

Composition and Protein Efficiency Ratio of Meat Samples Partially Defatted with Petroleum Ether, Acetone, or Ethyl Ether

MURIEL L. HAPPICH, STANLEY ACKERMAN, ARTHUR J. MILLER, CLIFTON E. SWIFT,¹ and MICHAEL R. GUMBMANN²

U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Philadelphia, PA 19118

Freeze-dried beef samples were partially defatted with either petroleum ether, acetone, or ethyl ether before determination of protein efficiency ratio (PER) to study the extraction effects on the composition and protein nutritional quality of the extracted beef. Defatting a protein source, such as meat or a meat product, may often be necessary to produce a test diet that contains 10% protein and 8% fat. Amino acid, carnosine, anserine, creatine, creatinine, inosine, and proximate compositions were determined on the extracted samples. Resulting data were compared to the composition and PER data of the beef that had no solvent treatment. Although the chemical analysis data from the study showed some variation between the proteins and other nitrogenous components of the unextracted and the extracted beef, these variations were too small to affect the protein nutritional quality of the beef as measured by PER.

AOAC method 43.212 (1) for determination of protein efficiency ratio (PER) states that the test diet fed to rats shall consist of 10% protein originating from the test protein sample, and 8% fat. In many meats, meat products, and meat food products, the fat content exceeds the protein content, necessitating an alteration in the official method to obtain the required protein-fat ratio in the test protein and ANRC casein diets. Possible solutions to the problem were reviewed and a procedure for removing excess fat by partial extraction with petroleum ether was used by the authors (2). Before partial extraction of the fat, the water content in a coarsely ground meat sample was reduced to about 1% by freeze-drying. To

prevent protein denaturation and oxidative changes, extractions were conducted at a low temperature with a nonpolar solvent. Because extraction of fat with a solvent might nevertheless affect the nutritional quality of the meat proteins, we investigated and compared the effects of 3 different solvents, acetone, ethyl ether, and petroleum ether, on extraction of lyophilized beef. PER, amino acid composition, and composition of nonprotein nitrogenous constituents of extracted beef samples were compared to those of unextracted beef to determine whether changes in composition or protein nutritional value had occurred.

Experimental

Reagents

(a) *Solvents*.—Nanograde ethyl ether, petroleum ether (30–60°C), and acetone (Mallinckrodt).

(b) *Perchloric acid*.—1M and 0.6N perchloric acid.

(c) *Alkaline picrate reagent*.—10 mL saturated picric acid + 2 mL 10% NaOH.

(d) *Diacetyl- α -naphthol reagent*.—Equal volumes of diluted diacetyl solution (stock solution of 1% diacetyl diluted 1:20 before use) and 1% α -naphthol in 2N NaOH. Prepare stock solution of diacetyl by heating 1.6 g dimethyl glyoxime with 200 mL 5N H₂SO₄ in an all-glass distilling apparatus and collecting first 50 mL of distillate. Dilute distillate to 100 mL with water. Amount of dimethyl glyoxime taken yields about 1 g diacetyl. Stock solution is stable at least one month (3).

(e) *Phosphate buffer, 0.1M*.—pH 7.0.

(f) *Potassium hydroxide*.—30% (w/w).

(g) *6N HCl*.

¹Deceased.

²Western Regional Research Center, Berkeley, CA 94710.

Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Received May 4, 1983. Accepted August 2, 1983.

- (h) 3N Mercaptoethanesulfonic acid.
- (i) 15% Sodium thiosulfate.
- (j) Sodium citrate buffer solutions for amino acid analyzer.—(1) 0.2N Na⁺, pH 2.90 for 126 min; (2) 0.2N Na⁺, pH 4.15 for 26 min; (3) 1.0N Na⁺, pH 5.90 for 100 min.

Apparatus

- (a) Meat grinder.—Butcher Boy, B-52, Lasar Manufacturing Co., Los Angeles, CA.
- (b) Buffalo ribbon blender.—John E. Smith's Sons, Buffalo, NY.
- (c) Freezer-dryer.—Stokes Model 338-F shelf dryer (Sharpley-Stokes Div., Pennwalt Corp., Warminster, PA).
- (d) Stainless steel trays.—26 × 9.5 × 88.9 cm.
- (e) Wiley mill.—Model No. 1, Arthur H. Thomas Co., Philadelphia, PA.
- (f) Soxhlet extractor.
- (g) Blender.—Waring, semi-micro, stainless steel jar.
- (h) Spectrophotometer.—Perkin-Elmer what model, 650-40.
- (i) Amino acid analyzer.—Beckman Model 119B (Beckman Instruments, Inc., 1117 California Ave, Palo Alto, CA 94304).

