National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1845a

Whole Egg Powder

This Standard Reference Material (SRM) is intended primarily for validation of methods for determining proximates, fatty acids, cholesterol, vitamins, elements, and amino acids in whole egg powder and similar materials. This SRM can also be used for quality assurance when assigning values to in-house reference materials. The SRM is a whole egg powder prepared by a commercial manufacturer. A unit of SRM 1845a consists of five heat-sealed aluminized pouches, each containing approximately 10 g of material.

Certified Mass Fraction Values: Certified mass fraction values for cholesterol, elements, and vitamins in SRM 1845a, reported on an as-received basis, are provided in Tables 1 through 3. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias and variability have been taken into account [1]. Analyses for value assignment were performed by NIST and collaborating laboratories. Certified values were calculated as the mean of the mean values from NIST methods and the median or mean of the means of results provided by collaborating laboratories, where appropriate. The associated uncertainties are expressed at an approximately 95 % level of confidence [2−4].

Reference Mass Fraction Values: Reference mass fraction values for additional analytes in SRM 1845a, reported on an as-received basis, are provided in Tables 4 through 8. A NIST reference value is a noncertified value that is the best estimate of the true value based on available data; however, the value does not meet the NIST criteria for certification [1] and is provided with associated uncertainties that may reflect only measurement reproducibility, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods. The reference mass fraction values were derived from results reported by NIST and/or collaborating laboratories.

Expiration of Certification: The certification of **SRM 1845a** is valid, within the measurement uncertainty specified, until **30 July 2029**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Storage and Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. 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.

Coordination of the technical measurements leading to the certification of this SRM was performed by M.M. Phillips and L.J. Wood of the NIST Chemical Sciences Division, K.E. Sharpless of the NIST Special Programs Office, S. Ehling of the Grocery Manufacturers Association (GMA, Washington, DC), and J. Roseland and K. Patterson of the US Department of Agriculture (USDA, Beltsville, MD).

Analytical measurements at NIST were performed by M. Bedner, C.E. Bryan, C.Q. Burdette, W.C. Davis, B.E. Lang, K.A. Lippa, C. Luvonga, M.A. Nelson, Y. Nuevo Ordóñez, R. Oflaz, D.J. O'Kelly, T.O. Okumu, R.L. Paul, M.M. Phillips, B.J. Porter, M.M. Schantz, L.T. Sniegoski, J.B. Thomas, B.E. Tomlin, M.J. Welch, and L.J. Wood of the NIST Chemical Sciences Division.

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Certificate Issue Date: 09 March 2020
Certificate Revision History on Last Page

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Office of Reference Materials

Analyses for value assignment were also performed by the following laboratories participating in a GMA Food Industry Analytical Chemists Share Group (FIACSG) interlaboratory comparison exercise: Campbell Soup (Camden, NJ); Conagra Foods (Omaha, NE); Covance Laboratories, Inc. (Madison, WI); Del Monte Foods (Walnut Creek, CA); Eurofins Central Analytical Laboratories (Metairie, LA); Eurofins Scientific (Des Moines, IA); Eurofins WEJ Contaminants (Hamburg, Germany); General Mills, Inc. (Golden Valley, MN); Hormel Foods Corporation (Austin, MN); Krueger Food Laboratories (Billerica, MA); Land O'Lakes (Arden Hills, MN); Schwan Food Company (Salina, KS); Silliker Illinois Analytical Laboratory (Crete, IL); The J.M. Smucker Co. (Orrville, OH); and The National Food Laboratory (Livermore, CA). Analyses for vitamin D and 25-hydroxyvitamin D value assignment were performed by the following laboratories participating in a second GMA FIACSG interlaboratory comparison exercise: Covance Laboratories, Inc.; Covance (Asia) Pte. Ltd. (The Synergy, Singapore); Eurofins Nutrition Analysis Center (Des Moines, IA); Eurofins Steins Laboratorium Vejen (Vejen, Denmark); General Mills, Inc.; Mérieux NutriSciences Brasil (Sao Paulo, Brazil); Mérieux NutriSciences Mexico (Mexico City, Mexico); Silliker Illinois Analytical Laboratory; and The National Food Laboratory. Additionally, the following laboratories participated in a USDA interlaboratory comparsion evaluating methods for 25-hydroxyvitamin D in foods: Covance Laboratories, Inc.; Health Canada (Longueil, QC, Canada); Heartland Laboratories (Ames, IA); Technical University of Denmark (DTU) (Kongens Lyngby, Denmark).

