National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1570a

Trace Elements in Spinach Leaves

This Standard Reference Material (SRM) is intended primarily for use in evaluating the reliability of analytical methods for the determination of major, minor, and trace elements; proximates; calories; and total dietary fiber in botanical materials, agricultural food products, and materials of similar matrix. A unit of SRM 1570a consists of 60 g of finely powdered dried spinach leaves.

Certified Concentration Values: The certified concentration values of the constituent elements are given in Table 1. These concentrations are based on the agreement of results from at least two independent analytical methods or from a method of known accuracy. Analytical methods are provided in Appendix A.

Reference Concentration Values: Reference concentration values of constituent elements are provided in Table 2; analytical methods are provided in Appendix A. Reference concentration values for selected proximates and total dietary fiber are provided in Table 3; analytical methods are provided in Appendix B. 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 constituent elements are provided in Table 4. Information values for carbohydrate, caloric content, fat, and individual fatty acids are provided in Table 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 SRM to quantitatively monitor method performance for analytes other than those with certified or reference concentration values in Tables 1 through 3 is not warranted.

Expiration of Certification: The certification of this SRM lot is valid until **31 August 2008**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate. Value assignment is nullified if the SRM is damaged, contaminated, or modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certified values before the expiration of this certificate, NIST will notify the purchaser. Return of the attached registration card will facilitate notification.

The original technical and support aspects involved in the certification and issuance of this SRM were coordinated through the NIST Standard Reference Materials Program by R.A. Alvarez and T.E. Gills. Revision of this certificate was coordinated through the NIST Standard Reference Materials Program by J.C. Colbert.

Willie E. May, Chief
Analytical Chemistry Division

Gaithersburg, MD 20899
Revised Certificate Issue Date: 31 August 2001
See Certificate Revision History on Last Page

John Rumble, Jr., Acting Chief
Standard Reference Materials Program

Coordination of analytical measurements for the characterization of this SRM was performed by D.A. Becker and K.E. Sharpless of the NIST Analytical Chemistry Division.

Analytical measurements at NIST were performed by E.S. Beary, D.A. Becker, C.M. Beck II, M.S. Epstein, J.D. Fassett, K.M. Garrity, R.R. Greenberg, R.M. Lindstrom, E.A. Mackey, P. Morales, K.E. Murphy, P.J. Paulsen, B.J. Porter, T.A. Rush, R. Saraswati, J.M. Smeller, G.C. Turk, R.D. Vocke, R.L. Watters, Jr., and L.J. Wood. Additional elemental analyses were performed by D.L. Anderson (Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Washington, DC), A.R. Byrne (Nuclear Chemistry Department, Jozef Stefan Institute, Ljubljana, Slovenia), and J. Kucera (Nuclear Physics Institute, Academy of Sciences of the Czech Republic, Rez, Czech Republic). Several elements were also measured in an International Atomic Energy Agency (IAEA) interlaboratory comparison exercise. Proximates, calories, fatty acids, and total dietary fiber were determined by Covance Laboratories (Madison, WI), Lancaster Laboratories (Lancaster, PA), Medallion Laboratories (Minneapolis, MN), and Southern Testing and Research Laboratories (Wilson, NC).

Statistical analysis of the experimental data was performed by W. Guthrie, S.B. Schiller, and L.M. Gill of the NIST Statistical Engineering Division.

NOTICE AND WARNINGS TO USERS

Stability: This material was radiation sterilized at an estimated minimum dose of 27.8 kGy for microbiological control; however, its stability has not been rigorously assessed. Spinach leaves have a tendency to rapidly bleach and to turn a tan or light brown color in the presence of visible light. Based on 15 years experience with the original SRM 1570, there is no evidence documenting any change in elemental concentrations as a result of that color change. However, NIST will monitor this material and will report any substantive changes in certified values to the purchaser.

Storage: The material should be kept tightly closed in its original bottle and stored in the dark at a temperature between 10 °C and 30 °C. It should not be exposed to intense sources of radiation. Ideally, the bottle should be kept in a desiccator under the conditions indicated above.

