45	3.92	5.67	79.38	76.24 ± 3.63	2789.6
mustard seed full-fat flour	5.52	4.23	23.37	5.07	27.99	21.47 ± 1.72	2384.7
			Cereal Grain Derived				
biscrum flour	2.40	1.97	17.92	5.71	13.70	11.27 ± 0.82	2103.8
vital wheat gluten	13.34	10.42	21.89	5.65	74.17	58.94 ± 2.23	2122.9
Hygrade flour H-82	3.27	3.39	3.54	5.42	17.72	3.68 ± 0.28	2437.9
Hygrade flour H-93	7.15	6.33	11.47	5.71	40.83	7.23 ± 0.49	2640.1
			Sensory Enhancers				
mixed: H-190 nonmeat binders and flavorings	0.17	0.197	15.88	5.10	0.87	0.48 ± 0.003	2355.7
flavorings: H-64 spices and spice extractives	2.23	2.18	2.24	5.46	12.18	2.38 ± 0.09	2119.
			Animal Derived				
milk solid nonfat powder	5.80	4.02	30.69	5.91	34.28	23.71 ± 0.28	2858.9
egg white solids	13.69	12.60	7.96	5.96	81.59	75.13 ± 1.72	3287.3
			Protein Supplements				
gluten bran	3.67	2.98	18.80	5.71	20.96	17.03 ± 0.90	2713.3
gluten feed	3.80	2.40	36.84	6.02	22.88	14.46 ± 0.18	2657.9
potato protein	9.97	8.04	19.36	5.94	59.22	47.75 ± 2.73	3115.9
alfalfa meal	2.76	2.17	21.38	5.76	15.90	12.52 ± 0.74	2757.3

[a] Calculated according to Heidelbaugh et al. (1975). [b] Calculated according to Horstmann (1979). Key: DWB, dry-weight basis.

sent from all of the nonmeat protein additives and ingredients used extensively today for processing (Ono, 1982; Terrell, 1982), accurate and detailed amino acid determinations were carried out in selected nonmeat protein additives and ingredients available to the processor. Samples of typical oilseed, cereal grain, and animal-derived nonmuscle protein additives and ingredients presently utilized in processed meats were selected from four major Canadian and one Greek manufacturer and subjected to proximate and complete amino acid analyses. All determinations were carried out by the single-column chromatographic methods developed in this laboratory for this purpose (Zarkadas et al., 1986, 1987b). The amino acid composition of two nonmeat sensory enhancers normally used industrially in the preparation of composite meat products and four typical protein supplements used in experimental diets were also included in this survey.

Proximate Composition. The average proximate compositions of representative commercial nonmeat additives and ingredients prepared by various separation and extraction processes (Wolf, 1982; Brink and Lofgren, 1982) are summarized in Tables I and II. The concentrations of Kjeldahl nitrogen, crude protein, moisture, fat, and ash are given on a dry-weight basis (DWB). The results obtained on the crude protein contents of the three edible soybean products (Table I) are in accord with those reported by Wolf (1982). The lipid profiles of textured soybean flour (Promate), concentrate, and isolates as presented in Table I appeared to be very similar. However, variation was found to be highly significant in the ash contents between the textured soybean flour Promate and the soybean concentrate and isolate. Mustard seed full-fat flour has a high content of lipid material (27.1%) and lower crude protein content (34–35%) compared to that reported for soybean full-fat flour (Wolf, 1982). Wheat (biscrum) flour appeared to be a poor source of protein, containing approximately 15.0% crude protein, compared to the high crude protein content (83.4%) found in vital wheat gluten

Table III. Comparison of Total Nitrogen and Amino Acid Composition (Grams of Amino Acid per Kilogram of Protein) of Selected Animal- and Plant-Derived Protein Additives and Ingredients from Two Different Commercial Sources (Manufacturers I-III) for Use in Meat Products

amino acid	milk solid nonfat powder (I): mean ± SEM[a]	cow's milk (FAO, 1965)	egg white solids (III): mean ± SEM[a]	hen's egg (FAO, 1965)	soybean products (I): mean ± SEM			mustard full-fat flour (II): mean ± SEM
					textured flour Promate	concentrate	isolate	
aspartic acid	75.89 ± 0.55		101.71 ± 0.97		113.35 ± 1.56	113.44 ± 0.62	114.09 ± 1.41	85.34 ± 0.63
threonine	40.26 ± 0.87	46	46.35 ± 0.41	51	35.17 ± 0.34	38.04 ± 0.68	34.38 ± 0.71	43.77 ± 2.22
serine	49.91 ± 1.20		64.97 ± 0.98		45.07 ± 1.06	49.38 ± 1.78	49.95 ± 0.84	48.31 ± 1.90
glutamic acid	213.08 ± 1.44		136.33 ± 1.78		183.34 ± 2.31	183.37 ± 0.74	197.01 ± 1.72	190.05 ± 3.10
proline	93.44 ± 0.98		30.80 ± 1.01		44.59 ± 0.99	47.85 ± 0.44	46.93 ± 0.80	60.33 ± 0.91
glycine	18.47 ± 0.32		29.15 ± 0.46		38.01 ± 0.32	36.29 ± 0.21	34.20 ± 0.31	53.56 ± 0.66
alanine	30.44 ± 0.97		53.38 ± 1.31		39.64 ± 0.43	39.21 ± 0.19	33.99 ± 0.53	38.33 ± 1.46
cysteine	6.10 ± 0.76	9	19.94 ± 0.33	24	8.08 ± 1.09	12.10 ± 0.09	11.85 ± 0.29	8.19 ± 0.10
valine	75.93 ± 1.04	69	68.46 ± 1.25	73	61.66 ± 0.78	63.18 ± 2.15	55.61 ± 0.67	57.25 ± 2.75
methionine	23.28 ± 1.78	24	37.73 ± 0.16	31	8.51 ± 1.15	15.39 ± 0.09	11.13 ± 0.27	6.11 ± 0.70
isoleucine	55.85 ± 1.97	64	53.05 ± 0.52	66	50.30 ± 0.77	53.86 ± 2.03	49.45 ± 0.40	47.15 ± 0.38
leucine	96.92 ± 1.25	99	83.88 ± 0.55	88	81.22 ± 0.87	81.68 ± 0.87	80.81 ± 0.89	80.45 ± 0.59
tyrosine	25.67 ± 1.60	41	40.85 ± 0.58	42	32.52 ± 0.78	34.01 ± 0.72	39.71 ± 0.93	31.85 ± 0.61
phenylalanine	47.23 ± 0.35	49	58.58 ± 1.16	58	54.43 ± 0.83	53.61 ± 0.42	56.39 ± 0.80	46.64 ± 0.32
histidine	34.65 ± 1.74		32.18 ± 0.40		37.59 ± 0.96	29.11 ± 0.38	31.64 ± 1.11	44.71 ± 1.11
lysine	78.12 ± 1.21	78	70.65 ± 1.23	64	76.65 ± 1.07	63.75 ± 0.69	64.70 ± 0.69	76.81 ± 2.20
arginine	34.76 ± 1.93		56.88 ± 0.78		77.48 ± 1.53	72.31 ± 0.71	77.14 ± 1.39	71.59 ± 1.71
tryptophan	nd	14	15.10[d]	16	12.40 ± 0.08	12.41 ± 0.08	11.02 ± 0.09	nd
ammonia	24.11 ± 0.26		14.18 ± 0.57		21.67 ± 2.85	23.56 ± 0.48	22.13 ± 0.33	39.80 ± 0.70
total AA N[b]	169.34		167.76		179.82	177.31	176.43	197.01
mean residue wt (WE),[c] μg/nmol	0.112235		0.111319		0.112570	0.112119	0.113410	0.109765
conversion factor F,[c] μg/nmol	0.112985		0.118427		0.114438	0.114482	0.115695	0.110730
conversion factor F',[d] μg/mol	0.126767		0.119168		0.120785	0.121454	0.122548	0.120119