Support for assignment of values for vitamin D_3 and 25-hydroxyvitamin D_3 was provided by the National Institutes of Health, Office of Dietary Supplements (NIH-ODS). Technical consultation was provided by J.M. Betz (NIH-ODS).

Statistical analysis was provided by J.H. Yen of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

NOTICE TO USERS:

SRM 1845a IS INTENDED FOR RESEARCH USE, NOT FOR HUMAN CONSUMPTION.

INSTRUCTIONS FOR STORAGE AND USE

Storage: The SRM should be stored under refrigeration (4 °C) in the original unopened packets. For elemental analyses, the packet can be resealed, stored under refrigeration, and test portions can be removed and analyzed until the material reaches its expiration date. For organic analyses, the packet can be resealed, stored under refrigeration, and test portions can be removed and analyzed for three weeks after the packet was initially opened.

Use: Before use, a packet should be allowed to warm to room temperature, and the contents of the packet should be mixed thoroughly. Allow the contents to settle for one minute prior to opening to minimize the loss of fine particles. Homogeneity of the material has not been evaluated for sample sizes smaller than those used by NIST methods described below. Therefore, the certified and reference values may not be valid for test portions smaller than those described in the sections below: 0.2 g to 0.5 g for elemental analyses, 0.3 g to 0.8 g for fatty acid analyses, 0.05 g for cholesterol analyses, 0.1 g for choline and carnitine analyses, and 1 g to 3 g for vitamin analyses. Results obtained in analyses should include their own estimates of uncertainty and can be compared to the certified values using procedures described in reference 5.

SOURCE, PREPARATION, AND ANALYSIS[(1)]

Source and Preparation: The SRM is a whole egg powder. The contents of a 50-pound bag of whole egg powder were prepared from USDA-inspected eggs by a commercial manufacturer of whole egg powder. The material was transferred to High-Purity Standards (Charleston, SC) where the material was blended and packaged. The egg powder was sealed in approximately 10 g aliquots in Mylar bags that had been flushed with nitrogen.

Analytical Approach for Determination of Cholesterol: Value assignment of the cholesterol mass fraction was based on measurements made by NIST using an isotope dilution gas chromatography mass spectrometry (ID-GC-MS) method.

NIST Analyses for Cholesterol Using ID-GC-MS: The mass fraction of cholesterol was measured using the ID-GC-MS method developed at NIST for serum cholesterol [6] and modified for the determination of cholesterol in

[(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.

food matrices using AOAC International Official Method 996.06 for hydrolysis [7]. Three sets of samples were prepared, each consisting of duplicate 0.05 g test portions from each of three packets of SRM 1845a weighed into round-bottom flasks. An aliquot of a solution containing a known mass of the internal standard, cholesterol-$^{13}C_3$, was added to each flask. Cholesterol esters were hydrolyzed by heating the samples in an alcoholic-KOH solution for 1 h at 100 °C. Cholesterol was extracted into hexane, and a portion of the hexane extract was evaporated to dryness prior to addition of N,O-bis(trimethylsilyl)acetamide to convert cholesterol to the trimethylsilyl (TMS) derivative. GC-MS was performed using a 30 m phenyl/methyl polysiloxane (5/95 mole fraction) non-polar fused silica column directly interfaced to the ion source. Cholesterol was determined in the electron ionization mode with selected ion monitoring at *m/z* 458 and *m/z* 461 for the unlabeled and labeled cholesterol-TMS, respectively. Calibrants were prepared gravimetrically from SRM 911c *Cholesterol* at levels intended to approximate the level of the cholesterol in the SRM following extraction. A single internal standard solution was used for the calibrants and samples. Calculations are based on linear regression analysis for the calibrants.

Analytical Approach for Determination of Elements: Value assignment of the mass fractions of the elements in SRM 1845a was based on the combination of results from NIST and collaborating laboratories, where appropriate. NIST provided measurements by using inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), instrumental neutron activation analysis (INAA), and radiochemical neutron activation analysis (RNAA).

