National Institute of Standards & Technology

Report of Investigation

Reference Material 8414

Bovine Muscle Powder

Agriculture Canada

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This Reference Material (RM) is intended for use in evaluating analytical methods and instruments used for the determination of major, minor, and trace constituent elements in meat, meat products, and other similar food, agricultural, and biological materials. RM 8414 was prepared and characterized by the Centre for Land and Biological Resources Research (CLBRR), Agriculture Canada, who is the sole authority for the information provided in this report including best estimate and other technical information. RM 8414 consists of 50 g of dry powdered beef muscle packaged in two glass bottles of 25 g each that are sealed in an aluminum-nylon pouch.

Expiration of Report: The Report of Investigation of RM 8414 is valid, within the measurement uncertainty specified, until **31 August 2011**, provided the RM is handled in accordance with instructions given in this report (see "Instructions for Use"). This report is nullified if the RM is damaged, contaminated, or otherwise modified.

Maintenance of RM Value Assignment: NIST will monitor this RM over the period of its value assignment. If substantive technical changes occur that affect the value assignment before the expiration of this report, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

Material Application: This material, together with other food-type RMs issued by NIST, covers a wide range of natural matrix compositions and elemental concentrations. By providing concentration values for a wide range of constituent elements of nutritional, toxicological, and environmental significance, RM 8414 is expected to be useful in assessing the role of nutrients in health and disease, establishing dietary requirements for nutrients, accumulating accurate baseline concentration data for nutrients and contaminants, and monitoring foods for nutrients and contaminants.

It is conceivable that some elemental contamination may have been picked up during preparation, processing, and handling, and also that alterations of original chemical composition of the bulk starting material may have resulted from differential sieving of component tissues with differing compositions. Hence, this material cannot be taken as strictly containing solely natural, physiological concentrations of inorganic constituent elements. It can, however, be considered to reflect a food product taken through preparation/processing, not unlike food materials processed commercially or in the analytical laboratory. This RM can thus be used for data quality control (DQC) in the analysis of such processed materials and can additionally be considered for DQC of methods measuring total concentrations of elements in food products with natural, uncontaminated, physiological element levels.

The material was prepared at Agriculture Canada under the direction of Milan Ihnat, Centre for Land and Biological Resources Research (CLBRR), who also coordinated the interlaboratory analytical campaign to characterize the material and performed evaluations/calculations of analytical data to arrive at best estimate and informational elemental concentration values. The former Food Research Centre and the Engineering and Statistical Research Centre, Agriculture Canada, kindly provided some facilities, experimental assistance, and construction of the blender.

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See Report Revision History on Page 7

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Statistical support, design, and consultation with computations for homogeneity estimates as well as best estimate and informational concentration values were provided by M.S. Wolynetz, Statistical Research Section, Research Program Service, Agriculture Canada.

Support aspects involved in the issuance of this RM were coordinated through the NIST Measurement Services Division.

Recommended Procedures for Use: RM 8414 should be kept at room temperature in its original bottle, tightly-capped and not exposed to intense direct light or ultraviolet radiation. Prior to each use, contents of the bottle should be well mixed by gentle shaking and rolling of the container. A recommended minimum subsample weight of 0.5 g should be taken for analysis. Moisture content should be determined on a separate subsample for conversion of analytical results to a dry weight basis. The recommended method of drying to relate analytical results to the best estimate values listed in Table 1 is drying for 4 h in an air oven at 85 $^{\circ}$C. Analyses reported in Table 1 represent total concentrations of elements in this RM. Dissolution procedures should be capable of rendering a completely dissolved sample appropriate to the method and should be designed to avoid losses of elements by volatilization or by retention on decomposition and processing containers and measuring equipment. Analytical methods should be capable of measuring total levels of analytes for comparison with best estimate values.

