# Assessment of the Protein Quality of Beefstock Bone Isolates for Use as an Ingredient in Meat and Poultry Products†

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The total protein, amino acids including 4-hydroxyproline, and estimated connective tissue proteins of three processed beefstock bone isolates were determined as potentially useful indices for evaluating their protein quality. Variations in amino acid composition were found among all three batches of beefstock bone isolates investigated. The total protein of demineralized beefstock bone powders, as determined by amino acid analysis, varied ($P < 0.05$) and ranged from 78 to 80% on a dry weight basis. Compared to the FAO/WHO essential amino acid ($EAA_9$) reference value of 33.9%, mean values for total EAA ranged from 22.2 to 22.9%, and the calculated mean protein efficiency ratio values (PER) ranged from 1.35 to 1.54. Total connective tissue proteins (72.3–81.1%) were determined from the amounts of 4-hydroxyproline present. It is concluded that this demineralized bone product may be used in limited amounts in meat mixtures without significant effect on the nutritive value.

**Keywords:** *Bone; beefstock; assessment; protein quality; amino acids; composition*

## INTRODUCTION

Collagen-based byproducts of the meat packing industry are becoming increasingly important for possible use as ingredients in various meat and poultry products. Such collagen byproducts include skins (rind) and skin trimmings, tendon, beef shank and residue sinew, and hand or mechanically separated bones from beef, pork, and poultry carcasses. These products have traditionally been utilized for the production of edible gelatin (Chvapil, 1979; Naghski, 1982; Nguyen et al., 1986) and for the manufacture of bone meal for pig, mink, and poultry diets but have constituted only a minor portion of the connective tissue animal protein supply. The use of commercially demineralized beef bone powders (termed beefstock bone isolates) as a feed supplement for animals and possibly as a food additive in various meat and poultry products for human consumption holds promise (Hegedus et al., 1983, 1990; Nelson et al., 1989). However, increased use of beefstock protein isolates will be related to both the economics of the processes required and its nutritional quality.

The extracellular organic matrix of bone constitutes approximately 35% of the tissue by weight, and the inorganic minerals constitute approximately 65% (Eastoe and Eastoe, 1954; Eastoe, 1956; Glimcher and Krane, 1968). Bone consists largely of a type I collagen, which accounts for 90–95% of the extracellular matrix along with other noncollagenous proteins with hydroxyapatite crystals deposited mostly on the fibril surfaces. The constituent noncollagenous proteins of the bone matrix include various glycoproteins, which includes osteonectin, sialoproteins I and II, phosphoproteins, γ-carboxyglutamic acid (Gla)-containing proteins, proteoglycans PGI and PGII, and serum-derived proteins.

Data on the total amino acid composition of bone meals and isolates have not been widely reported (Eastoe and Long, 1960; Hegedus et al., 1983, 1990), and there is little information on nitrogen and amino acid content of such collagen-based byproducts as beefstock bone isolates. Efforts to assess the protein quality of bone meals using rat bioassays have shown the product to be highly variable, mainly because of the extensive differences in both the chemical composition of the raw material and in variations in the heat treatment of individual batches (Hegedus et al., 1983, 1990; Jorgensen et al., 1984; Skilton et al., 1991). The protein quality of these collagen-based byproducts has become a subject of major interest to both food manufacturers and regulatory agencies concerned with the development of standards for such animal protein byproducts. An accurate assessment of the protein quality and nutritional adequacy of the commercially available beefstock bone isolates for use as an ingredient in meat and poultry product is therefore essential.

## MATERIALS AND METHODS

**Materials.** Type DC-5A (lot no. 746) and DC-5A (lot no. 775) cation-exchange spherical resins, sized to 6.0 ± 1.0 and 11.0 ± 0.5 μm, respectively, were purchased from Dionex Chemical Co., Sunnyvale, CA. The standard amino acid calibration mixture was purchased from Beckman Instruments, Inc., Palo Alto, CA. Other amino acids used as standards were obtained as follows: 4-hydroxyproline from Calbiochem-Behring Corp., La Jolla, CA; L-tryptophan from Schwarz/Mann, Orangeburge, NY; norleucine from Pierce Chemical Co., Rockford, IL; and 3-nitro-L-tyrosine from Aldrich Chemical Co., Milwaukee, WI. Octanoic acid was obtained from Eastman Kodak Co., Rochester, NY, and phenol was a product of J. T. Baker Chemical Co., Phillipsburg, NJ. Reagents and buffers were made with high-purity laboratory water as described previously (Zarkadas et al., 1987). All other chemicals and reagents were of the highest purity commercially available and were used without further purification.

**Experimental Procedures.** *Protein Ingredients.* The three batches of collagen based (beefstock) protein ingredients prepared from meat and poultry products, BS037, BS038, and BS126, were obtained from manufacturer I in eastern Canada in a relatively finely powdered state.

