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Comparison of the Amino Acid Composition of Two Commercial Porcine Skins (Rind)[1]

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The protein and amino acid contents of typical porcine skins (rind) produced in eastern and western Canada were compared to assess their protein quality and potential as a food or feed ingredient. Although wide ranges of values were found for moisture (43.1–76.3%) and each of the minerals analyzed, their total lipid content (12.8–47.4%) and the actual protein contents as determined by amino acid analysis did not differ significantly between eastern and western pig skins and ranged from 50.4 to 58.9% on a dry basis. The amino acid profiles from porcine skins from eastern and western Canada were similar, their calculated essential amino acid indices (32.9–35.9) were higher than previously reported, and all appeared limiting with respect to tryptophan, cyst(e)ine, tyrosine, and isoleucine. The mean residue weight for the amino acids in pig skin was 0.093777 μg/nmol, and correcting this mean residue weight for the absence of tryptophan and cyst(e)ine in protein hydrolysates resulted in a conversion factor, $F = 0.094021$ (μg). The chemical approach used in this study for evaluating protein quality of porcine skin was based on the direct chromatographic determination of its collagen and connective tissue contents. In this approach the content of collagen in pig skin (60–65.3%) was determined from the amounts of 5-hydroxylysine found and the content of total connective tissue proteins (70–82.5%) from the amounts of 4-hydroxyproline present.

INTRODUCTION

The definition of pork meat in the current Canadian Meat Inspection Regulations controlling meat products includes skin that normally accompanies the muscle after dressing, but excludes skin that has been detached primarily from the lard area of the back of dressed carcasses (Canada's Meat Inspection Act, 1979). Although such skins (rind) and skin trimmings have traditionally been utilized for the production of edible gelatin or for the manufacture of glue and luxury leather for shoes, garments, and upholstery (Heideman, 1979; Naghski, 1982), these uses consume only a minor portion of the pig skin supply.

According to Asghar and Henrickson (1982), about 65% of the annual production of hide collagen as gelatin in the United States is consumed in edible products such as desserts, marshmallows, jellied meat, bakery foods, ice cream, and other products, while the remaining 35% is used by the photographic, metallurgical, cosmetic, and pharmaceutical industries (for reviews see: Chvapil, 1979; Rose, 1977; Wood, 1977; Naghski, 1982). The use of collagen as a feed supplement for animals and possibly as a food additive (Battista, 1975; Henrickson, 1980) in various meat products for human consumption holds promise, but its use will be related mainly to the nutritional quality of pig skin proteins and to the economics of the processes required.

Although numerous studies have described the distribution and occurrence of collagen types in skin tissues from several species, primarily human, bovine, and avian (for reviews see: Eastoe, 1967; Bornstein and Sage, 1980; Epstein, 1974; Miller and Gay, 1982; Eyre et al., 1984; Weiss, 1984; Light, 1985), and the use of skin collagen in the form of edible gelatin, there is a paucity of nutritional and compositional data on pig skin and its constituent proteins. Eastoe (1955) reported the amino acid composition of pig skin gelatin, prepared after alkaline pretreatment of the skin, gelatinization, alcohol coacervation of the gelatin and purification on ion-exchange resins. Chapman et al. (1959)

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and Rama-Rao et al. (1964) indicated that gelatin is limited in all of the essential amino acids except arginine. Nutritional trials by Ashley and Fisher (1966) and Leung et al. (1968) have shown that when either collagen or gelatin is fed to rats or chicks, it causes nutritive amino acid imbalances with a consequent loss of body mass. Laser-Reutersward et al. (1982) have found that pig skin collagen fed to Sprague–Dawley rats has a digestibility of about 95%. Similarly Chvapil (1979) has shown that pig skin trimmings could be mixed with other proteins as a supplement for chicks to give a diet that provides a more balanced composition of essential amino acids. Collagen preparations for use in the human diets (Bistrian et al., 1976) have also attracted considerable interest recently (Grundel et al., 1981) mainly because of the incidence of sudden deaths of several obese individuals (Spencer, 1968; Michiel et al., 1978) who were consuming liquid protein diets containing collagen or gelatin hydrolysates (beef hide extract) as a means of reducing body weight.

The current Meat Regulations in Canada (Canada's Meat Inspection Act, 1979) limit the use of excessive amounts of pork skin as an ingredient in the manufacture of comminuted meats since it would affect both their amino acid composition and their nitrogen content, upon which the quality and nutritive value of such products is assessed. The regulations in the United States (U.S. Department of Agriculture, 1981) require a minimum PER value of 2.5 and a minimum essential amino acid content of 32% for most fabricated meat products. In France and the Federal Republic of Germany regulations restricting the use of connective tissue in meat products have been in force for some years (Centre Technique de la Charcuterie, 1978; Deutsches Lebensmittelbuch, 1975). The Swedish National Food Administration has also proposed legislation to limit the collagen content in meat products (Janson, 1978). Thus, the studies described in this paper were conducted to ascertain the protein content and detailed amino acid composition of commercially available pig skins (eastern vs. western) in order to fully assess their potential as a food or feed ingredient.

