Date of Issue:
02 February 2018

Standard Reference Material® 2385
Slurried Spinach
CERTIFICATE OF ANALYSIS

Purpose: This Standard Reference Material (SRM) is intended for use in validating methods for determining elements, vitamins, proximates, and calories in spinach and similar matrices, and for qualifying in-house control materials analyzed using those methods.

Description: A unit of SRM 2385 consists of four jars, each containing approximately 7 g of slurried spinach.

Certified Values: Certified values are provided in Table 1. A NIST certified value is a value for which NIST has the highest confidence in that all known or suspected sources of bias have been evaluated [1,2].

Table 1. Certified Values for Elements in SRM 2385

Values are metrologically traceable to the International System of Units (SI) derived unit for mass fraction expressed as milligrams per kilogram.

	Mass Fraction[a,b] (mg/kg)
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] 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 is believed to lie within the interval $x \pm U_{95\%}(x)$ with 95 % confidence. To propagate this uncertainty, treat the certified value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$. Values are based on a minimum required sample mass of 7 g.

[b] Determined at NIST as described in Appendix E.

Expiration of Certification: The certification of **SRM 2385** is valid, within the measurement uncertainty specified, until **30 June 2027**, provided the SRM is handled and stored in accordance with the instructions given in this certificate. The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

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Chemical Sciences Division

Steven J. Choquette, Director
Office of Reference Materials

Certificate Revision History on Page 4

Non-Certified Values: Tables 2 and 3 list values that do not meet the NIST criteria for certification. Non-certified values were formerly known as Reference and Information values.

Table 2. Estimated Mass Fraction Values for Constituents in SRM 2385[a]

Values are metrologically traceable to the measurement methods and the calibration procedures and standards used.

	Mass Fraction[b] (mg/kg)
Copper (Cu)[c]	0.90 ± 0.16
Sodium (Na)[c]	47 ± 1
trans-β-Carotene[d]	15.1 ± 3.1
	(g/100 g)
Solids[e]	5.28 ± 0.10

[a] Values are based in whole or in part on measurements made at NIST. Additional results were provided by the collaborating laboratories listed in Appendices C and D.

[b] Values are expressed as $x \pm U_{95\%}(x)$, where x is the estimated value and $U_{95\%}(x)$ is the expanded uncertainty of the value. As measured by the specific method(s), the true value of the analyte is believed to lie within the interval $x \pm U_{95\%}(x)$ with about a 95 % confidence. To propagate this uncertainty, treat the value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$.

[c] Values are based on a minimum required sample mass of 7 g.

[d] Values are based on a minimum required sample mass of 1.5 g.

[e] No minimum required sample mass is provided since mass used is dependent on individual drying protocols.

Table 3. Consensus Values for Constituents in SRM 2385[a]

Values are metrologically traceable to calibration procedures and standards used within this experienced measurement community.

	ν[b]	Mass Fraction[c] (mg/kg)
Niacin (Vitamin B_3)	7	2.99 ± 0.43
	ν[b]	(g/100 g)
Ash	12	0.97 ± 0.05
Fat (as sum of fatty acids as triglycerides)	7	0.20 ± 0.06
Protein	10	1.42 ± 0.13
Carbohydrates	10	2.73 ± 0.18
Total Dietary Fiber	5	1.55 ± 0.28
	ν[b]	(kcal/100 g)
Calories, reported calculations[d]	8	18.16 ± 0.50
Calories, estimated from proximates[e]	25	18.40 ± 0.80

[a] Values are based on results provided by the collaborating laboratories listed in Appendices C and D.

[b] Number of effective degrees of freedom, typically one less than the number of independent measurements.

[c] Values are expressed as $x \pm U_{95\%}(x)$, where x denotes the mean of participant mean results and $U_{95\%}(x)$ is the expanded uncertainty associated with the mean, evaluated as $(t_{0.975,\nu})(1.8582)(\mathrm{MAD}/\sqrt{n})$ where $t_{0.975,\nu}$ is the 97.5th percentile of the Student's t distribution with ν degrees of freedom and MAD is the median of the absolute differences between the participant means and their overall mean [3]. The true value of the analyte is believed to lie within the interval $x \pm U_{95\%}(x)$ with 95 % confidence. To propagate this uncertainty, treat the value as a Student's t random variable having ν degrees of freedom with mean x and standard deviation $s = (U_{95\%}(x)/t_{0.975,\nu})\sqrt{(\nu-2)/(\nu)}$.

