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Determination of the Myofibrillar and Connective Tissue Protein Contents and Amino Acid Composition of Selected Composite Meat Products[1]

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The contents of myosin, actin, and collagen of six composite meat products and hamburger have been determined by the use of analytical chromatographic methods developed to quantitate the unique amino acids that occur in those proteins. The proposed chemical approach for assessing protein quality in composite meats is based on the direct determination of their myofibrillar and collagen contents from the amounts of N^{τ}-methylhistidine and 5-hydroxylysine present, respectively. When the sum of the intracellular and extracellular skeletal muscle proteins is subtracted from the total protein of a composite meat sample, the difference is an accurate assessment of the nonmuscle protein additives present. The total protein content of each sample was calculated by the summation of the amino acid content as determined by detailed amino acid analysis. Mixed-meat products vary considerably in their myofibrillar (22.7–39.8%) and other soluble muscle proteins (14.7–32.2%), connective tissue proteins (6.7–21.6%), and nonmeat protein additives (2.4–52.8%), depending upon the type and amount of specific meat cuts and nonmeat protein ingredients used to formulate them.

The protein quality and nutritive value of processed meats and poultry products vary considerably depending upon the type and amount of specific meat cuts and nonmeat ingredients used to formulate them. Such composite meat products are prepared from cheaper meat cuts, which are frequently high in connective tissue, from the major meat-yielding species. Formulations usually include a number of other nonmuscle animal and plant protein additives including milk and egg powders, gelatin, soya, and other types of oilseed protein products, wheat gluten, etc., prepared by various separation and extraction processes. The actual levels of protein used in such processed meats vary and are tailored to meet the cost and nature of such ingredients available to the processor. These mixtures are then ground, chopped or comminuted, encased to retain defined shapes or forms, and then processed into a variety of meat products [reviewed by Terrell (1982) and Rust (1982)].

There has been a continuing interest in the development of standards for labeling prepackaged meats and reliable analytical methodology useful for the precise assessment of the skeletal muscle, connective tissue, and nonmuscle protein contents of composite meat products [reviewed by Pearson (1975), Olsman and Slump (1981), Ranken (1984), McNeal (1987), and Benedict (1987)]. Several of the electrophoretic and immunological methods including the enzyme-linked immunosorbent assay (ELISA), which have been described for determining the muscle and nonmuscle protein contents of processed meats (Menzel and Glatz, 1981; Hitchcock et al., 1981; Molander, 1982; Armstrong et al., 1982; Berkowitz and Webert, 1987), are limited mainly because of the extensive denaturation, decreased solubility, structural changes, and interactions that occur in these complex protein mixtures during processing. The more promising methods appear to be those based on the chromatographic separation and determination of specific peptides derived from actin (Anderson, 1976, 1981) or soya bean proteins (Llewellyn et al., 1978) and the unique basic amino acid N^{τ}-methylhistidine [His(τ-Me)] found in myosin and actin, the two principal muscle proteins (Elzinga et al., 1973; Elzinga and Collins, 1977; Huszar, 1984; Maita et al., 1987). Thus, the determination of His(τ-Me) has been proposed as an index for quantitating the lean meat content of various muscles and meats (Perry, 1970; Hibbert and Lawrie, 1972; Rangeley and Lawrie, 1976, 1977), and numerous studies have reported the distribution and occurrence of His(τ-Me) in a variety of muscle tissues and processed meats (Haverberg et al., 1975; Rangeley and Lawrie, 1977; Poulter and Lawrie, 1980; White and Lawrie, 1985; Olsman and Slump, 1981; Jones et al., 1982, 1985, 1987). However, until now, the multicolumn systems employed for these determinations have shown wide variation in the His(τ-Me) content of various muscles and meats, and complete separation of all these unusual basic amino acids from other ninhydrin-positive compounds found in meat hydrolysates has not been achieved [reviewed by Hancock and Harding (1984) and Ashworth (1987)]. To validate the use of His(τ-Me) as an index for assessing the myofibrillar protein content of processed meats, accurate and detailed determination of His(τ-Me) in selected composite meat products is essential.

The purpose of this investigation was to quantitatively establish the levels of all methylated basic amino acids, including the diastereoisomers of 5-hydroxylysine [Lys(5-OH)] and related compounds in commercially prepared

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composite meats, using the very sensitive and high-resolving-power chromatographic methods developed in this laboratory (Zarkadas et al., 1986b, 1987b). The experiment was also designed to establish the protein content of these composite meat products determined by detailed amino acid composition as described by Horstmann (1979) and Nguyen et al. (1986). An attempt was also made to relate the protein quality of these processed meats to their nutritive value by standard predictive tests based on their amino acid composition and by the direct determination of their myofibrillar and connective tissue contents from the amounts of His(τ-Me) and Lys(5-OH) found in their acid hydrolysates as described previously (Zarkadas, 1981; Zarkadas et al., 1988a).

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., Sunmnyvale, CA. The unusual amino acid standards were obtained as follows: diastereoisomer mixture of 5-hydroxyl-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 Commercially Processed Meats for Analysis.* The six major meat products and hamburger used in this study represent 60–70% of total sausage products produced under USDA inspection (Terrell, 1982; Ono, 1982). They included mixed-meat sausages, bologna, and frankfurters obtained from manufacturer I located in Montreal, Quebec; and all-beef sausages with (+) or without (−) condiments and wieners and hamburger supplied by manufacturers II and III, respectively, also located in eastern Canada (Toronto, Ontario). Three representative samples of each of these typical meat products originating from different batches were selected at random in 1.0-kg quantities. For amino acid analysis, approximately 200 g was sampled from each of these products, and after the casings were removed, the samples were cut into small cubes and ground separately, then frozen (−173 °C), and lyophilized. The freeze dried samples were then pulverized in a standard electrically driven end runner mill (coffee mill; Moulinex Canada Ltd., Weston, Ontario) and then stored at −20 °C in polypropylene bottles until needed. The mineral and proximate compositions of three of these products have been reported (Zarkadas et al., 1987a).

Extraction Procedures. To effectively remove the soluble histidine dipeptides including balenine from the lyophilized meat samples (Carnegie, 1984; Harris and Milne, 1987), the following three different extraction solvents were used: (a) distilled water, (b) a mixture of 75% ethyl alcohol in 0.1 M HCl (Rangeley and Lawrie, 1977), and (c) a mixture of methanol–chloroform as described by Bligh and Dyer (1959). The lyophilized sausage powder sample was thoroughly mixed and divided into four portions of approximately 10.0 g each. One of the samples was used as a control. Another two samples were suspended in 200 mL of the appropriate solvent (water or alcohol–0.1 M HCl, 3:1) 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 supernatants were removed and dried under vacuum (Buchi, Rotavapor R., Switzerland) at 45 °C. The pellet was suspended on the same solvent, and the extraction procedure was repeated a further two times. The final pellets were suspended in 20 volumes of acetone, and the suspension was again centrifuged as before. The pellets from the final centrifugation were dried at 50 °C overnight and then placed under vacuum to remove the last remnants of solvents. The dried pellets were finally ground to pass through a 40-mm screen and stored at −20 °C until needed.

The fourth meat sample was extracted with a mixture of chloroform, methanol, and water essentially as described by Bligh and Dyer (1959). The only alteration of the procedure was the use of a VirTis Model 45 homogenizer instead of a Waring blender. Since the moisture of the lyophilized meat samples was low, it was necesssary to adjust the final moisture content of the meat sample to 80 ± 1% by the addition of distilled water. The volumes of chloroform, methanol, and water, before and after dilution, were kept in the specified proportions, 1:2:0.8 and 2:2:1.8, respectively. Dilution with chloroform and water separtes the homogenate into two layers, the chloroform layer containing all the lipids and histidine dipeptides and the methanolic layer containing all the dilipidated proteins, which were recovered by filtration. The Bligh and Dyer (1959) extraction procedure was repeated two times on the insoluble protein fraction; the protein residue was dried overnight at room temperature, ground to pass through a 40-mm screen, and stored at −20 °C until needed.

