



National Institute of Standards & Technology

Report of Investigation

Reference Material 8415

Whole Egg Powder

A Joint Material of Agriculture Canada and NIST

(Distributed by the National Institute of Standards and Technology)

Reference Material (RM) 8415 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, fatty acids, and calories in egg, egg products and similar food, and agricultural and biological materials. This material can also be used for quality assurance when assigning values to in-house control materials. RM 8415 consists of 35 g of dry whole egg powder packaged in a glass bottle sealed in an aluminum-nylon pouch.

Reference Concentration Values: Reference concentration values for major, minor, and trace constituent elements are provided in Table 1. Reference concentration values for selected proximates 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, fatty acids, and water-soluble vitamins are provided in Tables 3, 4, and 5. 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 quantitatively monitor method performance for analytes other than those with reference concentration values in Tables 1 and 2 is not recommended.

Expiration of Certification: The certification of RM 8415 is valid, within the measurement uncertainty specified, until **24 February 2011**, provided the RM is handled in accordance with instructions given in this report (see "Instructions for Use"). This certification 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 certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

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

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

Stephen A. Wise, Chief
Analytical Chemistry Division

Robert L. Watters, Jr., Chief
Measurement Services Division

Gaithersburg, MD 20899
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See Report Revision History on Page 9

RM 8415 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, calories, and fatty acids. Selected individual fatty acids were measured in NIST's Analytical Chemistry Division in 2005 by T. Yarita. RM 8415 was also distributed in an interlaboratory comparison exercise in 1995; information values for the concentrations of several water-soluble vitamins have been assigned based on results reported by the laboratories listed in Appendix C.

NOTICE AND WARNING TO USERS

Storage: Until required for use, RM 8415 should be stored under refrigeration 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 minimum subsample size 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-mass basis. The recommended method of drying to relate analytical results to the reference values listed in the tables is drying for 4 h in an air oven at 85 °C. 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 ANALYSIS¹

Preparation: The source of material for RM 8415 was Canada grade A dried whole egg powder, containing added color and a maximum of 2 % Zeolex (sodium silico-aluminate anti-caking ingredient) obtained from Vanderpol's Eggs Ltd. (Surrey, BC, Canada). All preparatory work following acquisition of the commercial product was performed at the facilities of Agriculture Canada (Ottawa, Ontario, Canada) [1,2]. The dry bulk powder was sterilized with cobalt-60 gamma radiation to 2.0 megarads by Atomic Energy of Canada Ltd. Material sieving was through nylon monofilament sieve cloths supported in high-density white polyethylene holders. Pairs of sieves with openings of approximately 250 µm and 50 µm were used to yield a middle-cut fraction for use as the reference material. This fraction was blended in a polymethylmethacrylate V-configuration blender and packaged into clean 150 mL brim capacity, colorless glass bottles with triseal (polyethylene)-lined white polypropylene screw caps. Units were individually hermetically sealed in aluminum-nylon pouches and stored under refrigeration (0 °C to 5 °C) to enhance long-term stability. A total of 144 randomly selected units were used for physical and chemical characterization in the original analyses.

Homogeneity Assessment: Homogeneity testing was performed on randomly selected units for nine elements in three laboratories [3,4]. 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 aluminum, calcium, iron, magnesium, potassium, strontium, and zinc using acid digestion flame atomic absorption spectrometry [4–7]. Subsamples of 1.0 g to 2.0 g each, taken from a total of six units, were analyzed by R.W. Dabeka, Health and Welfare Canada, for lead by graphite furnace atomic absorption spectrometry following acid digestion and separation and preconcentration of the analyte using

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

coprecipitation with palladium and ascorbic acid [8]. Solid sampling graphite furnace atomic absorption spectrometric determinations were performed by M. Stoeppler and U. Bagschik, Nuclear Research Center, Jülich, Germany, on a total of 40 subsamples of 0.5 mg each, from a total of four units for copper [2]. In addition, the analytical results obtained from a large number of analysts (Appendix A) participating in an interlaboratory comparison exercise were assessed to provide homogeneity estimates for other elements [2,4]. No statistically significant heterogeneity was found for aluminum, calcium, iron, lead, magnesium, manganese, mercury, phosphorus, potassium, selenium, sodium, strontium, 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 and fatty acids) have been statistically 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 6. 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 “Instruction for Use” section of this report.