Instructions for Use: The contents of a bottle should be thoroughly mixed by rotating and/or rolling before each use. Allow the contents to settle for 1 minute prior to opening to minimize the loss of fine dust particles. A minimum sample mass of 150 mg of the material, dried as described in the section on "Instructions for Drying", should be used to relate analytical determinations to the certified values on this certificate. In some cases, especially for volatile elements such as mercury, it is preferable to analyze samples from the bottle without drying, determine the moisture content on a separate sample from the same bottle taken at the same time, and convert the analytical results to a dry-mass basis.

Digestion procedures should be designed to avoid loss of volatile elements, such as arsenic and mercury. Digestion of the SRM in nitric and perchloric acids was found to be incomplete, with a small residue of siliceous material remaining. This residue must be considered an integral part of this SRM and should be dissolved with a small amount of hydrofluoric acid to obtain total dissolution. All certified values are based on the total dissolution.

Instructions for Drying: Samples of this SRM must be dried by one of the following two procedures in order for certified values to be valid:

1. Drying in a desiccator at room temperature (approximately 22 °C) for 120 h over fresh anhydrous magnesium perchlorate. The sample depth should not exceed 1 cm.

2. Freeze-drying for 24 h at a pressure of 13.3 Pa or lower and a shelf temperature of –5 °C or lower after having frozen the sample (not to exceed 1 cm in depth) at –40 °C or lower for at least 1 h. At the end of the 24 h period, samples should be placed immediately in a desiccator with fresh anhydrous magnesium perchlorate. Samples should be weighed after allowing a minimum of 4 h to establish temperature equilibrium.

Note: Vacuum drying at room temperature and oven drying at elevated temperatures have resulted in excessive mass losses and therefore are **NOT** recommended.

Source and Preparation of Material: The material (approximately 2270 kg) for this SRM was obtained from commercial supplier Oregon Freeze-Drying Corp., Albany, OR. It consists of U.S. Grade A chopped frozen spinach. The material was thawed, placed in a ribbon mixer, thoroughly mixed, and blended. After mixing, the spinach was freeze-dried. The freeze-dried material was then ground in a stainless steel grinder and shipped to NIST. At NIST, the freeze-dried material was sieved through a polypropylene sieve having openings of 0.25 mm (equivalent to a U.S. Series 60 standard sieve). The sieved material was then jet milled and air classified to a particle size of approximately 75 μm (200 mesh). After mixing in a large blender, the spinach was irradiated with cobalt-60 radiation to a minimum absorbed dose of approximately 27.8 kGy for microbiological control and bottled.

Homogeneity Assessment: Samples from randomly selected bottles of SRM 1570a were tested for homogeneity by instrumental neutron activation analysis (INAA). No evidence of statistically significant inhomogeneity was observed.

Table 1. Certified Concentration Values of Constituent Elements[a,b]

Element	Mass Fraction (%)
Calcium	1.527 ± 0.041
Phosphorus	0.518 ± 0.011
Potassium	2.903 ± 0.052
Sodium	1.818 ± 0.043

Element	Mass Fraction (mg/kg)	Element	Mass Fraction (mg/kg)
Aluminum	310 ± 11	Mercury	0.030 ± 0.003
Arsenic	0.068 ± 0.012	Nickel	2.14 ± 0.10
Boron	37.6 ± 1.0	Selenium	0.117 ± 0.009
Cadmium	2.89 ± 0.07	Strontium	55.6 ± 0.8
Cobalt	0.39 ± 0.05	Thorium	0.048 ± 0.003
Copper	12.2 ± 0.6	Vanadium	0.57 ± 0.03
Manganese	75.9 ± 1.9	Zinc	82 ± 3

[a] The certified concentrations are equally weighted means of results from two or more different analytical methods or the mean of results from a single method of known high accuracy. In the case of two or more methods, each uncertainty is the sum of a 95 % confidence limit and an allowance for systematic error between the methods used. In the case of a method of known accuracy, each uncertainty is the sum of a 95 % confidence limit and the known systematic error of the method.

[b] These certified values are reported on a dry-mass basis. For certified values to be valid, the material must be dried according to the instructions provided above.