Preparation of Amino Acid Calibration Standards. The unusual basic amino acid calibration standards employed for peak identification and standardization of the amino acid analyzer were prepared essentially as described previously (Zarkadas, 1975, 1979), with 3-nitrotyrosine as the internal standard (Zarkadas et al, 1986b, 1987b). Since 3-nitrotyrosine was found to contain impurities that coeluted with histidine and ammonia, these were removed by gel filtration as described previously (Riordan and Giese, 1977; Zarkadas et al., 1987b).

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.

Amino acid analyses were carried out on both extracted and unextracted meat samples. Lyophilized and/or acetone-dried 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 recommended by Moore and Stein (1963) and Hunt (1985), as described previously (Zarkadas et al., 1987b).

The data reported for serine and threonine represent the average of values extrapolated to zero time of hy-

drolysis (Rees, 1946). Addition of phenol (10–15 μL) to the hydrolysates usually prevented chlorination of tyrosine (Sanger and Thompson, 1963; Hunt, 1985). The values for valine, isoleucine, leucine, and phenylalanine are averages of data from 48, 72, and 96 h of hydrolysis (Blackburn, 1978). The 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., 1986b). Recoveries of Pro(4-OH) were calculated relative to alanine. Tryptophan in meat samples (0.1 g) was also determined separately after alkaline hydrolysis (Hugli and Moore, 1972) by an improved chromatographic procedure (Zarkadas et al., 1986b) using 3-nitrotyrosine 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) with norleucine as the internal standard. Recoveries were calculated relative to alanine and leucine.

The determinations of 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.

Determination of Total Protein. Recoveries of the unique and other amino acids were calculated on the basis of the protein content of individual hydrolysates determined by the procedure described by Horstman (1979). According to this method, a mean residue weight (WE, μg/nmol) is calculated for the amino acids constituting the proteins in the composite mean products by

$$\mathrm{WE} = \sum_{i=1}^{20}(a_i b_i) \qquad (1)$$

where a_i is the mole fraction of a specific amino acid i found in the analyzed aliquot and b_i is the molecular weight of amino acid residue i. A conversion factor F', which is the apparent average residue molecular weight (μg/nmol) and is characteristic for each protein mixture, was used for determining the protein mass in each hydrolysate sample analyzed in the absence of tryptophane, cyst(e)ine, and 4-hydroxyproline and can be calculated as

$$F' = \mathrm{WE}/[1 - (a_{\mathrm{Trp}} + a_{\mathrm{Cys}} + a_{\mathrm{Pro}} + a_{\mathrm{Pro(4\text{-}OH)}})] \qquad (2)$$

where a_i is the mole fraction of the specific amino acid i per mole of total amino acid composition. The protein concentration [P] of each hydrolysate was then calculated from

$$[\mathrm{P}] = F\sum_{i=1}^{18}x_i \qquad (3)$$

where x_i are the nanomoles of each amino acid i found in the analyzed aliquot.

Determination of Connective Tissue Proteins in Meat Products. In this study, an attempt was made to relate the amounts of the unusual protein-bound amino acids, which occur exclusively in vertebrate connective tissue proteins, i.e., collagens and elastin, to the contents of these extracellular matrix proteins in composite meat products.

A method to calculate the amount of a specific protein j in processed meats has been described previously (Zarkadas, et al., 1988a) and is

$$P_j = C_i\frac{[1000]}{n_i'}\frac{\mathrm{WE}(P_j)}{M_r(i)} \qquad (4)$$

where $\mathrm{WE}(P_j)$ is the weight equivalent of a specific muscle protein j, determined from eq 1 according to Horstman (1979), n_i' is the number of residues of a unique amino acid per 1000 amino acid residues, and $M_r(i)$ is the anhydrous molecular weight of the unique amino acid i. In this chemical approach the total collagen and collagen-like proteins (Porter and Reid, 1978; Anglister et al., 1976) of the six major products and hamburger investigated (per kilogram total protein) could be determined from the amounts of the aLys(5-OH) diastereoisomer present in their acid hydrolysates. Since types I and III collagens accounted for 61.5% and 33.5%, respectively, of the recovered collagen in the muscle connective tissue's epimysium, perimysium, and endomysium, while type IV collagen (which is specific to basement membranes and epithelial cells) accounted for the remaining 5% (Light and Champion, 1984; Light, 1985; Light et al., 1985), a mean Lys(5-OH) content of n_i' = 10.0 residues/1000 total amino acid residues in muscle collagen could be computed from the relative distribution of collagen types and their respective Lys(5-OH) contents as described previously (Zarkadas et al., 1988a). The average residue weight (WE) for muscle collagen is 91.10 (g/mol), and Lys(5-OH) has an anhydrous M_r of 145.18. The following analytical convention derived from eq 4 can therefore be used for calculating collagen as grams per kilogram of total protein:

$$\text{amt of collagen } (P_C) = \text{amt of Lys(5-OH)} \times 63.3 \qquad (4a)$$

Similarly, the amount of total connective tissue in these meat products (in grams per kilogram of total protein) could also be calculated from the sum of collagen (P_C) and elastin (P_E) found in skeletal muscle tissues, as described previously (Zarkadas et al., 1988a). A mean value for Pro(4-OH) (n_i' 101.8) can be computed from the known Pro(4 OH) contents of muscle collagen (n_i' = 105.8) and elastin (n_i' = 22.0; Foster, 1982) and the relative distribution of these two proteins in the extracellular matrix of skeletal muscle (Bendall, 1967). The anhydrous M_r of Pro(4-OH) is 113.12. The average residue weight (WE) for amorphous elastin (Foster, 1982) is 85.06 (g/mol) and for muscle connective tissue is 90.81 (Zarkadas et al., 1988a). The following analytical convention can therefore be used for computing total connective tissue:

$$\text{amt of connective tissue } (P_{CT}) = \text{amt of Pro(4-OH)} \times 8.03 \qquad (4b)$$

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

Determination of Total Myofibrillar Proteins. The quantitation of protein bound His(τ-Me), a unique basic amino acid known to occur exclusively in myosin and actin (Elzinga et al., 1973; Elzinga and Collins, 1977; Huszar, 1984; Maita et al., 1987), can be used as an index for determining these two principal myofibrillar proteins in various muscles and prepared composite meats, provided considerable care is taken to extract all soluble sarcoplasmic histidine dipeptides, especially balenine (Carnegie et al., 1982–1984; Griffith, 1986), prior to acid hydrolysis of muscle tissues.

Sequence studies (Elzinga et al., 1973; Vandekerckove and Weber, 1979; Elzinga and Collins, 1977; Maita et al., 1987) have shown that 1 mol of actin (A) contains 1 mol of His(τ-Me) and that 1 mol of myosin (M) contains 2 mol of His(τ-Me). Thus, the total amount of His(τ-Me) in the composite meat products determined by the present method (Zarkadas et al., 1987b) represents the sum of the distribution of His(τ-Me) in the myosin and actin present in each of the meat products investigated according to

$$C_T = C_A + C_M \qquad (5)$$

where C_T is the total protein-bound His(τ-Me) in myosin and actin (in grams per kilogram of the total protein), C_A is the amount (g) of His(τ-Me) in actin, and C_M is the amount (g) of His(τ-Me) in myosin in 1 kg of total protein (Zarkadas et al., 1988a).