Beef Samples

Beef sample was 44 kg of 90% lean cow chuck obtained from a local slaughter house. Proximate composition of the beef as determined by AOAC methods 24.003(a) (moisture), 24.005(a) (petroleum ether extract), 24.027 (Kjeldahl nitrogen), and 24.062 (ash) (1) was moisture, 70.86%; fat, 7.4%; nitrogen, 3.32%; protein (N × 6.25), 20.74%; and ash, 1.0%.

Freeze-Drying

Grind 44.0 kg beef in meat grinder through ¾ in. plate. Spread ca 2 kg ground beef per stainless steel tray and freeze at -35°C. Place trays of frozen meat in a Stokes shelf dryer and dry 18-24 h at shelf temperature of 41.6-43.3°C under vacuum of 0.5 mm mercury. Tray of meat was considered dry when bottom of tray was warm to the touch. Freeze-dried meat weighed 13.4 kg, about 30.5% of original weight of beef.

Partial Extraction of Fat

Thoroughly mix freeze-dried beef and divide into 4 equal portions. Remove substantial proportion of fat from each of 3 portions by extraction with petroleum ether, ethyl ether, or acetone as described: Place 2 kg beef in double-layer cheesecloth bag and suspend for 3 h at 25°C in 20 L battery jar containing 10 L solvent. Raise bag occasionally, let it drain briefly, and return bag to the solvent. After 3 h, briefly drain solvent from bag and continue extraction for 1 h in battery jar containing 10 L fresh solvent. Then drain solvent from bag of beef and evaporate remaining solvent in flow of air inside fume hood at room temperature. Place meat in large vacuum chamber and remove last remnants of solvent. Thoroughly intersperse partially defatted meat with finely ground Dry Ice and grind mixture through Wiley Mill to pass 2 mm screen. Let CO₂ evaporate, thoroughly mix ground sample, and store in tightly closed air-impermeable plastic food-freezer bag or other air-tight container in refrigerator at 1°C.

Chemical Analyses

Determine moisture, fat, ash, and nitrogen by AOAC methods 24.003(a), 24.005(a) petroleum ether, 24.062 (31.012), and 24.027 (1), respectively, on 3 partially defatted samples and

on the unextracted beef sample (control). Calculate percent protein as percent nitrogen × 6.25.

Amino acid analysis of protein hydrolysates.—Weigh 10 g each of lyophilized control and the partially defatted beef samples into individual extraction thimbles and completely defat with petroleum ether (30-60°C, reagent grade) in Soxhlet extractor, using lowest setting on heater to keep solvent boiling. Continue extraction for 4 h, then evaporate solvent in air with stream of nitrogen flowing around thimble containing extracted beef, and finally, in a vacuum. Mix each sample well and store in glass jar with tight fitting plastic-lined screw cap at about 1°C. Determine nitrogen, protein (%N × 6.25), and moisture content.

Hydrolyze 0.1-0.15 g samples of defatted beef (0.09-0.135 g protein) by refluxing under nitrogen for 24 h with 75 mL 6N HCl, adding 0.75 mL 5% phenol solution to protect the sulfur amino acids. After hydrolysis, remove HCl and water under vacuum by using rotary evaporation with the hydrolysis flask rotating in 40°C water bath. Wash dried residue 3 times with 25 mL deionized water, evaporating the water after each wash. Dissolve residue and dilute to 50 mL with sample dilution buffer (0.2N Na⁺, pH 2.2). Add 0.1 or 0.2 mL diluted solution to sample holder on amino acid analyzer. Use sodium citrate, 3-buffer system (0.2N Na⁺, pH 2.90 for 126 min; 0.2N Na⁺, pH 4.15 for 26 min; and 1.0N Na⁺, pH 5.90 for 100 min) to elute 19 amino acids including hydroxylysine and hydroxyproline (4). Conditions: buffer flow, 70 mL/h; ninhydrin flow, 35 mL/h; column, 0.9 cm diameter, containing 31.5 cm Beckman cation exchange resin AA-20. Use computer system to integrate area of amino acid peaks and to calculate concentration of each amino acid in solution applied to column.