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**Table 1. Comparison of the Amino Acid (AA) Composition and Protein Contents (Grams of Amino Acids per Kilogram of Total Protein) of Three Collagen-Based (Beefstock) Protein Ingredients for Use in Meat and Poultry Products**

| | selected beefstock bone protein additives | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | BS037 | | BS038 | | BS126 | | | signif levels among ingredients | |
| AA | mean ± SEM[a] ($N = 3$) | CV | mean ± SEM[a] ($N = 3$) | CV | mean ± SEM[a] ($N = 3$) | CV | wtd mean ± SEM[a] ($N = 9$) | CV | $F$ |
| aspartic acid | 63.69 ± 0.37 | 1.00 | 60.59 ± 0.48 | 1.38 | 63.21 ± 1.89 | 5.21 | 62.49 ± 0.75 | 3.19 | 2.08[ns] |
| threonine | 20.59 ± 0.66[d] | 5.57 | 25.75 ± 1.19[b] | 8.06 | 23.86 ± 0.17[c] | 1.25 | 23.40 ± 0.85 | 5.90 | 10.73** |
| serine | 28.18 ± 1.13 | 6.93 | 31.28 ± 0.13 | 0.74 | 35.42 ± 3.42 | 16.72 | 31.62 ± 1.47 | 11.39 | 3.05[ns] |
| glutamic acid | 113.32 ± 0.38[b] | 0.58 | 111.84 ± 1.05[b,c] | 1.63 | 109.06 ± 0.99[c] | 1.57 | 111.41 ± 0.76 | 1.34 | 6.29* |
| proline | 117.83 ± 0.54[b] | 0.79 | 115.23 ± 0.76[b,c] | 1.06 | 111.98 ± 2.54[c] | 3.92 | 115.01 ± 1.15 | 2.33 | 3.57[ns] |
| glycine | 195.39 ± 1.20[c] | 1.07 | 204.04 ± 1.07[b] | 0.91 | 200.70 ± 1.87[b] | 1.62 | 200.04 ± 1.44 | 1.23 | 9.35** |
| alanine | 85.24 ± 0.18[b] | 0.37 | 83.30 ± 0.51[c] | 1.06 | 83.27 ± 0.21[c] | 0.43 | 83.93 ± 0.36 | 0.69 | 11.46** |
| cysteine | 2.48 ± 0.005 | 0.37 | 2.88 ± 0.35 | 21.07 | 2.41 ± 0.01 | 0.79 | 2.59 ± 0.12 | 13.53 | 1.56[ns] |
| valine | 32.25 ± 0.22[b] | 1.19 | 32.02 ± 0.07[b] | 0.42 | 31.46 ± 0.09[c] | 0.54 | 31.91 ± 0.13 | 0.79 | 7.68* |
| methionine | 15.09 ± 0.04[b] | 0.45 | 15.24 ± 0.08[b] | 0.96 | 14.66 ± 0.02[c] | 0.25 | 14.99 ± 0.09 | 0.64 | 29.96*** |
| isoleucine | 18.51 ± 0.05[b] | 0.43 | 18.03 ± 0.11[c] | 1.11 | 18.05 ± 0.09[c] | 0.88 | 18.19 ± 0.09 | 0.85 | 9.46** |
| leucine | 41.20 ± 0.09[b] | 0.36 | 41.38 ± 0.19[b] | 0.79 | 40.17 ± 0.12[c] | 0.52 | 40.91 ± 0.70 | 0.59 | 21.93** |
| tyrosine | 13.01 ± 0.59 | 7.97 | 13.25 ± 0.57 | 7.53 | 12.45 ± 0.59 | 8.26 | 12.91 ± 0.32 | 7.91 | 0.49[ns] |
| phenylalanine | 26.28 ± 0.23 | 1.53 | 26.38 ± 2.68 | 17.62 | 24.72 ± 0.10 | 0.74 | 25.79 ± 0.82 | 10.45 | 0.36[ns] |
| histidine | 12.98 ± 0.66 | 8.88 | 10.74 ± 0.04 | 0.71 | 12.59 ± 0.90 | 12.41 | 12.10 ± 0.47 | 9.27 | 3.40[ns] |
| lysine | 44.71 ± 0.20[b] | 0.79 | 38.62 ± 0.17[c] | 0.77 | 39.77 ± 1.49[c] | 6.52 | 41.03 ± 1.03 | 3.71 | 13.56** |
| arginine | 77.40 ± 0.78[b] | 1.75 | 69.77 ± 0.58[d] | 1.44 | 73.65 ± 0.59[c] | 1.39 | 73.62 ± 1.15 | 1.55 | 33.68*** |
| tryptophan | 1.82 ± 0.007[b] | 0.68 | 1.72 ± 0.01[c] | 1.31 | 1.57 ± 0.04[d] | 4.95 | 1.70 ± 0.03 | 2.78 | 21.02*** |
| 4-hydroxyproline | 90.03 ± 0.51[d] | 0.97 | 97.92 ± 0.45[c] | 0.79 | 100.98 ± 0.40[b] | 0.68 | 96.31 ± 1.65 | 0.81 | 155.28**** |
| ammonia | 15.54 ± 2.81 | 31.31 | 7.67 ± 0.67 | 15.15 | 12.51 ± 5.31 | 73.55 | 11.91 ± 2.09 | 50.79 | 1.29[ns] |
| total protein[e] g/kg dry mass | 780.08 ± 1.65[d] | 0.37 | 789.46 ± 5.05[c] | 1.11 | 800.95 ± 4.68[b] | 1.01 | 790.18 ± 3.65 | 0.89 | 6.57* |
| WE, μg/nmol[e] | 0.092105 ± 0.001 | 1.51 | 0.09207 ± 0.0001 | 0.22 | 0.092309 ± 0.0001 | 0.18 | 0.092162 ± 0.0002 | 0.89 | 0.07[ns] |
| CF, μg/nmol[e] | 0.093039 ± 0.001[b] | 0.13 | 0.09215 ± 0.0001[c] | 0.23 | 0.09238 ± 0.0009[c] | 0.18 | 0.092524 ± 0.0001 | 0.18 | 21.75*** |
| CF, μg/nmol[e] | 0.114424 ± 0.002 | 0.32 | 0.113647 ± 0.0002 | 0.33 | 0.11391 ± 0.0002 | 0.36 | 0.11399 ± 0.0002 | 0.34 | 3.09[ns] |