By substituting C_A and C_M by the amounts of each protein (P_A = amount of actin; P_M = amount of myosin) from eq 4, the following relationship has been derived (Zarkadas et al., 1988a):

$$\sum_{j=1}^{2}(P_A + P_M) = \frac{(P_A/P_M) + 1}{n_M'/\mathrm{WE}(P_M) + (P_A/P_M)n_A'/\mathrm{WE}(P_A)} \frac{1000C_T}{M_r(i)} \quad (6)$$

The amount of the two proteins ($P_A + P_M$) is dependent on the relative composition of the two proteins in the sample (P_A/P_M), the number of residues of the unique amino acid (n_i') in the respective proteins, and the amount of the unique amino acid (C_T). Equation 6 is analogous to eq 4 for one protein with a unique amino acid. As $P_A/P_M \rightarrow 0$, eq 6 will simplify to eq 4.

The relative amounts of actin (M_r(A) = 41 782; Elzinga et al., 1973) and myosin (M_r(M) = 521 000; Yates and Greaser, 1983) per unit of tissue are related to the distribution of His(τ-Me) in myosin and actin of these products. The total amount of actin and myosin in composite meats can thus be calculated from the amount (C_T) of His(τ-Me) and the molar ratio of actin and myosin per kilogram of total protein by the following equation derived previously (Zarkadas et al., 1988a), which is analogous to eq 6:

$$\sum_{j=1}^{2}(P_A + P_M) = \left[\frac{A/M}{A/(M+2)}\frac{41\,782}{151.2} + \frac{1}{A/(M+2)}\frac{521\,000}{151.2}\right]C_T \quad (6a)$$

or

$$\sum_{j=1}^{2}(P_A + P_M) = \left[\frac{276A}{A+2M} + \frac{3466M}{A+2M}\right]C_T \quad (6a')$$

Substituting the molar ratio of actin to myosin reported by Murakami and Uchida (1985) (A/M = 6) in eq 6, the sum of actin and myosin in meats and meat products can be made as follows:

$$\sum_{j=1}^{2}(P_A + P_M) = [207 + 431]C_T \quad (6b)$$

or

$$\sum_{j=1}^{2}(P_A + P_M) = 638C_T \quad (6c)$$

Since the sum of myosin and actin in the myofibril accounts for 65% of the total myofibrillar protein by weight (Yates and Greaser, 1983), the total myofibrillar protein in grams per kilogram of total meats and meat products can also be calculated as

$$\text{amt of myofibrillar protein} = \frac{\sum_{j=1}^{2}(P_A + P_M)}{0.65}C_T = 981C_T \quad (6d)$$

Since the mean value of other SDS soluble muscle proteins reported for the costal bovine diaphragm, a typical mammalian skeletal muscle, accounted for 42.5% of the total muscle protein (Zarkadas et al., 1988a), this soluble muscle protein fraction in meats and meat products could also be computed as follows:

$$\text{amt of other muscle SDS soluble proteins} = \frac{\sum_{j=1}^{2}(P_A + P_M)}{44.0/42.5}C_T = 616.22C_T \quad (6e)$$

Therefore, the sum of the myofibrillar and other intracellular muscle soluble proteins calculated from eq 6d and 6e, expressed in grams per kilogram of total muscle protein, represents the total intracellular muscle proteins found in composite meat products.

Since the average collagen content in 32 bovine skeletal muscles accounts for 4.2% of the total muscle proteins (Bendall, 1967; Dransfield, 1977; Light and Champion, 1984; Light et al., 1985), the amount of muscle connective tissue in composite meats can be calculated as follows:

$$\text{amt of muscle connective tissue} = \frac{\sum_{j=1}^{2}(P_A + P_M)}{44/4.25}C_T = 60.90C_T \quad (6f)$$

If the amount of muscle collagen is subtracted from the total collagen of a composite meat sample, the difference is an accurate estimate of the nonmuscle collagen added to this product.

If the transcellular insoluble matrix in skeletal muscle accounts for an estimated 1.8% of the total muscle proteins (Loewy et al., 1983; Zarkadas et al., 1988a), then the extracellular matrix (in grams per kilogram of total protein) of composite meats can also be calculated as

$$\text{amt of extracellular matrix} = \frac{\sum_{j=1}^{2}(P_A + P_M)}{44/(4.2 + 1.8)}C_T = 87.00C_T \quad (6g)$$

Therefore, when the sum of the intracellar and extracellular muscle proteins is subtracted from the total protein of a composite meat product, which was quantitated by amino acid analysis, the difference is an accurate assessment of the nonmuscle protein additives and ingredients present.

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 centre (VAX) at Datacrown, Inc., IBM, Toronto, Ontario.

RESULTS AND DISCUSSION

Of the great variety of fresh or cured sausages available in the market (Terrell, 1982), six typical sausage products were selected for this survey. Samples of these typical processed meats and hamburger, representing about 60–70% of the total volume of processed meats (Terrell, 1982), were randomly selected from three major meat product manufacturers in Canada. Processed meats, however, have been reported to contain variable amounts of soluble histidine dipeptides including carnosine (β-alanyl-L-histidine), anserine (β-alanyl-L-N^{π}-methylhistidine), and balenine (β-analyl-L-N^{τ}-methylhistidine) which on acid hydrolysis yield β-alanine, histidine, His(π-Me), and His(τ-Me). Although the physiological functions of anserine and balenine in muscle cells have not

Table I. Comparison of the Amino Acid of Lyophilized Mixed-Meat Sausage Samples (Manufacturer I) before and after Extraction and All-Beef Sausage Emulsions (Manufacturer II) with Condiments (+C) or without Condiments (-C) following Extraction with 0.1 M HCl in 75% Ethyl Alcohol