Determine tryptophan on separate sample as follows: Hydrolyze 4 mg lyophilized, defatted beef with 2 mL 3N mercaptoethanesulfonic acid (5) under vacuum in sealed tube for 24 h at 110°C. Neutralize mercaptoethanesulfonic acid with 4 mL 1N NaOH, dilute solution to 10 mL with sample dilution buffer, pH 4.25, and quantitate tryptophan by using a short column (0.9 cm diameter containing 5.75 cm Beckman cation exchange resin AA-20) and a sodium citrate buffer (0.2N Na⁺, pH 5.4) (6, 7).

Calculate each amino acid as g amino acid residue (molecular weight of amino acid minus molecular weight of 1 molecule of water) or as g amino acid (molecular weight) per 100 g protein or per g nitrogen, whichever is more appropriate for the experiment conducted. (We calculated results as g amino acid residue/100 g protein. Results also can be calculated to g of either amino acid residue or amino acid per weight of partially defatted beef or of the original beef if the proximate analysis of these stages is determined.)

Preparation of sample and analysis of free amino acids, anserine, carnosine, creatine, and creatinine.—Hydrate 10 g freeze-dried meat control and solvent-extracted beef samples individually by adding 20 mL deionized water to each. Mix well, cover tightly, and store in refrigerator overnight. The next morning, homogenize each hydrated sample with 50 mL 0.6N perchloric acid (8) for 30 min in semi-micro stainless steel jar of Waring blender (Cenco 17246B). Filter through Whatman No. 1 paper. Solution from control sample may filter more slowly if much fat is present. Extract remaining solids and filter paper in blender with second 50 mL aliquot of 0.6N perchloric acid, filter, and wash the residue with two 10 mL portions of deionized water. Combine the filtrates and washes, neutralize with 30% (w/w) potassium hydroxide, using phenolphthalein indicator and spot plate for testing near the end point. Let solution stand overnight to complete precipitation. Filter potassium perchlorate precipitate from solution,

wash residue with deionized water, combine filtrate and washes, and dilute to 200 mL with deionized water. Note: Potassium perchlorate is a highly explosive compound especially when dry. Therefore, immediately react the wet potassium perchlorate precipitate with a solution of 15% sodium thiosulfate to prevent explosion before discarding.

Creatinine determination.—Determine creatinine content by using a method described by Hawk et al. (9), and modified by Strange and Benedict (10). Combine 5, 2, and 1 mL aliquots of the potassium perchlorate filtrate with 0, 3, and 4 mL deionized water, respectively, and mix with 2.5 mL freshly prepared alkaline picrate reagent. After 15 min, read absorbance at 540 nm in Perkin Elmer spectrophotometer, using a 1-cm pathlength cuvet. Prepare blank for each run. Determine concentration of creatinine from prepared standard curve. Report results as mg creatinine/100 g moisture-free, fat-free beef.

Creatine determination.—Determine creatine content on potassium perchlorate filtrate by modified method of Eggleton et al. (3). Prepare diacetyl-naphthol reagent, immediately before use, by mixing equal volumes of diluted diacetyl solution (stock solution of 1% diacetyl diluted 1:20 before use) prepared by the method of Eggleton (3) and 1% α -naphthol in 2N NaOH (11). Mix 2 mL of 1:99 or 1:49 dilutions of potassium perchlorate filtrate with 2 mL of the reagent, and heat reaction mixture in boiling water for 10 min. Read absorbance at 550 nm, and determine absorbance of reagent blank for each run. Prepare standard curve for creatine; Beer's Law applies in range of concentration used. Give results as mg creatine/100 g moisture-free, fat-free beef.

Determination of free amino-acids.—Determine free amino acids in potassium perchlorate filtrate by ion exchange chromatography using amino acid analyzer and same buffer system as for acid hydrolysates. Inject 0.25 mL filtered extract per analysis onto column. The dipeptides carnosine and anserine were assumed to be in the extract, although individual peaks did not show on chromatogram for either one. Apparently, anserine co-elutes with lysine and carnosine with histidine under conditions and buffer system used. Peak in lysine position was calculated as mg lysine/100 g moisture-free, fat-free beef, and not as anserine (reasons discussed later). Calculate peak in histidine position as mg carnosine/100 g moisture-free, fat-free beef.