MATERIALS AND METHODS

Sampling and Preparation of Pig Skin (Rind) Tissues. The six randomly selected pig skin (rind) tissues that were used in this analytical work originated from two different geographical locations (east vs. west) in Canada. The western samples from Vancouver, British Columbia, were provided by the Meat Hygiene Division, Food Production and Inspection Branch, Agriculture Canada, and the Bureau of Nutritional Sciences, Health Protection Branch, Health and Welfare Canada, while the eastern samples were supplied by North Packers Ltd., Montreal, Quebec. The pig skin tissues (approximately 2 kg each), which had been detached primarily from the lard area of the back (butts) of dressed carcasses from mature porcine animals (sows) and freed from the underlying lipid layer, were cleaned of adhering fat, frozen (−50 °C), and transported to the laboratory. Representative samples (each approximately 200 g) from these tissues were cut into small cubes, frozen (−50 °C), and then pulverized in a standard electrically driven end-runner mill (Straub Co., Croydon, PA). The sample was maintained frozen with additions of dry ice throughout the grinding process. The pig skin powders were then stored at −20 °C in sealed polypropylene bottles to prevent oxidative deterioration of the lipids present.

Chemicals and Resins. The Beckman type AA-10 9.0 ± 1.0 μm spherical resin and type I standard amino acid calibration mixture were obtained from Beckman Instru-

ments Inc., Palo Alto, CA. The Durrum type DC-6A 11.0 ± 1.0 μm spherical resin was purchased from Dionex Corp., Sunnyvale, CA. L-Tryptophan, D-glucosamine hydrochloride, and DL-ornithine (5-aminorvaline) were purchased from Schwarz/Mann, Orangeburg, NY. N^6-Trimethyl-L-[U-^{14}C]lysine hydrochloride 1.49 mCi/mmol) was obtained from New England Nuclear, Boston, MA. The diastereoisomer mixture of 5-hydroxy-DL-lysine and allo-5-hydroxy-DL-lysine, N^6-methyl-L-lysine, N^6-dimethyl-L- and N^6-trimethyl-L-lysine bis[p-(hydroxyazo)benzenesulfonate] hydrate, N^G,N^G-dimethyl-L- and N^G,N'^G-dimethyl-L-arginines, N^G-methyl-L-arginine bis[p-(hydroxyazo)benzenesulfonate] monohydrate, N^τ-methylhistidine hydrate, and N^τ-methyl-L-histidine were purchased from Calbiochem-Behring Corp., La Jolla, CA. Desmosine and isodesmosine were isolated by the preparative method described by Zarkadas (1979) using bovine Ligamentum nuchae elastin purchased from Sigma Chemical Corp., St. Louis, MO. All other chemicals and reagents were of the highest purity commercially available and were used without further purification.

Proximate and Elemental 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). Preparation of quadruplicate samples for elemental analyses was carried out by the wet digestion procedure using a mixture of concentrated nitric (15 mL)–perchloric (8 mL) acids in a 100 mL of Kjeldahl flask as described by Parks and Dunn (1963). Phosphorus determinations were carried out by the molybdovanadate method (Parks and Dunn, 1963). Similarly zinc, iron, potassium, sodium, and magnesium were determined separately by the official lanthanum oxide method (section 2.109; AOAC, 1980) using a fully automated atomic absorption spectrophotometer (Varian Model AA-975) equipped with a programmable sample changer (Varian Model 55) and printer plotter (Hewlett-Packard Model HP 82905A).

Procedures for Amino Acid Analyses. Amino acid analyses were carried out on either a conventional (Beckman Spinco Model 120C) or a fully automated amino acid analyzer (Beckman Spinco Model 121MB). The automated instrument was equipped with a module control (Autolab Spectra-Physics GmbH, 61 Darmstadt, West Germany) and a companion Autolab system AA (Beckman Methodology Bulletins AA-TB-001–AA-TB-014) for computing peak concentrations. The preparation of the amino acid calibration standards was carried out as previously described (Zarkadas, 1975, 1979).

Pig skin (rind) samples (0.5 g) were hydrolyzed in Pyrex test tubes (18 × 150 mm) under vacuum (below 10 μm of mercury) with 15 mL of triple-glass-distilled constant-boiling HCl (6.0 M) at 110 °C in duplicate for each of four times, 24, 48, 72, and 96 h, respectively, with the usual precautions described by Moore and Stein (1963). The small amounts of insoluble materials formed during acid hydrolysis and the fat plug were removed by filtration (0.22-μm Millipore microfilters) and washed with the same acid (6.0 M HCl). Foaming of hydrolysates was suppressed during evacuation by the addition of 5–10 μL of octanoic acid. The clear filtrate and washings were combined, evaporated to dryness in a Rotary EvapoMix (Buchler Instruments, Fort Lee, NJ) at 45 °C, and brought to volume (usually to 5 mL) with 0.2 M sodium citrate buffer, pH 2.2. The data reported for serine, threonine, and

