

# Determination of the Proximate and Electrolyte Content of Beef

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RECENT STUDIES indicate that variations in meat composition, some so small as to appear superficial, may markedly affect meat quality. This is exemplified by the evidence that variation in proximate composition and ionic constituents, such as hydrogen and calcium, substantially influence the water retention of meat (7, 16, 17). In meats, as in many other foods, acquiring a comprehensive knowledge of the relation between composition and quality offers promise of developing new ways in which quality may be improved.

It has long been apparent in this Laboratory that a system of meat analysis was needed which would permit the collection of a large amount of data in as short a time as possible. It was our opinion that a system could be developed, improving on available methods (1, 6, 11), which would dispense with laborious and time-consuming separations, would use as far as possible a common ash solution for determination of electrolytes, and make extensive use of spectrophotometric methods. This paper describes such a system. It was successfully employed to collect the analytical data that has been reported (16) in a study on the variations in composition and properties among eight beef muscles.

## MATERIALS AND METHODS

**Preparation of samples.** Muscle was removed from the carcass and trimmed of all visible fat and connective tissue. The muscle tissue was cut into approximately 1 in cubes and the cubes thoroughly mixed by shaking in a large polyethylene jar. One hundred grams of the meat were then blended to a fine paste in a Serval Omnimixer<sup>a</sup> which, because of its stainless steel construction, high speed, and cutting action, yielded a finely comminuted sample with a minimum of metallic contamination. Heating during blending was prevented by immersing the mixer jar (polyethylene) in an ice bath.

**Determination of moisture.** Into tared, high-form Vitreosil crucibles (50 ml size C)  $15 \pm 0.1$  g portions of meat paste were weighed. The paste was spread to form a funnel shaped conduit to facilitate moisture loss. The crucibles were placed in a gravity convection oven, the temperature was slowly raised to 102–105° C, and the samples were held at this temperature overnight (16–18 hr) (18). After cooling, the crucibles were weighed and the moisture content was calculated. The dried samples were retained for subsequent ashing and preparation of the stock electrolyte solution.

**Determination of fat.** Content of fat was determined by a modified Babcock procedure. Nine grams of the original meat paste were weighed into 20% size, Paley-type Babcock bottles (Kimble Glass No. 508). Thirty ml of a 1:1 perchloric acid-acetic acid solution, prepared by mixing equal volumes of reagent grade perchloric acid of 60% strength and reagent grade glacial acetic acid, as recommended by Salwin *et al* (13),

were added. The contents were mixed by swirling, after which the bottles were immersed in a boiling water bath for 30–60 min. They were swirled occasionally in the bath. Satisfactory digestion was achieved when the meat solids had disintegrated into fine particles. The bottles were then removed, and additional acid mixture was added until the fat column rose into the calibrated neck of the bottles. The bottles were centrifuged for 2 min at 900 rpm in a head 15 in in diameter, and then placed in a 70° C water bath for 15 min. One half ml of the acid mixture was drained down the wall of the calibrated neck which momentarily separated the fat phase from the digest and permitted reading; otherwise, chromogens in the fat tended to obscure the fat-digest meniscus. From the amount of fat separated, the fat content as per cent wet weight of meat was calculated.

**Determination of nitrogen.** Nitrogen was determined by a micro-Kjeldahl procedure. Five grams of meat paste, plus 3 drops of octyl alcohol to prevent foaming, were blended with 100 ml of cold, ammonia-free water for 5 min in a Serval Omnimixer. Five ml of the slurry were transferred with a large bore pipette to a tared beaker and weighed. The amount of meat, in such an aliquot, was calculated, assuming the meat-water slurry to be homogeneous. Another 5 ml aliquot, containing approximately 8 mg of nitrogen, was placed in a 30 ml micro-Kjeldahl flask. Two ml of concentrated sulfuric acid, 2 drops of octyl alcohol, approximately 50 mg of a 1:1:3 mixture of mercuric oxide, potassium sulfate, and copper sulfate, and glass beads were added. The sample was gently boiled until frothing ceased, after which the sample was strongly heated until clear. After cooling, 15 ml of distilled water were added, and the solution, plus subsequent washings, was transferred to a steam distillation apparatus. Fifteen ml of 30% sodium hydroxide, plus 5% sodium thiosulfate, were added, and the alkaline mixture was distilled for 15 min. The ammonia was collected in a 125 ml Erlenmeyer flask receiver containing 5 ml of saturated boric acid solution and 2 drops of an indicator recommended by Ma and Zuayaga (10) (5 parts of 0.1% bromocresol green and 1 part of 0.1% methyl red, both in ethanol). The ammonia absorbed by the boric acid solution was titrated with 0.072 N sulfuric acid to a pink end point, 1 ml of the acid being equivalent to 1 mg of nitrogen. Protein was reported as  $N \times 6.25$ .

