National Institute of Standards and Technology
U.S. Department of Commerce

Date of Issue:
22 January 2021

Standard Reference Material® 2385
Slurried Spinach
CERTIFICATE OF ANALYSIS

Purpose: The certified values delivered by this Standard Reference Material (SRM) are intended for use in validation of methods used to inform nutritional labelling for selected elements. The non-certified values delivered by this SRM, provided in Appendix A, are intended for use in harmonizing measurements of other measurands.

Description: A unit of SRM 2385 consists of four jars, each containing approximately 70 g of slurried spinach.

Certified Values: A National Institute of Standards and Technology (NIST) certified value is a value for which NIST has the highest confidence in that all known or suspected sources of bias and imprecision have been accounted for [1].

Certified values for selected elements are provided in Table 1.

Table 1. Certified Mass Fraction Values for Elements in SRM 2385[a]

Element[b,c]	Mass Fraction (mg/kg)[d]
Calcium (Ca)	624 ± 40
Iron (Fe)	17.1 ± 1.9
Magnesium (Mg)	368 ± 30
Manganese (Mn)	3.81 ± 0.10
Phosphorus (P)	323.7 ± 6.6
Potassium (K)	3650 ± 250
Zinc (Zn)	8.37 ± 0.37

[a] These as-received values are metrologically traceable to the International System of Units (SI) through the purity evaluations and gravimetric procedures used to prepare the calibration solutions used in their assignment.
[b] Determined at NIST as described in Appendix B.
[c] Determined by collaborating laboratories as described in Appendix C.
[d] Values are expressed as $x \pm U_{95\%}(x)$, where x is the certified value and $U_{95\%}(x)$ is the expanded uncertainty of the certified value. The true value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with 95 % confidence. For guidance in propagating this uncertainty, see reference 2.

Non-Certified Values: Non-certified values for a variety of measurands are provided in Appendix A.

Period of Validity: The certified values of **SRM 2385** are valid, within the measurement uncertainty specified, until **30 June 2027,** provided the SRM is handled and stored in accordance with instructions given in this certificate. The certifications are nullified if the SRM is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Certified Values: NIST will monitor this SRM to the end of the period of validity. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Carlos A. Gonzalez, Chief
Chemical Sciences Division

Steven J. Choquette, Director
Office of Reference Materials

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Safety: SRM 2385 IS INTENDED FOR RESEARCH USE; NOT FOR HUMAN CONSUMPTION.

Storage: The SRM should be stored in the original, unopened jars under refrigeration (4 °C) until required for use.

Use: Prior to removal of a test portion for analysis, the contents of a jar of spinach should be mixed thoroughly and homogenized (e.g., using a rotor/stator type blender) for 1 min prior to removal of a test portion. Test portions used for analysis should be equal to or greater than 7 g for elements and 1.5 g for *trans*-β-carotene.

Source: SRM 2385 was prepared from a spinach crop that was held under refrigeration following harvesting, then washed lightly in a wash reel with cold water and blanched at 93 °C. The spinach was then pureed using a turbo cold extractor 0.635-cm screen for foods and vegetables. The puree was heated to 90.5 °C in a batch tank and the consistency was adjusted to 3 Bostwick units to 5 Bostwick units with water. The puree was then passed through a 0.084-cm screen, filled into jars to contain approximately 70 g of slurried spinach each, and capped. The spinach was processed in two retort loads. The jars were retorted at 121 °C and 207 kPa for 35 min.

Analysis: Measurements used to certify SRM 2385 values were performed at NIST using a variety of analytical techniques (Appendix B), by participants of interlaboratory comparison studies sponsored by the Grocery Manufacturers Association's Food Industry Analytical Chemists (FIAC) Share Group using their routine methods (Appendix C), and by participants of multiple interlaboratory comparison studies coordinated by NIST (Appendix C).