Following the original elemental determinations, NIST distributed RM 8415 to four laboratories (Appendix B) for measurement of proximates, fatty acids, and calories. Each laboratory analyzed one portion from each of three bottles of RM 8415 using their routine methods (Table 7). 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. RM 8415 was also analyzed by several laboratories participating in an interlaboratory comparison exercise in 1995; several of these laboratories (Appendix C) reported values for water-soluble vitamins, and these results are provided as information values in Table 5.

To assess stability, NIST measured selected individual fatty acids in 2005. Determinations were performed on three test portions taken from one jar and six test portions taken from a second jar. Fatty acids were extracted into hexane/acetone using pressurized-fluid extraction, were derivatized to their methyl esters, and were analyzed by gas chromatography (100-m poly-bis-cyanopropylsiloxane column) with flame ionization detection. Mass fractions reported on an as-received basis were converted to a dry-mass basis using the reference value for moisture.

Table 1. Reference Concentrations of Constituent Elements

Major Constituents		
Element	Mass Fraction (%) ^(a)	Methods ^(b)
Nitrogen ^(c)	6.30 ± 0.13	I01, J01, J02
Phosphorus	1.001 ± 0.032	B02, B03, F01, F02, M01
Sulfur	0.512 ± 0.050	B02, D04, J04, M02
Chlorine	0.508 ± 0.032	D01, D04, K02
Sodium	0.377 ± 0.034	A03, B01, B02, B03, D01
Potassium	0.319 ± 0.037	A01, A03, B02, B03, B04, D04
Calcium	0.248 ± 0.019	A01, B02, B04, D01

Minor and Trace Constituents		
Element	Mass Fraction (mg/kg) ^(a)	Methods ^(b)
Aluminum	540 ± 86	A05, B02, B03, B04, D01
Magnesium	305 ± 27	A01, A03, B02, B03, B04, D01
Iron	112 ± 16	A01, A03, B02, B03, B04, D02, D03
Zinc	67.5 ± 7.6	A01, A03, B02, B03, D02, D03, H01
Strontium	5.63 ± 0.46	A01, B02, B03
Copper	2.70 ± 0.35	A05, A06, B02, C06, D03, H01
Iodine	1.97 ± 0.46	D03, D05, F01, H05
Manganese	1.78 ± 0.38	A01, A03, A05, B02, B04, D01, D03
Selenium	1.39 ± 0.17	A08, C01, C04, D01, D02, D03, G01
Vanadium	0.459 ± 0.081	B02, D01, D03
Boron	0.41 ± 0.26	B02, C09, D04
Chromium	0.37 ± 0.18	A05, A06, A12, B02, C05, D02, D03
Molybdenum	0.247 ± 0.023	C06, D03, H06
Lead	0.061 ± 0.012	A16, H01
Cobalt	0.012 ± 0.005	D01, D02, D03, H01
Mercury	0.004 ± 0.003	A09, A10, D03

^(a) Reference values, expressed as mass fractions, 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 (B, Co, K, Mn, Mo, Zn) as an interval based on the entire range of accepted results for a single future determination, based on a sample mass 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. (NIST has replaced the previously used term “best estimate” with “reference value”.)

^(b) Analytical method codes and descriptions are provided in Table 6.

^(c) Nitrogen results have been updated to include results from four additional collaborating laboratories (Appendix B). Only the uncertainty has changed from that provided with the original assigned value.

Table 2. Reference Concentrations of Proximates

Analyte	Mass Fraction (as received) (%) ^{(a)(b)}	Mass Fraction (dry-mass basis) (%) ^(a)
Moisture	3.53 ± 0.54	0 (by definition)
Solids	96.47 ± 0.54	100 (by definition)
Ash	4.78 ± 0.53	4.96 ± 0.55
Protein ^(c)	37.8 ± 1.2	39.2 ± 1.1

^(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 and NIST Guides [9]. 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 7.

