Inorganic Elements in Beef Muscle and Their Relative Degree of Binding in Aqueous Beef Muscle Extracts

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The concentrations of 12 mineral elements in beef muscle were determined. Their relative degree of binding to meat extracts was evaluated from analysis of the concentration of each in dialyzed and undialyzed meat extracts and their dialyzates. The relative degree of binding was found to be Fe > Zn > Al > Ca > Cu > Mn > Mo > Mg > B > P > Na > K.

Berman and Swift (*4*) studied the action of NaCl on meat electrolyte binding. Using a centrifugation technique, the extent of the movement and binding of natural meat electrolytes following the addition of NaCl, the principal curing agent, was determined. Results show that little or no K, Na, or chloride was bound at 3° and 70° C. At 3° C., the addition of NaCl resulted in an increase in free Ca, Mg, and to a lesser extent, Zn. On heating, different results were obtained, and among the six elements studied, Zn was the only electrolyte that was substantially and strongly associated or bound with soluble proteins. The loosely bound elements are easily lost through drip and meat juices resulting from long periods of storage of fresh meat or repetitive freezing and thawing.

The oxidation rate of myoglobin present in crude aqueous beef extracts has been shown to decrease if the extracts were dialyzed before freezing and then displayed under light (*12*). In another study, the addition of metal ions to meat extracts was found to increase or decrease myoglobin oxidation rate depending on pH (*1*). The present study was carried out to determine the relative degree of binding of some of the elements present in high and low concentrations in muscle.

Experimental

Hanging beef tenderloin muscles were used in this study. As much fat as possible was removed, and the meat was homogenized with distilled deionized water in a Waring Blendor for 1 minute at full speed. Purification steps and preparation of dialyzed extracts and dialyzates are shown in Figure 1. The analysis of the dialyzed, dialyzate, undialyzed meat extracts, as well as meat tissue will be discussed. Iron was determined according to the method presented by Bandemer and Schaible (*2*) and outlined by Ullrey and coworkers (*20*). The flame photometric analysis procedure originally described by Dean (*7*) and outlined in detail by Kirton and Pearson (*10*) was used in determining K and Na.

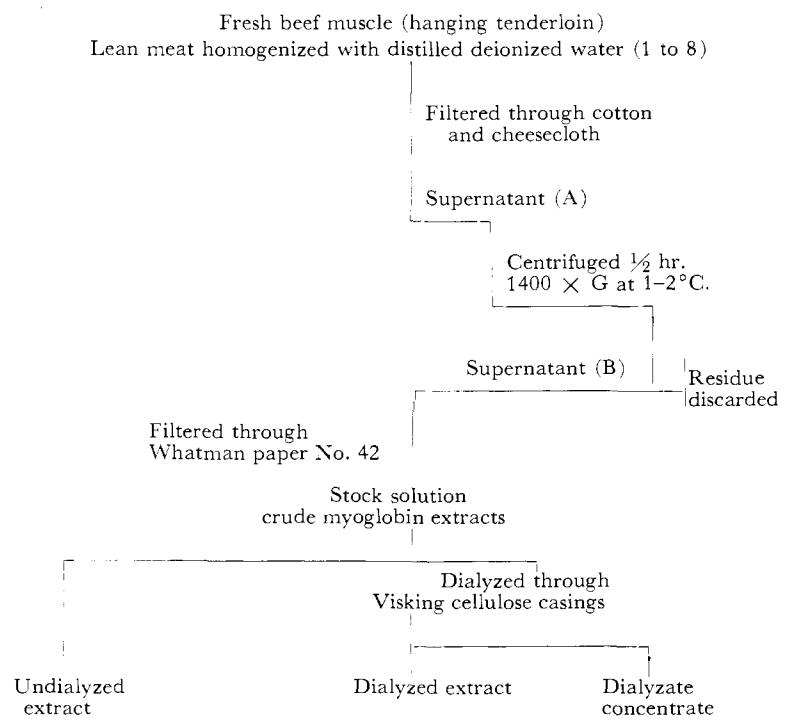

Figure 1. Extraction and general procedures

Phosphorus, Na, Mg, Ca, Fe, Zn, Al, Cu, Mn, B, and Mo were determined using spectrographic analysis. The raw meat samples, extracts or dialyzates intended for analysis were ashed at 550° C. for 12 hours. An acid solution containing 150 ml. of concentrated HCl per liter and 0.02% cobalt (used as an internal standard) was prepared. To buffer the rate of excitation of the different elements, 0.5% lithium and 1.0% potassium were added. The ash was then dissolved in ashing crucibles with a proper amount (2.5, 5, or 10 ml.) of the HCl-Co-Li-K solution. A portion of the ash solution large enough to prevent complete evaporation during the excitation was then transferred to a porcelain boat.