**Determination of chloride.** The method of Kerr (8) for chloride in meat products was adapted. Ten grams of meat paste were weighed into a 125 ml Erlenmeyer flask. Fifteen ml of 0.02 N silver nitrate were added, followed by 10 ml of concentrated nitric acid. The flask was placed on a hot plate and heated at low heat until foaming and frothing ceased. Then 1 ml of saturated (5%) potassium permanganate was added, and the sample was heated for 15 min or until clear. Ten ml of water were then added and the solution brought to a boil. The solution was cooled to room temperature, and 20 ml of water were added. Prior to titrating, 1 ml of mononitrobenzene, as recommended by Caldwell (5) to prevent fading of the end point, 10 ml of ethyl ether to dissolve the fat, and 1 ml of 1 N nitric acid saturated with ferric ammonium sulfate were added to the solution. The solution was then titrated with 0.02 N ammonium thiocyanate to a pink end point, and the chloride content of the sample was reported as mg per cent wet weight of meat.

**Preparation of meat ash.** Ashing conditions were chosen which minimized contamination and volatilization of the elements, prevented fusion of the determinants with the silica crucibles, and produced a clear, colorless solution of the ash.

<sup>a</sup> Mention of specific commercial materials or equipment does not constitute recommendation for their use above similar materials and equipment of equal value.

The samples dried for the moisture determination described above were placed in a gravity convection oven, the temperature was slowly raised to 325° C, and the samples held at this temperature overnight to char. The samples were then placed in a Tempeo muffle furnace (door closed), and the temperature was gradually raised to 400° C. At this stage, it was necessary to minimize air circulation, otherwise volatilizing fatty acids tend to ignite with possible loss of ash. After one hour at 400° C, the samples were cooled and the charred mass thoroughly wetted with 1 ml of concentrated nitric acid (prepared by distillation in an all-Pyrex, glass still). The samples were then placed on a hot plate and gently heated until the red-brown oxides of nitrogen were volatilized. The samples were replaced in the muffle furnace with the door slightly ajar to permit entrance of air. The temperature was raised to 525° C, and the samples were heated at this temperature for 2 hr. Some recalcitrant samples required two or three additions of nitric acid to produce the desired greenish-white ash. After cooling, the residues were weighed, and the ash was calculated and retained in the crucibles for analyses of electrolytes.

**Preparation of electrolyte solution.** Five ml of 0.1 N hydrochloric acid (prepared from all-Pyrex, glass-distilled acid) were added to each crucible. The solution of the ash was heated to boiling (to convert metal oxides to chlorides and pyrophosphate to orthophosphate) and then transferred to a 200 ml volumetric flask. Each crucible was washed repeatedly with 10 ml portions of hot, distilled water and the washings combined in the volumetric flask. The solution was adjusted to volume and transferred to polyethylene bottles.

**Determination of phosphorus.** Phosphorus present as the phosphate was determined by measurement of the molybdivanaphosphoric acid complex (9), which was stable for up to 2 days. Three ml of the stock electrolyte solution, containing approximately 150 mcg/ml of phosphorus, were transferred to a Beckman absorption tube (diameter 25 mm, length 150 mm). Fifteen ml of 1.7 N nitric acid and 4 ml of an ammonium molybdate-ammonium meta vanadate mixed reagent were added. The mixed reagent consisted of a 1:1 mixture of 5% ammonium molybdate and 0.125% ammonium meta vanadate in 6 N nitric acid. The contents were thoroughly mixed and the absorbance of the molybdivanadophosphoric acid complex was determined at 470 m $\mu$  in a Beckman B spectrophotometer. The amount of phosphorus present in the aliquot was determined from a standard curve. Phosphorus content of the sample was reported as mg per cent wet weight of meat.