REFERENCES

[1] Beauchamp, C.R.; Camara, J.E.; Carney, J.; Choquette, S.J.; Cole, K.D.; DeRose, P.C.; Duewer, D.L.; Epstein, M.S.; Kline, M.C.; Lippa, K.A.; Lucon, E.; Phinney, K.W.; Polakoski, M.; Possolo, A.; Sharpless, K.E.; Sieber, J.R.; Toman, B.; Winchester, M.R.; Windover, D.; *Metrological Tools for the Reference Materials and Reference Instruments of the NIST Material Measurement Laboratory*; NIST Special Publication 260-136; U.S. Government Printing Office: Washington, DC (2020); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2020.pdf (accessed Jan 2021).

[2] Possolo, A.M.; *Evaluating, Expressing, and Propagating Measurement Uncertainty for NIST Reference Materials*; NIST Special Publication 260-202; U.S. Government Printing Office: Washington, DC (2020); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-202.pdf (accessed Jan 2021).

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[4] Brown Thomas, J.; Kline, M.C.; Schiller, S.B.; Ellerbe, P.M.; Sniegoski, L.T.; Duewer, D.L.; Sharpless, K.E.; *Certification of Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum: Standard Reference Material 968b*; Fresenius' J. Anal. Chem., Vol. 356, pp. 1–9 (1996).

[5] Sander, L.C.; K.E. Sharpless; Craft, N.E.; Wise, S.A.; *Development of Engineered Stationary Phases for the Separation of Carotenoid Isomers*; Anal. Chem., Vol. 66, pp. 1667–1674 (1994).

[6] Sharpless, K.E.; Brown Thomas, J.; Sander, L.C.; Wise, S.A.; *Liquid Chromatographic Determination of Carotenoids in Human Serum Using an Engineered C_{30} and a C_{18} Stationary Phase*; J. Chromatogr. B: Biomed. Applications, Vol. 678, pp. 187–195 (1996).

Certificate Revision History: 22 January 2021 (Addition of non-certified nitrate value; correction of unit size to 70 g; editorial changes); **02 February 2018** (Change of expiration date; removal of values for total lutein, total β-carotene, and vitamin B_2 based on NIST's decision to no longer support these measurement capabilities in this matrix; editorial changes); **26 April 2012** (Change of expiration date; editorial changes); **09 December 2003** (Original certificate date).

Certain commercial equipment, instruments, or materials may be identified in this Certificate of Analysis 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.

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, Maryland 20899-2300; telephone (301) 975-2200; e-mail srminfo@nist.gov; or the Internet at https://www.nist.gov/srm.

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APPENDIX A

Non-Certified Values

Non-Certified Values: Non-certified values are best estimates based on currently available information. However, they do not meet NIST's criteria for certification [1]. Non-certified values are suitable for use in method development, method harmonization, and process control but do not provide metrological traceability to the International System of Units (SI) or other higher-order reference system.

Non-certified values for selected measurands are provided in Tables A1, A2, and A3 below.

Table A1. Non-Certified Values for Additional Analytes in SRM 2385[a]

Analyte	Mass Fraction (mg/kg)[b]	n[c]
Copper (Cu)[d,e]	0.90 ± 0.16	12
Sodium (Na)[d,e]	47 ± 1	13
trans-β-Carotene[d,e]	15.1 ± 3.1	32
Niacin (Vitamin B_3)[e]	2.99 ± 0.43	18
Nitrate (NO_3)[e]	130 ± 60	3

[a] These as-received values are metrologically traceable to the values assigned to calibration materials used by NIST and/or participants in interlaboratory comparison studies using their routine methods.
[b] These values are expressed as $x \pm 2u(x)$, where x is the value and $u(x)$ is the standard uncertainty of x. The standard uncertainty represents between-participant variability. While the best estimates of the values lie within the intervals $x \pm 2u(x)$, these intervals may not include the true values. For guidance in propagating this uncertainty, see reference 2.
[c] Number of contributors providing technically valid results.
[d] Determined at NIST as described in Appendix B.
[e] Determined by collaborating laboratories as described in Appendix C.