PREPARATION AND ANALYSIS[1]

Preparation: The source of material for RM 8414 was boneless hip, commonly denoted Canada grade A round steak (consisting of the major muscles: semimembranosus, semi-tendinosus, biceps femoris and adductor) from market weight beef cattle (Bos Taurus). All preparatory work was performed in-house at the facilities of Agriculture Canada, Ottawa. [1,2]. The meat was trimmed of all visible fat and connective tissues and the resulting lean muscle tissue was ground frozen in a stainless steel comminuting machine with a 1 mm stainless steel screen. The finely milled product was freeze-dried and the dry bulk powder was sterilized with cobalt-60 gamma radiation to 2.0 megarads by Atomic Energy of Canada Ltd. All subsequent processing was performed in a moderately clean room using plastic equipment. Additional reduction of particle size was effected by ball milling with Teflon-TFE balls in a 1 L Teflon-PFA screw cap jar. Material sieving was through nylon monofilament sieve cloths supported in high density white polyethylene holders. Pairs of sieves with openings of approximately 250 μm/50 μm or 250 μm/90 μm were used to yield suitable narrow middle cuts constituting the RM. This fraction was blended in a polymethylmethacrylate V-configuration blender and packaged into clean 150 mL brim capacity, clear glass bottles with pulp/Saran-lined black polypropylene screw caps. A total of 288 randomly selected units was segregated for physical and chemical characterization. Units were individually hermetically sealed in aluminum-nylon pouches to enhance long term stability.

Assessment of Homogeneity: Homogeneity testing was performed on randomly selected units for 12 elements by application of high precision analytical methods in three laboratories. [3] Subsamples of 0.5 g were taken from each of four units and analyzed by M. Ihnat, Agriculture Canada for calcium, iron, potassium, magnesium, sodium and zinc using a precise and reliable application of acid digestion flame atomic absorption spectrometry. [4,5] Subsamples of 0.3-1.5 g each, taken from a total of six units, were analyzed by R.W. Dabeka, Health and Welfare Canada for cadmium, cobalt, nickel, and lead by precise and reliable graphite furnace atomic absorption spectrometric methods following acid digestion and separation/preconcentration of the analytes using coprecipitation with ammonium pyrrolidine dithiocarbamate (all four elements) and additionally with palladium/ascorbic acid for lead. [6,8] Fluoride was determined by the same analyst in 0.1 g subsamples from six units by an acid-facilitated microdiffusion-ion specific electrode method. [9] Sensitive solid sampling graphite furnace atomic absorption spectrometric determinations were performed by M. Stoeppler and U. Bagschik, Nuclear Research Center, Julich, Federal Republic of Germany, on 0.0005 g (0.5 mg) subsamples for copper (50 subsamples from 5 units) and lead (280 subsamples from 8 units). [3] In addition, the extensive set of analytical results obtained from a large number of analysts participating in the interlaboratory characterization campaign was assessed to provide homogeneity estimates for other elements. [3] This material generally exhibits excellent homogeneity. Uncertainties associated with best estimate values reported in Table 1 include effects of material inhomogeneity.

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[1]Certain commercial materials and equipment are identified in order to adequately specify the experimental procedure. Such identification does not imply a recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment are the best available for the purpose.

Best Estimate Values for Constituent Elements: The population of analytical information from cooperating analysts using a wide variety of analytical methods was assessed using technical and statistical criteria as well as judgment to arrive at best estimate values and associated uncertainties listed in Table 1. These values, not certified by NIST, are based on results obtained by at least two, but typically several independent, reliable analytical methods. Concentration estimates for five other elements are provided in Table 2 as information values only, as they are based on results of limited determinations or only one analytical method.

Chemical Characterization: Chemical analyses to establish best estimate concentrations were conducted in an interlaboratory cooperative characterization campaign involving the initiating laboratory and a large number of selected expert analysts in other laboratories. A wide range of independent analytical methods listed in Table 3 were applied to provide analytical results for a large number of nutritionally, toxicologically, and environmentally pertinent elements. Typically, analyses were performed by each participant on duplicate subsamples from randomly selected (usually four) units of material using sample weights and methods left to the discretion of the analyst. Subsample sizes ranged from 0.002 to 5 g, typically 0.4 g. Elemental determinations were performed on "as received" material, with conversion of results to a dry matter basis using moisture values determined on separate 2 g samples by the procedure specified in this report. Analytical results obtained by Agriculture Canada and Health and Welfare Canada laboratories during homogeneity testing and analysis served a dual role of providing information for homogeneity assessment and quantitation.

