National Institute of Standards & Technology

Report of Investigation

Reference Material 8433

Corn Bran

A Joint Material of Agriculture Canada and NIST

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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 as well as proximates, total dietary fiber, and calories in corn, corn products, and other similar food, agricultural, and biological materials. This material can also be used for quality assurance when assigning values to in-house control materials. RM 8433 consists of 50 g of dry powdered corn bran packaged in a glass bottle.

Reference Concentration Values: Reference concentration values for major, minor, and trace constituent elements are provided in Table 1. Reference concentration values for proximates, total dietary fiber, and calories are provided in Table 2. The reference values in Tables 1 and 2 were derived from results reported in an interlaboratory comparison exercise and by four additional collaborating laboratories, respectively. Reference values are noncertified values that are the best estimates of the true values; however, the values do not meet NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods.

Information Concentration Values: Information concentration values for additional elements, fat, and fatty acids are provided in Tables 3 and 4. These are noncertified values with no reported uncertainties as there is insufficient information to assess uncertainties. The information values are given to provide additional characterization of the material. Use of this RM to evaluate method performance for analytes other than those with reference concentration values in Tables 1 and 2 is not warranted.

Expiration of Value Assignment: The value assignment of this RM lot is valid until **31 August 2008**, within the measurement uncertainties specified, provided the RM is handled and stored in accordance with the instructions given in this report. Value assignment is nullified if the RM is damaged, contaminated, or 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 its expiration, NIST will notify the purchaser. Return of the attached registration card will facilitate notification.

Statistical support was provided by M.S. Wolynetz, Statistical Research Section, Research Program Service, Agriculture Canada and L.M. Gill of the NIST Statistical Engineering Division.

Support aspects involved with the value assignment and issuance of this RM were coordinated through the NIST Standard Reference Materials Program by J.C. Colbert and W.R. Wolf of the U.S. Department of Agriculture.

Willie E. May, Chief
Analytical Chemistry Division

Thomas E. Gills, Director
Office of Measurement Services

Gaithersburg, MD 20899
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See Report Revision History on Page 8

RM 8433 was prepared at Agriculture Canada under the direction of M. Ihnat, Centre for Land and Biological Resources Research (CLBRR). Coordination of the technical measurements leading to the value assignment of this RM was performed by M. Ihnat of CLBRR, Agriculture Canada, and K.E. Sharpless and S.A. Wise of the NIST Analytical Chemistry Division. Following the original analyses for elemental value assignment by the laboratories listed in Appendix A, the material was distributed by NIST to Covance Laboratories (Madison, WI), Lancaster Laboratories (Lancaster, PA), Medallion Laboratories (Minneapolis, MN), and Southern Testing and Research Laboratories (Wilson, NC) for the measurement of proximates, fatty acids, calories, and total dietary fiber.

NOTICE AND WARNING TO USERS

Storage: Until required for use, RM 8433 should be stored at room temperature in its original bottle, tightly capped, and not exposed to intense direct light or ultraviolet radiation.

Warning: For laboratory use only. Not for human consumption.

Instructions for Use: Prior to each use, contents of the bottle should be well mixed by gentle shaking and rolling of the container. A recommended minimum subsample mass of 0.5 g should be taken for elemental analysis. Moisture content should be determined on a separate subsample for conversion of analytical results to a dry-mass basis. The recommended method of drying to relate analytical results to the assigned values listed in the tables is drying for 4 h in an air oven at 85 °C. Concentrations reported in Table 1 represent total concentrations of elements in this RM. Dissolution procedures for elemental analyses 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 elements for comparison with reference values.