Table A2. Non-Certified Values for Proximates in SRM 2385[a]

	Mass Fraction (g/100 g)[b]	n[c]
Ash[d]	0.97 ± 0.05	11
Solids[d,e]	5.28 ± 0.10	11
Fat (as sum of fatty acids as triglycerides)[d]	0.20 ± 0.06	9
Protein[d]	1.42 ± 0.13	12
Carbohydrates[d]	2.73 ± 0.18	11
Total Dietary Fiber[d]	1.55 ± 0.28	6

[a] These as-received values are metrologically traceable to the values assigned to calibration materials used by NIST and/or participants in interlaboratory comparison studies using their routine methods.
[b] These values are expressed as $x \pm 2u(x)$, where x is the value and $u(x)$ is the standard uncertainty of x. The standard uncertainty represents between-participant variability. While the best estimates of the values lie within the intervals $x \pm 2u(x)$, these intervals may not include the true values. For guidance in propagating this uncertainty, see reference 2.
[c] Number of contributors providing technically valid results.
[d] Values are based on results provided by the collaborating laboratories listed in Appendix C.
[e] Determined at NIST as described in Appendix B.

Table A3. Non-Certified Values for Calories in SRM 2385[a]

	(kcal/100 g)[b]	n[c]
Calories, reported calculations[d,e]	18.16 ± 0.50	11
Calories, estimated from proximates[d,f]	18.40 ± 0.80	18

[a] These as-received values are metrologically traceable to the values assigned to the materials used by participants in interlaboratory comparison studies using their routine methods.

[b] These values are expressed as $x \pm 2u(x)$, where x is the value and $u(x)$ is the standard uncertainty of x. The standard uncertainty represents between-participant variability. While the best estimates of the values lie within the intervals $x \pm 2u(x)$, these intervals may not include the true values. For guidance in propagating this uncertainty, see reference 2.

[c] Number of contributors providing technically valid results.

[d] Values are based on results provided by the collaborating laboratories listed in Appendix C.

[e] As reported by individual laboratories.

[f] Using consensus values in Table A2.

Period of Validity: The non-certified values are valid within the measurement uncertainty specified until **30 June 2027**. The value assignment is nullified if this material is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Non-Certified Values: NIST will monitor this material to the end of its period of validity. If substantive technical changes occur that affect the non-certified values during this period, NIST will update this Certificate of Analysis. Before using this material, users should obtain the most recent version of this documentation, available free of charge at https://www.nist.gov/srm.

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APPENDIX B

NIST Method Information

NIST Analytical Approach for Determination of Elements: Mass fractions of calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc were measured at NIST using ICP-OES. After homogenization, two 7 g test portions were taken from each of five jars of spinach and digested with nitric acid using a microwave sample preparation system. Quantification was based on the method of standard additions using calibration solutions prepared from the SRM 3100 series of single-element standard solutions.

NIST Analytical Approaches for Determination of *trans*-β-Carotene: The mass fraction of *trans*-β-carotene was measured at NIST by using two sample preparation approaches and two LC methods. For each jar sampled, the entire jar of spinach was homogenized for 1 min using a rotor/stator-type tissue homogenizer. One or two 1.5 g test portions were taken from homogenized jars. In the first sample preparation approach, test portions were combined with calcium carbonate, an ethanolic internal standard solution, tetrahydrofuran (THF), and methanol, and the mixture was homogenized for 1 min. This mixture was then vacuum-filtered and aqueous sodium chloride solution was added to the filtrate. The analyte was extracted into a mixture of hexane and diethyl ether, and the organic phase was washed with water and then evaporated under nitrogen. Dried extracts were reconstituted with 1 mL ethanol prior to analysis. In the second sample preparation approach, test portions were combined with ethanolic internal standard solution, THF, and methanol. The mixture was homogenized for approximately 45 s, and saponified at 40 °C for 30 min using methanolic potassium hydroxide. Glacial acetic acid was then added to neutralize any remaining potassium hydroxide, an aqueous sodium chloride solution was added, and the analyte was extracted into a mixture of hexane and diethyl ether. The organic phase was washed with water and then evaporated under nitrogen. The residue was redissolved in 500 µL ethanol prior to analysis. Measurements of all test portions were made using a C18 analytical column and a gradient consisting of acetonitrile, methanol, and ethyl acetate [3,4] and using a C30 analytical column and a gradient consisting of methanol, water, and methyl *tert*-butyl ether [5,6]. A programmable absorbance detector was used for measurement of the *trans*-β-carotene at 450 nm. The purity of the *trans*-β-carotene calibrant material was determined at NIST using spectrophotometry at 452 nm (molar extinction coefficient of 2560 $dL \cdot g^{-1} cm^{-1}$) in combination with LC-absorbance at 452 nm.