^(b) The “as received” values are based on the moisture content at the time the measurements for value assignment were performed. The amount of moisture in this material may change if moisture is transferred to or absorbed from the atmosphere during storage.

^(c) The protein concentration was calculated from the nitrogen values reported by the laboratories shown in Appendix B using a conversion factor of 6.25. 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.

Table 3. Information Concentrations of Constituent Elements

Element	Mass Fraction (mg/kg) ^(a)	Methods ^(b)
Antimony	0.002	D02, D03
Arsenic	0.01	D03
Barium	3	B02, B03, B04
Cadmium	0.005	A06, D03, H01

^(a) These analytical values, on a dry-mass basis, are estimates given strictly for information only, as they are based on results of a limited number of determinations or from only one method; no uncertainties are provided.

^(b) Analytical method codes and descriptions are provided in Table 6.

Table 4. Information Concentrations of Selected Fatty Acids (as Triglycerides)

Analyte	Mass Fraction (as received) (%) ^(a)	Mass Fraction (dry-mass basis) (%) ^(a)
Tetradecanoic Acid (C14:0) (Myristic Acid)	0.16	0.16
Pentadecanoic Acid (C15:0)	0.035	0.036
Hexadecanoic Acid (C16:0) (Palmitic Acid)	8.8	9.1
(Z)-9-Hexadecenoic Acid (C16:1 n-7) (Palmitoleic Acid)	1.8	1.8
Heptadecanoic Acid (C17:0) (Margaric Acid)	0.11	0.11
Octadecanoic Acid (C18:0) (Stearic Acid)	2.6	2.7
(Z)-9-Octadecenoic Acid (C18:1 n-9) (Oleic Acid)	17.0	17.6
(Z)-11-Octadecenoic Acid (C18:1 n-7) (Vaccenic Acid)	1.3	1.4
(Z,Z)-9,12-Octadecadienoic Acid (C18:2 n-6) (Linoleic Acid)	3.7	3.8
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3 n-3) (Linolenic Acid)	0.36	0.37
(Z)-11-Eicosenoic Acid (C20:1 n-9) (Gondoic Acid)	0.15	0.15

^(a) These information values, reported on an as-received or dry-mass basis, are the means of results provided by a single method (pressurized fluid extraction followed by gas chromatography with flame ionization detection) run at NIST and are included to provide additional characterization of the material; no uncertainties are provided.

Table 5. Information Concentrations of Selected Vitamins

Analyte	Mass Fraction, (as received) (mg/kg) ^(a)	Mass Fraction, (dry-mass basis) (mg/kg) ^(a)
Vitamin B ₁	2.8	2.9
Vitamin B ₂	12	13
Vitamin B ₆	3.7	3.8
Vitamin B ₁₂	0.068	0.070
Biotin	1.3	1.3
Folic Acid	1.8	1.8
Inositol	860	890
Niacin	2.4	2.5
Pantothenic Acid	91	94

^(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 C. These values are based on results from determinations by one to four laboratories and are included to provide additional characterization of the material; no uncertainties are provided. Analytical methodology information is provided in Table 8.

Table 6. 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, Fe, K, Mg, Mn, Sr, Zn
Dry Ashing Flame Atomic Absorption Spectrometry	A03	Fe, K, Mg, Mn, Na, Zn
Closed Vessel Acid Digestion Electrothermal Atomic Absorption Spectrometry	A05	Al, Cr, Cu, Mn
Dry Ashing Electrothermal Atomic Absorption Spectrometry	A06	(Cd), Cr, Cu
Dry Ashing Hydride Generation Atomic Absorption Spectrometry	A08	Se
Acid Digestion Cold Vapor Atomic Absorption Spectrometry	A09	Hg
Closed Vessel Acid Digestion Cold Vapor Atomic Absorption Spectrometry with Preconcentration	A10	Hg
Dry Ashing Digestion Electro-Thermal Atomic Absorption Spectrometry	A12	Cr
Acid Digestion Coprecipitation Electrothermal Atomic Absorption Spectrometry	A16	Pb
Acid Digestion Atomic Emission Spectrometry	B01	Na
Acid Digestion Inductively Coupled Plasma Atomic Emission Spectrometry	B02	Al, B, (Ba), Ca, Cr, Cu, Fe, K, Mg, Mn, Na, P, S, Sr, V, Zn
Closed Vessel Acid Digestion Inductively Coupled Plasma Atomic Emission Spectrometry	B03	Al, (Ba), Fe, K, Mg, Na, P, Sr, Zn
Dry Ashing Inductively Coupled Plasma Atomic Emission Spectrometry	B04	Al, (Ba), Ca, Fe, K, Mg, Mn,
Acid Digestion Isotope Dilution Mass Spectrometry	C01	Se
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
Neutron Activation Mass Spectrometry	C09	B
Instrumental Neutron Activation Analysis	D01	Al, Ca, Cl, Co, Mg, Mn, Na, Se, V