This Report of Investigation, prepared by Milan Ihnat, is contribution No. 92-46 from CLBRR, Research Branch, Agriculture, Canada.

Table 1. Best Estimate Concentrations of Constituent Elements

Major Constituents

Element	Content and uncertainty weight (%)[a]	Methods[b]
Nitrogen	13.75 ± 0.32	I01, J01, J02
Potassium	1.517 ± 0.037	A01, B02, D01, E01
Phosphorus	0.836 ± 0.045	B02, B03, B04,,F01, F02, M01
Sulfur	0.795 ± 0.041	B02, D04, J02, J04, L01, M02
Sodium	0.210 ± 0.008	A01, B02, D01
Chlorine	0.188 ± 0.015	D01, D04, F01, K02, L01

Minor and Trace Constituents

Element	Content and uncertainty mg/kg[a]	Methods[b]
Magnesium	960 ± 95	A01, A03, B02, B03, B04, D01
Calcium	145 ± 20	A01, A03, B02, B03, D01, E01, E02
Zinc	142 ± 14	A01, A03, B02, B03, B04, D01, D02, D03, E01
Iron	71.2 ± 9.2	A01, A03, B02, B03, D01, D02, D03, E01, E02
Rubidium	28.7 ± 3.5	D01, D02, E02
Copper	2.84 ± 0.45	A01, A05, B02, B03, C03, C06, D01, D03, E01, H01
Aluminum	1.7 ± 1.4	A05, B02, D01
Bromine	1.1 ± 0.5	D01, E01
Boron	0.6 ± 0.4	B02, D04
Lead	0.38 ± 0.24	A05, A06, A16, C03, H01
Manganese	0.37 ± 0.09	A01, A05, A06, B02, D01, D03, E01
Molybdenum	0.08 ± 0.06	C03, C06, C07, D03, F01, H06
Selenium	0.076 ± 0.010	B06, C01, C04, D02, D03, G01
Chromium	0.071 ± 0.038	A06, B02, C05, D03
Strontium	0.052 ± 0.015	B02, B03, C03
Nickel	0.05 ± 0.04	A16, C03, H01
Iodine	0.035 ± 0.012	D03, D05, D06, F02

Cadmium	0.013 ± 0.011	A04, A05, A06, A16, C03, D03, H01
Arsenic	0.009 ± 0.003	A11, D03
Cobalt	0.007 ± 0.003	A16, D01, D02, D03, H01
Mercury	0.005 ± 0.003	A09, A10, D03

[a] Best estimate values, weight percent or mg/kg (ppm), are based on the dry material, dried according to instructions in this report and are equally-weighted means of results from generally at least two, but typically several, different analytical methods applied by analysts in different laboratories. Uncertainties are imprecision estimates expressed either as a 95% confidence interval or occasionally (B, Ni) as an interval based on the entire range of accepted results for a single future determination, based on a sample weight of at least 0.5 g. These uncertainties, based on between-method and laboratory, between-unit and within-unit estimates of variances, include measures of analytical method and laboratory imprecisions and biases and material inhomogeneity.

[b] Analytical method codes and descriptions are provided in Table 3.

Table 2. Information Concentrations of Constituent Elements

Element	Estimated content, mg/kg[a]	Methods[b]
Antimony	0.01	D02, D03
Barium	0.05	C03, D02
Cesium	0.05	D01
Fluorine	0.22	H04
Vanadium	0.005	B02, D03

[a] These analytical values, on a dry matter basis, are estimates given strictly for information only as they are based on results of limited determinations or only one method; no uncertainties are provided. Use of this RM to quantitatively monitor method performance for elements other than those with best estimate concentration values in Table 1 is not warranted.

[b] Analytical method codes and descriptions are provided in Table 3.