**Determination of iron.** Iron was determined as the orange ferrous orthophenanthroline complex (4). Ten ml of the stock electrolyte solution, containing approximately 2 mcg/ml of iron, were transferred to a Beckman absorption tube. Five ml of 10% hydroxylamine hydrochloride were added to convert ferric iron to the ferrous state. The solution was mixed and allowed to stand for 15 min; subsequently, 10 ml of 0.1% orthophenanthroline and, as recommended by Bandemer and Schaible (2), 5 ml of 10% sodium citrate were added. The solutions were mixed and allowed to stand one hour for full color development. The absorbance of the orthophenanthroline-ferrous complex was determined at 520 m $\mu$  in a Beckman B spectrophotometer. The amount of iron present in the aliquot was determined from a standard curve and reported as mg per cent wet weight of meat.

**Determination of zinc.** Zinc was determined as the zinc dithizonate complex in carbon tetrachloride solutions. Two ml of the common stock electrolyte solution, containing approximately 2 mcg/ml of zinc, were transferred to a 100 ml polyethylene bottle. Fifteen ml of a mixed reagent, consisting of 10 parts water, 5 parts 2 N sodium acetate buffer pH 4.75, and 1 part 15% sodium thiosulfate, as recommended by Sandell (14), were added. The contents were mixed, and 15 ml of 0.001% dithizone (in redistilled carbon tetrachloride) were added. The bottle was capped and vigorously shaken on a shaking machine for 15 min. The mixture was transferred to an absorption tube, the two phases were allowed to separate, and the absorbance of the carbon tetrachloride solution at 530 m $\mu$  was determined. It was not necessary to separate the two phases, as the water phase was well above the light path of the Model B spectrophotometer. The amount of zinc present

in the aliquot was determined from a standard curve; the zinc content of the sample was calculated as mg per cent wet weight of meat.

**Determination of calcium.** Calcium was precipitated as the oxalate by the method of Sendroy (15), with subsequent oxidation of the oxalate by ammonium hexanitrate cerate as described by Berger (3). The excess cerate was titrated with ferrous ion employing orthophenanthroline as an oxidation-reduction indicator. Fifty ml of the stock electrolyte solution, containing approximately 125 mcg of calcium, were transferred to a 125 ml Erlenmeyer flask and slowly evaporated to dryness. Six ml of 1 N hydrochloric acid were added, and the solution was warmed. To 5 ml of this solution, in a 15 ml centrifuge tube, 2 ml of a 1:1 20% sodium acetate-saturated ammonium oxalate solution and 2 drops of bromocresol green indicator (0.1%) were added. The solution was adjusted to pH 4.5 and allowed to stand overnight to obtain complete precipitation. The tube was centrifuged at 3000 rpm for 15 min; the supernatant was decanted; and the precipitate was washed once with 2% ammonia and twice with a mixture of equal parts of ethyl alcohol, ethyl ether, and 2% ammonia. The precipitate was dried in an oven at 60° C. It was then dissolved by adding 2 ml of 2 N sulfuric acid and heating the mixture for 1 min in a boiling water bath. To the cooled solution, 2 ml of 0.014 N cerium ammonium nitrate were added. The solution was stirred and allowed to stand one hour. One drop of 0.005 M orthophenanthroline indicator was then added; and the excess cerate was titrated with 0.005 M ferrous ammonium sulfate, using a 5 ml microburette. The color change was from yellow to colorless, to a faint blue, and then to a sharp salmon-pink end point. The ferrous ammonium sulfate was standardized against 0.01 N sodium oxalate and the cerate solution against the iron solution. The amount of calcium present in the aliquot was determined from the titer. Total calcium content of the sample was reported as mg per cent wet weight of meat.