Calibration of the spectral lines was accomplished using a synthetic ash standard prepared according to the procedure described by Mathis (*14*) and outlined by Kenworthy (*9*). The synthetic ash solutions provided 11 different concentrations for the elements (P, Ca, Mg, Mn, Fe, Cu, B, Zn, Mo, Al, and Na). A synthetic ash which could conveniently be used has been suggested by Mitteldorf (*16*).

The photoelectric spectrograph used in these analyses involved a 1.5-meter Quantograph, which uses a diffraction grating that has 981 lines per millimeter. The excitation conditions were obtained from a Multiconice (Applied Research Laboratories) discharge with 900 volts output, 2 microfarads capacitance, 50 microhenries inductance, and residual resistance. The discharge was an interrupted arc producing a sparklike condition. In reading the samples, the authors found that room temperature fluctuation during the profiling proce-

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Table I. Total Concentrations[a] of Mineral Elements in Wet Raw Beef Muscle[b]

References	K	P	Na	Mg	Co	Fe	Zn	Al	Cu	Mn	B	Mo
Katz (8)	3.7×10^3	1.7×10^3	6.5×10^2	2.4×10^2	20	2.4×10^2	...	...	...	...	...	...
McCance, Widdowson (15)	3.3×10^3	2.8×10^3	6.9×10^2	2.4×10^2	154	43	...	...	...	...	...	...
Mitteldorf, Landon (17)	3.0×10^3	1.7×10^3	4.1×10^2	2.1×10^2	26	31	47	0.005–0.05	0.5	0.02	0.05	0.02
Berman (3)	4.2×10^3	2.0×10^3	4.4×10^2	2.4×10^2	34	23	39	...	...	...	...	...
Koch & Roesmer (11)												
Concn. in raw beef	...	1.3×10^3	...	...	...	41	20	...	...	...	...	...
Concn. in meat ash	...	1.9×10^3	...	...	...	5.8×10^3	2.9×10^3	...	...	...	...	...
Shirley et al. (18)	...	2.3×10^3	...	...	1.2×10^2	...	...	...	...	...	...	...
Present study												
Concn. in raw beef	3.5×10^3	*2.4×10^3[c]	6.5×10^3	2.1×10^2	1.2×10^2	48	17	0.9–9.0	2.0	1.7	0.04 0.1	0.05 0.90
Concn. in meat ash	3.8×10^3	*3.0×10^3[c]	7.3×10^3	2.6×10^4	1.3×10^4	6.0×10^3	2.1×10^3	$>0.9 \times 10^2$	2.5×10^2	2.2×10^2	1.3	11.0

[a] In p.p.m. ± 10%. All elements determined spectrographically except K by flame photometry.
[b] Hanging tenderloin.
[c] See also values in text.

Table II. The Concentration[a,b] of Free[c] Mineral Elements in the HCl Dissolved Dialyzate Ash and in the Water-Soluble and -Insoluble Dialyzate Ash Fractions

Element	Water Soluble	Nonwater Soluble	Dissolved in HCl (Total[c])
P	8.2×10^5	4.0×10^4	9.0×10^5
Na	8.0×10^4	5.0×10^3	9.0×10^4
Ca	2.0×10^4	5.0×10^3	2.5×10^4
Mg	7.5×10^4	1.6×10^3	9.0×10^4
Mn	3.0×10^2	1.4×10^2	4.6×10^2
Fe	2.5×10^3	1.8×10^3	4.4×10^3
Cu	3.3×10^2	2.7×10^2	6.8×10^2
B	7.0×10^2	0.85×10^2	8.0×10^2
Zn	1.5×10^3	6.0×10^2	4.0×10^3
Mo	25.0	6.0	30.0
Al	3.0×10^3	3.0×10^3	8.0×10^3

[a] In p.p.m. ± 15%.
[b] Based on 0.84 ash in the dialyzate.
[c] To find their respective concentration in meat, divide by 1000.