[a] Means and standard error of measurements for eight determinations; nd = not determined. [b] Calculated according to Heidelbaugh et al. (1975). [c] The WE and F constants were calculated as described by Horstmann (1979). [d] The conversion factor F' was also calculated according to eq 1, except in the absence of tryptophan, cyst(e)ine, proline, and 4-hydroxyproline.

(Table I), but the results obtained are comparable to those reported previously for Hygrade flours, which ranged from 20.4 to 44.7% crude protein (Zarkadas et al., 1987a). Egg white solids had a much higher total crude protein (85.5g%) and lipid content (14.4%) than milk solid nonfat powder, and the results obtained are comparable to those listed in the FAO Handbook No. 37 (FAO/WHO, 1965).

Protein Determination. For purposes of comparison, the total protein contents of the selected nonmeat plant and animal protein additives and ingredients used for this survey were also calculated from their amino acid nitrogen (Tables III–V) as described by Heidelbaugh et al. (1975) for Skylab foods. These authors have recommended that whenever accurate data on the protein content of individual foods are required, conversion factors based on the actual amino acid nitrogen content should be used. Table II summarizes the total nitrogen content and protein conversion factors calculated from the actual amino acid composition of each of these products. Differences between Kjeldahl nitrogen and nitrogen determined by the summation of the amino acid nitrogen contents of individual samples (Table II) were noted among the nonmeat plant and animal protein products surveyed. Variations in percent differences as a function of method of nitrogen determination ranged from 1.86 to 5.40% in soybean protein products to 30.7% and 36.8% in milk solid nonfat powder and gluten feed, respectively. To correct for this variation, new conversion factors based on the amino acid nitrogen content were calculated, which are characteristic for each product and can be used in all subsequent quantitations for converting Kjeldahl nitrogen into protein content. Significant differences in protein conversion factors were found among the various products evaluated, ranging from 5.07 in mustard seed full-fat flour to 5.94 and 6.02 in potato protein and gluten feed, respectively (Table II). These results are in accord with the National Research Council's (1963) recommendation that the commonly used protein conversion factor of 6.25 is useful only for a comparison of the crude or conventional protein content of different foods.

The data presented in Tables I and II indicate that the protein content in each of the 16 nonmeat protein products evaluated by the conventional Kjeldahl nitrogen procedure (AOAC, 1980), using the new conversion factors recommended by Heidelbaugh et al. (1975) and by quantitative amino acid analysis (Horstmann, 1979), differed significantly. For example, the typical commercial edible soybean protein products used in this survey are conventionally classified in the recent literature (Wolf, 1982; Berkowitz and Webert, 1987) on the basis of their protein content:

	min protein, % dry basis
grits and flours	40–50
concentrates	70
isoelectric isolates	90

This has been confirmed in the present study (Table I). However, when the new conversion factors (Table II) were used for converting the Kjeldahl nitrogen to total protein (Heidebauch et al., 1975), a more accurate approximation of the protein content in each of these soybean protein products was obtained. Such differences in protein content were also noted among the other products evaluated. These data suggest that a substantial quantity of Kjeldahl nitrogen is apparently derived from other nonprotein nitrogenous compounds present in these products (Benedict, 1987) and that the use of the conventional Kjeldahl nitrogen conversion factor, 6.25, for assessing the total protein quality of foods is limited. However, the best estimate of the protein content in each of these nonmeat protein additives and ingredients was made by the sum-

Table IV. Comparison of the Total AA Nitrogen and Amino Acid Composition (Grams of Amino Acid per Kilogram of Total Protein) of Selected Sensory Enhancers and Cereal- and Grain-Derived Protein Modifiers from Two Different Commercial Sources