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Table I. Average Values for Fat, Moisture, and Mineral Composition (g/kg of DM) of Pig Skin (Rind) Samples from Two Commercial Sources

	no. of determin	mean ● SEM	max	min	CV	$P > F^a$
moisture	18	597.83 ± 59.32	763.00	431.10	24.31	**
total lipid	18	306.81 ± 54.65	474.20	127.85	43.63	ns
total ash	18	8.869 ± 0.389	10.820	7.061	15.19	**
minerals						
calcium	9	0.161 ± 0.009	0.193	0.116	15.82	ns
phosphorus	9	0.538 ± 0.031	0.701	0.383	17.31	*
magnesium	9	0.187 ± 0.070	0.493	0.035	112.43	**
potassium	9	1.019 ± 0.073	1.452	0.774	21.61	**
iron	9	0.074 ± 0.012	0.126	0.045	45.74	**
zinc	9	0.007 ± 0.001	0.012	0.004	36.86	*
sodium	9	3.020 ± 0.142	3.661	2.581	14.11	**

$^a P > F$ test (eastern vs. western). Probability of a larger value of F. Significance: *, $P < 0.05$; **, $P < 0.01$; ns, not significant. Key: CV, coefficient of variation; SEM, standard error of means.

tyrosine 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). All others are reported as the average values from 24, 48, 72, and 96 h of hydrolysis (Zarkadas et al., 1982). 4-Hydroxyproline was determined separately from a concentrated hydrolysate (equivalent to 0.1 mg of protein/analysis) by the modified procedure of Piez and Morris (1960) using a single column (21 × 0.6 cm) packed with Dionex type DC-6A resin (Zarkadas et al., 1982). This column was eluted by a single buffer containing 0.20 M sodium citrate buffer, thiodiglycol (10 mL/L), 2-propanol (20 mL/L), and octanoic acid (0.1 mL/L) which was adjusted to pH 2.85 ± 0.01 at 25 °C. In this system, 4-hydroxyproline and aspartic acid were completely separated and emerged from the column at 28.5 and 32.7 min, respectively, at 30 mL/h and 44 °C. Recoveries of 4-hydroxyproline were calculated relative to alanine, which elutes at 68.5 min (Zarkadas et al., 1986; Zarkaolas et al., 1986).

Methionine and cyst(e)ine were determined separately (0.1 g) by the performic acid procedure of Moore (1963) as described previously (Hidiroglou and Zarkadas, 1976). Norleucine was added in the hydrolysates as an internal standard, and the recovery of cyst(e)ine as cysteic acid and methionine as the dioxide was calculated in proportion to the yields obtained by the performic acid treatment of standard solutions of these amino acids and relative to alanine and leucine present in the sample.

Tryptophan in pig skin samples (0.1 g) was determined separately after alkaline hydrolysis (Hugli and Moore, 1972) by an improved chromatographic procedure (Zarkadas et al., 1982). Samples were eluted from a 25 × 0.9 cm column of Dionex DC-6A resin amino acid analyzer (Beckman Model 120C) with 0.21 M sodium citrate buffer (pH 5.40) at 51 °C and a flow rate of 50 mL/h (827 kN/m²). Elution times for nitrotyrosine and tryptophan were 55 and 100 min, respectively (Zarkadas et al., 1986).

The determination of the diastereoisomers of 5-hydroxylysine and related compounds was carried out with concentrated hydrolysates (equivalent to 1–5 mg of protein) by the accelerated single-microcolumn (50 × 0.28 cm) system in the Beckman Model 121 MP amino acid analyzer as described previously (Zarkadas, 1979), so that peaks adequate for these compounds could be obtained.

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 design was carried out by the SAS (Statistical Analysis System) general linear model procedure (SAS, 1982).

RESULTS AND DISCUSSION

Animal skins are very complex tissues containing high levels of collagen (Bowes et al., 1955, 1957) and variable amounts of fat. Table I summarizes the overall fat and moisture contents and mineral composition of randomly selected pig skins from eastern and western Canada. Although significantly wide ranges were found for moisture (43.1–76.3%) and each of the minerals analyzed, their total lipid content (12.8–47.4%) on a dry matter basis (DM) did not differ significantly. The mean value (0.89%) for total ash (Table I) is comparable to that found in skin (0.8%) of other animal species (Ashgar and Henrickson, 1982). The data for the mineral composition of pig rind (Table I) show that each of the elements analyzed differed significantly ($P < 0.01$) for both products, with the exception of calcium. The least variability ($P < 0.05$) in mineral content for all pig skin samples was found in phosphorus and zinc. The coefficients of variation for the weighted means of phosphorus and zinc for all nine animals were 17.61 and 36.86, respectively. Coefficients of variation of 21.61, 45.74, and 112.43 were found ($P < 0.01$) for potassium, iron, and magnesium, respectively. The smallest coefficient of variation was found for the weighted mean of pig skin sodium concentrations ($P < 0.01$). Relative to variation between pork skins from different animals, variation due to quadruplicate sample determinations were small (Table I). This indicates that technical errors were small relative to biological variability.