NIST Analyses for Ba, Ca, Co, Cr, Cu, Fe, I, K, Mg, Mn, Mo, Na, Ni, P, Se, Sr, V, and Zn Using ICP-OES and/or ICP-MS: The mass fractions of barium, calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, strontium, and zinc were measured by ICP-OES. The mass fractions of barium, chromium, cobalt, molybdenum, nickel, selenium, strontium, and vanadium were measured by ICP-MS. For each technique, duplicate 0.5 g test portions were taken from each of 10 packets of SRM 1845a and were digested in a microwave sample preparation system with subsequent hot-plate digestion using a nitric acid/hydrofluoric acid mixture. The mass fraction of iodine was measured by ICP-MS in single 0.3 g test portions taken from each of six packets and in each of four packets one year later. Samples were digested in aqueous tetramethylammonium hydroxide (TMAH) using a microwave sample preparation system. Quantification for ICP-OES and ICP-MS was based on the method of standard additions using SRM 3100 series single element standard solutions.

NIST Analyses for Al, Cl, Co, Fe, Mg, Mn, Na, Se, and Zn Using INAA: The mass fractions of cobalt, iron, selenium, and zinc were measured by INAA in duplicate 0.22 g test portions taken from each of six packets of SRM 1845a. Powders were pressed into cylindrical pellets, and samples, controls, and standards, prepared from SRM 3100 series single element standard solutions, were packaged individually in clean polyethylene bags and irradiated individually. For determination of cobalt, iron, selenium, and zinc, samples were irradiated in polyethylene irradiation vessels for 4 h; irradiation capsules were then inverted 180 degrees, and materials were irradiated for another 4 h. Radioactive decay was counted for 8 h after a decay of more than 150 d. Mass fractions of aluminum, chlorine, magnesium, manganese, and sodium were measured by INAA in duplicate test portions of approximately 0.2 g taken from each of six packets of SRM 1845a. Samples, controls, and standards, prepared from SRM 3100 series single element standard solutions, were individually irradiated, together with one flux monitor foil, for 60 s. Radioactive decay for aluminum, chlorine, magnesium, manganese, and sodium was counted for 5 min after a decay of 5 min.

NIST Analysis for As Using RNAA: The mass fraction of arsenic was measured by RNAA using single 0.25 g test portions taken from each of six packets of SRM 1845a. Individual disks were formed from the test portions using a stainless-steel die and hydraulic press. Samples were packaged individually in clean polyethylene bags and irradiated in one polyethylene irradiation vessel for 5 h at 20 MW, which provided a thermal neutron fluence rate of $1x10^{14}$ $cm^{-2}s^{-1}$. Samples were combined with ^{77}As prior to chemical separation. Samples were dissolved in a mixture of nitric and perchloric acids, and arsenic was separated from the matrix [8]. The 559 keV line from decay of ^{76}As was used for quantification. The 239 keV line from decay of ^{77}As was evaluated for yield determination. Calibrants were prepared from SRM 3103a *Arsenic (As) Standard Solution*.

Analytical Approach for Determination of Vitamins: Value assignment of the mass fractions of the vitamins in SRM 1845a was based on the combination of results provided by NIST and by collaborating laboratories, where appropriate. NIST provided measurements by using isotope dilution (ID) with liquid chromatography (LC) and mass spectrometry (MS) or tandem mass spectrometry (MS/MS), by LC with absorbance detection, and by LC with fluorescence detection.

NIST Analyses for Vitamin D_3 and 25-Hydroxyvitamin D_3 Using ID-LC-MS/MS: The mass fractions of vitamin D_3 (cholecalciferol) and 25-hydroxyvitamin D_3 were measured in duplicate 2.0 g to 3.0 g test portions taken from each of ten packets of SRM 1845a. Vitamin D_3-$^{13}C_5$ and 25-hydroxyvitamin D_3-$^{13}C_5$ were added as internal standards. Prior to extraction, the samples of SRM 1845a were suspended in water and dipotassium oxalate solution (35 %, mass