Determination of nucleotides.—Determine nucleotides by method of Honikel and Fisher (12) after pretreatment to hydrate sample and remove water solubles as follows: Weigh 2 g sample of each of freeze-dried control, acetone, ethyl ether, or petroleum ether-extracted beef into centrifuge tubes. Add 14 mL deionized water to each tube, mix thoroughly, cover with plastic wrap, and place in refrigerator overnight. Next day, centrifuge at 19 000 rpm for 30 min at 0–4°C, and filter supernatant through glass wool to remove fat accumulated on top of solution. There is 6 or 7 mL of filtrate from each solution. To each sample, add 10 mL 1M perchloric acid, stir well, and filter out precipitate through glass wool. Dilute 0.3 mL portion of filtrate to 5 mL with 0.1M phosphate buffer, pH 7.0. Determine absorbance at 250 nm from peak maxima and concentration of nucleotides as inosine 5'-monobasic phosphoric acid, by reference to standard curve prepared with this nucleotide. Calculate inosine 5'-monobasic phosphoric acid as mg/100 g moisture-free, fat-free beef. (This method does have an error inherent from absorption at 250 nm of other low molecular components, such as peptides and amino acids.)

Protein Efficiency Ratio

Determine PER by AOAC method 43.212–43.216 (1) (5 rats per assay, 2 replicate determinations, or by 10 rats per assay) for the unextracted beef sample, for the 3 samples of solvent-extracted beef, and for the reference protein casein. Prepare each diet to contain 10% protein ($1.60 \times 100/\%N$ of test sample) supplied by test protein source and 8% fat supplied by test protein source and supplemented with corn oil. Use rats of Sprague-Dawley strain. Feed rats ad libitum for 28 days. Calculate PER (wt gain, g \div protein intake, g) for beef samples and for ANRC casein reference group. Also calculate by correcting to PER of casein at 2.5 (13).

$$\text{Corrected PER, test protein} = \frac{\text{actual PER, test protein}}{\text{actual PER, ANRC casein control}} \times 2.5$$

Apparent Nitrogen Digestibility Determination

Determine apparent nitrogen digestibility by using modified method of Mitchell (14, 15) during second week of PER test. Collect feces from each rat (5 rats per assay, 2 replicate determinations, or 10 rats per assay) on days 7–14 for the unextracted beef sample, for each of the 3 samples of solvent-extracted beef, and for the reference protein casein during PER test. Carefully separate feces from any spilled food or other extraneous matter. Composite feces from rats on an individual diet and dry feces overnight in 100°C oven. Equilibrate composited, dried feces sample(s) at room temperature and humidity, weigh each composite sample, grind in an Omni blender in metal cup immersed in ice water (or use other similar method). Mix thoroughly and determine nitrogen by the Kjeldahl method [1 (24.027)]. Determine feed intake by monitoring uneaten food. Carefully collect scattered food and uneaten food in feed cups, separate all extraneous matter, weigh, and determine Kjeldahl nitrogen (1). Weight of food offered to animals minus weight of uneaten food equals weight of food eaten. Calculate apparent nitrogen digestibility using following equation (15–18):

$$\text{Apparent nitrogen digestibility} = \frac{\text{N intake} - \text{fecal N} \times 100}{\text{N intake}}$$

Results and Discussion

The beef samples that were partially defatted with petroleum ether, acetone, or ethyl ether had less than half the fat and correspondingly higher moisture and protein than the control sample (Table 1). Ethyl ether was the most efficient solvent and petroleum ether was the least efficient in extracting fat at ambient temperature. The amount of protein lost with the fat extracted from 2000 g freeze-dried beef was small: 1.2 g (0.31%), 0.9 g (0.25%), or 2.0 g (0.48%) for the petroleum ether, acetone, or ethyl ether extraction, respectively.

Table 1. Proximate analysis of freeze-dried beef control and beef partially defatted with solvents

Solvent	Ash, %	Fat, %	Moisture, %	Protein, N \times 6.25	Total, %
None	3.1	30.2	3.9	63.8	101.0
Petroleum ether	3.6	14.7	8.9	73.0	100.2
Acetone	3.9	10.2	8.9	79.1	102.1
Ethyl ether	3.9	9.3	9.3	79.0	101.5