Table III. The Concentrations[a] of K, Na Determined by Flame Photometry and of Fe Determined Spectrophotometrically in Dialyzed, Undialyzed, and Dialyzates of Meat Extracts[b]

Sample	K	Na	Fe
Dialyzed	8	..	2.0
		..	..
Undialyzed	320	..	2.4
Dialyzate	..	..	0.4
Fe added to dialyzed (4.6)	..	..	8.0
Dialyzed	11	6	..
Dialyzed	15	5.5	..
Dialyzed	9	6	
Undialyzed	410	80	..
Undialyzed	390	73	..
Undialyzed	420	80	

[a] In p.p.m. ± 5%.
[b] Values reported here were obtained from different samples in different experiments.

dure affected the results, and a recheck was always made.

The recorded spectral lines of the various elements were based on the weight of the ash, the dilution factor, and compared with the standard curve of the mentioned synthetic ash. Reference is made here to the terminology used in the tables.

HCl–Dissolved Ash. This ash was dissolved with hot HCl before diluting in the HCl-Co-Li-K solution.

Water Soluble Ash. This was the ash filtrate obtained after filtering and washing the insoluble ash several times with hot distilled deionized water.

Total Concentrations of Elements in Ash. The values reported in Table I were obtained from ashing meat samples and were based on ash weight. Approximate values may be obtained if concentrations in the undialyzed extracts are multiplied by the dilution factor 9, since the extracts were made 1 to 8.

Water-Insoluble Ash. This was the residue obtained from the filtration of the water-soluble ash.

Bound Elements. Those elements present in 1-to-8 aqueous meat extracts and which did not dialyze out when the extracts were dialyzed against distilled, deionized water at 4° C. (Dialysis was considered complete 12 hours after the dialyzate gave a negative test for chlorine.) The dialyzed meat extracts were ashed for direct determination of the concentration of the bound elements. Concentrations of the bound elements were also calculated for recheck from the difference between the total and the free elements.

Free Elements. Those elements found in the collected dialyzate obtained from dialyzing the aqueous meat extracts. Usually the collected dialyzate was concentrated from a volume of 25 to 30 liters to approximately 2 ml. when 2 liters of the aqueous meat extracts were dialyzed in a 4-liter beaker for 4 to 5 days.

Free elements were also calculated for comparison of the difference between the concentration of the total and bound elements.

Results and Discussion

Since total element concentrations have been studied by several workers, they will be considered first. Although the methods of analysis or the muscle analyzed by various workers were not necessarily the same as those used in this study, comparisons will be made with the results of those workers who used muscles from beef. Table I gives the results of analyses obtained in this study as compared with those of other workers. All values reported are based on meat wet-weight basis.

Many of the results in this study agree with the values reported by most of these workers. Phosphorus, however, is somewhat higher than those values reported in the literature. It interfered with other elements in the sample, as diluting the samples changed the resultant phosphorus values. The value of sodium changed whenever an attempt was made to dilute the sample and bring the phosphorus spectral line within the scale. Koch and Roesmer (11) found that phosphorus interferes with the determination of chlorine and sulfur. A recheck using the chemical method of

Table IV. The Concentrations[a] of 12 Elements as Bound and Free in Meat Extracts

Sample	K	Na	P	B	Mg	Mo	Mn	Cu	Ca	Al	Zn	Fe
Undialyzed (total)	400.0	83.0	230.0	0.01	29.0	0.01	0.17	0.30	13.0	0.90	2.00	2.40
Dialyzed (bound)	10.0	6.0	24.0	0.002	9.00	0.004	0.07	0.15	7.00	0.50	1.20	2.00
Free dialyzable (by difference)	390.0	77.0	206.0	0.008	20.0	0.006	0.10	0.15	6.00	0.40	0.80	0.40
% Free	97.5	92.8	90.0	80.0	69.0	60.0	59.0	50.0	46.0	45.0	40.0	17.0

[a] In p.p.m. ± 10%.