Table 3.   Analytical Methods Used to Determine Best Estimate and
Information Concentration Values[a]

Analytical Method	Code	Elements Determined
Acid digestion flame atomic absorption spectrometry	A01	Ca, Cu, Fe, K, Mg, Mn, Na, Zn
Dry ashing flame atomic absorption spectrometry	A03	Ca, Fe, Mg, Zn
Acid digestion electrothermal atomic absorption spectrometry	A04	Cd
Closed vessel acid digestion electrothermal atomic absorption spectrometry	A05	Al, Cd, Cu, Mn, Pb
Dry ashing electrothermal atomic absorption spectrometry	A06	Cd, Cr, Mn, Pb
Acid digestion cold vapour atomic absorption spectrometry	A09	Hg
Closed vessel acid digestion cold vapour atomic absorption spectrometry with preconcentration	A10	Hg

Closed vessel acid digestion hydride generation atomic absorption spectrometry with preconcentration	A11	As
Acid digestion coprecipitation electrothermal atomic absorption spectrometry	A16	Cd, Co, Ni, Pb
Acid digestion inductively coupled plasma atomic emission spectrometry	B02	Al, B, Ca, Cr, Cu, Fe, K, Mg Mn, Na, P, S, Sr, (V), Zn
Closed vessel acid digestion inductively coupled plasma atomic emission spectrometry	B03	Ca, Cu, Fe, Mg, P, Sr, Zn
Dry ashing inductively coupled plasma atomic emission spectrometry	B04	Mg, P, Zn
Acid digestion hydride generation inductively coupled plasma atomic emission spectrometry	B06	Se
Acid digestion isotope dilution mass spectrometry	C01	Se
Closed vessel acid digestion isotope dilution inductively coupled plasma mass spectrometry	C03	As, (Ba), Cd, Cu, Mo, Ni Pb, Sr
Acid digestion dry ashing hydride generation isotope dilution inductively coupled plasma mass spectrometry	C04	Se
Dry ashing acid digestion isotope dilution mass spectrometry	C05	Cr
Acid digestion isotope dilution inductively coupled plasma mass spectrometry	C06	Cu, Mo
Dry ashing inductively coupled plasma mass spectrometry	C07	Mo
Instrumental neutron activation analysis	D01	Al, Br, Ca, Cl, Co, (Cs), Cu Fe, K, Mg, Mn, Na, Rb, Zn
Instrumental neutron activation analysis with acid digestion	D02	(Ba), Co, Fe, Rb, (Sb), Se Zn
Neutron activation analysis with radiochemical separation	D03	Cd, Co, Cr, Cu, Fe, Hg, I, Mn Mo, (Sb), Se, (V), Zn
Neutron capture prompt gamma activation analysis	D04	B, Cl, S

Epithermal instrumental neutron activation analysis	D05	I
Preconcentration neutron activation analysis	D06	I
Particle induced X-ray emission spectrometry	E01	Br, Ca, Cu, Fe, K, Mn, Rb, Zn
X-ray fluorescence	E02	Ca, Fe
Acid digestion light absorption spectrometry	F01	Mo, P
Dry ashing light absorption spectrometry	F02	Cl, I, P
Acid digestion fluorometry	G01	Se
Closed vessel acid digestion anodic stripping voltametry	H01	Cd, Co, Cu, Ni, Pb
Extraction ion selective electrode	H04	(F)
Dry ashing catalytic adsorption polarography	H06	Mo
Kjeldahl method for nitrogen -volumetry	I01	N
Combustion elemental analysis -thermal conductivity	J01	N
Combustion elemental analysis with chromatographic separation -thermal conductivity	J02	N, S
Combustion elemental analysis -fluorometry	J04	S
Dry ashing volumetry	K02	Cl
Closed vessel combustion ion chromatography	L01	Cl, S
Acid digestion gravimetry	M01	P
Dry ashing gravimetry	M02	S

[a] Letter codes refer to classes of similar methods; number codes refer to specific variants. Elements in parentheses have only information values for this RM.

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APPENDIX A

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