Amino Acid Analysis

Comparison of the amino acid analysis data from the beef and from the beef samples partially defatted with any 1 of 3 solvents (Table 2) indicates that the beef extracted with acetone had the highest values for each amino acid quantified, except for ½ cystine, tryptophan, and hydroxylysine (Table 2), and the highest value for the total indispensable amino acids and their sparing amino acids, histidine through valine (38.33, 38.11, 39.69, and 37.50 g/100 g protein for the control), and for beef extracted with petroleum ether, acetone, or ethyl ether, respectively [mean = 38.41 g; SD 0.92 g; CV 2.40%]. The *F*-value by an analysis of variance, 4.33, was significant for *P* < 0.05 but not for *P* < 0.01). The individual residue values were less than 0.30 g higher/100 g protein, with exceptions for glutamic acid, proline, and aspartic acid, which had higher differences. However, a one-way analysis of variance over the replicate amino acid residue values obtained for the 4 beef samples showed no statistically significant (*P* < 0.05) difference between the individual amino acid residue content of beef and of the beef samples extracted with any 1 of the 3 solvents (Table 2, *F*-values) except for phenylalanine and hydroxylysine. The value for phenylalanine in the acetone-extracted beef is significantly higher than for phenylalanine in the beef extracted with ethyl ether or petroleum ether or in the unextracted beef. The value for hydroxylysine is significantly higher for the unextracted beef sample. The acetone-extracted beef had the highest total amino acid residues (86.66 g/100 g protein). Data for beef extracted with either petroleum ether or ethyl ether were in closest agreement with data for the unextracted beef. An analysis of variance over the total amino acid residue values obtained for the 4 beef samples showed no statistically significant difference between the total amino acid residue content of the 4 samples of beef ($F_{0.05} = 1.43$).

Table 2. Amino acid content (g residue/100 g protein) of freeze-dried beef control and partially defatted beef samples

Amino acid	Solvent treatment				<i>F</i> -value ^d
	None ^a	Petroleum ether ^b	Acetone ^a	Ethyl ether ^c	
His	2.95	2.75	2.96	2.69	1.56
Ile	3.61	3.67	3.72	3.59	0.20
Leu	6.54	6.57	6.75	6.43	0.45
Lys	7.10	7.00	7.31	6.85	0.90
Met	2.39	2.35	2.52	2.38	0.57
½ Cys	1.09	0.97	1.05	0.99	0.15
Phe	3.50	3.59	3.79	3.54	4.62**
Tyr	3.09	3.09	3.20	3.05	0.71
Thr	3.29	3.47	3.58	3.44	1.27
Trp	0.88	0.81	0.79	0.84	— ^e
Val	3.89	3.85	4.02	3.70	0.46
Ala	4.80	4.89	5.03	4.83	0.54
Arg	5.68	5.66	5.98	5.58	1.08
Asp	7.23	7.44	7.70	7.39	0.84
Glu	12.69	12.89	13.40	12.64	1.66
Gly	4.83	4.78	5.07	4.93	1.27
Hyl	0.46	0.39	0.39	0.39	3.50**
Hyp	1.38	1.34	1.57	1.48	3.11
Pro	3.77	4.26	4.48	4.31	1.98
Ser	3.06	3.09	3.31	3.10	1.24
Total ^f	82.24	82.86	86.66	82.15	

^aMean of 5 replicates.

^bMean of 4 replicates.

^cMean of 3 replicates.

^d**Variance, ratio significantly different at the 5% level. Reference value for significance is 3.41 where F_1 is 13 and F_2 is 3.

^e*F*-value was indeterminate because the within-treatment variation was zero (i.e., perfect agreement between replicates). Mean of tryptophan values = 0.83, SD = 0.039, CV = 4.7%.

^fMean = 83.48, SD = 2.14, CV = 2.6%.