[a] Mean values and standard error of measurements (SEM) for 3 replications ($N = 3$) and 48 determinations. Significance: $F$ values; ****, $P < 0.0001$; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; CV, coefficient of variation; ns, not significant. [b–d] Means along a horizontal column with different superscripts are significantly different (Duncan, 1955). [e] Protein mass was determined according to the method of Horstmann (1979), and WE, CF, and CF′ constants were calculated according to the methods of Horstmann (1979) and Zarkadas et al. (1988a–c) using eqs 1–3.

*Procedures for Amino Acid Analyses.* Amino acid analyses were carried out on either a conventional (Beckman Model 120C) or a fully automated amino acid analyzer (Beckman Model 121MB) as desccribed previously (Zarkadas et al., 1986, 1987, 1990). The conventional instrument was equipped with a module control Autolab System AA (Beckman Methodology Bulletins AA-TB-001 to AA-TB-014) for computing peak concentrations (Zarkadas, 1975). The automated instrument was interfaced with a Beckman Model 406 analog interface module, the system Gold (Beckman Instrument, Inc., Altex Division, San Ramon, CA) chromatographic data reduction system, and an IBM (AT-series) compatible personal computer, which was obtained from Microcom AL Computer, Ottawa, ON. The incorporation of these components to the system increased the sensitivity of the analysis and enabled quantitation of amino acids at the picomole level as described previously (Zarkadas et al., 1987).

Complete amino acid analyses were carried out on each of three replicate samples of the bone (beefstock) protein isolate (50.0 mg) according to the standard chromatographic procedures described previously (Zarkadas et al., 1986, 1987). Each of the three dried replicate samples was divided into two subsamples, i.e., A and B, which were then hydrolyzed in duplicate in Pyrex test tubes (18 × 150 mm) under vacuum (below 10 mmHg) with 5.0 mL of triple-glass-distilled constant-boiling HCl (6.0 M; 20.5% v/v) at 110 °C for 24, 48, 72, and 96 h, respectively, following the precautions described previously (Zarkadas et al., 1987, 1988a). The data reported for serine, threonine, and ammonia in Tables 1 and 2 represent the average values of 48 determinations extrapolated to zero time of hydrolysis, carried out by linear regression analysis of the results. The values for valine, isoleucine, leucine, and phenylalanine are the average of 24 determinations obtained from 72 and 96 h of hydrolysis. All other values are reported as the average values of 48 determinations obtained from 24, 48, 72, and 96 h of hydrolysis.

Methionine and cyst(e)ine were determined separately (50.0 mg samples) according to the performic acid procedure of Moore (1963). Norleucine was added in the hydrolysates as an internal standard, and recoveries of cyst(e)ine as cysteic acid and methionine as the dioxide were calculated in proportion to the yields obtained by the performic acid treatment of standard solutions of these amino acids and relative to alanine, valine, leucine, and isoleucine present in the sample (Zarkadas et al., 1988a).

Tryptophan in these animal protein ingredients (50.0 mg) was determined separately after alkaline hydrolysis (Hugli and Moore, 1972) by an improved chromatographic procedure using 3-nitrotyrosine [Tyr($NO_2$)] as an internal standard (Zarkadas et al., 1986).

Determination of the 4-hydroxyproline [Pro(4-OH)] content of the commercial animal protein ingredients was carried out separately from a concentrated hydrolysate (equivalent to 50.0 μg of protein per analysis) using a single column (21 × 0.6 cm) packed with Dionex DC-6A resin (Zarkadas et al., 1986). Recoveries of Pro(4-OH) were calculated relative to alanine, isoleucine, and leucine. The Pro(4-OH) data represent the average values of 24 determinations.