amino acid (AA)	mixed-meat sausages: control mean ± SEM[a]	CV[a]	mixed-meat sausages: extracted mean ± SEM[a]	CV[a]	F^a between treatments	USDA[b] Handbook No. 8-7	extracted all-beef sausages: sausage (-C) mean ± SEM[a]	CV[a]	extracted all-beef sausages: sausage (+C) mean ± SEM[a]	CV[a]
aspartic acid	77.29 ± 0.81	2.56	95.23 ± 0.56	1.43	109.53**	84.78	86.86 ± 0.52	1.69	85.63 ± 0.57	3.33
threonine	44.92 ± 0.83	4.50	41.04 ± 0.37	2.22	34.60*	34.70	39.33 ± 0.30	2.13	41.08 ± 0.16	0.26
serine	41.23 ± 0.86	5.10	36.73 ± 0.55	3.65	45.17*	38.06	36.41 ± 0.16	0.16	39.38 ± 0.36	1.43
glutamic acid	137.02 ± 1.26	2.25	148.17 ± 0.90	1.49	34.59*	148.81	138.87 ± 1.44	1.44	155.83 ± 1.01	2.92
proline	59.50 ± 1.49	6.10	62.02 ± 0.99	3.90	6.98[ns]	50.22	56.43 ± 0.59	2.97	61.73 ± 0.62	3.47
glycine	70.45 ± 2.33	8.08	69.65 ± 1.84	6.47	0.37[ns]	63.96	77.68 ± 0.95	3.47	65.27 ± 17.74	36.77
alanine	56.08 ± 0.64	2.82	59.15 ± 0.34	1.38	24.62*	57.76	65.91 ± 1.11	4.77	60.29 ± 0.31	1.48
cysteine	8.25 ± 0.49	14.32	6.92 ± 0.21	7.14	10.39[ns]	7.84	16.88[g]		16.87[g]	
valine	52.00 ± 0.54	2.54	51.20 ± 0.35	1.64	16.68[ns]	34.63	54.84 ± 1.07	5.02	55.12 ± 0.99	5.08
methionine	23.82 ± 0.54	5.58	24.37 ± 0.32	3.14	0.59[ns]	27.16	14.03 ± 1.20	24.29	23.95 ± 3.51	13.84
isoleucine	48.84 ± 0.57	2.85	46.03 ± 0.36	1.92	20.48*	36.19	45.89 ± 0.43	2.63	50.41 ± 1.84	10.29
leucine	75.21 ± 0.83	2.69	77.07 ± 0.40	1.27	0.23[ns]	60.45	78.73 ± 0.34	1.23	83.48 ± 1.29	4.36
tyrosine	41.18 ± 0.65	3.86	35.61 ± 0.46	3.13	109.51**	28.81	35.62 ± 0.41	3.23	35.23 ± 0.10	0.82
phenylalanine	40.52 ± 0.79	4.76	40.09 ± 0.19	1.25	1.11[ns]	30.22	41.20 ± 0.46	3.18	41.16 ± 0.19	1.27
histidine	36.58 ± 0.45	3.03	24.62 ± 0.42	4.18	1120.11**	29.48	30.02 ± 0.16	1.48	31.37 ± 0.19	1.75
lysine	73.36 ± 0.68	2.26	68.05 ± 0.51	1.85	166.70**	67.39	82.89 ± 0.91	3.10	66.58 ± 0.72	3.07
arginine	68.62 ± 0.35	1.26	73.95 ± 0.32	1.05	118.43**	57.09	67.32 ± 0.59	2.49	58.30 ± 0.73	3.55
tryptophan	12.64 ± 0.48	9.18	10.43 ± 0.40	9.39	5.34[ns]	7.99	12.39[g]		12.23[g]	
4-hydroxyproline	29.21 ± 1.39	11.64	26.95 ± 0.89	8.06	6.92[ns]		15.74 ± 0.98	12.48	13.77 ± 1.27	18.43
N^{τ}-methylhistidine	0.57 ± 0.09	38.01	0.003 ± 0.001	143.94	117.85**		0.147 ± 0.003	10.19	0.350 ± 0.01	
ammonia	25.84 ± 1.27	12.05	17.69 ± 0.89	6.50	13.85[ns]		17.88 ± 0.83	13.19	19.72 ± 1.01	14.35
total AA N[c]	187.28		178.88				181.75		177.70	
total EAA,[d] mg/g of N	2613.04		nd				2691.8		2725.0	
EAA index[d]	71.83		nd				72.32		75.03	
protein score[d]	60.91		nd				58.72		77.97	
WE,[e] μg	0.107278		0.107101				0.105811		0.107062	
F,[e] μg	0.109007		0.108533				0.108452		0.107062	
F',[f] μg	0.120440		0.129948				0.117639		0.119679	

[a] Mean values and standard error of measurements (SEM). Significance denoted by F values: **, $P < 0.01$; *, $P < 0.05$; ns, not significant; nd, not determined; CV, coefficient of variation. [b] Values converted from Richardson's et al. (1980) USDA Handbook No. 8-7 (07075). [c] Calculated according to Heidelbaugh et al. (1975). [d] From Oser (1951) and Block and Mitchell (1946). [e] The WE and F constants were calculated from the amino acid composition of each hydrolysate according to Horstmann (1979) and Tristram and Smith (1963). [f] The F' value was also calculated according to eq 2 except for determining protein mass in the absence of tryptophan, cyst(e)ine, proline, and 4-hydroxyproline. [g] Values from all-beef weiner determinations.

been established, both β-alanine and carnosine are now considered as putative neurotransmitters (Griffith, 1986), these dipeptides must be extracted from composite meats prior to acid hydrolysis.

To quantitatively establish the levels of protein-bound His(τ-Me) and Lys(5-OH) in processed meats, the first objective was to find which of the existing solvent extraction procedures would quantitatively extract all soluble compounds including the histidine dipeptides from the lyophilized meat samples prior to acid hydrolysis. Three solvent systems were compared, distilled water, a mixture of 75% ethanol in 0.1 M HCl (Rangeley and Lawrie, 1976), and a mixture of methanol–chloroform–water (Bligh and Dyer, 1959). The results obtained indicated that both the Bligh and Dyer (1959) and Rangeley and Lawrie (1976) solvent extraction procedures were equally effective in extracting soluble amino acids and peptides including histidine dipeptides from processed meats at ambient temperature, while distilled water was the least efficient. Other extraction procedures, i.e. 0.6 M perchloric acid (Happich et al., 1984) and 0.9% saline in 8% 5-sulfosalicylic acid (Carnegie et al., 1984), have also been reported to be effective in extracting these histidine dipeptides. The only limitation of these two procedures, however, would be to free the extracted samples from these two solvents prior to amino acid analysis.

Another problem often encountered in meats during extraction by any of these procedures is the incidence of unemptied muscle cell segments observed during homogenization (McCollester and Semente, 1964). Although the mechanisms responsible for the emptying of the muscle cell are not known, it appears that the high incidence of these unemptied cell segments is related to irreversible contraction of muscle cells during homogenization. McCollester (1962) has shown that the presence of traces of heavy metals during muscle extraction (i.e., 50 mM $CaCl_2$, pH 7.4) will completely prevent the emptying phenomenon and will avoid the incidence of unemptied muscle cell segments, as may be the case in processed meats.

Protein, Nitrogen, and Amino Acid Contents of Composite Meats. The amino acid composition of mixed-meat sausage samples before and after extraction and of extracted all beef sausages emulsions with condiments (+C) or without (-C), and levels of statistical significance obtained from analysis of variance presented in Table I, represent the average values of three replicates and duplicate determination obtained from duplicate 24-, 48-, 72-, and 96-h hydrolysates. The results (Table I) are expressed as grams of amino acid residues per kilograms of total protein. The main advantage of this unit of expressing the composition of a protein mixture, i.e., moisture-, fat-, and ash-free basis (Tristram and Smith, 1963; Eastoe, 1967), is that the usual practice of subtracting the percentage of connective tissue present in such complex protein mixtures (Olsman and Slump, 1981) is no longer required. Thus, protein determinations were carried out in each acid hydrolysate as described previously (Horstman, 1979; Nguyen et al., 1986). This method is based upon knowledge of the amino acid composition of the protein or protein mixture and yields accurate estimates of the amount of protein present (eq 1–3). The constants, weight equivalent (WE, μg/nmol) and conversion factors (F and F', μg/nmol), for each of the sausages and other meat products investigated

Table II. Elution Times and Contents (Nanomoles per Milligram of Protein, $N = 6$) of Four Major Unknown Ninhydrin-Positive Peaks Separated from Processed Meat Hydrolysates by the Method of Zarkadas et al. (1987a)

compound[a]		elution time, min	composite meat products: sausages mean ± SEM[b]	sausages CV[b]	bologna mean ± SEM[b]	bologna CV[b]	frankfurter mean ± SEM[b]	frankfurter CV[b]
unknown	1	38.0	nd		nd		nd	
	5	78.0	15.698 ± 0.619	9.66	17.513 ± 0.226	3.16	8.902 ± 0.302	8.30
	13	216.0	5.826 ± 0.713	27.37	2.186 ± 0.908	101.75	4.222 ± 0.241	13.97
	17	354.0	7.133 ± 0.384	13.18	13.927 ± 0.775	12.45	7.822 ± 0.358	11.21
ornithine		190.0	5.757 ± 0.349	14.85	8.267 ± 0.359	10.65	7.809 ± 1.002	31.44

[a] Each of the unknown peaks is assigned an arabic number to indicate its relative order of elution from the microcolumn. [b] Mean values and standard error of measurements (SEM) of six determinations: CV, coefficient of variation; nd, not determined. The relative concentrations of the various unknown peaks were calculated by assuming a relative response factor equal to that of the internal standard 3-nitrotyrosine.