fractions) was added. The analytes and internal standards were extracted from the sample into 10:5:7 ethanol:tert-butylmethylether:petroleum ether (volume fractions) by rotational agitation for 30 min. The samples were centrifuged, the supernatants were decanted, and an additional aliquot of 5:7 tert-butylmethylether:petroleum ether (volume fractions) was added. Samples were extracted further by 30 min of mixing/rotation. Two additional cycles of sonication were conducted, for a total of four extractions. The pooled organic layers dried by addition of magnesium sulfate and evaporated to dryness under nitrogen. The analytes were derivatized with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) and reconstituted in 1:1:1 isopropanol:methanol:ethyl acetate (volume fractions) for analysis by positive-ion mode LC-MS/MS. A gradient method with a water/methanol mobile phase and a pentafluorophenyl column, as well as a gradient method with a water/methanol mobile phase and a C18 column, were used for LC-MS/MS determination. Vitamin D_3+PTAD and vitamin D_3-$^{13}C_5$+PTAD were measured at transitions *m/z* 560 → *m/z* 298 and *m/z* 565 → *m/z* 298, respectively. 25-Hydroxyvitamin D_3+PTAD and 25-hydroxyvitamin D_3-$^{13}C_5$+PTAD were measured at transitions *m/z* 558 → *m/z* 298 and *m/z* 563 → *m/z* 298, respectively. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the vitamins in the SRM following extraction. The purity of the neat vitamin D_3 calibrant material was determined by the manufacturer and confirmed at NIST using spectrophotometry at 265 nm. The purity of the neat 25-hydroxyvitamin D_3 calibrant material was determined at NIST using LC-absorbance, Karl Fischer titration, thermogravimetric analysis, and quantitative proton nuclear magnetic resonance spectroscopy. A single internal standard solution was used for the calibrants and samples.

NIST Analyses for Choline and Carnitine Using ID-LC-MS: The mass fractions of choline and carnitine were measured in duplicate 0.1 g test portions taken from each of 10 packets of SRM 1845a. $^2H_9$-choline chloride and $^2H_9$-carnitine hydrochloride were added as internal standards. The analytes and internal standards were extracted and hydrolyzed by microwave digestion in dilute hydrochloric acid for analysis by positive-ion mode LC-MS. A gradient method with an ammonium formate/acetonitrile mobile phase and a mixed-mode C18 column were used for LC-MS determination. Choline and $^2H_9$-choline were measured at *m/z* 104 and *m/z* 113, respectively. Carnitine and $^2H_9$-carnitine were measured at *m/z* 162 and *m/z* 171, respectively. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the vitamins in the SRM following extraction. The purity of neat calibrant materials was determined at NIST using quantitative proton nuclear magnetic resonance spectroscopy. A single internal standard solution was used for the calibrants and samples.

Analytical Approach for Determination of Fatty Acids: Value assignment of the mass fractions of fatty acids in SRM 1845a was based on the combination of measurements made at NIST and by collaborating laboratories, where appropriate. NIST provided measurements by using gas chromatography (GC) with flame ionization detection (FID) and GC-MS as described below.

NIST Analyses for Fatty Acids Using GC-FID: Mass fractions of fatty acids were determined by GC-FID from two 0.3 g to 0.5 g test portions from each of 10 packets of SRM 1845a. The egg powder and internal standard solution (octacosanoic acid and myristic-d_{27} acid) were mixed with diatomaceous earth in pressurized fluid extraction (PFE) cells. Following PFE with hexane:acetone (4:1 volume fraction), extracts were combined with methanolic (*m*-trifluoromethylphenyl) trimethylammonium hydroxide (1:1 volume fraction), vortexed, and allowed to stand for at least 30 min prior to analysis by GC-FID. GC-FID was performed using a 0.25 mm × 100 m biscyanopropyl polysiloxane fused silica capillary column. Calibrants were prepared gravimetrically from SRM 2377 *Fatty Acid Methyl Esters in 2,2,4-Trimethylpentane*, at levels intended to approximate the levels of the fatty acids in the SRM following extraction. A single internal standard solution was used for the calibrants and samples. Calculations are based on average response factors for the calibrants.