	Hygrade sensory enhancers: mean ± SEM[a]		nonmeat flour binders: mean ± SEM[a]			
			Hygrade flours		manufacturer I flours	
amino acid	mixed: nonmeat binders, flavorings (H-190)	flavorings: spices, spice extr (H-64)	H-82	H-93	biscrum flour	wheat gluten
aspartic acid	151.46 ± 0.62	45.32 ± 0.42	55.33 ± 1.84	49.50 ± 0.67	43.88 ± 0.82	29.37 ± 0.52
threonine	40.41 ± 0.03	26.03 ± 0.36	27.67 ± 0.71	31.59 ± 0.02	23.55 ± 2.60	23.35 ± 1.44
serine	39.20 ± 1.54	42.98 ± 1.41	47.16 ± 1.62	54.02 ± 0.75	36.15 ± 2.44	40.33 ± 1.36
glutamic acid	176.76 ± 1.17	311.01 ± 0.57	205.66 ± 2.46	159.14 ± 1.92	356.25 ± 2.77	361.15 ± 3.96
proline	54.18 ± 0.73	122.80 ± 0.90	135.16 ± 0.38	181.78 ± 0.02	108.22 ± 0.67	113.48 ± 1.03
glycine	44.46 ± 0.80	33.52 ± 0.24	40.01 ± 1.17	26.38 ± 1.75	36.83 ± 1.75	29.92 ± 0.59
alanine	44.98 ± 0.36	29.54 ± 0.33	35.29 ± 1.41	35.35 ± 0.35	32.19 ± 0.44	21.24 ± 0.39
cysteine	18.72 ± 0.41	20.34 ± 0.34	22.85 ± 0.39	25.39 ± 0.51	21.20 ± 1.17	23.29 ± 1.46
valine	46.14 ± 0.33	46.42 ± 0.30	58.07 ± 0.01	59.55 ± 0.46	71.34 ± 0.68	67.48 ± 2.28
methionine	12.16 ± 0.70	14.87 ± 0.76	16.97 ± 0.74	22.27 ± 0.74	13.56 ± 0.79	20.59 ± 1.29
isoleucine	36.78 ± 0.08	41.63 ± 0.30	46.46 ± 0.33	56.27 ± 0.69	35.56 ± 0.99	35.44 ± 0.14
leucine	70.53 ± 0.05	73.45 ± 0.46	84.29 ± 0.72	56.39 ± 0.85	65.76 ± 1.56	64.13 ± 0.51
tyrosine	37.16 ± 1.92	37.12 ± 1.37	40.02 ± 1.42	48.19 ± 1.74	17.23 ± 0.65	28.37 ± 1.25
phenylalanine	43.95 ± 0.07	55.96 ± 0.34	62.20 ± 0.83	80.49 ± 2.14	48.89 ± 2.14	52.76 ± 0.42
histidine	20.02 ± 0.68	24.55 ± 0.14	28.87 ± 0.02	30.18 ± 0.49	17.97 ± 2.75	29.06 ± 2.16
lysine	48.98 ± 0.36	23.57 ± 0.21	31.20 ± 0.21	25.32 ± 0.04	29.51 ± 0.97	26.17 ± 1.94
arginine	99.64 ± 1.23	44.21 ± 0.64	54.12 ± 0.92	51.32 ± 0.64	41.85 ± 2.14	33.88 ± 1.27
tryptophan	7.03 ± 0.12	4.64 ± 0.23	6.10 ± 0.10	5.54 ± 0.05	nd	nd
4-hydroxyproline	6.61 ± 0.12	0.96 ± 0.08	1.71 ± 0.01	0.82 ± 0.01	nd	nd
ammonia	40.30 ± 4.22	47.16 ± 2.32	40.38 ± 4.21	30.91 ± 3.12	38.19 ± 3.20	43.09 ± 0.95
total AA N[b]	195.91	183.20	184.56	175.12	175.21	176.86
mean residue wt (WE),[c] μg/nmol	0.111266	0.112198	0.109971	0.110283	0.111206	0.113040
conversion factors, F,[c] μg/nmol	0.114048	0.115066	0.113136	0.113746	0.113809	0.116001
conversion factors, F',[d] μg/nmol	0.122672	0.134816	0.134557	0.144671	0.130343	0.134195

[a] Means and standard error of measurements for eight determinations. [b] Calculated according to Heidelbaugh et al. (1975). [c] The WE and F constants were calculated as described by Horstmann (1979). [d] The conversion factor F' was also calculated according to eq 1 but in the absence of tryptophan, cyst(e)ine, proline, and 4-hydroxyproline.

Table V. Total Amino Acid Nitrogen and Amino Acid Composition of Protein Supplements Used as Ingredients for Experimental Diets

	mean ± SEM,[a] g amino acid/kg protein			
	gluten bran	gluten feed	potato protein	alfalfa meal
aspartic acid	83.01 ± 1.60	67.94 ± 0.63	111.69 ± 0.89	140.00 ± 1.23
threonine	42.93 ± 0.57	41.87 ± 0.30	52.34 ± 0.34	51.01 ± 0.30
serine	49.17 ± 0.62	46.24 ± 0.75	53.99 ± 0.57	47.51 ± 0.57
glutamic acid	172.31 ± 2.04	181.10 ± 1.39	110.13 ± 1.15	114.70 ± 2.14
proline	62.21 ± 0.47	103.00 ± 0.47	57.69 ± 0.75	50.19 ± 0.35
glycine	58.92 ± 0.31	46.99 ± 0.16	62.31 ± 1.26	51.90 ± 0.60
alanine	61.67 ± 0.42	72.34 ± 0.49	49.15 ± 0.82	62.53 ± 0.61
cysteine	12.72 ± 0.58	18.12 ± 0.70	14.90 ± 0.80	6.95 ± 0.50
valine	73.53 ± 0.55	61.69 ± 0.24	64.05 ± 1.04	69.43 ± 0.44
methionine	11.71 ± 0.82	10.65 ± 1.02	23.64 ± 0.58	10.10 ± 0.44
isoleucine	42.58 ± 0.60	35.59 ± 0.58	48.91 ± 0.51	53.80 ± 0.54
leucine	88.54 ± 0.65	106.24 ± 0.48	90.69 ± 0.73	89.35 ± 0.35
tyrosine	30.48 ± 0.41	32.62 ± 0.59	50.71 ± 1.23	30.49 ± 0.53
phenylalanine	47.78 ± 0.34	39.24 ± 0.30	55.89 ± 1.07	58.20 ± 0.58
histidine	37.65 ± 0.43	40.81 ± 1.46	20.62 ± 0.77	25.32 ± 0.51
lysine	46.67 ± 1.06	41.83 ± 0.83	71.03 ± 0.90	60.41 ± 0.69
arginine	78.11 ± 1.55	53.74 ± 1.23	52.45 ± 2.95	49.24 ± 0.98
4-hydroxyproline	nd	nd	9.86 ± 0.78	28.87 ± 1.97
ammonia	12.81 ± 13.38	8.73 ± 9.21	13.70 ± 14.97	21.02 ± 22.56
total AA N[b]	175.09	166.14	168.37	173.72
mean residue wt, WE,[c] μg	0.106976	0.106318	0.106848	0.106630
conversion factor, F,[c] μg	0.108407	0.108343	0.108524	0.107402
conversion factor, F',[c] μg	0.116499	0.122411	0.117182	0.117115

[a] Means and standard error of measurements (SEM) for eight determinations; nd = not determined. [b] Calculated according to Heidelbaugh et al. (1975). [c] The WE and F constants were calculated from the amino acid composition found in the hydrolysates according to Horstmann (1979).

mation of the weights of the amino acid residues of which each of these products are composed, as described by Horstmann (1979). The results summarized in Table II show that this method yields accurate estimates of the absolute amount of protein present among the products evaluated.