Free Amino-Acids, Dipetides, and Other Nitrogenous Components

In addition to the total amino acids, free amino acids and several other nitrogenous components were determined in the control and solvent-extracted samples of beef. These were 2 dipeptides found in meat, anserine and carnosine; creatine; creatinine; and nucleotides reported as inosine 5'-monophosphoric acid. The results are shown in Table 3. These components account for 4.2–5.5% of the nitrogen in the samples of partially defatted, freeze-dried beef (Table 4). About 4.9% was found in the undefatted freeze-dried beef. Seventeen free amino acids were found in measurable amounts, including taurine, an oxidation product of cysteine metabolism (19) (Table 3). The free amino acids serine and alanine were found in the highest quantities. A small amount of free histidine was present but could not be quantified. Free hydroxyproline, hydroxylysine, and tryptophan were not measurable and possibly were completely lacking. There was considerable evidence that the 2 dipeptides anserine (β-alanyl-1-methylhistidine) and carnosine (β-alanylhistidine) (20) were present and co-eluting with other amino acids under the chromatographic separation conditions used in these experiments, i.e., anserine with lysine and carnosine with histidine. The 440/570 nm peak height ratios of the substances eluting at the time of lysine and histidine supported this conclusion (Table 5). The 440/570 nm peak height ratio for anserine was 4.9 times that for lysine, and the ratio for carnosine was about 3 times that for histidine (Table 5). Data obtained from the chromatogram and the peak height ratios from the filtrate of the potassium perchlorate precipitate of the control and of the 3 partially defatted beef samples, indicated that the peak eluted at 191.5–192 min, in the lysine position, was produced mainly by free lysine (size of peak plus 440/570 peak height ratio), but undoubtedly had a small amount of the dipeptide, anserine (Table 5). However, the anserine/carnosine ratio in beef is low, 0.06–0.2 (21, 22), and the peak was calculated as lysine. Similarly, the data obtained (440/570 peak height ratio) (Table 5) on the peak in the histidine position (195 min) was expected to be nearly all carnosine and was calculated as carnosine. This value may be high because there may be a small amount of free histidine present. Nevertheless, the lysine–anserine and histidine–carnosine content of the beef samples, control or solvent-extracted, were similar.

Nitrogen Recovery

A comparison of the nitrogen recovery data for the total amino acid residues in the unextracted beef (control sample), the petroleum ether- and ethyl ether-extracted beef (Table 4) is similar to that found by Happich et al. (2) in a sample of lean beef.

Data for the total recovery of nitrogen by all analyses from each of the 4 beef samples (Table 4) indicated that nitrogen recovery was highest from acetone-extracted beef. Nitrogen recovery data from analyses of petroleum ether- and ethyl ether-extracted beef, although lower than recovery data from the control beef, agreed more closely with it.

Protein Efficiency Ratio (PER)

An analysis of variance (ANOVA) indicated that the PER values of the individual rats (5 per test) by 5 protein sources, by 2 experiments, or by interactions of protein source and experiment were not significantly different. Estimation of within-diet variability was performed (ANOVA) on PER data for each replicate and there was no evidence of any significance between the error variances. Thus the data from the 2 replicates were combined and analyzed statistically.

Table 3. Nonprotein nitrogenous components of freeze-dried beef and partially defatted beef (mg/100 g fat-free, moisture-free beef)

Chemical component	Solvent treatment			
	None	Petroleum ether	Acetone	Ethyl ether
Inosine	388	430	405	360
Creatine	1968	1574	2212	1636
Creatinine	105.2	98.4	116.3	137.0
<i>Free dipeptides:</i>				
Anserine ^a	UQ	UQ	UQ	UQ
Carnosine	1182	1045	1096	1102
<i>Free amino acids:</i>				
Taurine	170.0	145.0	160.0	155.0
Aspartic acid	8.9	5.1	4.9	4.9
Threonine	17.3	23.8	17.7	22.5
Serine	219.0	298.6	305.4	248.3
Glutamic acid	95.3	46.9	45.2	57.8
Proline	9.3	7.0	7.3	7.9
Glycine	37.4	45.0	42.8	40.8
Alanine	216.0	189.2	154.8	178.3
Valine	22.3	25.1	26.9	24.9
½ Cystine	34.3	19.3	24.4	23.7
Methionine	32.8	14.8	26.9	22.5
Isoleucine	32.0	19.9	31.0	24.9
Leucine	48.4	41.2	52.5	42.0
Tyrosine	43.2	46.9	45.8	39.5
Phenylalanine	32.8	38.6	39.1	31.6
Lysine	81.9	65.0	64.1	61.4
Histidine ^b	UQ	UQ	UQ	UQ
Arginine	49.2	44.4	51.3	40.8
Total free amino acids	1150.1	1075.8	1100.1	1026.8
Ammonia	90.9	45.7	53.1	60.8

^aUQ = unable to quantify completely in the system used. Lysine and anserine eluted together.

^bUQ = unable to quantify completely in the system used. Histidine and carnosine eluted together.