*Protein Determination.* Precise quantitation of the protein content in each of the animal protein ingredients hydrolysate was carried out according to the method of Horstmann (1979) as described previously (Zarkadas et al., 1988a–c). According to this method a mean residue weight (WE, in micrograms per nanomole) is calculated for the 18 standard amino acid residues plus Pro(4-OH) constituting the proteins in the bone protein isolates using the expression

$$WE = \sum_{i=1}^{19} (a_i b_i) \qquad (1)$$

where $a_i$ is the mole fraction of a specefic amino acid $i$ found in the analyzed aliquot and $b_i$ is the molecular weight of the amino acid residue $i$. A conversion factor CF (in micrograms per nanomole) was used for determining the protein mass in each hydrolysate sample analyzed in the absence of tryptophan and cyst(e)ine. Similarly, CF, which is the apparent average residue molecular weight in micrograms per nanomole, was

**Table 2. Amino Acid (AA) Composition and Nitrogen Contents of Selected Beefstock Protein Ingredients (Collagen Based) for Use in Composite Meats (Grams of Amino Acids per 16 g of Nitrogen)**

| AA | beefstock bone protein ingredients | | | | signif levels among ingredients | | Hegedus et al. (1983) | Eastoe and Long (1960) |
|---|---|---|---|---|---|---|---|---|
| | BS037 mean ± SEM | BSO38 mean ± SEM | BS126 mean ± SEM | wtd (N-9) mean ± SEM | CV | F | | |
| aspartic acid | 5.30 ± 0.09 | 5.27 ± 0.04 | 5.34 ± 0.07 | 5.31 ± 0.04 | 2.46 | 0.26$^{ns}$ | 6.83 | 6.5 |
| threonine | 1.17 ± 0.06$^{c}$ | 2.24 ± 0.11$^{b}$ | 2.02 ± 0.03$^{b}$ | 1.99 ± 0.08 | 6.75 | 11.56*** | 2.43 | 2.5 |
| serine | 2.34 ± 0.07$^{c}$ | 2.72 ± 0.00$^{b,c}$ | 2.99 ± 0.23$^{b}$ | 2.68 ± 0.11 | 8.79 | 5.61* | 3.47 | 3.7 |
| glutamic acid | 9.44 ± 0.14 | 9.73 ± 0.09 | 9.24 ± 0.28 | 9.47 ± 0.12 | 3.49 | 1.65$^{ns}$ | 11.09 | 10.1 |
| proline | 9.81 ± 0.08 | 10.02 ± 0.03 | 9.49 ± 0.42 | 9.78 ± 0.14 | 4.37 | 1.17$^{ns}$ | 9.93 | 11.7 |
| glycine | 16.28 ± 0.30$^{c}$ | 17.75 ± 0.09$^{b}$ | 17.01 ± 0.52$^{b,c}$ | 17.01 ± 0.27 | 3.58 | 4.38$^{ns}$ | 17.55 | 22.2 |
| alanine | 7.10 ± 0.16 | 6.91 ± 0.37 | 7.05 ± 0.17 | 7.03 ± 0.12 | 5.96 | 0.16$^{ns}$ | 8.38 | 9.2 |
| cysteine | 0.20 ± 0.002 | 0.25 ± 0.02 | 0.20 ± 0.004 | 0.22 ± 0.01 | 13.58 | 2.25$^{ns}$ | 0.24 | |
| valine | 2.69 ± 0.55 | 2.78 ± 0.005 | 2.66 ± 0.06 | 2.71 ± 0.03 | 3.03 | 1.84$^{ns}$ | 3.70 | 3.1 |
| methionine | 1.24 ± 0.004$^{c}$ | 1.33 ± 0.003$^{b}$ | 1.24 ± 0.03$^{c}$ | 1.27 ± 0.02 | 2.23 | 8.38* | 1.14 | 0.5 |
| isoleucine | 1.54 ± 0.02 | 1.57 ± 0.005 | 1.53 ± 0.03 | 1.55 ± 0.01 | 2.19 | 1.06$^{ns}$ | 2.13 | 1.7 |
| leucine | 3.43 ± 0.04$^{b,c}$ | 3.60 ± 0.008$^{b}$ | 3.40 ± 0.07$^{b,c}$ | 3.48 ± 0.04 | 2.55 | 4.32* | 4.70 | 4.0 |
| tyrosine | 1.08 ± 0.06 | 1.15 ± 0.05 | 1.05 ± 0.03 | 1.09 ± 0.03 | 7.84 | 1.07$^{ns}$ | 1.44 | 0.95 |
| phenylalanine | 2.19 ± 0.008 | 2.29 ± 0.24 | 2.09 ± 0.04 | 2.19 ± 0.07 | 11.13 | 0.53$^{ns}$ | 2.50 | 2.6 |
| histidine | 1.08 ± 0.057 | 0.93 ± 0.24 | 1.07 ± 0.09 | 1.02 ± 0.04 | 10.94 | 1.57$^{ns}$ | 1.13 | 0.94 |
| lysine | 3.72 ± 0.05$^{b}$ | 3.36 ± 0.002$^{c}$ | 3.36 ± 0.07$^{c}$ | 3.48 ± 0.06 | 2.52 | 17.00*** | 4.38 | 4.0 |
| arginine | 6.44 ± 0.02 | 6.07 ± 0.034 | 6.24 ± 0.18 | 6.25 ± 0.07 | 3.02 | 2.99$^{ns}$ | 7.35 | 7.3 |
| tryptophan | 0.15 ± 0.001$^{b}$ | 0.14 ± 0.00$^{b}$ | 0.13 ± 0.006$^{c}$ | 0.14 ± 0.003 | 4.75 | 6.34* | 0.30 | |
| 4-hydroxyproline | 7.49 ± 0.05$^{c}$ | 8.52 ± 0.01$^{b}$ | 8.56 ± 0.21$^{b}$ | 8.19 ± 0.18 | 2.72 | 21.62*** | | 10.3 |
| ammonia | 1.29 ± 0.21 | 0.66 ± 0.005 | 1.04 ± 0.42 | 0.99 ± 0.17 | 48.31 | 1.26$^{ns}$ | | |
| total AA nitrogen (N)$^{d}$ | | | | | | | | |
| g of AAN/kg of protein | 192.13 ± 2.39 | 183.91 ± 0.79 | 189.05 ± 4.06 | 188.36 ± 1.83 | 2.54 | 2.26$^{ns}$ | | |
| g of AAN/kg of dry sample | 149.74 ± 1.83 | 145.06 ± 0.30 | 151.32 ± 3.54 | 148.71 ± 1.49 | 2.69 | 1.99$^{ns}$ | | 43.8 |
| g of AAN/16 g of N | 83.30 ± 1.04 | 87.00 ± 0.37 | 84.71 ± 1.82 | 85.01 ± 0.81 | 2.50 | 2.31$^{ns}$ | | |