PREPARATION AND ANALYSIS

Preparation: The source of material for RM 8433 was refined corn bran, G-fine mesh, obtained from A.E. Staley Manufacturing Co., Decatur, IL, USA. All preparatory work following acquisition of the commercial product was performed at the facilities of Agriculture Canada, Ottawa [1,2]. The dry bulk powder was sterilized with cobalt-60 gamma radiation to 2.0 Mrad by Atomic Energy of Canada Ltd. The material was sieved through nylon monofilament sieve cloths supported in high-density white polyethylene holders. Pairs of sieves with openings of approximately 200 µm and 90 µm were used to yield a middle-cut fraction for use as the RM. This fraction was blended in a polymethylmethacrylate V-configuration blender and packaged into clean 150 mL brim capacity, clear glass bottles with triseal (polyethylene)-lined white polypropylene screw caps. A total of 144 randomly selected units were used for physical and chemical characterization in the original analyses.

Assessment of Homogeneity: Homogeneity testing was performed on randomly selected units for 12 elements by three laboratories [2,3]. Subsamples of 0.5 g and 2.0 g were taken from a total of four units and analyzed by M. Ihnat, Agriculture Canada, for calcium, potassium, magnesium, sodium, strontium, and zinc using acid digestion flame atomic absorption spectrometry [4,5]. Subsamples of 0.8 g to 1.6 g each, taken from a total of six units, were analyzed by R.W. Dabeka, Health and Welfare Canada, for cadmium, cobalt, lead, and nickel by graphite furnace atomic absorption spectrometric (GFAAS) methods following acid digestion and separation and preconcentration of the analytes using coprecipitation with ammonium pyrrolidine dithiocarbamate (all four elements) and additionally with palladium and 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 [8]. Solid sampling GFAAS determinations were performed by M. Stoeppler and U. Bagschik, Nuclear Research Center, Jülich, Federal Republic of Germany, on a total of 40 subsamples of 0.0005 g (0.5 mg) each, from four units for copper [2,3]. In addition, the analytical results obtained from a large number of analysts (Appendix A) participating in the interlaboratory characterization campaign were assessed to provide homogeneity estimates for other elements [2,3]. No statistically significant heterogeneity was found for aluminum, barium, boron, bromine, cadmium, calcium, chlorine, copper, iodine, iron, lead, magnesium, manganese, mercury, molybdenum, nickel, nitrogen, phosphorus, potassium, rubidium, selenium, sodium, strontium, sulfur, vanadium, and zinc in sample sizes required by the analytical technique ranging from 0.1 g to 2 g. Data for all analytes (including the proximates) have been treated as though they are homogeneous, although the homogeneity of other analytes has not been investigated.

Value Assignment: Chemical analyses to establish reference concentrations of elements were conducted in an interlaboratory comparison exercise involving Agriculture Canada and selected analysts in other laboratories (Appendix A) using analytical methods listed in Table 5. Analyses were performed by each participant on duplicate subsamples from randomly selected (typically four) units of material; subsample sizes and methods were left to the discretion of the analyst. Subsample sizes ranged from 0.001 g to 5 g, typically 0.4 g. Elemental determinations were performed on the material "as received," with conversion of results to a dry-mass basis using moisture values determined on separate 2 g subsamples by the drying procedure specified in the "Instructions for Use" section of this report.

Following the original elemental determinations, NIST distributed RM 8433 to four laboratories (Appendix B) for measurement of proximates, fatty acids, calories, and total dietary fiber. Each laboratory analyzed one portion from each of three bottles of RM 8433 using their routine methods (Table 6). Determinations were performed on the material "as received," with conversion of results to a dry-mass basis using moisture values determined on separate subsamples taken from each of the three bottles. Standard Reference Material (SRM) 1846 Infant Formula was analyzed for quality assurance.