**Determination of magnesium.** The determination of magnesium was based on titrating with a standard solution of disodium ethylenediamine tetraacetate, employing a mixture of Eriochrome black T and methyl red as the indicator. Twenty-five ml of the common stock electrolyte solution, containing approximately 500 mcg of magnesium, were transferred to a 500 ml Erlenmeyer flask and diluted to 275 ml with distilled water. Dilution of the sample avoided interaction of meat phosphate components with the Eriochrome black T and resulted in a sharper end point. Ten ml of a monoethanolamine hydrochloric acid buffer pH 10.3, as recommended by Patton and Reeder (12), were added, together with 50 mg hydroxylamine hydrochloride and 100 mg potassium cyanide. The buffer solution consisted of 55 ml concentrated hydrochloric acid diluted to 400 ml, plus 310 ml redistilled monoethanolamine, the mixture diluted to 1 liter. Fifty mg of indicator, consisting of 0.2 g Eriochrome black T and 0.1 g methyl red triturated with 50 g of potassium chloride, were added. The solution was titrated with 0.02 N disodium ethylenediamine tetraacetate to a pale green end point. The disodium ethylenediamine tetraacetate solution was standardized by titrating separately, under similar conditions, against 500 mcg of magnesium and 500 mcg of calcium.

The calcium content of the 25 ml aliquot (determined separately as the oxalate) was subtracted from the titer, and the quantity of magnesium present was determined. The magnesium content of the sample was reported as mg per cent wet weight of meat.

**Determination of sodium and potassium.** Sodium and potassium were determined by flame photometry, employing a Beckman spectrophotometer equipped with a photomultiplier and a Model 9200 flame attachment. Interfering specific spectral emission was minimized by dilution of samples. Use of the photomultiplier permitted the selection of slit widths which minimized non-specific spectral emission.

Ten ml of the common stock electrolyte solution, containing 25–50 mcg/ml of sodium and 200–300 mcg/ml of potassium, were diluted to 50 ml. Sodium was determined at 589 m $\mu$ , using photomultiplier tube (IP28); slit width, 0.02 mm; sensitivity, 0.1; photomultiplier position, 4; oxygen pressure, 10 lb;

**TABLE 1**  
Eighteen analyses on a meat paste

Replicates <sup>1</sup>	% Wet weight of meat				Mg/100 g wet weight of meat							
	Moisture	Ash	Protein (N x 6.25)	Fat	Chloride	Magnesium	Calcium	Zinc	Sodium	Phosphorus	Potassium	Iron
1	72.95	1.18	23.00	2.0	40.2	23.6	3.38	3.84	42.6	197.1	407.0	2.24
2	72.95	1.20	23.21	2.0	41.2	23.3	3.38	3.61	46.4	197.3	419.5	2.20
3	72.68	1.22	22.97	2.2	41.2	24.4	3.37	3.98	42.5	198.9	416.0	2.28
4	72.89	1.17	22.80	2.0	40.0	22.9	3.39	3.98	46.5	197.4	425.9	2.23
5	72.73	1.19	22.94	2.1	40.3	23.1	3.36	3.84	41.6	197.3	420.8	2.17
6	71.50	1.22	23.32	1.9	39.8	22.9	3.39	3.98	46.8	197.4	421.4	2.17
7	72.82	1.23	23.15	2.0	39.5	23.0	3.38	3.99	41.6	199.4	414.7	2.31
8	72.62	1.19	23.06	2.0	41.3	23.4	3.38	3.83	43.7	202.1	420.7	2.29
9	72.70	1.13	23.23	2.2	39.6	23.6	3.35	3.84	42.6	199.8	420.1	2.24
10	72.99	1.21	23.29	2.1	36.0	23.5	3.35	4.10	42.4	199.7	417.9	2.29
11	72.96	1.24	23.00	2.2	40.4	23.2	3.39	3.83	46.7	199.4	426.0	2.23
12	72.72	1.21	23.23	1.9	40.3	23.6	3.38	4.08	44.8	196.1	422.0	2.23
13	72.24	1.24	23.02	2.2	40.1	23.3	3.32	3.84	45.2	201.1	425.0	2.24
14	71.31	1.22	22.97	2.0	40.7	24.4	3.41	4.06	45.1	197.9	425.5	2.24
15	71.93	1.29	23.21	2.1	40.6	23.9	3.39	3.96	46.6	200.0	423.8	2.29
16	71.93	1.21	22.91	1.9	40.6	24.3	3.36	4.00	46.6	202.6	423.4	2.29
17	71.56	1.23	23.23	2.0	40.0	23.6	3.34	3.95	42.8	197.6	427.4	2.37
18	69.58	1.18	23.38	2.2	39.5	23.5	3.34	3.71	45.3	200.1	422.8	2.38
Average value	72.28	1.21	23.11	2.1	40.1	23.5	3.37	3.91	44.4	198.9	421.1	2.26
Coef. of variation	1.21%	2.94%	0.69%	5.62%	2.89%	2.05%	0.68%	3.15%	4.21%	0.92%	1.18%	0.82%