A summary of the amino acid composition of pig skins produced in eastern and western Canada is presented in Table II. Calculations of the maximum, minimum, and mean values obtained from the overall amino acid composition of the pig skin (rind) samples, summarized in Table III, were based on the actual protein determined by the procedure described by Horstmann (1979). This method is based upon knowledge of the amino acid composition of the protein or protein mixture and yields accurate estimates of both the protein quality and the amount of protein present. According to this approach, a mean residue weight is calculated for the amino acids constituting the proteins in pig skin as

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

where a_i is the mole fraction of a specific amino acid i found in the analyzed aliquot and b_i is the weight (μg) of 1 nmol of amino acid residue i. Table III also lists the average values for the mole fraction (a_i) and the mean residue weight (WE, μg) for the amino acids in pig skin.

Table II. Amino Acid Composition (g of Amino Acids/kg of Protein) of Six Eastern- and Western-Produced Pig Skin (Rind) Samples (Sows)

	eastern		western		
	mean ± SEM[a]	CV	mean ± SEM[a]	CV	$P > F^b$
phenylalanine	24.65 ± 0.51	3.56	25.15 ± 1.80	12.43	ns
tyrosine	12.12 ± 0.94	13.38	16.20 ± 1.22	13.11	ns
histidine	12.42 ± 0.96	13.40	22.50 ± 2.65	20.41	*
isoleucine	18.17 ± 0.99	9.48	18.11 ± 1.50	14.15	ns
leucine	37.03 ± 0.98	4.61	40.06 ± 3.42	14.79	ns
methionine	15.74 ± 2.41	26.56	14.37 ± 0.74	8.88	ns
cysteine	1.81 ± 0.08	8.58	4.88 ± 0.19	6.97	**
valine	28.20 ± 0.57	3.48	37.28 ± 3.89	18.10	ns
arginine	85.12 ± 3.77	7.69	78.68 ± 4.41	9.72	ns
lysine	49.95 ± 1.60	5.56	43.06 ± 0.52	2.09	*
threonine	23.72 ± 0.69	5.09	22.58 ± 0.88	6.74	ns
tryptophan	0.93 ± 0.10	19.97	nd		
total essential AA	297.74		311.52		
total EAA, mg/g of N	1548.06		1650.68		
aspartic acid	64.58 ± 1.43	3.83	62.83 ± 1.89	4.68	ns
glutamic acid	97.53 ± 3.04	5.40	96.56 ± 2.12	3.81	ns
serine	37.87 ± 0.89	4.10	33.92 ± 1.19	6.11	ns
glycine	182.32 ± 3.17	3.01	186.72 ± 8.24	7.64	ns
alanine	69.42 ± 2.00	4.99	76.75 ± 3.37	7.61	ns
proline	133.13 ± 1.02	1.32	109.60 ± 5.02	7.93	*
4-hydroxyproline	100.66 ± 0.73	1.25	84.80 ± 4.19	8.57	*
5-hydroxylysine	6.31 ± 0.76	21.06	nd		
total	1001.68		977.02		
ammonia	5.48 ± 2.03		8.74 ± 2.3		
total nitrogen	185.13		184.80		
total protein, g/kg of DM	589.73 ± 92.92	27.29	504.47 ± 52.27	17.94	
EAA index[c]	32.96		35.73		
protein score,[d] %	14.20		13.60		
amino N, % of total protein	99.97		94.22		
connective tissue proteins,[e] %	82.50[f]		69.60[f]		
	81.92[g]		70.82[g]		
collagen,[e] %	65.30[f]		nd		
	60.00[g]		nd		

[a] Mean of 48 determinations ± standard error of means (SEM). For Ser, Thr, and Tyr the standard error of the estimates is used. [b] $P > F$ test (eastern vs. western). Probability of a larger value of F. Significance: *, $P < 0.05$; **, $P < 0.01$; ns, not significant. Key: nd, not determined, CV, coefficient of variation. [c] Calculated as described by Oser (1951). [d] Calculated as described by Block and Mitchell (1946). [e] Amino acid N as percent total protein N. [f] Data for pig skin gelatin from Eastoe (1955) according to eq 3. [g] Calf skin (9-month-old) collagen from Bailey and Sims (1976) according to eq 3.

A conversion factor F (μg) for determining the protein mass in each hydrolysate sample analyzed in the absence of tryptophan and cyst(e)ine was calculated according to

$$F = \frac{\sum_{i=1}^{20} (a_i b_i)}{1 - (a_{Trp} + a_{Cys})} \quad (2)$$

where a_i is the mole fraction of the specific amino acid i per mole of total amino acid composition. The F value, also listed in Table III, is a constant characteristic for pig skin and can be used in all subsequent quantitations of this tissue following standard procedures as described by Horstmann (1979) and Peterson (1983). It was found that the actual protein content as determined by amino acid analysis did not differ significantly between eastern and western pig skins and ranged from 50.4 to 58.9% on a dry basis.