Table 2. Reference Concentration Values of Constituent Elements[a,b,c]

Element	Mass Fraction (%)
Nitrogen (Total)[d]	6.06 ± 0.20
Nitrogen (Organic)[d]	6.20 ± 0.25
Nitrogen (Protein)[d]	5.68 ± 0.13

Element	Mass Fraction (mg/kg)	Element	Mass Fraction (mg/kg)
Europium	0.0055 ± 0.0010	Rubidium	12.7 ± 1.6
Scandium	0.0055 ± 0.0006	Uranium	0.155 ± 0.023

[a] NIST has replaced the previously used term "non-certified" with "reference value" or "information value," as appropriate.

[b] Each reference concentration value, expressed as a mass fraction on a dry-mass basis, is an equally weighted mean of results provided by NIST and/or collaborating laboratories. The uncertainty in the reference concentration values is calculated as $U = ku_c$. The quantity u_c is the combined standard uncertainty calculated according to the ISO Guide [1], which accounts for the combined effect of the within-laboratory variance for all participating laboratories at one standard deviation and bias between methods. 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.

[c] These reference values are reported on a dry-mass basis. In order for these reference values to be valid, the material must be dried according to the instructions provided above.

[d] Data from three methods for the determination of nitrogen have been treated separately. Total nitrogen was determined by prompt gamma activation analysis; "organic" nitrogen was determined by the Dumas method; and "protein" nitrogen was determined by the Kjeldahl method.

Table 3. Reference Concentration Values of Selected Proximates and Total Dietary Fiber[a]

Analyte	Mass Fraction, as received (%)	Mass Fraction, dry-mass basis (%)[b]
Moisture[c]	3.45 ± 0.25	0 (by definition)
Solids[c]	96.55 ± 0.25	100 (by definition)
Ash	14.66 ± 0.38	15.18 ± 0.38
Protein[d]	35.8 ± 3.0	37.0 ± 3.1
Total dietary fiber	30.5 ± 4.3	31.6 ± 4.4

[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 C. (NIST and one of these laboratories provided results used in value assignment of mass fractions of moisture and solids; see footnote c.) 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 [1]. 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 Appendix B.

[b] Results have been converted to a dry-mass basis using the reference value for solids. Uncertainty in the solids determination has been included in the uncertainties provided for the mass fractions on a dry-mass basis.

[c] Moisture has been determined by NIST (using freeze-drying and desiccation) and one of the collaborating laboratories (using desiccation) as specified in this certificate. Drying in a forced-air or vacuum oven by three laboratories resulted in a moisture value of 6.3 % ± 1.5 %.

[d] The protein concentration was calculated from the nitrogen values reported by the laboratories (two laboratories using the Dumas method, two laboratories using Kjeldahl) using a conversion factor of 6.25. The value for protein is the mean of the individual protein calculations reported by the laboratories shown in Appendix C. If the mean nitrogen values above are used for calculation, the mean protein concentrations are 35.8 % and 37.1 % on an as-received and dry-mass basis, respectively.

Table 4. Information Concentration Values of Constituent Elements[a]

Element	Mass Fraction (%)
Magnesium	0.89
Sulfur	0.46
Element	**Mass Fraction (mg/kg)**
Lead	0.20

[a] NIST has replaced the previously used term "non-certified" with "reference value" or "information value," as appropriate.

Table 5. Information Concentration Values of Carbohydrate, Fat, Caloric Content, and Selected Fatty Acids (as Triglycerides)[a]

Analyte	Mass Fraction, as received (%)	Mass Fraction, dry-mass basis (%)
Carbohydrate[b]	45	46
Fat	2	2
Calories[b,c]	340 kcal/100g	350 kcal/100g
Pentadecanoic Acid (C15:0)	0.010	0.011
Hexadecanoic Acid (C16:0) (Palmitic Acid)	0.61	0.64
Heptadecanoic Acid (C17:0) (Margaric Acid)	0.006	0.006
Octadecanoic Acid (C18:0) (Stearic Acid)	0.031	0.032
(Z)-9-Octadecenoic Acid (C18:1) (Oleic Acid)	0.25	0.26
(Z,Z)-9,12-Octadecadienoic Acid (C18:2) (Linoleic Acid)	0.27	0.28
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3) (Linolenic Acid)	0.63	0.65
Linolenic Acid (C18:3)	0.048	0.050
Docosanoic Acid (C22:0) (Behenic Acid)	0.028	0.029
Tetracosanoic Acid (C24:0) (Lignoceric Acid)	0.044	0.046

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

[b] These information values are calculated from the results reported by one laboratory.