<sup>1</sup> Analyses were made on eighteen 15-g samples, except that for the protein and chloride determinations separate samples, 5 and 10 g, respectively, were used.

**TABLE 2**  
Recovery of ions added to meat

Aliquot	Mg/100 g wet weight							
	Chloride	Magnesium	Calcium	Zinc	Sodium	Phosphorus	Potassium	Iron
1.....	114.6	40.36	6.73	6.95	82.8	363.2	804.6	5.73
2.....	114.6	40.74	6.49	6.95	70.4	364.6	815.0	5.81
3.....	114.4	41.84	6.68	7.19	77.8	364.9	827.0	5.65
4.....	116.9	40.52	6.75	7.22	77.6	366.5	827.4	5.60
5.....	115.7	40.62	6.60	7.00	73.4	363.2	819.0	5.61
6.....	116.6	39.96	6.54	7.15	77.2	362.7	819.4	5.59
Average.....	115.5	40.7	6.64	7.08	76.6	364.2	818.6	5.67
Amount in meat.....	401.1	23.5	3.37	3.91	44.4	198.9	421.1	2.26
Amount added.....	71.0	16.7	3.33	3.33	33.3	166.6	376.3	3.33
Per cent recovery.....	106%	103%	97%	94%	97%	99%	103%	102%

and acetylene pressure, 3.5 lb. To compensate for the enhancement of radiation due to the potassium present in meat samples, 60 mcg of potassium were incorporated in each ml of the sodium standards.

Potassium was determined at 768 m $\mu$ , using a cesium oxide, red sensitive phototube, slit width, 0.25 mm; sensitivity, 0.2; resistor, 2000 ohms; oxygen pressure, 10 lb; and acetylene pressure, 3.5 lb. At the level of sodium present, approximately 10 ppm, interference from this ion could not be detected in determining potassium.

#### RECOVERY EXPERIMENTS

To a residue, prepared by drying a 15 g sample of meat paste, a solution was added containing amounts of magnesium, calcium, zinc, sodium, phosphorus, and iron approximately equivalent to that in tissue. The solution was slowly evaporated to dryness, and the residue was charred and then ashed. The ash was dissolved in 0.1 N hydrochloric acid and diluted to 400 ml. Subsequent analysis for each electrolyte was performed as previously described.

To a 10 g sample of meat paste, 10 ml of 0.02 N sodium chloride were added and thoroughly mixed. Thirty ml of 0.02 N silver nitrate were added, followed by 10 ml of concentrated nitric acid, and the sample was analyzed for chloride as described.

#### ANALYTICAL RESULTS

Table 1 gives the results of the analysis of 18 replicate analyses of the same meat paste. Quantitative

results are given for 12 constituents. The coefficient of variation of the determinations ranged from 0.68% to 5.6%.

Table 2 gives the results of the recovery of 6 replicate analyses of the same meat paste. The percentage recovery ranged from 94% to 106%.

#### SUMMARY

A convenient plan of analysis for the determination of the proximate and major electrolyte content of beef has been described. With this procedure, the coefficient of variation for the constituents was as follows: moisture, 1.21%; ash, 2.94%; protein, 0.69%; fat, 5.62%; chloride, 2.89%; magnesium, 2.05%; calcium, 0.68%; zinc, 3.15%; sodium, 4.21%; phosphorus, 0.92%; potassium, 1.18%; and iron, 2.26%. The average recovery of various ions was as follows: chloride, 106%; magnesium, 103%; calcium, 97%; zinc, 94%; sodium, 97%; phosphorus, 99%; potassium, 103%; and iron, 102%.

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