Table II also compares the nitrogen contents of pig skins produced in eastern and western Canada calculated from their amino acid composition as described by Heidelbaugh et al. (1975) for Skylab foods. These authors found that the best estimate of the protein content of a food is the summation of the amino acid nitrogen content and recommended that whenever accurate data on the protein content of individual foods is required, conversion factors based on the actual amino acid composition should be used. In this study the protein conversion factor, calculated for pig skin from the mean amino acid nitrogen content given in Table II, was 5.40. The low conversion

factor obtained for pig skin indicates the high connective tissue protein contents in these tissues.

The values obtained for the amino acid contents of both pig skin samples (eastern vs. western) show high reproducibility, and within the precision of the chromatographic procedure (100 ± 3.0%), recoveries were found to be quantitative for all amino acids (Table II) except for arginine and glycine, which showed a slightly higher error and as shown in Table II do not differ significantly. The cyst(e)ine, however, shows highly significant variation ($P < 0.01$), being much lower in the eastern pig skins than in the western product. The lower cyst(e)ine content in the eastern pig skin compared with that of the western product (Table II) may therefore reflect a lower content of epidermal α-keratins in these samples (Sun and Green, 1978; Green et al., 1982). Significant variations ($P < 0.05$) were also noted in the case of histidine, lysine, proline, and 4-hydroxyproline. A comparison between the essential amino acid (EAA) profile of the eastern and western samples and the EAA profile of the whole egg indicated that pig skin was limited with respect to tryptophan, cyst(e)ine, tyrosine, and isoleucine and that the EAA indices (eastern 32.9; western 35.9) were higher than previously believed. These values, however, are considerably higher than the chemical score value of 30 reported by Laser-Reutersward et al. (1982). The low protein score values found for both pig skin samples reflects their high percentage deficit in tryptophan as shown in Table II. In such calculations, the high cyst(e)ine deficit (19–47.6%) can be disregarded, in view of the known relationship of cysteine and methionine

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Table III. Average, Maximum, and Minimum Values ± Standard Error of Means (SEM) and Coefficient of Variability (CV) for Total Protein, Nitrogen, and Amino Acid Composition of Six Pig Skin (Rind) Samples from Two Different Commercial Sources

| | g of AA/kg of protein | | | | mean[b] mole fraction (a_i) | mean[b] wt equiv ($a_i b_i$) |
	mean ± SEM[a]	max	min	CV		
aspartic acid	63.71 ± 2.61	66.48	59.52	4.10	0.052 352	0.006 026
threonine	23.15 ± 1.38	24.96	20.82	5.85	0.021 657	0.002 190
serine	35.89 ± 2.71	39.64	31.91	7.56	0.038 973	0.003 395
glutamic acid	97.05 ± 4.10	100.71	91.45	4.22	0.071 100	0.009 179
proline	121.37 ± 14.05	134.99	103.04	11.58	0.118 221	0.011 479
glycine	184.52 ± 9.97	195.10	170.24	5.40	0.305 639	0.017 452
alanine	73.09 ± 5.88	80.25	67.08	8.05	0.097 228	0.006 913
cysteine	3.34 ± 1.70	5.16	1.60	50.81	0.003 064	0.000 516
valine	32.74 ± 6.59	43.52	27.29	20.12	0.031 247	0.003 097
methionine	15.05 ± 2.86	19.89	11.53	19.02	0.010 849	0.001 423
isoleucine	18.14 ± 1.95	20.55	15.44	10.77	0.015 157	0.001 716
leucine	38.55 ± 4.24	46.51	34.86	10.99	0.032 209	0.003 646
tyrosine	14.16 ± 2.80	18.49	11.06	19.78	0.008 206	0.001 339
phenylalanine	24.90 ± 2.07	28.22	21.97	8.32	0.015 999	0.002 355
histidine	17.46 ± 6.33	25.91	10.69	36.25	0.012 036	0.001 651
lysine	46.51 ± 4.20	52.86	42.42	9.03	0.034 313	0.004 399
arginine	81.90 ± 7.28	90.35	70.07	8.87	0.049 59	0.007 746
tryptophan	0.93 ± 0.18	1.14	0.81	19.96	0.000 472	0.000 088
4-hydroxyproline	92.73 ± 9.85	102.09	76.67	10.63	0.077 546	0.008 770
5-hydroxylysine	6.31 ± 1.33	7.71	5.07	21.06	0.004 139	0.000 597
total AA	991.50				1.000 000	
ammonia	7.11 ± 2.31	8.74	5.48			
total nitrogen, g/kg of DM	101.36					
protein, g/kg of DM	547.1 ± 60.3	589.73	504.47			
mean residue wt (WE)[b] μg (defined by eq 1)						0.093 777
conversion factor F,[b] μg (defined by eq 2)						0.094 021
connective tissue proteins,[c] %	75.98[d]					
	77.31[e]					
collagen,[c] %	65.30[d]					
	60.00					