Table III. N^{τ}-Methylhistidine and 5-Hydroxylsine Contents (Grams of Amino Acid per Kilogram of Protein) of Commercially Prepared Mixed-Meat Products, Blended All-Beef Sausage Emulsions with Condiments (+C) or without (-C), and Hamburger before (un) and after (ex) Solvent Extraction with 0.1 M HCl in 75% Ethyl Alcohol

product	N^{τ}-methylhistidine mean ± SEM[a]	range, min–max	CV[a]	F^a between treatments	5-hydroxylysine mean ± SEM[a]	range min–max	CV[a]	F^a between treatments
mixed meat ($R = 3$)								
sausages ($N = 6$)								
protein-bound	0.303 ± 0.013[c]	0.268–0.351	10.85		2.628 ± 0.221	1.927–3.269	20.56	
total (un)	0.759 ± 0.010[b]	0.715–0.789	3.26	429.43**	2.237 ± 0.093	1.839–2.485	10.17	3.06[ns]
bologna ($N = 6$)								
protein-bound	0.266 ± 0.020	0.219–0.335	18.45		1.171 ± 0.053[c]	1.017–1.332	11.01	
total (un)	0.297 ± 0.014	0.261–0.356	11.32	0.78[ns]	1.594 ± 0.060[b]	1.414–1.759	9.21	36.78*
frankfurters ($N = 6$)								
protein-bound	0.378 ± 0.013	0.341–0.419	8.34		1.737 ± 0.078[c]	1.586–2.081	11.02	
total (un)	0.473 ± 0.017	0.417–0.525	8.90	17.95[ns]	2.067 ± 0.045[b]	1.960–2.235	5.32	87.15*
wieners (ex) ($N = 2$)								
protein-bound	0.231 ± 0.010	0.221–0.241	6.03		1.531 ± 0.050	1.480–1.581	4.60	
all-beef (ex)								
sausage (-C) ($N = 4$, $R = 2$)								
protein-bound	0.522 ± 0.011	0.498–0.534	3.07		2.529 ± 0.167	2.362–2.696	9.35	
sausage (+C) ($N = 4$, $R = 2$)								
protein-bound	0.405 ± 0.025	0.380–0.431	8.85		1.950 ± 0.162	1.788–2.230	11.73	
hamburger (ex) ($N = 6$, $R = 3$)								
protein-bound	0.533 ± 0.027	0.480–0.572	10.17		1.755 ± 0.035	1.632–1.794	3.97	

[a] Mean values and standard error of measurements (SEM). Significance within and between treatments is denoted by F values: **, $P < 0.01$; *, $P < 0.05$; ns, not significant; CV, coefficient of variation; R, number of replicates; N, number of determinations. (b, c) Means within a column with different superscripts are significantly different.

have been determined (Table I) and can be used in all subsequent quantitations of these products following standard procedure as described by Horstman (1979), Peterson (1983), and Zarkadas et al. (1988b).

The composition of lyophilized mixed-meat sausage samples before and after solvent extraction, given in Table I, shows that only 1.77% of the total amino acid residues was extracted by this method (Rangeley and Lawrie, 1976) prior to acid hydrolysis. A sizable proportion of the total amino nitrogen extracted from processed meats was free ammonia, which accounts for 4.04%, 6.01%, and 5.21% of the total nitrogen in sausages. Practically all of the His(π-Me) found in composite meats was extracted at ambient temperature by this procedure. It was found that as much as 34.14% of the total histidine present in untreated sausages (Table I) could thus be extracted from the lyophilized mixture. Similarly the soluble histidine found in bologna accounts for 32.7%. Carnegie et al. (1982–1984) and Olsman and Slump (1981) have reported that the ratio of carnosine to anserine or vice versa could be used as an index for the identification of the meat-yielding species used in processed meats. Thus, if all of the soluble histidine and His(π-Me) in these products has originated from carnosine and arserine, respectively, then the ratio of soluble histidine and His(π-Me) calculated from the data in Table I could also be used as a quantitative index for meat species identification. The mixed meat sausage samples had a high ratio (23.9) of soluble histidine to His(π-Me) and appear to contain a high proportion of pork meat.

The data presented in Table I indicate that each of these sausage products has a characteristic amino acid profile depending upon the amounts of specific meats or meat cuts and nonmuscle ingredients used to formulate each product. The following features between values for individual amino acids, however, seem to be common to all products. Glutamic acid, for example, is the most abundant amino acid in all products and accounts for almost 12–15% of all residues. Aspartic acid, proline, glycine, leucine, lysine, and arginine, which are the next most abundant amino acids in processed meats, when taken together account for a further 43–45%. Thus, seven amino acids account for approximately 55–60%, so that only one-third of the positions in the polypeptide chains of processed meats is available for the remaining 40 amino acids and the ninhydrin-positive unknown compounds determined in this study (Tables I–III). Serine and threonine account for 8.0–8.6% and tyrosine for another 4.0% of the total amino acid residues. The Pro(4-OH) together with small amounts of Lys(5-OH) (Table III) bring the total composition of residues with hydroxyl groups to nearly 16.0%, which is relatively frequently. The amino acids with hydrophobic side chains (Barrantes, 1973; Bigelow, 1967; Nozaki and Tanford, 1971) ranged between 27 and 29%. Leucine

accounts for about 7.5%, valine for a little more than 5%, tryptophan for only 1.25%, and tyrosine, isoleucine, and phenylalanine for approximately 13.0%. Methionine accounts for only 2–2.5%. This sulfur amino acid has been reported to become oxidized into methionine *S*-oxide during processing (Spindler et al., 1984) and was determined both as methionine *S,S*-dioxide (Moore, 1963) and in regular hydrolysates as methionine (Zarkadas et al., 1987b). Both methods gave identical results. Thus, the frequency of hydrophobic groups in processed meats greatly exceeds that of hydroxyl groups.

The following differences between values for individual amino acids were noted among the extracted and untreated composite meats evaluated in this study. The aspartic acid values of the extracted sausages were some 9.9–19.0% higher than the corresponding untreated samples (Table I). USDA Handbook No. 8-7 (Richardson et al., 1981) recalculated mean value for aspartic acid of 91.12 g/kg of protein for mixed-meat sausages is considerably higher than the untreated sample used in this study, but lower than the corresponding extracted samples. The mean values obtained for arginine in the extracted composite meats ranged from 58.30 to 73.95 g/kg of protein (Table I), with the mixed-meat sausages being much higher in arginine (73.95 g/kg of protein) compared to 68.62 g/kg of protein found in the untreated sample. Richardson et al. (1980) reported arginine values of 62.60 g/kg of protein for sausages (Table I). The glutamic acid values for the extracted products exceed those of the untreated samples but are in reasonable agreement with recalculated data of Richardson et al. (1980). It is possible that the values obtained for aspartic and glutamic acids and arginine in the extracted samples are slightly augmented by the presence of unknown compounds in the regions of these peaks, but this would probably be insufficient to account for the difference, since the low coefficient of variations and high resolution achieved between these peaks (Zarkadas et al., 1987b) have surpassed any existing methods to date. Finally, the mean values obtained for the remaining amino acids in the extracted samples are slightly lower than the corresponding untreated samples, except as mentioned earlier for histidine. The weighted mean values obtained for the amino acid composition of all products investigated are consistently higher compared to those recalculated from USDA Handbook No. 8-7 (Richardson et al., 1981).