Table 4. Nitrogen recovered by analysis of beef samples partially defatted with petroleum ether, acetone, or ethyl ether^a

Component	Solvent extraction			
	None	Petroleum ether	Acetone	Ethyl ether
Total nitrogen	15254.2	15239.9	15247.6	15245.9
Recovered amino acid residue N	12476.1	12492.7	13088.1	12393.2
% of total nitrogen	81.8	82.0	85.8	81.3
Recovered ammonia N	1192	1115	1125	1164
Inosine 5' monobasic phosphoric acid (I)	81.0	89.9	84.6	75.2
Creatine (C)	630.5	504.3	708.7	524.2
Creatinine (Cr)	39.0	36.6	43.2	50.9
Total I + C + Cr	750.5	630.8	836.5	650.3
% of total N	4.93	4.20	5.52	4.30
Total N recovered from 5 categories	14141.9	14238.5	15049.6	14207.5
% of total N	94.6	93.4	98.7	93.2
% N unaccounted for	5.4	6.6	1.3	6.8

^aNitrogen (N), mg/100 g fat-free, moisture-free beef.

Table 5. Elution time and peak height ratios of selected amino acids and dipeptides, and elution data from analysis of beef samples

Std soln	Elution time, min	Peak height ratio, 440/570 nm	Beef sample	Peak height ratio, 440/570 nm	
				191.5–192.0 min	195.0 min
Lysine	191.5–192.2	0.28–0.30	Control	0.46	0.64
Histidine	194.0–195.3	0.21 and 0.22	Acetone ext	0.45–0.47	0.65–0.66
1-Methyl His	197.2	0.26	Ethyl ether ext	0.46–0.48	0.63–0.65
3-Methyl-L-His	195.8	0.18	Pet. ether ext	0.46–0.49	0.65–0.67
Anserine	191.7	1.41			
Carnosine	195.9–196.2	0.68 and 0.69			

There were no significant differences between the actual PER (10 rats) determined by bioassay for the control beef and those determined for the beef samples partially extracted with any 1 of 3 solvents (Table 6). An ANOVA (10 rats, 5 diets) showed no significant differences between diets by an *F* test ($F = 1.367$) at 95 or 99% levels of probability. A

comparison of the actual PER value for the untreated beef with that for each solvent-extracted beef sample shows them to have a range of differences no larger than 0.11 PER, values within experimental limits. Adjusting the PER to that of casein at 2.5, decreased the range of differences, but only slightly to 0.08 PER.

Table 6. PER^a and apparent nitrogen digestibility of freeze-dried beef control and partially defatted beef samples

Dietary source of protein ^b	Final body weight, g ± SE ^{c,d}	Total feed consumption, g ± SE ^c	PER ^e		Apparent nitrogen digestibility, ^g %
			Actual ± SE ^c	Adjusted ^f	
ANRC casein	162 ± 4.7 ^{bb}	314 ± 11 ^{bb}	3.41 ± 0.06 ^{aa}	2.50	94.2 ± 0.45 ^{aa}
Beef	185 ± 7.4 ^{aa}	391 ± 18 ^{aa}	3.34 ± 0.07 ^{aa}	2.45	90.4 ± 0.10 ^{bb}
Beef, pet. ether extd	180 ± 4.4 ^{ab}	388 ± 14 ^{aa}	3.23 ± 0.04 ^{aa}	2.37	91.5 ± 0.70 ^{ab}
Beef, acetone extd	194 ± 4.5 ^{aa}	411 ± 13 ^{aa}	3.41 ± 0.03 ^{aa}	2.50	91.5 ± 0.95 ^{ab}
Beef, ethyl ether extd	195 ± 7.1 ^{aa}	418 ± 13 ^{aa}	3.34 ± 0.09 ^{aa}	2.45	90.8 ± 0.30 ^{bb}

^a28-day feeding tests; mean of 2 assays, 5 rats/assay.

^bDiets contained 10% protein (N × 6.25).

^cMean ± SE. Duncan's multiple range test: Means without a superscript letter in common are significantly different. Lower case, P < 0.05; upper case, P < 0.01. N = 10.

^dAverage initial body weight of the rats was 54.5 g.

^ePER = protein efficiency ratio = wt gain (g)/protein intake (g).

^fAdjusted to PER of casein, assumed to be 2.5.

^gApparent nitrogen digestibility = N intake - fecal N/N intake × 100. Feces collected on days 7-14 during each 28-day PER feeding test; mean of 2 assays, 5 rats/assay.

Comparing the amino acid content (Table 2) and the actual PER values (Table 6) for each beef sample, the beef extracted with acetone had the highest total value (39.69 g/100 g protein) for the 11 indispensable and their sparing amino acids, histidine through valine, a value only 1.4 g higher than for the control. The acetone-extracted beef also had the highest adjusted PER value, although not significantly higher than the other values. The differences in PER values are considered within experimental error.