Chen, Toribara, and Warner (*6*), gave phosphorus values of 1400 and 140,000 p.p.m. in meat and meat ash, respectively. Thus, the values for P obtained by the spectrograph should be considered as only approximate. From a general observation on the percentage concentration of the major elements in meat ash, there was 38.0% K, 14.0% P, 7.3% Na, 2.6% Mg, 1.3% Ca, 0.6% Fe, and 0.21% Zn. The minor elements did not make up more than 0.25%, and the total percentages of the major and minor elements together do not add to 100%. This may be explained because the elements determined here are not present as free elements, but occur in ash as chlorides, oxides, etc. Phosphates and oxides are stable at the ashing temperature, but since the ash is soluble (Table II), the phosphates are the most dominant ash form. Average per cent ash was 1.16% which closely agrees with values reported by Koch and Roesmer (*11*) and Shirley and coworkers (*18*).

Concentrations of elements in the water-soluble ash fraction and the non-water-soluble fraction are shown in Table II. The sum of these two fractions may be easily compared with HCl-dissolved ash in the same table. P is the most dominant element of the dialyzable ions present. The phosphorus value is exceedingly high due to the interference previously discussed; however, even after correction for interference, phosphorus ranks first in Table II and second to potassium in the over-all concentration. Based on the per cent solubility—i.e., concentration % of H_2O soluble in the acid soluble—Table II also shows that the order in solubility of the dialyzable metal ions in the ash is as follows: P > Mg > Na > B > Mo > Ca > Zn > Mn > Fe > Cu > Al.

Solubility of these elements should be taken into consideration in analytical methods utilizing extracts or aqueous dilutions of muscle rather than on the muscle itself. This order of elements' solubility was measured after ashing, after which one would expect that the chemical and physical change introduced will affect the solubility characteristics of these different elements.

Relative degree of binding of the elements K, Na, and Fe is shown in Table III. The data show that the average concentration of K and Na in the dialyzed extracts was 9.5 and 5.9 p.p.m., respectively. The average concentration of these two elements in the undialyzed control was 388 p.p.m. for K and 62 p.p.m. for Na. Since the meat extracts were made in a meat-to-water ratio of 1 to 8, the calculated concentrations of these elements with respect to raw dialyzed meat is 85.5 p.p.m. for K and 53 p.p.m. for Na, whereas the undialyzed meat would have 3.49×10^3 p.p.m. K, and 5.58×10^2 p.p.m. Na, which closely agree with values reported in Table I for both elements in direct analysis of raw meat samples. Table III also shows that Fe was much more tightly bound than the monovalent ions K^+ and Na^+. Thus, whereas 80% of the iron in meat is bound, only 3% of the potassium and 9% of the sodium are bound. Stone and Shapiro (*19*) reported that concentration of K in the ash of meat residue and meat filtrate were proportionally in close agreement, indicating the high water solubility of K. They did not determine, however, the degree to which K was bound in meat filtrate (extract) or in the residue of meat tissue. The great difference in the binding of Fe, on the one hand, and K, P, and Na, on the other hand, was thought to be related to their effect on the oxidation or stability of the meat pigment (myoglobin) present in the meat extracts. However, the ash form—viz., oxides, chlorides, or phosphates, etc.—in which the elements occur and the pH of the added ashed elements were found to be the most important factors in controlling the formation of brown acidic metmyoglobin in meat and meat extracts (*1*).

Analyses for the concentration of 12 elements in the dialyzed extracts, the undialyzed extracts, and dialyzate of meat extracts are summarized in Table IV. The relative degree of binding is in this order: Fe > Zn > Al > Ca > Cu > Mn > Mo > Mg > B > P > Na > K.

Binding stability constant tables for metals were presented by Martell and Calvin (*13*) and Chaberek and Martell (*5*). Whenever the data presented in this study are compared, it should be kept in mind that the system used to obtain these data involves more than one soluble protein.

Acknowledgment

The authors are grateful to A. L. Kenworthy and Frank Hager of the Horticulture Department, Michigan State University, for facilitating the use of a spectrograph and to Harry E. Snyder of Iowa State University for his critical comments during the preparation of this manuscript.

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Received for review September 7, 1965. Accepted May 31, 1966. Presented in part before the Division of Agricultural and Food Chemistry, 148th Meeting, ACS, Chicago, Ill., August 1964. Journal Article No. 3705, Michigan Agricultural Experiment Station.