[c] If the mean proximate values in Tables 2 and 4 are used for calculation, with caloric equivalents of 9, 4, and 4 for fat, protein, and carbohydrate, respectively, the mean caloric content is 340 kcal/100 g and 350 kcal/100g on an as-received and dry-mass basis, respectively.

REFERENCE

[1] *Guide to the Expression of Uncertainty in Measurement,* ISBN 92-67-10188-9, 1st Ed. ISO, Geneva, Switzerland, (1993); see also Taylor, B.N. and Kuyatt, C.E., "Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results," NIST Technical Note 1297, U.S. Government Printing Office, Washington, DC, (1994); available at http://physics.nist.gov/Pubs/.

Certificate Revision History: 31 August 2001 (This technical revision reports the addition of reference and information values for proximates, calories, total dietary fiber, and fatty acids and a change from non-certified to reference and information values for several inorganic constituents); 15 July 1996 (editorial change); 20 October 1994 (original certificate date).

Users of this SRM should ensure that the certificate 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 http://www.nist.gov/srm.

Appendix A. Methods Used in Elemental Determinations

Element	Method Code[a]
Aluminum	ICP INAA
Arsenic	FI-HGAAS RNAA
Boron	IDICPMS PGAA
Cadmium	IDICPMS PGAA RNAA
Calcium	IDTIMS INAA
Cobalt	INAA RNAA
Copper	ICP RNAA
Europium	IAEA INAA
Lead	IAEA IDICPMS
Magnesium	IDICPMS INAA
Manganese	INAA LEAFS
Mercury	CVAAS RNAA
Nickel	IDICPMS RNAA
Nitrogen	DUMAS KJEL PGAA
Phosphorus	COLOR ICP
Potassium	IDTIMS INAA
Rubidium	IAEA INAA
Scandium	IAEA INAA
Selenium	FI-HGAAS INAA RNAA
Sodium	PGAA INAA
Strontium	IDTIMS INAA
Sulfur	PGAA IAEA
Thorium	INAA RNAA
Uranium	RNAA
Vanadium	IDTIMS INAA
Zinc	ICP INAA

[a] Acronyms for analytical methods:

CVAAS = Cold-Vapor Atomic Absorption Spectrometry
FI-HGAAS = Flow Injection Hydride Generation Atomic Absorption Spectrometry
IAEA = International Atomic Energy Agency Interlaboratory Comparison Exercise
ICP = Inductively Coupled Plasma Optical Emission Spectrometry
IDICPMS = Isotope Dilution, Inductively Coupled Plasma Mass Spectrometry
IDTIMS = Isotope Dilution, Thermal Ionization Mass Spectrometry
INAA = Instrumental Neutron Activation Analysis
KJEL = Kjeldahl Nitrogen Determination
LEAFS = Laser-Excited Atomic Fluorescence Spectrometry
PGAA = Prompt Gamma Activation Analysis
RNAA = Radiochemical Neutron Activation Analysis

Appendix B. Methods Used in the Determination of Proximates, Caloric Content, Fatty Acids, 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 at room temperature in a desiccator (1 laboratory + NIST); freeze-drying (NIST)
Nitrogen – Dumas (1 laboratory); modified Dumas (1 laboratory); Kjeldahl (2 laboratories + NIST)
Protein – calculated from nitrogen reported by 4 laboratories using a factor of 6.25
Solids – calculated; (sample mass – moisture)
Total dietary fiber – enzymatic digestion followed by gravimetry

Appendix C. Collaborating Laboratories for Proximate, Fatty Acid, Total Dietary Fiber, 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