Apparent nitrogen digestibility data (means for 10 rats) for all beef samples were similar, with casein exhibiting slightly higher nitrogen digestibility than the beef.

Conclusions

Although the chemical analysis data obtained in this study showed some variations between the proteins and other nitrogenous components of the unextracted beef and the beef extracted with petroleum ether, acetone, or ethyl ether, these variations were too small to significantly affect the protein nutritional quality of the extracted beef as measured by PER. The PER data suggest that any one of these 3 solvents could be used to extract excess fat from samples of meat before determination of PER.

Acknowledgement

The authors thank J. G. Phillips, Biometrical and Statistical Services, Eastern Regional Research Center, for consultation and statistical analysis for this study.

REFERENCES

- (1) *Official Methods of Analysis* (1980) 13th Ed., AOAC, Arlington, VA, secs 24.003(a), 24.005(a), 24.027, 24.062, 43.212-43.216
- (2) Happich, M. L., et al. (1975) *J. Food Sci.* **40**, 35-39
- (3) Eggleton, P., Elsdon, S. R., & Gough, N. (1943) *Biochem. J.* **37**, 526-529
- (4) Happich, M. L., Bodwell, C. E., & Phillips, J. G. (1981) in *Protein Quality In Humans: Assessment and In Vitro Estimation*, C. E. Bodwell, J. S. Adkins, & D. T. Hopkins (Eds), Avi Publishing Co., Inc., Westport, CT, Chap. 12, pp. 197-220
- (5) Penke, B., Ferenczi, R., & Kovacs, K. (1974) *Anal. Biochem.* **60**, 45-50
- (6) Moore, S., Spackman, D. H., & Stein, W. H. (1958) *Anal. Chem.* **30**, 1185-1190
- (7) Hughli, T. E., & Moore, S. (1972) *J. Biol. Chem.* **247**, 2828-2834
- (8) Macy, R. L., Jr, Naumann, H. D., & Bailey, M. E. (1970) *J. Food Sci.* **35**, 78-80
- (9) Hawk, P. B., Oser, B. L., & Summerson, W. H. (1954) in *Practical Physiological Chemistry*, 13th Ed., The Blakiston Co., Inc., New York, NY, p. 801
- (10) Strange, E. D., & Benedict, R. C. (1978) *J. Food Sci.* **43**, 1652-1655, 1661
- (11) Smith, I. (1960) in *Chromatographic and Electrophoretic Techniques*, Vol. 1, 2nd Ed., Interscience Publishers, Inc., New York, NY, p. 227
- (12) Honikel, K. O., & Fisher, C. (1977) *J. Food Sci.* **42**, 1633-1636
- (13) Chapman, D. G., Castillo, R., & Campbell, J. A. (1959) *Can. J. Biochem. Physiol.* **37**, 679-686
- (14) Mitchell, H. H. (1924) *J. Biol. Chem.* **58**, 873-903
- (15) National Academy of Sciences (1963) *Evaluation of Protein Quality*, Publication 1100, National Research Council, Washington, DC, p. 63
- (16) Harper, A. E. (1974) in *Improvement of Protein Nutriture*, National Academy of Sciences, National Research Council, Washington, DC, pp. 6-7
- (17) Bressani, R. (1977) in *Evaluation of Proteins for Humans*, C. E. Bodwell (Ed.), Avi Publishing Co., Inc., Westport, CT, pp. 86-89
- (18) Pellett, P. L., & Young, V. R. (Eds) (1980) *Nutritional Evaluation of Protein Foods*, WHTR-3/UNUP-129, The United Nations University, Shibuya-ku, Tokyo 150, Japan, pp. 123, 125-126
- (19) Mahler, H. R., & Cordes, E. H. (1971) *Biological Chemistry*, 2nd Ed., Harper and Row, New York, NY, p. 50
- (20) West, E. S., & Todd, W. R. (1964) *Textbook of Biochemistry*, 3rd Ed., The Macmillan Co., New York, NY, pp. 1114-1115
- (21) Tinbergen, B. J., & Slump, P. (1976) *Z. Lebensm. Unters. Forsch.* **161**, 7-11
- (22) Slump, P., Bremmer, J. N., & Jongerius, G. C. (1974) CIVO-Report No. R4376, Central Institute for Nutrition and Food Research, TNO, Zeist, The Netherlands