Table 1. Reference Concentration Values of Constituent Elements

Major Constituent	Mass Fraction (%)[a]			Methods[b]
Nitrogen[c]	0.882	±	0.027	I01, I02, J01, J02
Minor and Trace Constituents	**Mass Fraction (mg/kg)[a]**			**Methods[b]**
Sulfur	860	±	150	B02, B03, J02, J03
Magnesium	818	±	59	A01, A03, B02, B03, D01
Potassium	566	±	75	A01, B01, B02, B03, D01, E01
Sodium	430	±	31	A01, B01, B02, B03, D01
Calcium	420	±	38	A01, B02, D01, E02
Phosphorus	171	±	11	B02, B03, F01, F02
Chlorine	31	±	21	D01, D04, K02
Zinc	18.6	±	2.2	A01, A03, B02, B03, D01, D02, D03, E01, H01
Iron	14.8	±	1.8	A01, B02, B03, D02, D03, E01
Strontium	4.62	±	0.56	A01, B02, B03, C03, E01
Boron	2.8	±	1.2	B02, B03, D04
Manganese	2.55	±	0.29	A01, A05, B02, B03, D01, D03, E01, E02
Copper	2.47	±	0.40	A01, A05, A06, B02, C03, C06, D01, D03, E01, H01
Barium	2.40	±	0.52	B02, B03, C03, D01
Bromine	2.3	±	0.5	D01, E01
Aluminum	1.01	±	0.55	A05, A06, D01
Rubidium	0.5	±	0.3	D01, D02, E01
Molybdenum	0.252	±	0.039	B02, C03, C06, C07, D01, D03
Nickel	0.158	±	0.054	A05, A16, C03, H01
Lead	0.140	±	0.034	A04, A05, A16, C01, C03, H01
Selenium	0.045	±	0.008	C01, C04, D02, D03, G01
Iodine	0.026	±	0.006	D03, D05, D06, F01
Cadmium	0.012	±	0.005	A05, A16, C03, D03, H01
Vanadium	0.005	±	0.002	B02, D01, D03
Mercury	0.003	±	0.001	A09, A10, A15, D03

[a] Reference values are based on the dry material, dried according to instructions in this report and are equally weighted means of results from 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 (aluminum, boron, mercury) 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 among-method, among-laboratory, among-unit, and within-unit estimates of variances, include measures of analytical method and laboratory imprecisions and biases and material inhomogeneity. **NOTE:** NIST has replaced the previously used term "best estimate" with "reference value."

[b] Analytical method codes and descriptions are provided in Table 5.

[c] Nitrogen results have been updated to include results from four additional laboratories (Appendix B). Each reference concentration value, expressed as a mass fraction on a dry-mass basis, is a weighted mean of the two group means from the

laboratories shown in Appendices A and B; results were weighted at 75 % and 25 %, respectively, based on the number of laboratories that provided data in the two studies. The uncertainty in the reference values is expressed as an expanded uncertainty, U, at the 95 % level of confidence, and is calculated according to the method described in the ISO Guide [10]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte.

Table 2. Reference Concentration Values of Proximates, Total Dietary Fiber, and Calories

	Mass Fraction, as received (%)[a]	Mass Fraction, dry-mass basis (%)[a]
Moisture	7.12 ± 0.60	0 (by definition)
Solids	92.88 ± 0.60	100 (by definition)
Ash	0.437 ± 0.050	0.471 ± 0.054
Protein[b]	5.093 ± 0.052	5.484 ± 0.057
Carbohydrate	86.37 ± 0.88	92.99 ± 0.82
Total Dietary Fiber	83.6 ± 2.7	90.0 ± 2.5
Calories[c]	(374.7 ± 4.8) kcal/100 g	(403.4 ± 4.0) kcal/100 g

[a] Each reference concentration value, expressed as a mass fraction on an as-received or dry-mass basis, is an equally weighted mean of results from the laboratories shown in Appendix B. The uncertainty in the reference values is expressed as an expanded uncertainty, U, at the 95 % level of confidence, and is calculated according to the method described in the ISO Guide [10]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. Analytical methodology information is provided in Table 6.

[b] The protein concentration was calculated from the nitrogen values reported by the laboratories shown in Appendix B using a conversion factor of 6.25; subsequent calculations of carbohydrates and calories were also based on these protein concentrations. The nitrogen values reported by the laboratories shown in Appendix B were combined with the original data for calculation of the reference value for nitrogen provided in Table 1.