Processed sausage meats contained significant amounts of all amino acids commonly found in proteins with the exception of cyst(e)ine and methionine and possibly isoleucine and phenylalanine. A comparison between the EAA profile of mixed-meat sausages and all-beef sausage emulsions (Table I) with the total EAA (mg/g of N) of reference proteins showed that all of these products were much lower than cow's milk (3200 mg/g of N) or hen's whole egg (3215 mg/g of N) protein (FAO/WHO, 1965). Similar results were obtained from EAA indices and protein scores calculated from the amino acid composition of these products (Table I) by the methods of Oser (1951) and Block and Mitchell (1946). Although these predictive tests are based on the known amino acid composition of these selected processed meats, which afford a valuable guide in compounding the protein mixtures or meat products (Pellet and Young, 1984), they fail to take into account differences in the digestibility and availability of individual amino acids and the quality of the various proteins present. Because of this, Sarwar (1984), Sarwar et al. (1985), Happich et al. (1984), and McLaughlan et al. (1980) developed reliable and rapid methods of assessing the nutritive value and protein quality of foods including available amino acid score, net protein ratio (NPR), and relative NPR (RNPR). The Expert Work Group (FSIS, 1984) has presented the considerations, procedures, and recommendations for evaluating the protein quality of meats and meat products.

Unique Basic Amino Acid Content of Composite Meats. To validate the use of His(τ-Me) as an index for assessing the myofibrillar protein content of processed meats, accurate and detailed determination of His(τ-Me) in a variety of selected composite meat products was carried out. The major objective of this survey was to provide experimentally and statistically sound data on the occurrence and variation of His(τ-Me) and other unique basic amino acids found in the selected major meat products. All determinations were carried out by the single-column chromatographic methods developed to quantitate the unique amino acids that occur in proteins and tissue (Zarkadas et al., 1986b, 1987b). The chromatograms illustrated in Figure 1 are typical of the separations obtained by one of these methods (Zarkadas et al., 1987b). Good separations from a standard containing all the unique basic amino acids likely to be encountered in a biological system were obtained (Figure 1A). The unusual basic amino acid components of processed meats were determined from concentrated 96-h hydrolysate samples (200–800 μg/100 μL) so that reasonably sized peaks (50–250 pmol/100 μg of protein) for these components could be obtained.

As may be seen in Figure 1B,C, the analysis of sausage hydrolysate (96 h) before and after solvent extraction by this method (<100 pmol/100 μg of protein) enabled the complete separation of all methylated basic amino acids including the diastereoisomers of Lys(5-OH) and revealed four major (peaks 1, 5, 13, and 17) and 13 minor as yet unidentified ninhydrin-positive components. Each of the 17 unknown peaks was assigned an Arabic number to indicate its relative order of elution from the microcolumn, followed by its retention time in minutes. The elution times and relative concentrations of the four major unknown peaks are presented in Table II, while the retention times (min) of the minor components shown in Figure 1B,C are as follows: peak 2, 44.0; 3, 47.0; 4, 55.0; 6, 105.0; 7, 124.0; 8, 130.0; 9, 135.0; 10, 142.0; 11, 179.0; 12, 183.2; 14, 258.0; 15, 267.0; 16, 270.0. The relative concentrations of the four major unknown peaks found in extracted sausage samples are presented in Table II. These were calculated by assuming a relative response factor equal to that of the internal standard, 3-nitrotyrosine. However, further detailed studies to ascertain the nature, function, and actual concentrations of all these unknown compounds are required.

The elution profiles obtained in the analysis of typical acid hydrolysates of extracted and untreated sausage samples shows that His(π-Me) and unknown 13 have been completely extracted with 0.1 M HCl in 75% ethanol, prior to hydrolysis (Figure 1C), compared to the untreated sample (Figure 1B) while unknown 6 increased considerably. Since the positions of both His(π-Me) and His(τ-Me) are very sensitive to pH, rigid control of the pH of the second eluting buffer (pH 4.501 ± 0.002) is also necessary to obtain good resolution (Zarkadas et al., 1987b). Thus, as shown in Figure 1A, by introducing the second buffer (pH 4.501) just before Lys(Me) and by increasing the temperature to 73 °C, both His(π-Me) and His(τ-Me) were completely separated following the ammonia peak. It should be noted (Figure 1B,C) that at pH 4.501 His(τ-Me) is completely separated from an unknown compound (17),