$^{a}$ Mean values and standard error of measurements (SEM) for 3 replicates ($N = 3$) and 48 determinations. Significance: $F$ values; ***, $P < 0.001$; *, $P < 0.05$; ns, not significant; CV, coefficient of variation. $^{b,c}$ Means along a horizontal column with different superscripts are significantly different (Duncan, 1955). $^{d}$ Total amino acid nitrogen was determined according to the methods of Heidelbaugh et al. (1975), Horstmann (1979), and Zarkadas (1988a–c).

also used to calculate protein concentration in the absence of tryptophan, cyst(e)ine, proline, and Pro(4-OH) and can be calculated as

$$CF' = \sum_{i=1}^{15}(a_i b_i)/[1 - (a_{Trp} + a_{Cys} + a_{Pro} + a_{Pro(4\text{-}OH)}] \quad (2)$$

These factors, WE, CF, and CF', can be used in all subsequent quantitations of a given sample. The protein concentration $P$ (in micrograms) of each hydrolysate was calculated by multiplying CF or CF' by the total nanomoles ($X_i$) of amino acids found (Horstman, 1979; Peterson, 1983) as follows:

$$P = CF' \sum_{i=1}^{15} X_i \quad (3)$$

*Determination of Connective Tissue Proteins in the Product.* A method for calculating the amount of total connective tissue proteins in these collagen-based byproducts (in grams per kilogram of total protein) is based on the known Pro(4-OH) and amino acid composition of purified collagen [$n$ = 105.8; see Zarkadas et al. (1988a); Miller and Gay (1982, 1987); Light (1985, 1987)] and amorphous elastin ($n_i$ = 22) (Foster, 1982) and the anhydrous molecular weight of Pro(4-OH) ($M_{r(i)}$ = 113.12). The following analytical convention described previously (Zarkadas et al., 1988a) can therefore be used for computing total connective tissue proteins (in grams per kilogram of total protein):

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

**Statistical Analysis.** Data processing and statistical analysis of the results were carried out by a FORTRAN computer program developed for this purpose. Analysis of variance conducted on the amino acid data for a completely randomized block design (factorial) was carried out according to the Statistical Analysis System (SAS) (1982) by the general linear model procedure. Differences among sample means were also tested for significance with Duncan's multiple-range test (Duncan, 1955).

## RESULTS AND DISCUSSION

Accurate amino acid determinations were carried out on three batches of collagen-based byproducts (beefstock bone isolates) to ascertain whether the amino acid profiles and/or collagen contents in such animal protein products could be used as potentially useful indices for assessing their protein quality [Expert Work Group (FSIS), 1984; Pellett and Young, 1984, 1988; Young and Pellett, 1984; Lee et al., 1978; Nguyen et al., 1986; FAO/WHO/UNU, 1985; FAO/WHO, 1990]. Although the actual levels of various animal bone derived protein ingredients being used and their decalcification treatment were not disclosed by the manufacturer, the mechanically separated beefstock bone powder preparation starts with commercially demineralized bone (ossein) which may include bone marrow and, in some cases, spinal cord and very small amounts of muscle tissue. Demineralization is often achieved with acids, frequently 1.0 M HCl or 0.5 M acetic acid. A more suitable method is the use of ethylenediaminetetraacetic acid (EDTA), which in the form of its sodium salts can be used as buffered solution at neutral pH. Results of the amino acid analyses and levels of statistical significance obtained from analysis of variance are presented in Table 1. The data represent the average values of three replicates ($N = 3$). Duplicate 24-, 48-, 72-, and 96-h hydrolysates were prepared, and each was analyzed in duplicate (48 determinations). The least variability occurred when the amino acid data were expressed as grams of anhydrous amino acids per kilogram of anhydrous fat- and ash-free tissue protein. The main advantage of this unit of expressing the composition of a complex protein mixture is that it allows comparisons to be made between the results from this study, with

those given in food compositional tables, and the recommended FAO/WHO/UNU (1985) and FAO/WHO (1990) reference amino acid patterns for humans. The values obtained for the amino acid contents of the three batches of beefstock bone protein isolates show deviations of less than 100 ± 2.5% from the average values obtained among replicates within the same batch. The mean residue weight (WE) and conversion factors CF and CF' (all in micrograms per nanomole) of these samples determined by the summation of the weights of the amino acids present, as described by Horstsmann (1979), are also given in Table 1.