When comparisons of the essential amino acid profiles of these nonmeat protein products were made (Table II), as recommended by Pellet and Young (1984), the results indicated significant variations in their total essential amino acid contents. Mean values for total essential amino acids ranged from 2100 to 2850 mg/g of N in oilseed, cereal

grain, sensory enhancers, and supplements evaluated, compared to 3287.3 mg/g of N found in egg white solids and 3115.9 mg/g of N in potato protein supplements. The results are in agreement with those listed in the FAO Handbook No. 37 (FAO/WHO, 1965).

Amino Acid Composition. The results on the amino acid composition of 10 representative nonmuscle plant and animal protein additives and ingredients, two typical sensory enhancers, and four protein supplements selected for this survey are presented in Tables III–V. The features of the overall amino acid composition among these products show considerable variation. A comparison of different methods of expressing results (Eastoe, 1967) indicated that, within any given product, the least variability occurred when the data is expressed on a moisture-, fat-, and ash-free basis. Results have therefore been calculated as grams of amino acid residues (anhydro amino acid) per kilogram of total protein. This unit, introduced by Tristram and Smith (1963) for the calculation of the amino acid content of proteins or protein mixtures, allows comparisons to be made between the present results (Tables III–V) and those reported by others or those given in food compositional tables. Values for all determinations show a reproducibility of 100 ± 3% for all amino acids and better than 100 ± 2% for amino acids present in amounts greater than 3% in any given sample.

The amino acid profiles of the three edible soybean protein products evaluated in this survey, i.e., texture flour Promate, concentrate, and isolate (Table III), appeared to be very similar in composition. The acidic amino acids are present in substantially high quantities in the three soybean products analyzed and when taken together account for almost 29.5–31.1% of all residues. The amino acids with hydrophobic side chains account for a further 19.3%. The aromatic amino acids tyrosine and phenylalanine are present in approximately similar amounts. The soybean protein isolates, however, tend to have slightly lower amounts of threonine, glycine, alanine, valine, and isoleucine than the other two soybean products. Agreement between the mean values obtained in the present study with those published values that have appeared more recently (Wolf, 1982; Berkowitz and Webert, 1987) is good both in the amino acid composition as a whole and in many of the individual values.

Mustard full-fat seed flour, which is often being marketed as a sensory enhancer for meat processing, is rich in glutamic acid (19.0%), proline (6.0%), valine (8%), and the basic amino acids, which account for a further 19.3% of all residues (Table III). Aspartic acid accounts for 8.5% of the amino acid residues in mustard seed flour compared to 11.4% in soybean flours.

A summary of the amino acid composition of milk and egg white powders, which are often utilized as nonmeat animal protein additives in processed meats, is presented in Table III. Both of these products were found to contain high levels of aspartic and glutamic acids, leucine, and lysine, and overall amino acid profiles which distinguished them from all other oilseed and cereal grain derived proteins investigated. Although the data reported in Table III are in reasonable agreement with those reported by FAO/WHO (1965) and Sarwar (1984), some differences have been noted. The threonine and cystine contents in both products were lower than the corresponding cow's milk and hen's egg (Table III) quoted in Table III from FAO/WHO (1965). The tyrosine content of milk solid nonfat powder does not approach that of cow's milk. There is a lower content of the long-chain amino acids leucine and isoleucine in these two products compared to the reference pattern (FAO/WHO, 1965). Both lysine and tryptophan were found in approximately the same amount to those cited in the reference pattern. Methionine is present in substantially higher quantities in egg white solids than in hen's eggs (Table III). A comparison between the essential amino acid profiles (Table II) indicated that only the milk solid nonfat powder appeared to be lower in essential amino acids (2859 mg/g of N) than cow's milk (3200 mg/g of N).

Somewhat less information is available concerning the amino acid composition of the other two typical sensory enhancers used in the preparation of processed meats. The results for one of these Hygrade products, the mixed non-meat binders and flavorings H-190 (Table IV) are interesting as relating to the only mixed sensory enhancer studied. They do not suggest any significant departure from the general oilseed-derived protein product pattern, except for a rather higher lysine value. The most consistent and characteristic feature of the amino acid composition of the other flavoring enhancer sample containing spices and spice extractives (Table IV) is the very high glutamic acid and proline contents, which when taken together account for 43.3% of the total residues present. It is not known whether other potentiators or enhancers such as monosodium glutamate were added to this product in the process of its preparation. Comparable data on these two sensory enhancers with which to compare each of the amino acid values measured in this study appear to be unavailable.

The amino acid profiles of the cereal grain derived protein additives and ingredients and protein supplements such as gluten feed and gluten bran, as presented in Tables IV and V, show close similarities in composition. The following features between values for individual amino acids seem to be common to all cereal grain products. Glutamic acid is the most abundant amino acid and ranged from 15.9% in Hygrade H-93 flour to 36.1% in vital wheat gluten. Proline, the next most abundant amino acid, accounts for a further 10.8–18.2%, except in gluten bran flour where it accounts for 6.2%. The present mean values for total aromatic amino acids ranged from 66.1 to 81.1 g/kg of protein in wheat (biscrum) flour and gluten and from 102.2 g to 129.6 g/kg of protein in Hygrade flour fillers (Table IV). Differences in aspartic acid values were found among the cereal grain protein products evaluated. These results are in good agreement with those reported by Sarwar et al. (1983) for whole wheat flour.