[c] The value for calories is the mean of the individual caloric calculations. If the mean proximate values are used for calculation, with caloric equivalents of 9, 4, and 4 for fat, protein, and carbohydrate, respectively, the mean caloric content is 374.7 kcal/100 g and 403.8 kcal/100 g on an as-received and dry-mass basis, respectively.

Table 3. Information Concentration Values of Constituent Elements

	Mass Fraction, dry-mass basis (mg/kg)[a]	Methods[b]
Antimony	0.004	D01, D02, D03
Arsenic	0.002	A11, D03
Chromium	0.11	A06, B02, C05, D02, D03
Cobalt	0.006	A16, D01, D02, D03, H01

[a] This analytical value, on a dry-mass basis, is an estimate given strictly for information only as it is based on the result of limited determinations or lack of agreement among results; no uncertainty is provided.

[b] Analytical method codes and descriptions are provided in Table 5.

Table 4. Information Concentration Values of Fat and Selected Fatty Acids (as Triglycerides)

	Mass Fraction, as received (%)[a]	Mass Fraction, dry-mass basis (%)[a]
Fat	0.98	1.1
Hexadecanoic Acid (C16:0) (Palmitic Acid)	0.22	0.24
Octadecanoic Acid (C18:0) (Stearic Acid)	0.041	0.044
(Z) - 9 - Octadecenoic Acid (C18:1) (Oleic Acid)	0.31	0.33
9 - Octadecenoic Acid (C18:l) (Elaidic Acid)	0.036	0.039
(Z,Z) - 9, 12 - Octadecadienoic Acid (C18:2) (Linoleic Acid)	0.13	0.14
Eicosanoic Acid (C20:0) (Arachidic Acid)	0.012	0.013
Docosanoic Acid (C22:0) (Behenic Acid)	0.0080	0.0086
Tetracosanoic Acid (C24:0) (Lignoceric Acid)	0.017	0.018

[a] These information values, reported on an as-received or dry-mass basis, are the equally weighted means of results reported by the laboratories shown in Appendix B. These values are based on results from determinations by three or four of the laboratories and are included to provide additional characterization of the material; no uncertainties are provided. Analytical methodology information is provided in Table 6.

Table 5. Analytical Methods Used by Collaborating Laboratories (Appendix A) to Determine Reference and Information Concentration Values of Elements[a]

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

Closed vessel acid digestion cold vapor atomic absorption spectrometry with preconcentration	A10	Hg
Closed vessel acid digestion hydride generation atomic absorption spectrometry with preconcentration	A11	(As)
Acid digestion cold vapor atomic absorption spectrometry with preconcentration	A15	Hg
Acid digestion coprecipitation electrothermal atomic absorption spectrometry	A16	Cd, (Co), Ni, Pb
Acid digestion atomic emission spectrometry	B01	K, Na
Acid digestion inductively coupled plasma atomic emission spectrometry	B02	B, Ba, Ca, (Cr), Cu, Fe, K, Mg, Mn, Mo, Na, P, S, Sr, V, Zn
Closed vessel acid digestion inductively coupled plasma atomic emission spectrometry	B03	B, Ba, Fe, K, Mg, Mn, Na, P, S, Sr, Zn
Acid digestion isotope dilution mass spectrometry	C01	Pb, Se
Closed vessel acid digestion isotope dilution inductively coupled plasma mass spectrometry	C03	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, Ba, Br, Ca, Cl, (Co), Cu, K, Mg, Mn, Mo, Na, Rb, (Sb), V, Zn
Instrumental neutron activation analysis with acid digestion	D02	(Co), (Cr), Fe, Rb, (Sb), Se, Zn
Neutron activation analysis with radiochemical separation	D03	(As), Cd, (Co), (Cr), Cu, Fe, Hg, I, Mn, Mo, (Sb), Se, V, Zn