The Joint FAO/WHO Expert Consultation Group (FAO/WHO, 1990) have recommended that amino acid data be also reported as milligrams of amino acids per g of nitrogen. For purposes of comparison, the data from this study have also been calculated in this way, as grams of amino acid per 16 g of total nitrogen, and are presented in Table 2.

The data on the nitrogen content of the collagen-based beefstock bone isolates are in reasonably good agreement with those reported by Eastoe and Eastoe (1954), Eastoe and Long (1960), and Eastoe (1967) for the total nitrogen content of mammalian compact bone and for collagen and gelatin (18.0–18.6%) isolated from the extracellular matrices of a variety of connective tissues. The total nitrogen of these samples ranged from 18.4 to 19.2%, which is considerably higher than the 16.0% value frequently assessed for proteins and which serves as the basis for the factor of 6.25 used to convert total nitrogen to crude protein. The protein conversion factors among these samples varied from 5.21 to 5.43. These results give further support to the recommendations of Benedict (1987) and Khanizadeh et al. (1992) that the protein conversion factor of 6.25 be used for calculating only the crude protein content of different foods.

The amino acid profiles of processed beefstock bone proteins as presented in Tables 1 and 2 appeared to be very similar. However, among batches variation was found to be highly significant for threonine, glycine, alanine, methionine, isoleucine, leucine, lysine, arginine, tryphophan, and 4-hydroxyproline. These three batches of connective tissue-based proteins appeared to have amino acid compositions close to that of type I collagen from bone but completely different from that of any other mammalian protein or group of proteins. Glycine, the simplest amino acid, is the most abundant in processed beefstock bone and accounts for almost 20.0% of the total amino acid residues. Proline and 4-hydroxyproline taken together account for a further 20.0% of all amino acids. Alanine makes up 1 in 11 of all residues. Thus, four amino acids, glycine, proline, 4-hydroxyproline, and alanine, account for about 50% of all amino acids present. Glutamic acid occupies 1 position in 10 in the polypeptide chains and aspartic acid approximately 1 in 15, giving a total carboxyl group frequency of about 17.5% of the total amino acids. Since there are 40–44 amide groups per 1000 residues in collagen (Eastoe, 1967), the frequency of free carboxyl groups is approximately 8.0%. The frequency of total basic amino acids (including arginine, lysine, and histidine) is approximately 12.7%, which slightly exceeds that of the free carboxyl groups. This indicates that the major bone proteins are basic proteins and suggests that the isoionic point of native bone collagen is above pH 9.

Serine accounts for 0.32% and threonine for approximately 0.23% of the total amino acids. The content of 4-hydroxyproline, together with the small amounts of serine and tyrosine, bring the total content of bone amino acids with hydroxyl groups to nearly 15.1% of the total amino acids, which is relatively frequent, compared with meat proteins. The content of hydrophobic amino acids, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine, is approximately 14.5% of the total amino acids, which is lower than that of meat proteins. Since the frequency of hydroxyl groups and charged basic and acidic groups exceeds that of hydrophobic groups of processed bone proteins, these proteins can be regarded as hydrophilic.

The least common protein residues in processed bones are cysteine and tryptophan (Tables 1 and 2). Eastoe (1967) has pointed out that these two amino acids are absent from collagen isolated from a variety of connective tissues. However, Hoermann and Mancewicz (1964) did find 0.31% of tryptophan in bone following demineralization with EDTA. They used a very sensitive fluorometric method, following hydrolysis with barium hydroxide. The possibility exists that these two amino acids are constituents of the glycoprotein component of this hard tissue rather than the constituents of bone collagen.

The essential amino acid (EAA) profiles of the three collagen-based ingredients ranged from 1496 to 1550 mg of EAA/g of dietary nitrogen (Table 3). The data indicated that these ingredients contained significantly lower amounts of all EAA required for human nutrition than either whole egg (3215 mg of EAA/g of nitrogen) or cow's milk protein (3200 mg of EAA/g of nitrogen) (FAO/WHO, 1965). The low EAA indices (35.2–36.6) of these three connective tissue-based ingredients reflect their deficits in tryptophan and the sulfur-containing and aromatic amino acids, as shown in Table 3. These results are in close agreement with earlier findings by Hegedus et al. (1983) on bone meal produced from industrial raw bone, which indicated that bone meal proteins were limiting with respect to tryptophan.

Despite the obvious advantages of simplicity and widespread use of these chemical scoring methods (Block and Mitchell, 1946; Oser, 1951; FAO/WHO, 1965), an even more accurate assessment of the protein quality of foods, especially those containing connective tissue proteins, was recommended by the U.S. Department of Agriculture [Expert Work Group (FSIS), 1983; Alsmeyer et al., 1974; Happich et al., 1975; Lee et al., 1978; Pellett and Young, 1984, 1988]. It involves the determination of the complete amino acid composition, EAA content, calculated protein efficiency ratio (PER), and total collagen content. Lee et al. (1978) defined total EAA in two ways, consisting of either 7 or 10 amino acids, the seven ($EAA_7$) being threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine, and the ten ($EAA_{10}$) being these seven plus histidine, arginine, and tryptophan. On the other hand, the Joint FAO/WHO Expert Consultation Group (FAO/WHO/UNU, 1985; FAO/WHO, 1990) have recommended that, in conjunction with the *in vitro* protein digestibility data, all of the above essential EAA minus arginine ($EAA_9$) and the reference amino acid pattern for the 2–5-year-old child (Table 4) be used as the reference pattern in the evaluation of foods for all persons except infants. It was also recommended that since cystine and tyrosine can partially replace methionine and phenylalanine, respectively, the two sulfur-containing (methionine plus cystine) and two aromatic amino acids (phenylalanine plus tyrosine) be considered together.