Table 6 (continued)

Analytical Method	Code	Elements Determined
Instrumental Neutron Activation Analysis with Acid Digestion	D02	Co, Cr, Fe, (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, K, S
Epithermal Instrumental Neutron Activation Analysis	D05	I
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 Voltammetry	H01	(Cd), Co, Cu, Pb, Zn
Acid Digestion Differential Pulse Polarography	H05	I
Dry Ashing Catalytic Adsorption Polarography	H06	Mo
Kjeldahl Method for Nitrogen-Volumetry	I01	N ^(b)
Combustion Elemental Analysis-Thermal Conductivity	J01	N ^(b)
Combustion Elemental Analysis with Chromatographic Separation-Thermal Conductivity	J02	N ^(b)
Combustion Elemental Analysis-Fluorometry	J04	S
Dry Ashing Volumetry	K02	Cl
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 in this RM. (NIST has replaced the previously used term “best estimate” with “reference value”.)

^(b) See Table 7 for additional information.

Table 7. Methods Used by Collaborating Laboratories (Appendix B) for the Determination of Nitrogen and Selected Proximates

Analyte	Methods
Ash	mass loss after ignition in a muffle furnace
Moisture	mass loss after drying in a vacuum oven (3 laboratories); mass loss after drying in a forced-air oven (1 laboratory)
Nitrogen	Dumas (1 laboratory); modified Dumas (1 laboratory); Kjeldahl (2 laboratories). Note that in the original elemental determinations 12 laboratories provided results (one laboratory provided results using two techniques): Kjeldahl (7); combustion - thermal conductivity (4), and combustion - chromatographic separation - thermal conductivity (2).
Protein Solids	calculated from nitrogen using a factor of 6.25 calculated; (sample weight – moisture)

Table 8. Methods Used by Collaborating Laboratories (Appendix C) for the Determination of Vitamins

Analyte	Methods
Vitamin B ₁	microbiological (1 laboratory); digestion with fluorescence detection (3 laboratories)
Vitamin B ₂	microbiological (1 laboratory); digestion with fluorescence detection (2 laboratories)
Vitamin B ₆	microbiological (2 laboratories)
Vitamin B ₁₂	microbiological (2 laboratories)
Biotin	microbiological (2 laboratories)
Folic Acid	microbiological (3 laboratories)
Inositol	microbiological (1 laboratory)
Niacin	microbiological (1 laboratory); acid digestion with absorption spectrophotometry (1 laboratory)
Pantothenic Acid	microbiological (2 laboratories)