NIST Analytical Approach for Determination of Solids: The mass fraction of solids was calculated after determination of moisture content at NIST by using samples from twelve jars of SRM 2385. The contents of the jars were emptied into Teflon vessels and placed into a freeze-dryer with a shelf temperature initially set at –20 °C. The shelf temperature was adjusted daily until it reached 5 °C, and remained at this temperature until constant mass was achieved, at nine days. The percent moisture mass loss during freeze drying was subtracted from 100 % to obtain the percent total solids.

Homogeneity Assessment: The homogeneity of calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, zinc, and *trans*-β-carotene was assessed at NIST using the methods and test portions described above. Analysis of the variance showed statistically significant heterogeneity in some cases, and a component of inhomogeneity has been added to the uncertainty for calcium, copper, magnesium, and potassium. Homogeneity of constituents measured solely by collaborating laboratories was not assessed, although the data were treated as though these analytes were homogeneously distributed.

Value Assignment: The value assignments in this SRM were derived from results of analyses performed by NIST and the collaborating institutions listed in Appendix C. For calculation of assigned values for analytes measured only by NIST, the mean of the mean values from NIST results was used. For calculation of assigned values for analytes that were measured only by collaborating laboratories, the mean or median of the laboratory means was used. For analytes that were also measured by NIST, the mean of the individual sets of NIST data were averaged with the mean of the individual collaborating laboratory means.

Responsibilities: Material coordination was conducted by M.M. Phillips, L.J. Wood, and K.E. Sharpless of the NIST Chemical Sciences Division and H.B. Chin and D.W. Howell of GMA (Dublin, CA, and Washington, DC). Analytical measurements were conducted by K.E. Sharpless and L.J. Wood of NIST and J.M. Brown Thomas and B.J. Porter formerly of NIST. Statistical analysis was conducted by J.H. Yen of the NIST Statistical Engineering Division.

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APPENDIX C

Additional Method Information

Analyses by Collaborating Laboratories: Data from four additional sources were used for value assignment of this material: an interlaboratory comparison exercise organized by the GMA's FIACSG including 14 participating laboratories, and three separate interlaboratory comparison exercises organized by NIST in which carotenoids, vitamins, and nitrate were measured by 22 laboratories. Not every laboratory measured every analyte. The laboratories participating in the GMA FIACSG interlaboratory comparison exercise were asked to use AOAC methods or their equivalent, to make single measurements from each of two jars, and to report the analytical method that was used. The laboratories participating in interlaboratory comparison exercises organized by NIST were asked to use their usual methods to make single measurements of nitrate, carotenoids, and/or vitamins in each of two or three jars. Data from these exercises were combined with NIST data, where appropriate, for assignment of values (methods used by NIST are listed in Appendix B and acronyms are defined below).