Neutron capture prompt gamma activation analysis	D04	B, Cl
Epithermal instrumental neutron activation analysis	D05	I
Preconcentration neutron activation analysis	D06	I
Particle induced X-ray emission spectrometry	E01	Br, Cu, Fe, K, Mn, Rb, Sr, Zn
X-ray fluorescence	E02	Ca, Mn
Acid digestion light absorption spectrometry	F01	I, P
Dry ashing light absorption spectrometry	F02	P
Acid digestion fluorometry	G01	Se
Closed vessel acid digestion anodic stripping voltametry	H01	Cd, (Co), Cu, Ni, Pb, Zn
Kjeldahl method for nitrogen -volumetry	I01	N[b]
Kjeldahl method for nitrogen-light absorption spectrometry	I02	N[b]
Combustion elemental analysis -thermal conductivity	J01	N[b]
Combustion elemental analysis with chromatographic separation -thermal conductivity	J02	N[b], S
Combustion elemental analysis-infrared spectrometry	J03	S
Dry ashing volumetry	K02	Cl

[a] Letter codes refer to classes of similar methods; number codes refer to specific variants. Elements in parentheses have only information values in this RM. **NOTE:** NIST has replaced the previously used term "best estimate" with "reference value".

[b] See Table 6 for additional information.

Table 6. Methods Used by Collaborating Laboratories (Appendix B) for the Determination of Proximates, Fatty Acids, Calories, and Total Dietary Fiber

Ash	mass loss after ignition in a muffle furnace
Calories	calculated; [(9 × fat) + (4 × protein) + (4 × carbohydrate)]
Carbohydrate	calculated; [solids – (protein + fat + ash)]
Fat	sum of individual fatty acids
Fatty acids	hydrolysis followed by gas chromatography
Moisture	mass loss after drying in a vacuum oven (2 laboratories); mass loss after drying in a forced-air oven (2 laboratories)
Nitrogen	Dumas (1 laboratory); modified Dumas (1 laboratory); Kjeldahl (2 laboratories). Note that in the original elemental determinations, laboratories provided results using Kjeldahl, combustion - thermal conductivity, and combustion - chromatographic separation - thermal conductivity.
Protein	calculated from nitrogen using a factor of 6.25
Solids	calculated; (sample mass – moisture)
Total dietary fiber	enzymatic digestion followed by gravimetry

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Report Revision History: 04 October 99 (This technical revision reports the addition of reference and information values for proximates, calories, fatty acids, and total dietary fiber.); 24 September 93 (original report date).

Users of this RM should ensure that the report in their possession is current. This can be accomplished by contacting the SRM Program at Telephone: (301) 975-6776 (select "Certificates"), Fax: (301) 926-4751, e-mail srminfo@nist.gov, or via the Internet at http://ts.nist.gov/srm.

Appendix A. Collaborating Analysts for Elemental Determinations

G. Alfthan, National Public Health Institute, Helsinki, Finland.

P. Allain and Y. Mauras, Laboratoire de Pharmacologie et toxicologie, Centre de Pharmacovigilance, Centre Hospitalier Regional et Universitaire d'Angers, Angers Cedex, France.

D.L. Anderson, Division of Contaminants Chemistry, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Washington, DC, USA.

R. Beine, D.E. Lichtenberg, E. Denniston, and M. Peralta, Division of Regulatory Services, University of Kentucky, Lexington, KY, USA.

W.T. Buckley, G. Wilson, and D. Godfrey, Agassiz Research Station, Agriculture Canada, Agassiz, BC, Canada.

A.R. Byrne, M. Dermelj, M. Horvat, N. Prosenc, and D. Konda, Nuclear Chemistry Department, J. Stefan Institute, E. Kardelja University, Ljubljana, Slovenia.

A. Chatt and R.R. Rao, Slowpoke-2 Facility, Trace Analysis Research Centre, Department of Chemistry, Dalhousie University, Halifax, NS, Canada.

J.G. Crock, Branch of Geochemistry, U.S. Geological Survey, Denver, CO, USA.

W.C. Cunningham, Division of Contaminants Chemistry, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Washington, DC, USA.