Potato protein isolates and alfalfa leaf proteins are largely untapped protein resources of the farm of great economic potential, which could be used in both human and animal diets. The use of potato and alfalfa proteins as feed supplements for experimental animals and possibly as food additives in various meat products for human consumption hold promise, but it will depend mainly upon the nutritional value and protein quality of these products and on the economics of the processes required for their preparation. Potato protein contained significant amounts of all amino acids commonly found in proteins with the exception of cyst(e)ine and possibly isoleucine and leucine (Table V). A comparison of the essential amino acid profile of potato protein given in Table II (3115 mg/g of N) with the essential amino acid profiles of the whole egg indicated that this protein supplement was only slightly lower than either hen's whole egg (3215 mg/g of N) or cow's milk (3200 mg/g of N) proteins (FAO/WHO, 1965). Moreover, when comparisons of the essential amino acid profile of alfalfa (2752 mg/g of N) were made with other proteins (Table II), it was found that alfalfa leaf protein was higher

Table VI. Unique Basic Amino Acid Contents and Unknown Compound No. 17 in Hydrolysates (96 h) of Nonmeat Protein Additives and Ingredients[a]

nonmeat protein form modifiers	mean ± SEM,[b] nmol/mg protein (N = 4)				
	Orn	Lys(Me)+	$Lys(Me_2)$	$Lys(Me_3)$	17
Oilseed Derived					
mustard seed full-fat flour	2.78 ± 0.63		1.57 ± 0.04	7.37 ± 0.61	43.52 ± 2.31
textured soybean flour, Promate	3.43 ± 0.09		1.15 ± 0.01	1.11 ± 0.03	25.92 ± 1.11
soybean protein concentrate	0.83 ± 0.01		tr	tr	12.63 ± 0.94
soybean protein isolate	1.66 ± 0.03		1.05 ± 0.03	1.54 ± 0.01	6.52 ± 0.33
Cereal Grain Derived					
biscrum flour	tr		tr		
vital wheat gluten	1.81 ± 0.02	tr	1.75 ± 0.04		9.12 ± 0.30
Hygrade flour H-82	8.03 ± 0.94				22.09 ± 1.47
Hygrade flour H-93	5.35 ± 0.77				15.26 ± 0.81
Animal Derived					
milk solid nonfat powder	5.32 ± 0.49		tr		tr
egg white solids	2.01 ± 0.09				4.59 ± 0.63
Sensory Enhancers					
mixed: nonmeat binders, flavorings (H-190)	7.80 ± 1.00				18.16 ± 0.68
flavorings: spices, spice extr (H-64)	9.88 ± 1.07				35.45 ± 1.80
Protein Supplements					
gluten bran	6.07 ± 1.01	0.19 ± 0.01	0.91 ± 0.81	2.55 ± 0.21	16.30 ± 0.48
gluten feed	8.04 ± 0.31	0.35 ± 0.01		0.87 ± 0.01	0.43 ± 0.01
potato protein	7.63 ± 0.74		0.56 ± 0.01	0.80 ± 0.11	10.32 ± 0.11
alfalfa meal	5.63 ± 1.21	0.18 ± 0.02		0.65 ± 0.12	7.63 ± 0.54

[a] Determined by the methods described previously (Zarkadas et al., 1988).

than that of cereal grain derived proteins but lower than those of casein (Drouliscos, 1976) and whole-egg protein (FAO/WHO, 1965). Similar results were obtained from the essential amino acid indices and protein scores (data not shown) calculated from their amino acid composition (Table V) by the methods of Block and Mitchell (1946) and Oser (1951). The earlier finding by Hulan et al. (1982a,b) that chicks fed a 20% potato waste meal plus 0.05% methionine, 20% corn, and 5% fish meal diet had weight gains equal to those fed on a 58% corn diet plus 0.05% methionine provides support for use of potato protein isolates as an economical feed supplement or as a plant protein additive for meat products. According to Wang and Kinsella (1975), fresh alfalfa leaves contained about 20–30% protein depending upon the amount of stems included in the samples. These authors showed that leaf protein extracts are very suitable as feed supplements for ruminants and that the whey or liquid portion from alfalfa leaf protein precipitation is an excellent source of protein for supplementing human diets (Woodham, 1971; Waterlow, 1962), especially cereal foods, which are low in lysine and threonine. The results obtained on the amino acid composition of alfalfa leaf protein (Table V) are in good agreement with those presented by Wang and Kinsella (1975) for alfalfa leaf isoelectric protein isolates.

4-Hydroxyproline Content of Additives and Ingredients. From the results presented in Tables IV and V, it is apparent that mustard seed flour and the alfalfa meal contained, respectively, 1.0% and 2.9% Pro(4-OH). Small amounts of Pro(4-OH) were also found to be present in both sensory enhancers (Table IV), soybean samples (Table III), and the Hygrade flours (Table IV) analyzed by the present methods (Zarkadas et al., 1986). Pro(4-OH) was once thought to be a unique to collagen amino acid except for the comparatively small amounts (1.3–1.6%; Eastoe, 1967) found in elastin preparations. However, evidence has been obtained to indicate that this hydroxylated unique amino acid seems to be a constituent of the 4-hydroxyproline-rich glycoproteins found in the primary cell walls of plants (Angiosperms) and seeds (Lamport, 1977; Lamport and Epstein, 1983; McNeil et al., 1984) including potato waste meal and oat groats (Zarkadas et al., 1982; Hulan et al., 1982a), corn pericarp (Boundy et al., 1967), mung bean, broadbean, and soybean seedlings (Chao and Dashek, 1973; Clarke and Ellinger, 1967), and lectins (Allen and Neuberger, 1973), but its occurrence is very restricted. This unique amino acid is known to occur in all three classes of extracellular matrices of cell wall glycoproteins, i.e., extensins, arabinogalactan proteins, and salt-extractable glycoproteins and agglutinins (Lamport, 1977; Fincher et al., 1983; McNeil et al., 1984; Cooper et al., 1987). Only the 86-kDa carrot extensin monomer has been the best characterized, consisting of 35% protein and 65% carbohydrate (Stuart and Varner, 1980; Van Holst and Varner, 1984; Stafstrom and Staehelin, 1986). The 30-kDa protein moiety contains 306 amino acids in its primary sequence (Chen and Varner, 1985a,b; Showalter et al., 1985; Smith et al., 1986; Staftsrom and Staehelin, 1986), and Pro(4-OH) makes up 45.5% of the polypeptide backbone. For these reasons, caution must therefore be exercised in interpreting the data available in the literature concerning the connective tissue contents of composite meats. If for example Pro-(4-OH) has been used as the basis for determining the connective tissue contents of processed meats (Expert Work Group (FSIS), 1984; Ashworth, 1987), which contained plant protein additives and ingredients, such calculations could give overestimated connective tissue values for processed meats than the actual levels present. The present results are in accord with those of Zarkadas et al. (1982), who have recommended that the use of the Pro-(4-OH) as an index for determining connective tissue content in composite meats should be discontinued.