[d] As reported by individual laboratories.

[e] Using consensus values in Table 3.

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

Use: The minimum sample mass indicated in Tables 1 through 3 should be used to relate analytical determinations to the certified values in this Certificate of Analysis. Results obtained in analyses should include their own estimates of uncertainty and can be compared to the listed values using procedures described in references 3 and 4. NIST strives to maintain the SRM inventory supply, but NIST cannot guarantee the continued or continuous supply of any specific SRM. Accordingly, NIST encourages the use of this SRM as a benchmark for the quality and accuracy of the user's in-house reference materials and working standards. As such, the SRM should be used to validate the more routinely used reference materials in a laboratory. Comparisons between the SRM and in-house reference materials or working measurement standards should take place at intervals appropriate to the conservation of the SRM and the stability of relevant in-house materials. For further guidance on how this approach can be implemented, see reference 3 or contact NIST by email at srms@nist.gov.

Safety: SRM 2385 is intended for research use; not for human consumption.

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 Bertocchi cold extractor 0.635-cm (0.250-inch) screen. The puree was heated to 90.5 °C in a batch tank and the consistency was adjusted to 3 to 5 Bostwick units with water. The puree was then passed through a Fitzmill 0.084-cm (0.033-inch) screen, filled into jars to contain approximately 70 g (2.5 oz) 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.

REFERENCES

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Certificate Revision History: 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.

NIST will monitor this SRM until its certification expires. If substantive technical changes occur that affect the certified values before this certificate expires, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. Contact the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, Maryland 20899-2300; telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or the Internet at https://www.nist.gov/srm.

APPENDIX A

Responsibilities for SRM 2385

Coordination: M.M. Phillips, L.J. Wood, and K.E. Sharpless of NIST; H.B. Chin and D.W. Howell of GMA (Dublin, CA, and Washington, DC).

Analytical measurements: J.M. Brown Thomas, B.J. Porter, K.E. Sharpless, and L.J. Wood of NIST; analysts at organizations participating in the GMA FIACSG interlaboratory comparison exercise (see Appendix C for the list of participating organizations); analysts at organizations participating in NIST interlaboratory comparison exercises for value assignment of vitamins and carotenoids (see Appendix D for the list of participating organizations).

Statistical analysis: J.H. Yen

APPENDIX B

The following abbreviations are 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
LC	liquid chromatography
NPLC	normal phase liquid chromatography
RPLC	reversed phase liquid chromatography
USDA	United States Department of Agriculture

APPENDIX C

Analysts at the following laboratories analyzed SRM 2385 as blind samples 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)

APPENDIX D

Analysts at the following laboratories analyzed SRM 2385 as blind samples in three separate interlaboratory comparison exercises organized by NIST in which vitamins and carotenoids were measured.

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)
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)

APPENDIX E

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. 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 [5,6] and using a C30 analytical column and a gradient consisting of methanol, water, and methyl *tert*-butyl ether [7,8]. 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.

Analyses by Collaborating Laboratories: Data from four additional sources was 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 and vitamins were measured by 19 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 carotenoids and/or vitamins in each of two or three jars. Data from these

exercises were combined with NIST data for assignment of values (acronyms are defined in Appendix B and methods used are listed in Appendix F).

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 certified values in this SRM were derived from results of analyses performed by NIST and the 33 collaborating institutions listed in Appendices B and C [1]. For calculation of assigned values for analytes that were measured only by NIST, the mean of the mean values from NIST results was used. The collaborating laboratories reported the individual results for each of their analyses for a given analyte. The mean of each laboratory's results was then determined. For calculation of assigned values for analytes that were measured only by the GMA FIACSG laboratories, the mean 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 GMA FIACSG laboratory means.

APPENDIX F

Methods Used for Analytical Determinations

	Analyte	Method
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.
	Moisture	Freeze drying, forced air oven, and 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; and Kjeldhal 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 absorbance, acid digestion followed by absorption detection.
	trans-β-carotene	Extraction and/or saponification followed by RPLC absorbance or NPLC absorbance.