NIST Analyses for Fatty Acids Using GC-MS: Mass fractions of fatty acids were determined by GC-MS from one 0.5 g to 0.8 g test portion from each of six packets of SRM 1845a. The egg powder and internal standard solution (octacosanoic acid and myristic-d_{27} acid) were mixed with diatomaceous earth in glass extraction thimbles containing glass wool. Following a 22-h Soxhlet extraction with methylene chloride:methanol (2:1 volume fraction), extracts were concentrated, methanolic sodium hydroxide was added, and the sample was heated at 100 °C for 30 min with gentle shaking every 10 min. Extracts were cooled to room temperature, methanolic BF_3 was added, and the samples were heated to 100 °C for 30 min. Extracts were cooled to 40 °C and fatty acids were extracted with 40 mg/L butylated hydroxytoluene (BHT) in hexane and saturated aqueous sodium chloride solution. The hexane/BHT layer was removed and the hexane/BHT extraction repeated twice and combined with the first extracted portion. A subsample of the combined extracts was analyzed by GC-MS using a 0.25 mm × 60 m fused silica capillary column containing a cyanopropyl:methylpolysiloxane (50:50 mole fraction) phase. Calibrants were prepared gravimetrically from SRM 2377 *Fatty Acid Methyl Esters in 2,2,4-Trimethylpentane*, at levels intended to approximate the levels of the fatty acids in the SRM following extraction. A single internal standard solution was used for the calibrants and samples. Calculations are based on average response factors for the calibrants.

Collaborating Laboratories' Analyses: The GMA FIACSG laboratories were asked to use their usual methods to make single measurements of proximates, calories, vitamins, elements, fatty acids, and amino acids on test portions taken from each of two packets of SRM 1845a. Because of variability among data provided by laboratories participating in an interlaboratory comparison exercise, the median of laboratory means is used, with the uncertainty estimated using the median absolute deviation (MADe) [9]. The laboratories participating in the USDA interlaboratory study were asked to use their usual methods to make single measurements of vitamin D and metabolites on test portions taken from each of three packets of SRM 1845a. The mean of laboratory means is used, with the uncertainty estimated using the standard error of the mean of laboratory means. The methods used by collaborating laboratories are indicated in the tables below.

Homogeneity Assessment: The homogeneity of fatty acids, cholesterol, elements, and vitamins was assessed at NIST using the methods and test portion sizes described above. Analysis of the variance showed statistically significant heterogeneity in some cases, and the uncertainties for oleic acid, calcium, manganese, molybdenum, phosphorus, vanadium, and zinc all incorporate an additional component for possible heterogeneity. Homogeneity of constituents measured solely by collaborating laboratories (e.g., proximates, amino acids) was not assessed, although the data were treated as though these analytes were homogeneously distributed.

Value Assignment: For calculation of assigned values for analytes that were measured only by NIST, the mean of the mean values from NIST results were used. For calculation of assigned values for analytes that were measured only by the GMA FIACSG laboratories, the 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 median of the individual GMA FIACSG laboratory means or the mean of the USDA laboratory means, as appropriate.

Certified Mass Fraction Value for Cholesterol: The certified mass fraction for cholesterol is the mean of results obtained by NIST using ID-GC-MS. 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. To propagate this uncertainty, the certified value should be treated as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [2-4]. The uncertainty incorporates a Type A component for the difference between the certification set of data and a confirming set of data using a different GC column and different ions and Type B components for purity of the reference compound, completeness of hydrolysis, and stability of cholesterol in base. The measurand is the total mass fraction of cholesterol in whole egg powder as listed in Table 1 on an as-received basis. Metrological traceability is to the SI measurement unit for chemical mass fraction, expressed as milligrams per gram.

Table 1. Certified Mass Fraction Value for Cholesterol in SRM 1845a

	Mass Fraction (mg/g)
Cholesterol	17.67 ± 0.29

Certified Mass Fraction Values for Elements: Each certified mass fraction value is the combined mean from the mean of results from analyses by NIST and the median of the means of results provided by collaborating laboratories, where appropriate. 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. To propagate this uncertainty, the certified value should be treated as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [2-4]. The uncertainties for calcium, manganese, phosphorus, and zinc also incorporate an additional component for possible inhomogeneity. The measurands are the total mass fraction of elements in whole egg powder as listed in Table 2 on an as-received basis. Metrological traceability is to the SI measurement unit for chemical mass fraction, expressed as milligrams per kilogram.