Unique Basic Amino Acid Content of Nonmeat Ingredients. The data presented in Table VI indicate the absence of His(τ-Me), Lys(5-OH), and Des from the acid hydrolysates of the 16 nonmeat plant and animal protein products investigated by the chromatographic methods described previously (Zarkadas et al., 1986, 1987c). The resolving power of the methods was indicated by the complete separation of all methylated basic amino acids, the diastereoisomers of Lys(5-OH), and all stable elastin

cross-linking amino acids (data not shown) and related compounds, including a major unknown ninhydrin-positive peak, designated 17. The Arabic number assigned to this unknown peak indicates its relative elution times (364.1 min) from the microcolumn. Although the identity of this unknown compound is not yet established, it has been found in variable amounts in all products investigated. It was also found that unless the second eluting buffer was adjusted at pH 4.501, this unknown peak (17) coeluted from the microcolumn with His(τ-Me) in composite meats (Zarkadas et al., 1987b; Karatzas and Zarkadas, 1988). The values in Table VI show that both oilseed-derived protein products and protein supplement samples contained variable amounts of methylated lysines and ornithine.

The data presented in this paper show that both His(τ-Me) and Lys(5-OH) were absent from 16 typical nonmeat protein additives and ingredients used to formulate processed meats and that these two unique amino acids can be used as markers for determining, respectively, the myofibrillar and collagen contents of composite meats. Since Pro(4-OH) was found to be present in oilseed and cereal-derived nonmeat protein additives as well as in sensory enhancers, potato protein isolate, and alfalfa meal proteins, the use of this unique amino acid as an index for determining the connective tissue proteins, i.e., collagen and elastin, in composite meats is limited. Variations in amino acid and protein contents have also been established among typical nonmeat protein additives and ingredients used to formulate meat products, and it was found that the determination of the protein content of these 16 nonmeat ingredients, when based upon knowledge of the amino acid composition of those foods or feeds, yields accurate values of the amount of protein present. This information would be of value for assessing the overall protein quality and nutritive value of meats and meat products.

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Registry No. DL-Lys(5-OH), diastereomer 1, 3506-26-1; DL-Lys(5-OH), diastereomer 2, 52153-41-0; L-Lys(Me), 1188-07-4; L-Lys(Me_2), 2259-86-1; L-Lys(Me_3), 74886-62-7; L-His(τ-Me), 24886-03-1; L-Pro(4-OH), 51-35-4; nitrogen, 7727-37-9; ammonia, 7664-41-7.

LITERATURE CITED

Allen, A. K.; Neuberger, A. "The Purification and Properties of the Lectin from Potato Tubers, a 4-Hydroxyproline-containing Glycoprotein". Biochem. J. **1973**, *135*, 307–314.

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.

Asatoor, A. M.; Armstrong, M. D. "N^{τ}-Methylhistidine, a Component of Actin". *Biochem. Biophys. Res. Commun.* **1967**, *26*, 168–174.

Ashworth, R. B. "Amino Acid Analysis for Meat Protein Evaluation". *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 80–85.

Association of Official Agricultural Chemists. *Official Methods of Analysis of the Association of Official Agricultural Chemists*, 13th ed.; AOAC: Washington, DC, 1980.

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

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

Blackburn, S. "Sample Preparation and Hydrolytic Methods". In *Amino Acid Determination: Methods and Techniques*, 2nd ed.; Blackburn, S., Ed.; Marcel Dekker: New York, 1978.

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.

Boundy, J. A.; Wall, J. S.; Turner, J. E.; Woychick, J. H.; Dimler, R. J. "A Mucopolysaccharide Containing 4-Hydroxyproline from Corn Pericarp". *J. Biol. Chem.* **1967**, *242*, 2410–2415.

Brink, M. F.; Lofgren, P. A. *Composition and Nutritional Value of Milk and Dairy Products*; Wolff, I. A., Ed.; CRC Handbook of Processing and Utilization in Agriculture I; CRC: Boca Raton, FL, 1984; pp 291–313.

Chao, H. Y.; Dashek, W. V. "4-Hydroxyproline Metabolism During Mung Bean and Soybean Seedling Growth". *Ann. Bot.* **1973**, *37*, 95–105.

Chen, J.; Varner, J. E. "Isolation and Characterization of cDNA Clones for Carrot Extensin and a Proline-rich 33 kDa Protein". *Proc. Natl. Acad. Sci. U.S.A.* **1985a**, *82*, 4399–4403.

Chen, J.; Varner, J. E. "An Extracellular Matrix Protein in Plants: Characterization of Genomic Clone for Carrot Extensin". *EMBO J.* **1986b**, *4*, 2145–2152.

Clarke, E. M. W.; Ellinger, G. E. "Fractionation of Plant Material. II. Amino Acid Composition of Some Fractions Obtained from the Broad Bean Plant (*Vicia faba* L.) and Chromatographic Differentiation of 4-hydroxyproline Isomers". *J. Sci. Food Agric.* **1967**, *18*, 536–540.

Cooper, J. B.; Chen, J. A.; Van Holst, G. J.; Varner, J. E. "4-hydroxyprolines-rich Glyproteins of Plant Cell Walls". *TIBS* **1987**, *12*, 24–27.

Crampton, E. W. In *Applied Animal Nutrition*; Crampton, E. W., Ed.; W. H. Freeman: San Francisco, CA, 1956.

Drouliscos, N. J. "Nutritional Evaluation of the Protein of Dried Tomato Pomace in the Rat". *Br. J. Nutr.* **1976**, *36*, 449–456.

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

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

Elzinga, M.; Collins, J. H. "Amino Acid Sequence of a Myosin Fragment that Contains SH1, SH2 and N^{τ}-methylhistidine." *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 4281–4284.

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.

Expert Work Group. "Final Report and Recommendations: Food Safety and Inspection Service (FSIS), USDA". *Am. J. Clin. Nutr.* **1984**, *70*, 675–684.