[a] Mean values and standard deviations for 92 determinations (six samples). Cv, coefficient of variation. [b] The WE and F constants were calculated from the amino acid composition found in the hydrolysates according to eq 1 and 2, respectively, and represent the means of 92 determinations (Horstmann, 1979). [c] Amino acid N as percent of total protein N. [d] Data for pig skin gelatin from Eastoe (1955) according to eq 3. [e] Calf skin (9-month-old) collagen from Bailey and Sims (1976) according to eq 3.

in metabolism, whereby methionine is convertible to cysteine but the reverse reaction does not occur (Cooper, 1983). The calculated protein score for the sum of methonine plus cyst(e)ine in both samples was over 75% compared to only 34% reported by Laser-Reutersward et al. (1982). Similarly, the limiting amino acids tyrosine and especially isoleucine showed relatively narrow ranges of values and corresponding low coefficients of variability (Tables II and III), and their chemical scores were over 65% compared to only 34% reported by Laser-Reutersward et al. (1982). The results presented in Table II suggest that pig skin (rind) or skin trimmings can be utilized, within limits, as a suitable food additive, provided the protein quality (PER of 2.5) and essential amino acid balance (32% of total AA) of the product are maintained as recommended by the U.S. Department of Agriculture (1981). Although the specified levels for maintaining the protein quality can be quite readily achieved through manipulation of the proportion of the other protein sources in the product, Bodwell (1977) indicated that maintaining such high levels of PER values (2.5) and percentage of the seven essential amino acids (32%) would economically be costly. However, the earlier finding by Ashley and Fisher (1966) that chicks fed on a 10% gelatin plus 3% casein diet had weight gains equal to those fed on a 13% soy protein plus 0.2% methionine diet provides support for use of collagen as an economical feed supplement. Satterlee et al. (1973) found that when enzymatic hydrolysates of beef or pork skin were used in sausage formulation, it gave the sausage emulsion increased stability during cooking and improved the water and fat holding capacity of the product. Similar results were reported by Puolanne and Ruusunen (1981) for raw pig skin used in sausage formulation.

The data presented in Tables II–IV indicate that pig skin is rich in glycine (30%), proline (12.9%), alanine (9.2%), and 4-hydroxyproline (8.4%). About 62.1% of the amino acid residues in pig skin is nonpolar (hydrophobic) compared to 63.5% in type I calf skin collagen (Table IV). These differences suggest that, in addition to collagen, pig skin must contain various other proteins that are relatively rich in tyrosine, threonine, and serine and possibly leucine, isoleucine, and aspartic acid, which have but little or no 4-hydroxyproline.

Bowes et al. (1955, 1957) have shown that fresh calf skin consists largely of water (60–65%), some 30–35% protein, excluding the hair, 1–10% grease, and a small amount of carbohydrate (0.5–1.0%; Moss, 1955). According to these authors, of the 30–35% protein, about 4–6% is made of the interfibrillary proteins such as albumins and globulins of the tissue fluids, about 0.5–1.0% represents the epidermis, and 0.5% represents insoluble protein after autoclaving and there are small amounts of muscle proteins, elastin, and reticulin, but by far the greater part of the bovine adult or calf skin protein is collagen, ranging from 90 to 95%. Their data, however, appear to reflect the connective tissue protein content that includes collagen, elastin, etc., rather than collagen alone. Laser-Reutersward et al. (1981) have reported the essential amino acids and chemical scores for pig skin and found that it contains 78.9% collagen. Since their collagen value was based upon the amounts of 4-hydroxyproline found in pig skin multiplied by a factor of 7.1 as recommended by Prandl et al. (1967), their data may also reflect the total connective tissue content of pig skin that includes collagen. Although quantitation of 4-hydroxyproline (Piez and Morris, 1960; Berg, 1982; Lindblad and Diegelmann, 1984) was once the

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Table IV. Comparison of the Amino Acid (AA) Composition of Pig Skin (Rind) with Other Mammalian and Avian Skin Collagens and Their Subunits (Number of AA Residues/1000 Total AA Residues)

| amino acid | porcine skin | | bovine skin collagen | | | total collagen | | |
	rind[a]	gelatin[b]	total[c]	type I[d] $[\alpha(I)_2\alpha2]$	type III[d] $[\alpha(III)]_3$	avian[e] skin	guinea[f] skin	human[g] dermis
4-hydroxyproline	83.5	95.5	87.0	93	118	106	100	93
aspartic acid	57.7	46.8	45.2	45	45	45	49	45
threonine	22.1	17.1	17.8	17	15	19	20	17.5
serine	40.9	36.5	37.1	33	37	29	39	35.6
glutamic acid	71.0	72.0	70.9	74	70	73	69	73
proline	128.8	130.4	133.8	132	105	118	123	128
glycine	300.2	326.0	324.1	323	344	332	331	330
alanine	91.8	92.1	110.8	111.7	90	116	103	110
cysteine	1.7			0	1.5			
valine	26.7	21.9	22.3	21	14	18	23	24.4
methionine	11.3	5.4	6.4	5	5	7.4	5.8	6.2
isoleucine	15.1	9.6	11.7	13	12	10	9.7	9.5
leucine	30.8	23.7	24.6	24	18	24	23	24.3
tyrosine	7.0	3.2	3.0	2	2	1.6	2.7	2.8
phenylalanine	15.7	14.4	13.2	12	8	12.0	10	12.0
5-hydroxylysine	4.1	5.9	7.4	7	6	6.9	5.5	5.8
lysine	36.6	26.2	26.8	29	28	27	33	26.9
histidine	8.5	6.0	4.9	5	8	4.2	4.8	4.8
arginine	51.2	48.2	52.4	52	46	51	48	51
tryptophan	0.5							
Pro(4OH)/(Pro + Pro(4OH))	0.39	0.42	0.39	0.41	0.53	0.47	0.45	0.42
Lys(5OH)/(Lys + Lys(5OH))	0.10	0.18	0.22	0.19	0.18	0.20	0.14	0.18