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Report Revision History: 25 January 2008 (Update of expiration date and editorial changes); 14 June 05 (This technical revision reports the removal of reference values for fat, carbohydrate, and calories, and the change from reference to information values for selected fatty acids. Storage conditions have also been updated in this version. The material is to be stored under refrigeration by the purchaser, and no longer stored at room temperature as the previous version of this Report stated. This revised Report applies to all RM 8415 units, including those originally stored at room temperature as previously instructed. From the initiation, RM 8415 has been stored under refrigeration at NIST and the "Preparation" section has been updated to reflect this.); 28 April 99 (This technical revision reports the addition of reference values for fat, protein, carbohydrate, calories, ash, moisture, solids, and fatty acids.); 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; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet at <http://www.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, US 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.
- P.R. Beljaars and Th. Rondags, Governmental Food and Commodities Inspection Service, Maastricht, The Netherlands.
- M. Bouraly, N. Texier, and A. Couty, Centre d'Application de Levallois, Atochem, Levallois-Perret Cedex, France.
- 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.
- W.B. Clarke, Department of Physics, McMaster University, Hamilton, ON, Canada.
- J.G. Crock, Branch of Geochemistry, US Geological Survey, Denver, CO, USA.
- W.C. Cunningham, Division of Contaminants Chemistry, Center for Food Safety and Applied Nutrition, US 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), Wageningen, The Netherlands.
- A. Farina Mazzeo, R. Piergallini, E.P. Salsano, and F. Abballe, Laboratory of Pharmaceutical Chemistry, Istituto Superiore di Sanita, Rome, Italy.
- 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.
- K. Frank, J. Denning, and L. Hayne, Institute of Agriculture and Natural Resources, Department of Agronomy, University of Nebraska-Lincoln, Lincoln, NE, USA.
- E.S. Gladney and E.M. Hodge, Health and Environmental Chemistry Group, Los Alamos National Laboratory, Los Alamos, NM, USA.
- D.C. Gregoire, K. Church, and J.L. Bouvier, Analytical Chemistry Laboratory, Geological Survey of Canada, Energy Mines and Resources Canada, Ottawa, ON, Canada.
- R.D. Hauck and R.H. Scheib, Office of Agricultural and Chemical Development, Tennessee Valley Authority, Muscle Shoals, AL, USA.
- G.U. Hesselius, Mikro Kemi AB, Uppsala, Sweden.
- W. Holak, New York Regional Laboratory, US Food and Drug Administration, Brooklyn, NY, USA.
- M. Ihnat, Centre for Land and Biological Resources Research, Agriculture Canada, Ottawa, ON, Canada.
- J.L. Imbert and M. Olle, Service Central d'Analyse, Centre National de la Recherche Scientifique, Vernaison, France.
- L.L. Jackson, Branch of Geochemistry, US Geological Survey, Denver, CO, USA.
- D.L. Jeffress and S. Allison, Feed Control Laboratory, Missouri Department of Agriculture, Jefferson City, MO, USA.
- L. Jorhem, E. Ericsson, and C.A. Yates, National Food Administration, Uppsala, Sweden.
- 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.
- D. Kuik and P. Heida, Governmental Food and Commodities Inspection Service, Leeuwarden, The Netherlands.
- J. Kumpulainen, Central Laboratory, Agricultural Research Center of Finland, Jokioinen, Finland.
- G.W. Latimer Jr., W. Iglar, L. Park, H. Hinojosa, C. Upton, and D. Arvelo, Agricultural Analytical Services, Office of the Texas State Chemist, College Station, TX, USA.
- 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, US Department of Agriculture, Beltsville, MD, USA.
- I. Olmez, Nuclear Reactor Laboratory, Massachusetts Institute of Technology, Cambridge, MA, USA.
- I.S. Palmer, O.E. Olson Biochemistry Laboratories, Chemistry Department, South Dakota State University, Brookings, SD, USA.

Appendix A (continued)

J.B. Reust, H.R. Lang, and A. Janchen, Analytical Research and Development, Project/Product Coordination, Sandoz Pharma Ltd., Basle, 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 Ökologische Chemie, Gesellschaft für Strahlen-und Umweltforschung GmbH, Neuherberg, Federal Republic of Germany.

T.R. Shuler and F.H. Nielsen, Grand Forks Human Nutrition Research Center, US 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, and U. Bagschik, 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, US 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, US Department of Agriculture, Beltsville, MD, USA.

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

R.F. Walker, K.J. Thurlow, and G. Holcombe, Laboratory of the Government Chemist, Teddington, Great Britain.

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, and Caloric Determinations

Covance Laboratories, Madison, WI, USA.

Lancaster Laboratories, Lancaster, PA, USA.

Medallion Laboratories, Minneapolis, MN, USA.

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

APPENDIX C

Collaborating Laboratories for Water-Soluble Vitamin Determinations

Food and Drug Administration, Center for Food Safety and Applied Nutrition, Washington, DC, USA.

Covance Laboratories, Madison, WI, USA.

Lancaster Laboratories, Lancaster, PA, USA.

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