R.W. Dabeka, Food Research Division, Health Protection Branch, Health and Welfare Canada, Ottawa, ON, Canada.

J. de Jong and E. Boers, State Institute for Quality Control of Agricultural Products (RIKILT), Wageningin, The Netherlands.

J.F. Dlouhy, Analytical Services Division, River Road Environmental Technology Centre, Environment Canada, Ottawa, ON, Canada.

R. Dybczynski and B. Danko, Department of Analytical Chemistry, Institute of Nuclear Chemistry and Technology, Warsaw, Poland.

A. Farina Mazzeo, R. Piergallini, E.P. Salsano, and F. Abballe, Laboratory of Pharmaceutical Chemistry, Istituto Superiore di Sanita, Rome, Italy.

M. Ferguson, W.R. Musick, S.A. MacIntyre, and W.R. Laing, Analytical Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA.

C.T. Figueiredo and W.B. McGill, Department of Soil Science, University of Alberta, Edmonton, AB, Canada.

P.W.F. Fischer and A. Giroux, Bureau of Nutritional Sciences, Food Directorate, Health and Welfare Canada, Ottawa, ON, Canada.

A.R. Flegal and D.R. Smith, Institute of Marine Sciences, University of California-Santa Cruz, Santa Cruz, CA, USA.

K. Frank, J. Denning, and L. Hayne, Institute of Agriculture and Natural Resources, Department of Agronomy, University of Nebraska-Lincoln, Lincoln, NE, USA.

F.L. Fricke, C. Gaston and K.A. Wolnik, National Forensic Chemistry Center, U.S. Food and Drug Administration, Cincinnati, OH, USA.

G.A. Gill, Institute of Marine Sciences, University of California-Santa Cruz, Santa Cruz, CA, USA.

E.S. Gladney and E.M. Hodge, Health and Environmental Chemistry Group, Los Alamos National Laboratory, Los Alamos, NM, USA.

G.U. Hesselius, Mikro Kemi AB, Uppsala, Sweden.

K. Heydorn, E. Damsgaard, and N. Lavi, Isotope Division, Riso National Laboratory, Roskilde, Denmark.

E.L. Hoffman, Activation Laboratories Ltd., Ancaster, ON, Canada.

M. Ihnat, Centre for Land and Biological Resources Research, Agriculture Canada, Ottawa, ON, Canada.

J.L. Imbert and M. Olle, Service Centrale d'Analyse, Centre National de la Recherche Scientifique, Vernaison, France.

L.L. Jackson, Branch of Geochemistry, U.S. Geological Survey, Denver, CO, USA.

D.L. Jeffress and S. Allison, Feed Control Laboratory, Missouri Department of Agriculture, Jefferson City, MO, USA.

P.F. Kane and N. Suttles, Indiana State Chemist Laboratory, Purdue University, West Lafayette, IN, USA.

F.J. Kasler, Department of Chemistry, University of Maryland, College Park, MD, USA.

B. Kratochvil and N. Motkosky, Department of Chemistry, University of Alberta, Edmonton, AB, Canada.

S.S. Krishnan and J. Lin, Medical Physics Laboratory, Toronto General Hospital, Toronto, Ontario, Canada.

D. Kuik and P. Heida, Governmental Food and Commodities Inspection Service, Leeuwarden, The Netherlands.

G.W. Latimer Jr., W. Igler, L. Park, H. Hinojosa, C. Upton, and D. Arvelo, Agricultural Analytical Services, Office of the Texas State Chemist, College Station, TX, USA.

J.W. McLaren, S.N. Willie, V.J. Boyko, and S.S. Berman, Measurement Science, Institute for Environmental Chemistry, National Research Council of Canada, Ottawa, ON, Canada.

W. Maenhaut, and G. Hebbrecht, Laboratory for Analytical Chemistry, Instituut voor Nucleaire Wetenschappen, Rijksuniversiteit Gent, Gent, Belgium.

B. Magyar, B. Aeschlimann, and H.R. Elsener, Institute of Inorganic Chemistry, Swiss Federal Institute of Technology, Zurich, Switzerland.