[a] Means of 92 determinations (sows), present study. [b] Pig skin gelatin from Eastoe (1955). [c] Calf skin collagen from Piez et al. (1960). [d] Nine-month-old steer skin collagen from Bailey and Sims (1976). [e] One-day-old white Leghorn chick skin collagen from Kang et al. (1969). [f] Guinea pig skin collagen from Nold et al. (1970). [g] Infant skin collagen from Bornstein and Piez (1964).
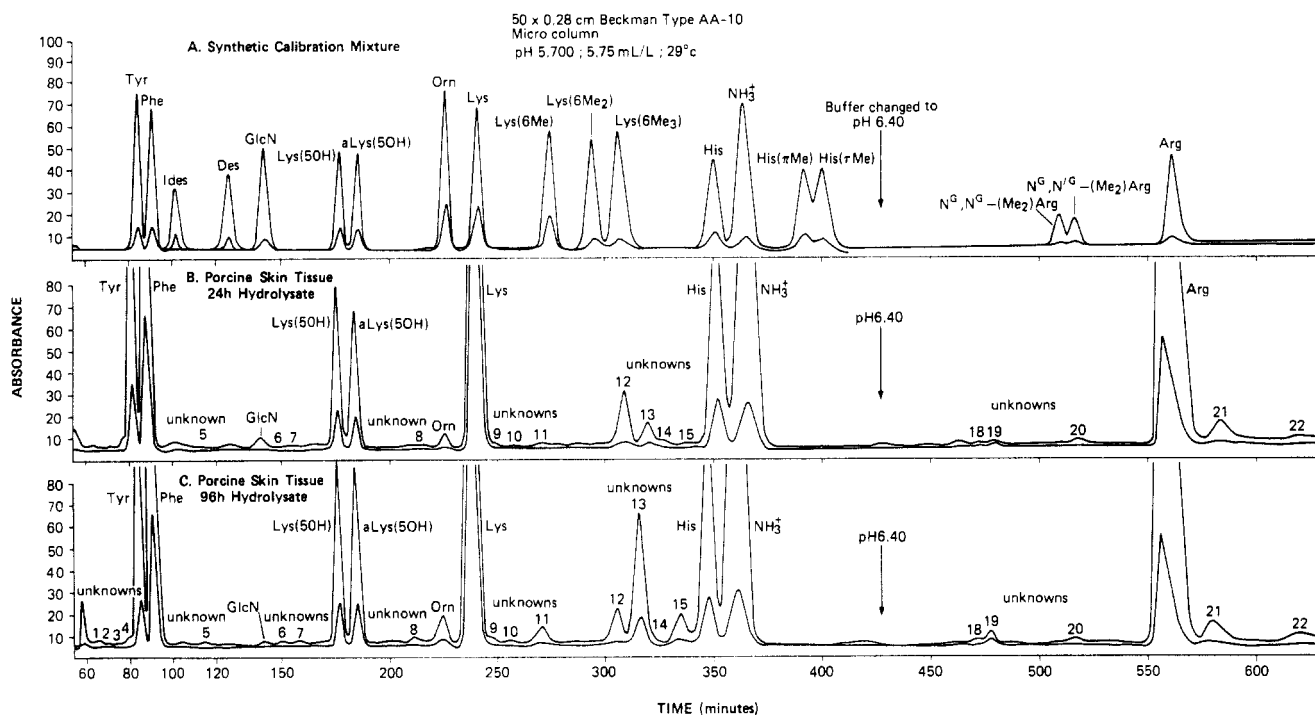
Figure 1. Chromatographic separations of all methylated basic amino acids, the diastereoisomers of 5-hydroxylysine, and related compounds in porcine skin (rind) tissue hydrolysates: (A) separation of a synthetic amino acid calibration mixture; (B) typical chromatographic separation of a 24-h hydrolysate of porcine skin tissue; (C) analysis of a 96-h porcine skin tissue hydrolysate. The upper curve shows absorbance at 570 nm and the lower curve the absorbance of 440 nm. Key: Ides, isodesmosine; Des, desmosine; GlcN, glucosamine; Lys(5OH), 5-hydroxylysine; aLys(5OH), *allo*-5-hydroxylysine; Orn, ornithine; Lys(6Me), N^6-methyl-L-lysine; Lys(6Me₂), N^6-dimethyl-L-lysine; Lys(6Me₃), N^6-trimethyl-L-lysine; His(πMe), N^{π}-methylhistidine; His(τMe), N^{τ}-methylhistidine; N^G,N^G-(Meu)Arg, ω-N^G,N^G-dimethylarginine (unsymmetrical); N^G,N'^G-(Mes)Arg, ω-N^G,N'^G-dimethylarginine (symmetrical).