Table 2. Certified Mass Fraction Values for Elements in SRM 1845a

	Mass Fraction (mg/kg)		
Barium (Ba)[a,b]	1.458	±	0.026
Calcium (Ca)[a,c]	2369	±	91
Copper (Cu)[a,c]	2.40	±	0.11
Iron (Fe)[a,c,d]	83.0	±	2.3
Magnesium (Mg)[a,c,d]	406	±	12
Manganese (Mn)[a,c,d]	1.15	±	0.11
Phosphorus (P)[a,c]	8640	±	610
Potassium (K)[a,c]	4110	±	400
Selenium (Se)[b,d]	1.554	±	0.054
Sodium (Na)[a,c,d]	4576	±	93
Strontium (Sr)[a,b]	1.6962	±	0.0060
Zinc (Zn)[a,c,d]	55.9	±	2.3

[a] NIST ICP-OES
[b] NIST ICP-MS
[c] Collaborating laboratories. Reported methods included atomic absorption spectroscopy (AAS), direct current plasma atomic emission spectrometry (DCP-AES), ICP-OES, ICP-MS, and colorimetry.
[d] NIST INAA

Certified Mass Fraction Values for Vitamins: Each certified mass fraction value is the mean from the combination of the mean results from analyses by NIST ID-LC-MS/MS and the median or mean of the means of results provided by collaborating laboratories using ID-LC-MS/MS and LC-absorbance. 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. To propagate this uncertainty, the certified value should be treated as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [2-4]. The measurands are the total mass fractions of the vitamins in whole egg powder as listed in Table 3 on an as-received basis. Metrological traceability is to the SI measurement unit for chemical mass fraction, expressed as milligrams per kilogram.

Table 3. Certified Mass Fraction Values for Vitamins in SRM 1845a

	Mass Fraction (mg/kg)		
Cholecalciferol (Vitamin D_3)	0.0488	±	0.0047
25-Hydroxyvitamin D_3	0.0122	±	0.0015

Reference Mass Fraction Values for Elements: Each reference mass fraction value is the mean result of NIST analyses from a single method or the mean from the combination of the mean results from multiple analyses by NIST. Values are expressed as $x \pm U_{95\%}(x)$, where x is the reference value and $U_{95\%}(x)$ is the expanded uncertainty of the value. The method-specific value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with about a 95 % confidence [2-4]. The uncertainties for molybdenum and vanadium incorporate an additional component for possible inhomogeneity. The measurands are the total mass fraction of each element listed in Table 4 on an as-received basis as determined by the methods indicated. Metrological traceability is to the SI measurement unit for chemical mass fraction, expressed as milligrams per kilogram, as realized by the method used.

Table 4. Reference Mass Fraction Values for Elements in SRM 1845a

	Mass Fraction (mg/kg)		
Aluminium (Al)[a]	973	±	11
Arsenic (As)[b]	0.00635	±	0.00028
Chlorine (Cl)[a]	5699	±	57
Chromium (Cr)[c]	0.112	±	0.011
Cobalt (Co)[a,c]	0.0089	±	0.0018
Iodine (I)[c]	3.03	±	0.10
Molybdenum (Mo)[c]	0.418	±	0.012
Nickel (Ni)[c]	0.0753	±	0.0044
Vanadium (V)[c]	0.0461	±	0.0022

[a] NIST INAA
[b] NIST RNAA
[c] NIST ICP-MS

Reference Mass Fraction Values for Choline and Carnitine: Each reference mass fraction value is the mean from the combination of the mean results from analyses by NIST and the median of the mean of results provided by collaborating laboratories, where appropriate. 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. The method-specific value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with about a 95 % confidence [2-4]. The measurands are the total mass fractions of each analyte listed in Table 5 on an as-received basis as determined by the methods indicated. Metrological traceability is to the SI measurement unit for chemical mass fraction, expressed as milligrams per kilogram, as realized by the method used.

Table 5. Reference Mass Fraction Values for Choline and Carnitine in SRM 1845a

	Mass Fraction (mg/kg)		
Choline[a,b]	16400	±	3800
Carnitine[a]	6.15	±	0.56

[a] NIST ID-LC-MS
[b] Collaborating laboratories. Reported methods included acid digestion followed by absorbance spectrophotometry.