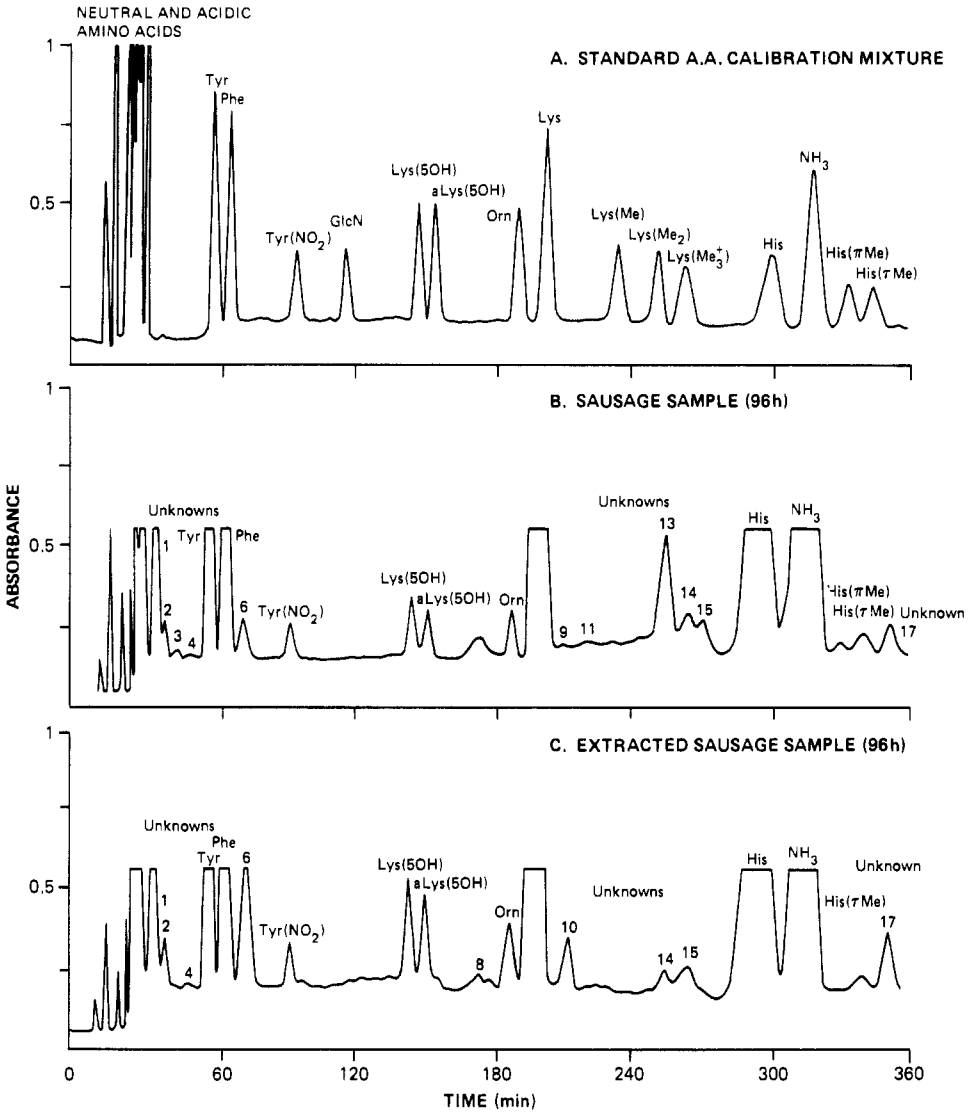

Figure 1. Chromatographic separation of methylated lysines and histidines, the diastereoisomers of 5-hydroxylysine and related compounds in mixed-meat sausages: (A) separation of a synthetic amino acid calibration mixture; (B) typical separation of an untreated 96-h hydroysate of sausage; (C) analysis of an extracted 96-h hydrolysate of a sausage sample. The curve shows absorbance at 570 nm. Key: Tyr(NO_2), 3-nitrotyrosine; GlcN, glucosamine; Lys(5-OH), 5-hydroxylysine; aLys(5-OH), *allo*-5-hydroxylysine; Orn, ornithine; Lys(Me), N^6-methyl-L-lysine; Lys(Me_2), N^6,N^6-dimethyl-L-lysine; Lys(Me_3^+), N^6,N^6,N^6-trimethyl-L-lysine; His(π-Me), N^π-methylhistidine; His(τ-Me), N^τ-methylhistidine.

which appeared to occur in variable amounts (Table II) in most meat and plant tissue hydrolysates investigated. It was also found that when the second eluting buffer was adjusted at either higher or lower (i.e., pH 4.100) pH values, this unknown compund (17) coeluted from the microcolumn with His(τ-Me), giving variable results for His(τ-Me) in composite meats.

As may be seen in Figure 1, an accurate determination of the 5-hydroxylysine content of composite meats was made from the values obtained for one of its diastereoisomers, aLys(5-OH), after epimerization. Although previous work (Zarkadas, 1975) showed that the determination of Lys(5-OH) can be made from the sum of the values obtained from its diastereoisomers (Figure 1A) after epimerization in 6 M HCl at 110 °C for 96 h, in the present study it was found that another unknown ninhydrin-positive compound(s), which appears to be present in meats, coeluted with one of the diastereoisomers of Lys-(5-OH), thus interfering with its quantitation (Figure 1). Because of this, the aLys(5-OH) diastereoisomer after epimerization (96 h) is now being routinely used in the quantitation of this amino acid in collagen as well as in meats and meat products (Zarkadas et al., 1988a).

For the above reasons, caution must therefore be exercised in interpreting the data available in the literature concerning the contents of His(τ-Me) and Lys(5-OH) of various muscles from different meat-yielding species and processed meat products.

Table III compares the His(τ-Me) and Lys(5-OH) contents of four commercially prepared mixed-meat products, two blended all-beef sausage emulsions with or without condiments, and hamburger before and after solvent extraction with 0.1 M HCl in 75% ethyl alcohol. The range and levels of statistical significance obtained from analysis of variance of the His(τ-Me) and Lys(5-OH) contents of the six processed meats and hamburger are also presented in Table III. The values obtained for His(τ-Me) and Lys(5-OH) of all composite meat products and hamburger 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 for both amino acids. Each of these meat products has a typical His(τ-Me) and Lys(5-OH) profile depending upon the amounts of specific meats or meat cuts and nonmuscle

Table IV. Myofibrillar and Connective Tissue Contents (Grams of Protein per Kilogram of Total Protein) of Selected Mixed-Meat Products, Blended All-Beef Sausage Emulsions with (+C) or without (-C) Condiments, and Hamburger

protein class	composite meat product[a]						
	all-beef sausage		mixed-meat				hamburger[a] (all-beef)
	-C	+C	sausages	frankfurters	bologna	wieners	
Skeletal Muscle Proteins							
i. intracellular (a + b)[b]	834.14	647.47	484.04	603.86	424.93	369.03	851.63
a. myofibrillar[b]	512.23 ± 10.79	397.72 ± 24.53	297.33 ± 12.41	370.93 ± 12.41	261.02 ± 19.63	226.68 ± 9.81	523.12 ± 14.12
actin	108.27 ± 2.28	84.00 ± 5.17	62.80 ± 2.69	78.34 ± 2.69	55.13 ± 4.15	47.87 ± 2.07	110.48 ± 5.59
myosin	224.83 ± 4.73	174.57 ± 10.75	130.51 ± 5.37	162.81 ± 5.60	114.57 ± 8.61	99.50 ± 4.31	229.72 ± 11.61
actomyosin	333.12 ± 7.01	258.57 ± 15.93	193.31 ± 8.07	241.15 ± 8.94	169.70 ± 12.76	147.37 ± 6.37	340.20 ± 9.18
b. other soluble proteins[b]	321.91 ± 6.78	249.75 ± 15.41	186.71 ± 7.32	232.93 ± 8.01	163.91 ± 12.32	142.35 ± 6.16	328.51 ± 8.87
ii. extracellular matrix[c]	45.47 ± 0.76	35.26 ± 2.18	26.36 ± 1.13	32.89 ± 1.13	23.14 ± 1.74	20.10 ± 0.87	46.38 ± 1.25
a. collagen	32.76 ± 0.71	24.71 ± 1.52	18.48 ± 0.90	23.06 ± 0.78	16.20 ± 1.21	14.09 ± 0.61	32.52 ± 0.88
total (i + ii)	879.61	682.73	510.40	636.75	448.07	389.13	898.01
Nonmuscle Ingredients and Additives							
iii. connective tissue[d]	126.39 ± 7.75	110.57 ± 10.05	216.41 ± 7.04	113.28 ± 0.63	127.11 ± 1.98	66.97 ± 0.08	88.89 ± 0.0
iv. total collagen and collagen-like proteins[e]	158.71 ± 10.48	122.37 ± 10.17	164.91 ± 13.37	109.00 ± 4.89	73.48 ± 4.61	96.07 ± 3.14	110.13 ± 3.85
v. added collagen (iv - iia)	125.95	97.66	146.43	85.94	57.28	81.98	77.61
$\sum^3$(i + ii + v)	1005.56	780.39	656.83	722.69	505.35	471.11	975.62
added nonmuscle proteins	0.0	219.61	343.17	277.31	494.65	528.89	24.38

[a] Samples extracted with a mixture of 75% ethyl alcohol in 0.1 M HCl (Rangeley and Lawrie, 1977). [b] Calculated from eq 6f, 6g, and 8–8d and His(τMe) data (Table VIII). [c] Calculated from eq 8e and 8f and His(τMe) data (Table VIII). [d] Calculated from the Pro(4-OH) data (Tables II–IV) using eq 5b and 6 and substituting $n_i' = 105.8$. [e] Calculated from the Lys(5-OH) data of Table III using eq 5a and substituting $n_i' = 10.0$.

ingredients used to formulate each product. A comparison, for example, between different types of meat sausages (mixed-meat sausages, all-beef sausages with or without condiments, bologna, frankfurters, and wieners (Table III)) indicates that significant differences exist among these products with respect to their His(τ-Me) and Lys(5-OH) contents. The mixed-meat and all-beef sausages had high levels of Lys(5-OH) and appear to contain a higher proportion of connective tissue compared to bologna and wieners, which appeared to contain lower levels of myofibrillar and connective tissue proteins but higher levels of nonmuscle proteins than the corresponding mixed-meat sausages. The hamburger and all-beef sausage without condiments meat samples contained the highest levels of His(τ-Me), indicating that both had the highest levels of myofibrillar protein contents. Both of these products and frankfurters were found to contain about the same amounts of connective tissue. Although the data reported in Table III are in reasonable agreement with those reported by other authors (Rangeley and Lawrie, 1977; Poulter and Lawrie, 1980; Olsman and Slump, 1981), some differences have been noted (Jones et al., 1985, 1987). These may arise from the fact that other methods were employed for these determinations or because of variations in the levels of muscle or nonmuscle proteins used in the preparation of such products.