standard procedure for assessing the collagen content in tissues or foods (Eastoe, 1967; Pearson, 1975; Etherington and Sims, 1981; Etherington et al., 1984), its use as an index for determining collagen in such tissue is limited, since this hydroxylated amino acid has also been found in a limited number of residues in animal proteins other than collagen [e.g., elastin, Bentley and Hanson (1969), Franzblau (1971); acetylcholinesterase, Anglister et al.

(1976); Clq complement protein, Porter and Reid (1978)] and in plant proteins [e.g., lectins, Allen and Neuberger (1973); extensin, the structural glycoprotein of dicotyledonous primary cell walls, Adams and Frank (1980), McNeil et al. (1984)] as well as in oat groats, corn, and potato tissues (Zarkadas et al., 1982; Hulan et al., 1982).

In this study, an attempt was made to relate the protein quality of pig skin to its amino acid composition by the

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direct determination of its connective tissue and collagen contents by the accelerated single-column chromatographic method developed in this laboratory (Zarkadas, 1979). In this chemical approach, collagen is determined from the amounts of 5-hydroxylysine found in pig skin acid hydrolysates. Connective tissue, which includes collagen, elastin, etc., can also be determined from the amounts of 4-hydroxyproline present. Analysis of pig skin hydrolysates by this method (Figure 1) enabled the complete separation of the diastereoisomers of 5-hydroxylysine and revealed four major (peaks 12, 13, 15, and 21) and 18 minor as yet unidentified stable components. It should be noted that the relative size of two major unknown peaks, designated as 13 and 15, increased with time (96 h) of hydrolysis (Figure 1C) while another unknown (12) decreased considerably (Figure 1B,C). As may be seen in Figure 1C, an accurate determination of the 5-hydroxylysine content of pig skin can be made from the sum on the values obtained for its diastereoisomers after epimerization in 6 M HCl at 110 °C for 96 h (Zarkadas, 1975). This chemical approach (Zarkadas, 1975, 1979, 1981) is now being routinely used in this laboratory to assess the collagen and connective tissue contents and protein quality of various animal tissues including pig skin as follows:

$$\frac{\text{amt Lys(5OH) N as \% total pig skin protein} - N}{\text{amt same AA N as \% total pig skin gelatin}} \times$$
$$100 \quad (3)$$

The accuracy of such calculations, however, will depend on the purity of the collagen on which their 5-hydroxylysine and 4-hydroxyproline contents are based. Although the structure and amino acid composition of purified skin collagens from various species (Table IV) has been investigated quite thoroughly (Bornstein and Sage, 1980; Miller and Gay, 1982; Eyre et al., 1984), the only biochemical studies that have been done on collagen of porcine skin are those of Eastoe (1957, 1967) who reported on gelatin, a relatively pure form of skin collagen. If his results, as summarized in Table IV, are used to calculate the present pig skin data (Table III), the collagen averages 65.3% and connective tissue averages 75.98% of pig skin (Table III). On the other hand, if the 5-hydroxylysine (1.10 N as percent total collagen N) and 4-hydroxyproline (8.02 N as percent total collagen N) data of Bailey and Sims (1976) for the two distinct collagen isotypes (types I and III) purified from calf skin are used (Table IV), the values for collagen and connective tissue proteins averaged 60.0 and 77.31%, respectively (Table III). The ratio of type I to type III collagen is normally 4:1 (Bailey and Sims, 1976; Epstein, 1974). Although the data reported for the connective tissue content for pig skin in Table III (75.98–77.31%) are in reasonable agreement with the value (78.9%) reported by Laser-Reutersward et al. (1981), it differs considerably from that reported for bovine hide collagen (90–95%) by Bowes et al. (1957). The higher collagen value (65.3%) determined from Eastoe (1967) pig skin gelatin data, compared to 60.0% collagen calculated from the data of Bailey and Sims (1976), may be attributed to the purity of the pig skin gelatin since the calculated connective tissue values in both cases appear to be very similar. The possibility remains, of course, that there exist minor differences in the extent of lysine hydroxylation between the collagens of pig and calf skin.

From the foregoing results, it is evident that the collagen content of pig skin (Tables II–IV) can be determined accurately from the amounts of 5-hydroxylysine present in its acid hydrolysates and that this direct approach could be easily applied on a routine basis for industrial control

and formulation or for enforcing meat regulations. This approach has the additional advantage that it can be applied to the determination of the collagen content of fresh, cooked, or processed pig skin proteins as well as processed meats and bone meals and other animal tissue including fish (Zarkadas et al., 1986a).

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