**Table 3. Protein Quality Evaluation of Selected Beefstock Protein Ingredients Based on Their Amino Acid (AA) Composition**

| AA | beefstock bone protein ingredients | | | | signif levels among ingredients | |
|---|---|---|---|---|---|---|
| | BSO37 mean ± SEM | BSO38 mean ± SEM | BS126 mean ± SEM | wtd ($N = 9$) mean ± SEM | CV | $F$ |
| (i) essential AA (EAA) | | | | | | |
| total EAA,[b] mg/g N | 1527.4 ± 19.39 | 1550.2 ± 22.07 | 1496.9 ± 24.28 | 1525.0 ± 13.42 | 2.50 | 1.47$^{ns}$ |
| EAA[c] index | 35.99 ± 0.51 | 36.60 ± 2.67 | 35.29 ± 0.91 | 35.96 ± 0.39 | 3.31 | 0.91$^{ns}$ |
| $EAA_7$,[d] % of total protein | 18.35 ± 0.07 | 18.22 ± 0.36 | 17.80 ± 0.18 | 18.12 ± 0.14 | 2.23 | 1.51$^{ns}$ |
| $EAA_{10}$,[d] % of total protein | 27.57 ± 0.12 | 26.44 ± 0.35 | 26.59 ± 0.04 | 26.87 ± 0.21 | 1.39 | 8.20$^{ns}$ |
| (ii) protein efficiency ratio (PER)[e] predicted by | | | | | | |
| eq 4 ($PER_7$)[e] | 1.37 ± 0.005 | 1.36 ± 0.02 | 1.33 ± 0.01 | 1.35 ± 0.01 | 2.41 | 1.51$^{ns}$ |
| eq 5 ($PER_{10}$)[e] | 1.59 ± 0.007 | 1.52 ± 0.02 | 1.53 ± 0.002 | 1.54 ± 0.01 | 1.53 | 8.19* |
| eq 6 ($PER_{10}$)[e] | 1.49 ± 0.004 | 1.35 ± 0.003 | 1.30 ± 0.003 | 1.47 ± 0.001 | 0.89 | 6.57* |
| (iii) connective tissue content[f] | | | | | | |
| % total protein | 72.29 ± 0.41 | 78.63 ± 0.36 | 81.09 ± 0.32 | 77.34 ± 1.33 | 0.81 | 155.28*** |

[a] Mean values and standard error of measurements (SEM) for 3 replicates ($N = 3$) and 48 determinations. Significance: $F$ values; ***, $P < 0.001$; *, $P < 0.05$; ns, not significant. [b] Computed from reference protein standards (FAO/WHO, 1965, 1973). [c] Computed according to the methods of Block and Mitchell (1946) and Oser (1951). [d] Calculated according to the method of Lee et al. (1978). $EAA_7$: threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine. $EAA_{10}$: $EAA_7$ plus histidine, arginine, and tryptophan. [e] PER values were calculated according to the method of Lee et al. (1978) from eq 5 ($PER = 0.08084(EAA_7) - 0.1094$), eq 6 ($PER = 0.06320(EAA_{10}) - 0.1539$), and eq 7 ($PER = -0.02290(\text{collagen}) + 3.1528$). [f] Total connective tissue proteins were calculated from the amounts of Pro(4-OH) present according to the method of Zarkadas et al. (1988a).

**Table 4. Comparison of the Essential Amino Acid (EAA) Composition of Selected Beefstock Bone Protein Ingredients (Collagen Based) for Composite Meats with the Suggested EAA Pattern of Requirements for Humans**

| EAA | EAA pattern of requirement[a] | | processed beefstock bone protein ingredients | | | | other animal products[c] | | |
|---|---|---|---|---|---|---|---|---|---|
| | preschool child (2–5 years) | adult | BS037 | BS038 | BS126 | wtd means | egg | cow's milk | beef |
| | Milligrams of Amino Acid per Gram of Total Protein | | | | | | | | |
| histidine | 19 | 16 | 13.0 | 10.7 | 12.6 | 12.1 | 22 | 27 | 34 |
| isoleucine | 28 | 13 | 18.5 | 18.0 | 18.1 | 18.2 | 54 | 47 | 48 |
| leucine | 66 | 19 | 41.2 | 41.1 | 40.2 | 40.9 | 86 | 95 | 81 |
| lysine | 58 | 16 | 44.7 | 38.6 | 39.8 | 41.0 | 70 | 78 | 89 |
| methionine and cystine | 25 | 17 | 17.6 | 18.1 | 17.0 | 17.5 | 57 | 33 | 40 |
| phenylalanine and tyrosine | 63 | 19 | 39.3 | 39.6 | 37.2 | 38.7 | 93 | 102 | 80 |
| threonine | 34 | 9 | 20.6 | 25.7 | 23.8 | 23.4 | 47 | 44 | 46 |
| tryptophan | 11 | 5 | 1.8 | 1.7 | 1.6 | 1.7 | 17 | 14 | 12 |
| valine | 35 | 13 | 32.3 | 32.0 | 31.5 | 31.9 | 66 | 64 | 50 |
| total | | | | | | | | | |
| including His | 339 | 127 | 229.0 | 225.8 | 221.8 | 225.4 | 512 | 504 | 479 |
| minus His | 320 | 111 | 216.0 | 215.1 | 209.2 | 213.3 | 490 | 477 | 445 |
| | Percent Protein Digestibility in Man | | | | | | | | |
| | | | 90 | 90 | 90 | 90 | 95 | 97 | 98 |
| | Percent Amino Acid Score Adjusted for Digestibility (Percentage of Adequacy)[b] | | | | | | | | |
| | | | 16.4 | 15.5 | 14.4 | 15.45 | 100 | 100 | 94 |