T.P. Mawhinney, R. Boles, R. Cathey, and P. Eggeman, Experimental Station Laboratories, College of Agriculture, University of Missouri-Columbia, Columbia, MO, USA.

N.J. Miller-Ihli and F.E. Greene, Nutrient Composition Laboratory, Beltsville Human Nutrition Research Center, U.S. Department of Agriculture, Beltsville, MD, USA.

Nuclear Research Reactor Facility, Department of Nuclear Engineering and Engineering Physics, University of Virginia, Charlottesville, VA, USA.

I. Olmez, Nuclear Reactor Laboratory, Massachussets Institute of Technology, Cambridge, MA, USA.

I.S. Palmer, O.E. Olson Biochemistry Laboratories, Chemistry Department, South Dakota State University, Brookings, SD, USA.

J.B. Reust, H.R. Lang, and A. Janchen, Analytical Research and Development, Project/Product Coordination, Sandoz Pharma Ltd., Basel, Switzerland.

R. Schelenz and E. Zeiller, Chemistry Unit, International Atomic Energy Agency-Seibersdorf, Vienna, Austria.

L.J. Schmidt and U. Soni, Mass Spectrometry and Elemental Analysis Department, Shell Development Company, West Hollow Research Center, Houston, TX, USA.

J. Schoenau, Department of Soil Science, University of Saskatchewan, Saskatoon, SK, Canada.

P. Schramel, Institut für Okologische Chemie, Gessellschaft für Strahlen- und Umweltforschung mbH, Neuherberg, Federal Republic of Germany.

T.R. Shuler and F.H. Nielsen, Grand Forks Human Nutrition Research Center, U.S. Department of Agriculture, Grand Forks, ND, USA.

R.J. Stevens and A. Beattie, Food and Agricultural Chemistry Research Division, Department of Agriculture (Northern Ireland), Belfast, Northern Ireland.

M. Stoeppler, K. May, P. Ostapczuk, M. Froning, U. Bagschik, and V. Arenas, Forschungszentrum Jülich GmbH, Institut für Angewandte Physikalische Chemie, Jülich, Federal Republic of Germany.

J.T. Tanner and K.K. Cook, Nutrient Surveillance Branch, Division of Nutrition, U.S. Food and Drug Administration, Washington, DC, USA.

C. Veillon, K.Y. Patterson, and N. Hardison, Vitamin and Mineral Nutrition Laboratory, Beltsville Human Nutrition Research Center, U.S. Department of Agriculture, Beltsville, MD, USA.

J. Versieck, L. Vanballenberghe, and A. Wittoek, Department of Internal Medicine, Division of Gastroenterology, University Hospital, Gent, Belgium.

J.H. Watkinson and A.A. Judge, MAFTech Ruakura, Ruakura Agriculture Centre, Ministry of Agriculture and Fisheries, Hamilton, New Zealand.

G.M. Whitford, School of Dentistry, Department of Oral Biology - Physiology, Medical College of Georgia, Augusta, GA, USA.

P.C. Williams, Grain Research Laboratory, Canadian Grain Commission, Winnipeg, MB, Canada.

J.R.W. Woittiez, M.dC. Tangonan, K. Kroon, and A. Lagerwaard, Interfaculty Reactor Institute, Department of Radiochemistry, Delft University of Technology, Delft, The Netherlands.

J.R.W. Woittiez and M. Geusebroek, Netherlands Energy Research Foundation ECN, Petten, The Netherlands.

W. Yuen, Saskatchewan Research Council, Saskatoon, SK, Canada.

Appendix B. Collaborating Laboratories for Proximate, Fatty Acid, Total Dietary Fiber, and Calorie Determinations

Covance Laboratories, Madison, WI, USA.

Lancaster Laboratories, Lancaster, PA, USA.

Medallion Laboratories, Minneappolis, MN, USA.

Southern Testing and Research Laboratories, Wilson, NC, USA.