Methods Used for Analytical Determinations

	Analyte	Method(s)
Elements	Ca	DCP-AES, FAAS, ICP-OES
	Cu	DCP-AES, FAAS, ICP-OES
	Fe	DCP-AES, FAAS, ICP-OES
	K	DCP-AES, FAAS, FAES, ICP-OES
	Mg	DCP-AES, FAAS, ICP-OES
	Mn	DCP-AES, FAAS, ICP-OES
	Na	ICP-OES
	P	Absorption Spectrophotometry, FAAS, ICP-OES
	Zn	DCP-AES, FAAS, ICP-OES
Proximates	Ash	Mass loss after ignition in muffle furnace
	Calories	Calculation [9×Fat + 4×Protein + 4×Carbohydrates]
	Carbohydrates	Calculation [solids – (protein + fat (as the sum of fatty acids) + ash)]
	Fat	Sum of fatty acids as triglycerides as determined by hydrolysis with GC
	Solids	Freeze drying, forced air oven, vacuum oven
	Protein	Nitrogen determination using thermal conductivity or pyrolysis with thermal conductivity, nitrogen determination using pyrolysis with GC or pyrolysis with thermal conductivity and GC, Kjeldahl nitrogen determination. A factor of 6.25 was used to convert nitrogen to protein.
	Total dietary fiber	Enzymatic hydrolysis followed by gravimetry
Vitamins	Niacin	Microbiological assay, extraction followed by RPLC with absorbance detection, acid digestion followed by absorbance detection
	trans-β-carotene	Extraction and/or saponification followed by RPLC or NPLC with absorbance detection
Contaminants	Nitrate	Ion chromatography, ISE, LC with absorbance detection, spectrophotometry

Laboratories Participating in a GMA FIACSG Interlaboratory Comparison Exercise

Campbell Soup Company (Camden, NJ, USA)
Centro de Investigación y Asistencia Técnica a la Industria (Provincia de Río Negro, Argentina)
Covance, Inc. (Madison, WI, USA)
Del Monte Foods (Walnut Creek, CA, USA)
General Mills, Inc. (Golden Valley, MN, USA)
Gerber Products Company (Fremont, MI, USA)
Hormel Foods Corporation (Austin, MN, USA)
Kraft Foods, Inc. (Glenview, IL, USA)
Nabisco, Inc. (East Hanover, NJ, USA)
National Food Laboratory (Dublin, CA, USA)
Nestlé Food Corporation (Dublin, OH, USA)
Novartis Nutrition Technical Center (St. Louis Park, MN, USA)
TPC Labs/Pillsbury (St. Paul, MN, USA)
USDA, Food Composition Laboratory (Beltsville, MD, USA)

Laboratories Participating in NIST Interlaboratory Comparison Exercises

Advanced Botanical Consulting & Testing, Inc. (Tustin, CA, USA)
AgriQuality (Auckland, New Zealand)
Anchor Products (Waitoa, New Zealand)
Harvard School of Public Health (Boston, MA, USA)
Inspectorate for Health Protection and Veterinary Public Health (Maastricht, The Netherlands)
Institute of Nutrition, Directorate of Fisheries (Bergen, Norway)
Institut National de la Recherche Agronomique (Saint-Genès Champanelle, France)
Laboratoire Marcel Merieux (Lyon, France)
Livsmedelsverket (Uppsala, Sweden)
Metametrix Clinical Laboratory, Inc. (Norcross, GA, USA)
Nestlé Research Center (Lausanne, Switzerland)
New Zealand Dairy Research Institute (Palmerston North, New Zealand)
Puerta de Hierro (Madrid, Spain)
Roche Vitamins Ltd. (Basel, Switzerland)
Technology Laboratory of Uruguay (Montevideo, Uruguay)
Thermo Fisher Scientific (Sunnyvale, CA, USA)
TNO Nutrition and Food Research (Zeist, The Netherlands)
University of Illinois at Chicago (Chicago, IL, USA)
University of Ulster (Coleraine, Ireland)
USDA, BHNRC (Beltsville, MD, USA)
USDA – Human Nutrition Research Center on Aging at Tufts University (Boston, MA, USA)
U.S. Food and Drug Administration (College Park, MD, USA)

Abbreviations Used in this Certificate of Analysis

BHNRC	Beltsville Human Nutrition Research Center
DCP-AES	direct current plasma atomic emission spectrometry
FAAS	flame atomic absorption spectrometry
FAES	flame atomic emission spectrometry
FIACSG	Food Industry Analytical Chemists Share Group
GC	gas chromatography
GMA	Grocery Manufacturers Association
ICP-OES	inductively coupled plasma optical emission spectrometry
ISE	ion-selective electrode
LC	liquid chromatography
NPLC	normal phase liquid chromatography
RPLC	reversed phase liquid chromatography
USDA	United States Department of Agriculture

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