[a] Data from FAO/WHO/UNU (1985), FAO/WHO (1990), and Bodwell (1987). [b] Calculation of protein rating was carried out by comparison of the amino acid composition of the selected collagen-based beefstock protein ingredients with that of the reference pattern established by FAO/WHO/UNU (1985) for preschool child (2–5 years) and adult. [c] Data taken from Bodwell (1987).

The connective tissue-based beefstock bone ingredients had mean values for total $EAA_7$ and $EAA_{10}$ of 18.1 and 26.9%, respectively, and a calculated PER value close to 1.5 (Table 3). These results show that the calculated mean PER values for connective tissue-based ingredients can vary with the amount of collagen present. Collagen is entirely lacking in tryptophan and, when compared with most food proteins, is deficient to different degrees in all other EAA except arginine (Asghar and Henrickson, 1984). A comparison of the EAA composition of processed beefstock bone protein ingredients with that of the selected FAO/WHO (1990) reference pattern is shown in Table 4. The $EAA_9$ in the beefstock bone ingredients listed represent only 20.5–22.5% of the total amino acids, which is considerably lower than the 33.9% $EAA_9$ reference pattern recommended for the 2–5-year-old child (FAO/WHO/UNU, 1985). In particular, the values for total sulfur amino acids are quite low and only a small amount of tryptophan is present. Mean values for the protein digestibility corrected amino acid scores ranged from 14.4 to 16.4% (Table 4), with tryptophan as the first limiting amino acid in bone proteins.

In the present study an attempt was also made to relate the protein quality of the three connective tissue-based bone ingredients for meats and poultry products to the amount of the protein-bound 4-hydroxyproline using the single-column chromatographic method developed in this laboratory (Zarkadas et al., 1986). Total connective tissue proteins, which include collagen, etc., are determined from the amounts of 4-hydroxyproline present (Berg, 1982; Zarkadas et al., 1988a–c, 1994) multiplied by 8.03 (eq 4). The results, summarized in Table 3, show that in the three connective tissue-based bone ingredients evaluated by this method there were significant differences in their total connective tissue contents ($P < 0.001$). Mean values for total connective tissue protein in those ingredients ranged from 72.3 to 81.% (Table 3) of the total bone proteins. The differences noted in connective tissue proteins and glycine,

alanine, arginine, and 4-hydroxyproline contents (Tables 1–3) suggest that, in addition to collagen, the beefstock bone ingredients must contain small amounts of various other proteins that are relatively higher in certain amino acids, which have little or no 4-hydroxyproline. These results are in good agreement with those reported for processed bone meal collagen (83%) by Eastoe and Long (1960). Similar values have been found by Hegedus et al. (1983, 1990) for industrial raw bone meal, which contained 24.9% crude protein corresponding to approximately 80.8% connective tissue proteins of the total bone proteins.

The data presented in this paper show the amino acid and collagen contents among selected beefstock bone protein ingredients from mature bovine animals that are being considered possible protein additives in meat processing. The data obtained in this study suggest that collagen-based ingredients such as beefstock bone products can be added to meat and poultry products for human consumption without significantly decreasing their protein nutritional value. Laser-Reutersward et al. (1982, 1985) have shown that over 90% of the protein present in pig skin or bovine tendon was digestible by the rat and that the true digestibility values for various mixtures of beef and pig skin were 95–100%, regardless of the heat treatment during processing or the age of the animal from which these tissues were taken. Lee et al. (1978) and Pellett and Young (1984) have shown that replacement of lean beef with 10% collagen (protein basis) decreased the digestibility slightly (from 93 to 92%) and the PER value from 2.8 to 2.5. Increasing the replacement level to 50% caused a marked decrease in PER (to 1.7) but only a small decrease in digestibility (to 89%). However, at the 25% collagen replacement level, with a further 25% of the beef replaced with whey protein concentrate, the decrease was much smaller (PER value of 2.4). Bodwell (1985) recommended an upper limit of 35% collagen-based ingredients (4-hydroxyproline × 8.03) which seems to be appropriate. From the present study it becomes apparent that significant levels of collagen-based ingredients may be added to meat and poultry products without significantly reducing the protein quality of meat products.

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