Assessment of Protein Quality in Composite Meats. In this study, an attempt was made to relate the protein quality of six major meat products and hamburger to their amino acid composition by the direct determination of their myofibrillar and connective tissue contents by the very sensitive and high-resolving-power chromatographic method that has been developed (Zarkadas et al., 1987b).

From the results presented in Table III on the His(τ-Me) and Lys(5-OH) contents of six composite meat products and hamburger samples, it has been possible to determine their myofibrillar and connective tissue protein contents, and the results are summarized in Table IV. In this chemical approach, the actin, myosin, actomyosin, and total myofibrillar protein contents of hamburger and prepared composite meats can be determined from the amounts of His(τ-Me) found in their acid hydrolysates from eq 6a.

Myofibrillar Protein Contents of Composite Meats. The data presented in Table IV indicate that the myofibrillar protein content of hamburger accounts for an estimated 52.3% of the total muscle proteins compared to 51.2% found for all beef sausages without condiments. Actin accounts for 11.05% while myosin for another 22.9% of the total muscle protein in hamburger. These results are in accord with those reported for skeletal muscle by Hanson and Huxley (1957) and Yates and Greaser (1983) who have shown that the Psoas major from rabbits contains 57.71% myofibrillar proteins and that actin accounts for an estimated 12.69% while myosin accounts for 24.82% of the total muscle proteins, but differed from those of Dutson and Calkins (1982). In contrast, the content of the myofibrillar proteins in mixed-meat sausages, bologna, and wieners ranged from 22.0 to 28.9% compared to 36.0% and 39.4% found, respectively, in frankfurters and all beef sausage emulsions with condiments. These results indicate that the actual levels of meat cuts used to formulate each of the composite meat evaluated varied significantly and that certain of these products contained substantial amounts of nonmeat protein ingradients and additives. These results also show that the direct approach used in this study for evaluating protein quality in composite meats has the advantage over other methods in that it is based on the determination of at least two classes of high-quality muscle proteins: the myofibrillar myosin and actin, and other intracellular soluble muscle proteins (Table IV), which can be compared in each of the meat products evaluated.

Other Soluble Muscle Proteins in Meats. Since the mean value of other muscle proteins reported for the costal region of the bovine diaphragm, a typical mammalian skeletal muscle, accounts for 42.5% of the total muscle protein (Zarkadas et al., 1987b, 1988a), this muscle protein

fraction in composite meat products could also be calculated by eq 6e, and the results are summarized in Table IV. This intracellular muscle protein fraction includes sarcoplasmic proteins, organelles, and Z-band and other membrane proteins, etc., which are soluble in 2% sodium dodecyl sulfate as described by McCollester (1962) and Laurent et al. (1981).

Connective Tissue Contents of Composite Meats. The weighted mean collagen and connective tissue contents of commercially prepared composite meats are presented in Table IV. In this approach the content of total collagen in meats and meat products was determined from the amounts of Lys(5-OH) found in their acid hydrolysates and the content of total connective tissue proteins from the amounts of Pro(4-OH) present from eq 4. The accuracy of such calculations, however, will depend on the purity of the collagen or elastin on which their Lys(5-OH) and Pro(4-OH) contents are based. Although Pro(4-OH) was once thought to be unique to collagen (Eastoe, 1967) except for the comparatively small amounts (1.3–1.6%) found in elastin (Bendall, 1967), and this amino acid can be used as an index for determining the connective tissue content of various muscle tissues such as hamburger and all-beef sausage without condiments, its application to composite meats is limited for the following reasons. Recent evidence has shown that Pro(4-OH) seems to be a constituent of certain seed proteins (Zarkadas et al., 1982), including lectins (Allen and Neuberger, 1973) and extensin, which is found in the primary cell walls of plants and seeds (Lamport, 1977; Fincher et al., 1983; McNeil et al., 1984; Cooper et al., 1987). Therefore, the values reported for the connective tissue contents of processed meats in Table IV, which usually include plant protein additives such as soya bean and other types of oilseed proteins, could be an overestimate of the actual levels of connective tissue proteins found in composite meats.

For these reasons, the determination of Lys(5-OH) is now being routinely used in this laboratory as an index for quantitating the collagen content of various muscles and composite meats, since this amino acid remains relatively constant among the various genetic forms of collagen (Miller and Gay, 1982; Cheah, 1985; Light, 1985) and because the relative distribution of the various collagen isoforms among normal skeletal muscles also appears to be constant (Light and Champion, 1984; Light et al., 1985; Zarkadas et al., 1988a). The average collagen content of 32 bovine skeletal muscle tissues accounts for 4.2% of the total muscle proteins (Bendall, 1967; Dransfield, 1977; Light and Champion, 1984; Light et al., 1985), and the transcellular insoluble matrix in skeletal muscle accounts for 1.8% of the total muscle proteins (Loewy et al., 1983). Thus, the higher collagen value (11.01%) determined in the hamburger samples (Table IV), compared to 4.2% collagen calculated for skeletal muscles, may be attributed to cheaper meat cuts being used in this product. These data correspond closely to those of Terrell (1982) for beef plate and cow meat.

Added Nonmuscle Proteins. From the data presented in Table IV it is apparent that differences in the collagen composition exist among the meat products analyzed. Mean values for total collagen ranged from 7.35% to 16.5% in meat products. If the amount of collagen normally associated with muscle 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, mixed-meat sausages contained 16.5% total collagen (Table IV), 14.6% of which was added as nonmuscle collagen to this product. Values for collagen being added to all-beef sausages without condiments and frankfurters were high and ranged from 5.7 to 14.6 g/kg of total protein. The bologna and wiener samples contained the lowest levels of collagen of all the products studied. Similarly the total extracellular matrix proteins, which include collagen, elastin, transcellular proteins, fibronectin, etc., of composite meats was calculated from eq 6g, and the results are presented in Table IV.

From the foregoing results, it is evident that this proposed direct approach for evaluating the protein quality of composite meats is 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 chemical approach the myofibrillar myosin and actin contents of muscles and prepared composite meats marketed today can be determined from the amounts of His(τ-Me) found in their acid hydrolysates, and collagen and collagen-like proteins can be calculated from the amounts of Lys(5-OH) present. Therefore, when the sum of the intracellular and extracellular matrix skeletal muscle proteins is subtracted from the total protein of a composite meat hydrolysate sample, the difference is an accurate assessment of the nonmuscle proteins present (Table IV). This direct approach has the advantage over other methods that complete separation of these unique basic amino acids is possible in a single analysis in less than 5.7 h and that the determination of all these classes of proteins can be carried out in both fresh muscles or processed meats, as well as animal protein supplements (Hulan et al., 1979; Zarkadas et al., 1986a; Nguyen et al., 1986). In addition, this method may prove especially valuable for industrial control and formulation and could be easily applied for enforcing meat regulations.

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Registry No. His(τ-Me), 332-80-9; Lys(5-OH), 1190-94-9.

Supplementary Material Available: Information on the amino acid composition and protein content of bologna, frankfurters, wieners, and hamburger (Tables V–VII) (6 pages). Ordering information is given on any current masthead page.

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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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[2]In memoriam.

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