FAO/WHO. *Protein Requirements*; Food and Agriculture Organization/World Health Organization: Rome, 1965; FAO/WHO Nutrition Meetings, Report Series No. 37.

Fincher, G. B.; Stone, B. A.; Clarke, A. E. *Arabinogalactan-Proteins: Structure, Biosynthesis, and Function*; Briggs, W. R., Jones, R. L., Walbot, V., Eds.; Annual Review of Plant Physiology 34; Annual Reviews: Palo Alto, CA, 1983; pp 47–70.

Foster, J. A. *Methods Enzymol.* **1982**, *82*, 559–570.

Hancock, W. S.; Harding, D. R. K. "Rapid Analysis of N^{τ}-Methylhistidine in Urine, Plasma, Muscle and Amniotic Fluid with a Single High Performance Liquid Chromatographic System but with Different Ion-Pairing Reagents". *J. Chromatogr.* **1982**, *228*, 273–278.

Hancock, W. S.; Harding, D. R. K. *Review of Separation Conditions*; Hancock, W. S., Ed.; CRC Handbook of HPLC for the Separation of Amino Acids, Peptides and Proteins I; CRC: Boca Raton, FL, 1984; pp 235–262.

Haverberg, L. N.; Onstedt, P. T.; Munro, H. N.; Young, V. R. "N^{τ}-methylhistidine Content of Mixed Proteins in Various Rat Tissues". *Biochem. Biophys. Acta* **1975**, *405*, 67–71.

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

Hirs, C. H. W.; Moore, S.; Stein, W. H. "Peptides Obtained by Tryptic Hydrolysis of Performic Acid Oxidized Ribonuclease".

J. Biol. Chem. **1956**, *219*, 623–642.

Horstmann, J. H. "A Precise Method for the Quantitation of Proteins Taking into Account Their Amino Acid Composition". *Anal. Biochem.* **1979**, *247*, 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.

Hulan, H. W.; Proundfoot, F. G.; Zarkadas, C. G. "Protato Waste Meal. I. Compositional Analyses". *Can. J. Anim. Sci.* **1982a**, *62*, 1161–1169.

Hulan, H. W.; Proudfoot, F. G.; Zarkadas, C. G. "Potato Waste Meal. II. The Nutritive Value and Quality for Broiler Chicken". *Can. J. Anim. Sci.* **1982b**, *62*, 1171–1180.

Huszar, G. *Methods Enzymol.* **1984**, *106*, 287–295.

Jones, A. D.; Homan, A. C.; Favell, D. J.; Hitchcock, C. H. S. "Investigation of the Levels of N^{τ}-methylhistidine in a Range of Beef Cuts and Offals". Meat Sci. **1985**, *15*, 137–147.

Jones, A. D.; Homan, A. C.; Favell, D. J. "Investigation of the Levels of N^{τ}-methylhistidine in a Range of Pork and Chicken Meats". *Meat Sci.* **1987**, *19*, 197–205.

Karatzas, C. N.; Zarkadas, C. G. "The Determination of the Myofibrillar and Connective Tissue Protein Contents and Amino Acid Composition of Selected Composite Meat Products". *J. Agric. Food Chem.* **1988**, comp paper in this issue.

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

Lamport, D. T. A.; Epstein, L. *A New Model for the Primary Cell Wall: A Concatenated Extensin-Cellulose Network*; Randall, D. D., Blevine, D. J., Larson, R. L., Rupp, B. J., Palavoly, C. J., Nelson, J. C., Polacco, J. C., George, M. F., Eds.; Current Topics in Plant Biochemistry and Physiology 2; University of Missouri: Columbia, MO, 1983; pp 73–83.

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". *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *44*, 416–420.

McNeil, 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*; Richardson, D. D., Boyer, P. D., Meister, A., Eds.; Annual Review of Biochemistry 53; Annual Reviews: Palo Alto, CA, 1984; pp 625–663.

Moore, S. "On the Determination of Cysteine and Cysteic Acid". *J. Biol. Chem.* **1963**, *238*, 235–237.

Moore, S.; Stein, W. N. *Methods Enzymol.* **1963**, *6*, 819–831.

Mounts, T. L. *Soybean Oil Processing and Products*; Wolff, I. A., Ed.; Handbook of Processing and Utilization in Agriculture 2; CRC: Boca Raton, FL, 1982; pp 57–82.

National Research Council. "Evaluation of Protein Quality". National Academy of Sciences: Washington, DC, 1963; Publication 1100.

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 Commerical 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 2; Applied Science: NJ, 1981; pp 197–240.

Ono, K. *Composition and Nutritional Values of Meat and Meat Products*; Wolff, J. A., Ed.; CRC Handbook of Processing and Utilization in Agriculture; CRC: Boca Raton, FL, 1982; pp 29–82.

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

Pellet, 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.

Peterson, G. L. *Methods Enzymol.* **1983**, *91*, 95–119.

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

Poulter, N. H.; Lawrie, R. A. "Rapid Determination of the N^{τ}-Methylhistidine in Determining the Lean Meat Content of Food Products". *Meat Sci.* **1980**, *4*, 21–31.

Rangeley, W. R. D.; Lawrie, R. A. "Methylated Amino Acids as Indices in Meat Products. I. Development and Validity of an Analytical Procedure". *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 3; Elsevier Applied Science: New York, 1984; pp 1–32.

Rees, M. W. "The Estimation of Threonine and Serine in Proteins". *Biochem. J.* **1946**, *40*, 632–640.

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.

Sanger, F.; Thomson, E. O. P. "Halogenation of Tyrosine during Acid Hydrolysis". *Biochim. Biophys. Acta* **1963**, *71*, 468–471.

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

Sarwar, G.; Christensen, D. A.; Finlayson, A. J.; Friedman, M.; Hackler, L. R.; Mackenzie, S. L.; Pellet, P. L.; Tkachuk, R. "Inter- and Intra-Laboratory Variation in Amino Acid Analysis of Food Proteins". *J. Food Sci.* **1983**, *48*, 526–531.

Showalter, A. M.; Bell, S. N.; Cramer, C. L.; Bailey, S. A.; Varner, J. E.; Lamb, C. J. "Accumulation of Hydroxyproline-rich Glycoprotein mRNAS in Response to Fungal Elicitor and Infection". *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 6551–6555.