Reference Values for Proximates and Calories: Each reference value is the median of the means of results provided by collaborating laboratories. 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. The method-specific value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with about a 95 % confidence [2-4]. For proximates and fiber, the measurands are the mass fractions listed in Table 6 on an as-received basis as determined by the methods indicated. For calories, the measurand is the caloric content listed in Table 6 on an as-received basis as determined by the method indicated. Metrological traceability is to the measurement processes and standards used by the collaborating laboratories.

Table 6. Reference Values for Proximates and Calories in SRM 1845a

	Mass Fraction (g/100 g)
Solids[a]	95.87 ± 0.37
Ash[b]	5.498 ± 0.078
Nitrogen[c]	6.933 ± 0.072
Protein[c]	43.32 ± 0.47
Carbohydrates[d]	4.6 ± 1.8
Fat (as the sum of fatty acids as triglycerides)	43.4 ± 1.4
	Energy (kcal per 100 g)
Calories[e]	581.2 ± 5.3

[a] Solids were determined by collaborating laboratories using drying in a forced-air oven and drying in a vacuum oven.

[b] Ash was determined by collaborating laboratories using weight loss after ignition in a muffle furnace and thermogravimetric analysis.

[c] Nitrogen was determined by collaborating laboratories using Kjeldahl, thermal conductivity, and combustion (LECO). A factor of 6.25 was used to convert nitrogen results to protein.

[d] Carbohydrates were determined by collaborating laboratories by difference (solids less the sum of protein, fat, and ash).

[e] Calories were determined by collaborating laboratories as the median of the lab mean caloric calculations from the interlaboratory comparison exercise. If the mean proximate values above are used for calculation with caloric equivalents of 9, 4, and 4 for fat (as the sum of fatty acids as triglycerides), protein, and carbohydrate, respectively, the mean caloric content is 582.3 kcal per 100 grams.

Reference Mass Fraction Values for Fatty Acids as Free Fatty Acids: Each reference mass fraction value is the mean from the combination of the mean results from analyses by NIST and the median of the mean of results provided by collaborating laboratories, where appropriate. 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. The method-specific value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with about a 95 % confidence [2-4]. For fatty acids values containing NIST data, the uncertainty incorporates a component for possible inhomogeneity based on the standard deviation. The uncertainty for oleic acid includes a component for possible heterogeneity. The measurands are the total mass fraction of each fatty acid listed in Table 7 on an as-received basis as determined by the method indicated. Metrological traceability is to the measurement processes and standards used by NIST and the collaborating laboratories.

Table 7. Reference Mass Fraction Values for Fatty Acids (as Free Fatty Acids) in SRM 1845a