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.

Stafstrom, J. P.; Staehelin, L. A. "Cross-linking Patterns in Salt-extractable Extensin from Carrot Cell Walls". *Plant Physiol.* **1986**, *81*, 234–241.

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

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.

Terrell, R. N. *Sausage Products*; Wolff, I. A., Ed.; CRC Handbook of Processing and Utilization in Agriculture 1; 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: New York, 1963; pp 227–318.

Van Holst, G. J.; Varner, J. E. "Reinforced Polyproline II Conformation in a Hydroxyproline-rich Cell Wall Glycoproteins from Carrot Root". *Plant Physiol.* **1984**, *74*, 247–251.

Vandekerckove, J.; Weber, K. "At Least Six Different Actins are Expressed in a Higher Mammal: An Analysis Based on the Amino Acid Sequence of the Amino-terminal Tryptic Peptide". *J. Mol. Biol.* **1978**, *126*, 783–802.

Vandekerckove, J.; Weber, K. "The Complete Amino Acid Sequence of Actins from Bovine Aorta, Bovine Heart, Bovine Fast Skeletal Muscle, and Rabbit Slow Skeletal Muscle". *Differentiation* **1979**, *14*, 123–133.

Wang, J.; Kinsella, J. E. "Composition of Alfalfa Leaf Protein Isolates". *J. Food. Sci.* **1975**, *40*, 1156–1161.

Waterlow, J. C. "The Absorption and Retention of Nitrogen from Leaf Protein by Infants Recurring from Malnutrition". *Br. J. Nutr.* **1962**, *16*, 531–540.

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.

Woodham, A. A. In *Leaf Protein: HS Agronomy, Preparation, Quality and Use*; Pirie, N. Y., Ed.; Blackwell Scientific: Oxford, England, 1971; p 115.

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. "Accelerated Methods for Determining Methylated Basic Amino Acid in Proteins and Muscle Tissues and Stable Crosslinks in Elastin and Collagen". *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; pp 572–575.
Zarkadas, C. G.; Hulan, H. W.; Proudfoot, F. G. "A Comparison of the Amino Acid Composition of Two Commercial Oat Groates". *Cereal Chem.* **1982**, *159*, 323–327.
Zarkadas, C. G.; Zarkadas, G. C.; Karatzas, C. H.; Khalili, A. D.; Nguyen, Q. "Rapid Method for Determining Desmosine, Isodesmosine, 5-Hydroxylysine, Triptophan, 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.; 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-Hydroxylsine, Elastin Crosslins, Other Amino Acids and the Amino Sugars in Proteins and Tissues". *Anal. Biochem.* **1987b**, *160*, 251–266.
Zarkadas, C. G.; Meighen, E. A.; Zarkadas, G. C.; Karatzas, C. N.; Rochemont, J. A.; Berthelet, M. "The Determination of the Myofibrillar and Connective Tissue Proteins in the Bovine Diaphragm". *J. Agric. Food Chem.* **1988**, companion paper in this issue.

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Quantitative Determination of the Myofibrillar Proteins and Connective Tissue Content in Selected Porcine Skeletal Muscles[1]

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The new analytical chromatographic methods developed to quantitate the unique amino acids that occur in proteins have been successfully applied for the determination of the myofibrillar and connective tissue content of both select porcine skeletal muscles and the intracellular and extracellular protein fractions. The proposed chemical approach is based on the direct determination of the myofibrillar myosin and actin contents of skeletal muscles from the amounts of protein bound N^{τ}-methylhistidine present. Collagen and collagen-like proteins can be calculated from the amounts of 5-hydroxylysine found and the elastin content from the amounts of desmosine or isodesmosine present. These quantitations are based on the total protein content of the selected porcine muscles determined by their detailed amino acid composition. Actin accounts for an estimated 10.2–11.5% of the total porcine muscle mass or about 21.1% of the total myofibrillar protein, while myosin ranged from 21.3 to 24.0% of the total muscle protein corresponding to 43.9% of the myofibrillar proteins (52.14% of protein). Total porcine muscle collagen ranged from 2.84 to 5.89% in select porcine muscles, while elastin accounts for an estimated 0.063–0.143%.

The possibility of using N^{τ}-methylhistidine [His(τ-Me)] as an index for determining the absolute mass of the myofibrillar proteins myosin and actin in skeletal muscles and composite meat products has stimulated considerable interest recently (Hibbert and Lawrie, 1972; Olsman and Slump, 1981; Ranken, 1984; Expert Work Group, FSIS, 1984; Benedict, 1987; Ashworth, 1987; McNeal, 1987). This quantitation is based on the following findings. Sequence studies have shown that actin contains 1 mol of His(τ-Me) at position 73 in its amino sequence (Elzinga et al., 1973; Vanderkerckhove and Weber, 1978, 1979) and that myosin isolated from adult fast-twitch white skeletal muscles contains 1 mol of His(τ-Me) at position 755 in each of the two heavy chains of this protein (Okamoto and Yount, 1975; Maita et al., 1987). Other studies have indicated that His(τ-Me) is absent from all other muscle and nonmuscle proteins (Huszar, 1984). In addition, Yates and Greaser (1983) have shown that the psoas skeletal muscle myosin accounts for 43% and actin for an estimated 22% of the myofibrillar protein mass (57.7%) of skeletal muscle. Moreover, the in situ molar ratio of actin to myosin in the myofibrils of skeletal muscle has been shown to be 6/1 (Murakami and Uchida, 1985).

Measurements of the levels of His(τ-Me) in several experimental animals (Asatoor and Armstrong, 1967; Johnson et al., 1967; Haverberg et al., 1975; Holbrook et al., 1979) and in bovine, ovine, and avian skeletal muscle tissues (Rangeley and Lawrie, 1976; Olsman and Slump, 1981; White and Lawrie, 1985; Jones et al., 1985, 1987), using a variety of chromatographic methods (Hancock and Harding, 1984; Ashworth, 1987), have yielded variable amounts of His(τ-Me) among the muscle tissues studied, ranging from 2.24 to 10.6 μmol of His(τ-Me)/g of tissue. Although some of this variation was attributed to the distribution of porcine muscle fiber types or the presence of variable amounts of balenine (Carnegie et al., 1982, 1984; Harris and Milne, 1981, 1987), a histidine dipeptide, β-

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