	Common Name	Mass Fraction (g/100 g)		
Tetradecanoic Acid (C14:0)[a,b,c]	Myristic Acid	0.118	±	0.011
(Z)-9-Tetradecenoic Acid (C14:1)[a,b,c]	Myristoleic Acid	0.0218	±	0.0035
Pentadecanoic Acid (C15:0)[c]		0.0285	±	0.0013
Hexadecanoic Acid (C16:0)[a,b,c]	Palmitic Acid	8.75	±	0.89
(Z)-9-Hexadecenoic Acid (C16:1 n-7)[a,b,c]	Palmitoleic Acid	0.904	±	0.063
Heptadecanoic Acid (C17:0)[c]	Margaric Acid	0.0955	±	0.0098
Heptadecenoic Acid (C17:1)[c]	Margaroleic Acid	0.056	±	0.013
(E)-9-Octadecenoic Acid (C18:1 n-9)[b]	Elaidic Acid	0.0053	±	0.0016
(E)-11-Octadecenoic Acid (C18:1 n-7)[b]	Transvaccenic Acid	0.0123	±	0.0039
Octadecanoic Acid (C18:0)[a,b,c]	Stearic Acid	2.98	±	0.28
(Z)-9-Octadecenoic Acid (C18:1 n-9)[a,b,c]	Oleic Acid	12.2	±	2.2
(Z)-11-Octadecenoic Acid (C18:1 n-7)[a,b,c]	Vaccenic Acid	0.556	±	0.034
(Z,Z)-9,12-Octadecadienoic Acid (C18:2 n-6)[a,b,c]	Linoleic Acid	5.73	±	0.41
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3 n-3)[a,b,c]	α-Linolenic Acid	0.1581	±	0.0084
(Z,Z,Z)-6,9,12-Octadecatrienoic Acid (C18:3 n-6)[a,b,c]	γ-Linolenic Acid	0.0472	±	0.0034
Eicosanoic Acid (C20:0)[b]	Arachidic Acid	0.0047	±	0.0020
(Z)-9-Eicosenoic Acid (C20:1 n-11)[c]	Gadoleic Acid	0.113	±	0.012
(Z)-11-Eicosenoic Acid (C20:1 n-9)[b]	Gondoic Acid	0.043	±	0.020
(Z)-11,14-Eicosadienoic Acid (C20:2 n-6)[c]	Eicosadienoic Acid	0.097	±	0.029
(Z,Z,Z)-8,11,14-Eicosatrienoic Acid (C20:3 n-6)[c]	Dihomo-γ-linolenic Acid	0.108	±	0.040
(Z,Z,Z,Z)-5,8,11,14-Eicosatetratrienoic Acid (C20:4 n-6)[a,b,c]	Arachidonic Acid	0.679	±	0.054
(Z,Z,Z,Z,Z)-7,10,13,16,19-Docosapentaenoic Acid (C22:5 n-3)[a,b,c]	DPA	0.0212	±	0.0013
(Z,Z,Z,Z,Z,Z)-4,7,10,13,16,19-Docosahexaenoic Acid (C22:6 n-3)[a,b,c]	DHA	0.181	±	0.013
Tetracosanoic Acid (C24:0)[c]	Lignoceric Acid	0.0092	±	0.0015
(Z)-15-Tetracosenoic Acid (C24:1 n-9)[b]	Nervonic Acid	0.0595	±	0.0017

[a] NIST GC-MS
[b] NIST GC-FID
[c] Collaborating laboratories. Reported methods included GC-FID and GC-MS.

Reference Mass Fraction Values for Amino Acids: Each reference mass fraction value is the median of the means of results provided by collaborating laboratories. 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. The method-specific value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with about a 95 % confidence [2-4]. The measurands are the mass fractions of each amino acid listed in Table 8 on an as-received basis as determined by the collaborating laboratories. Metrological traceability is to the measurement processes and standards used by the collaborating laboratories.

Table 8. Reference Mass Fraction Values for Amino Acids in SRM 1845a

	Mass Fraction (g/100 g)
Alanine	2.42 ± 0.45
Arginine	3.0 ± 1.1
Aspartic Acid	4.37 ± 0.20
Cysteine	1.02 ± 0.65
Glutamic Acid	5.39 ± 0.49
Glycine	1.40 ± 0.12
Histidine	0.95 ± 0.14
Isoleucine	2.13 ± 0.36
Leucine	3.53 ± 0.65
Lysine	3.02 ± 0.74
Methionine	1.37 ± 0.18
Phenylalanine	2.20 ± 0.22
Proline	1.63 ± 0.62
Serine	3.35 ± 0.52
Threonine	2.01 ± 0.26
Tryptophan	0.63 ± 0.03
Tyrosine	1.69 ± 0.40
Valine	2.43 ± 0.89

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Certificate Revision History: 09 March 2020 (Extension of expiration date; certified values for fatty acids downgraded to reference values to properly reflect traceability and moved from Table 1 to Table 7; removal of certified values for riboflavin, niacinamide, pantothenic acid, pyridoxal, pyridoxamine, and total vitamin B_6 and reference values for thiamine, total vitamin B_6 by microbiological assay, total folate by microbiological assay, total biotin by microbiological assay, retinol, α-tocopherol, and γ-tocopherol based on NIST's decision to no longer support these measurement capabilities in this matrix; editorial changes); **07 May 2018** (Correction to nomenclature of gadoleic acid in Table 5; editorial changes); **27 June 2017** (Added certified values for vitamin D_3 and 25-hydroxyvitamin D_3; converted values for fatty acids to free fatty acids; converted values for vitamin B_6 to the non-salt form; information added about calibrants for each NIST method; information added about methods used by collaborating laboratories for each table of values; editorial changes); **04 September 2014** (Original certificate date).

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