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: The certified mass fraction values for selected fatty acids and cholesterol, selected elements, and selected vitamins in SRM 1845a, reported on an as-received basis, are provided in Tables 2 through 4. 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 have been investigated or 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 of the mean results provided by collaborating laboratories, where appropriate. The associated uncertainties are expressed at an approximately 95 % level of confidence [2–4]. The measurand in each case is the total mass fraction of each analyte. Metrological traceability is to the SI unit as indicated in Tables 2-4, respectively.

Reference Mass Fraction Values: Reference mass fraction values, reported on an as-received basis, are provided for additional elements (Table 5), additional vitamins, choline, and carnitine (Table 6), proximates, additional fatty acids and calories (Table 7), and amino acids (Table 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 or collaborating laboratories. The measurand in each case is the mass fraction of each analyte as determined by the methods indicated in the text. Metrological traceability is to the SI unit as indicated in Tables 5-8, respectively.

Expiration of Certification: The certification of **SRM 1845a** is valid, within the measurement uncertainty specified, until **30 July 2019**, 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, K.E. Sharpless, and L.J. Wood of the NIST Chemical Sciences Division and S. Ehling of the Grocery Manufacturers Association (GMA, Washington, DC).

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Analytical measurements at NIST were performed by C.E. Bryan, W.C. Davis, C. Luvonga, 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 Chemical Sciences Division and B.E. Lang of the Biosystems and Biomaterials Division. Analyses for value assignment were also performed by the following laboratories participating in a GMA Food Industry Analytical Chemists Committee (FIACC) 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, Madison, WI; The J.M. Smucker Co., Orville, OH; and The National Food Laboratory, Livermore, CA.

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 LABORATORY USE ONLY, 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 removed and analyzed until the material reaches its expiration date. For organic analyses, the packet can be resealed, stored under refrigeration, and test portions 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. For certified values to be valid, test portions of the following masses should be used: 0.05 g for cholesterol analysis, a minimum of 0.3 g for fatty acid analysis, between 0.2 g and 0.5 g for elemental analysis, and between 1 g and 2.5 g for vitamin analysis. 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 Fatty Acids and Cholesterol: Value assignment of the mass fractions of fatty acids in SRM 1845a was based on the combination of measurements made using two extraction procedures and two different analytical methods at NIST and by collaborating laboratories, where appropriate. NIST provided results using gas chromatography (GC) with flame ionization detection (FID) and GC with mass spectrometry (MS) as described below. Value assignment of the cholesterol mass fraction was based on measurements made by NIST using an isotope dilution (ID) GC/MS method.

NIST Analyses for Fatty Acids Using GC/FID: Two 0.3 g to 0.5 g test portions from each of 10 packets of SRM 1845a were added to pressurized-fluid extraction cells that were half filled with Hydromatrix (Varian, Palo Alto, CA). The egg powder was mixed with the Hydromatrix and additional Hydromatrix was added to fill the cell. The mixtures were spiked with an internal standard (IS) solution containing octacosanoic acid and myristic-d_{27} acid and 0.2 g water. The egg powder was extracted into a hexane:acetone (4:1 volume fraction) solution. Samples were analyzed by GC/FID, performed using a 0.25 mm × 100 m biscyanopropyl polysiloxane fused silica capillary column. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the fatty acids in the SRM. The same internal standard solution was used for the calibrants and samples. Calculations are based on average response factors for the calibrants.

[(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.

NIST Analyses for Fatty Acids Using GC/MS: Single 0.5 g to 0.8 g test portions from each of six packets of SRM 1845a were combined with wet Hydromatrix and transferred to a glass extraction thimble containing glass wool. An internal standard solution containing octacosanoic acid and myristic-d_{27} acid was added, and samples were extracted for 22 h using a methylene chloride:methanol (2:1 volume fraction) solution. Following extraction, 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. The sample was cooled to room temperature, methanolic BF_3 was added, and the samples were heated to 100 °C for 30 min. Butylated hydroxytoluene (an antioxidant) in hexane was added, and the sample was mixed for 30 s while still warm. Saturated aqueous sodium chloride solution was added, and the sample was mixed for 1 min and cooled to room temperature. The hexane layer was removed to another tube, and the hexane extraction was repeated twice. The three hexane layers were combined, and a portion was transferred to an autosampler vial for GC/MS analysis. GC/MS was performed using a 0.25 mm × 60 m fused silica capillary column containing cyanopropyl/phenylpolysiloxane (50/50 mole fraction) phases. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the fatty acids in the SRM. The same internal standard solution was used for the calibrants and samples. Calculations are based on average response factors for the calibrants.

NIST Analyses for Cholesterol: 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 food matrices using AOAC International Official Method 996.06 for hydrolysis [7]. Three sets of samples were prepared. Each set consisted 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 tube. Hydrolysis of cholesterol esters was accomplished by heating the samples in an alcohol-KOH solution for 1 h at 100 °C. Hexane was then used to extract the cholesterol. A portion of the hexane extract was evaporated to dryness and N,O-bis(trimethylsilyl)acetamide was added to convert cholesterol to the trimethylsilyl (TMS) derivative. Analyses were performed on a GC/MS system operated 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. The GC was equipped with a 30 m (phenyl/methyl polysiloxane, 5/95 mole fraction) non-polar fused silica column directly interfaced to the ion source. Standards consisting of mixtures of known quantities of pure unlabeled cholesterol (SRM 911c Cholesterol) and cholesterol-$^{13}C_3$ were run before and after the samples to generate composite linear regressions for calculation of the quantity of cholesterol in the samples.

Analytical Approach for Determination of Elements: Value assignment of the mass fractions of the elements in SRM 1845a was based on the combination of measurements from two different analytical methods at 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. Samples 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 a year later. Samples were digested in aqueous tetramethylammonium hydroxide using a microwave sample preparation system. Quantitation for ICP-OES and ICP-MS was based on the method of standard additions.

NIST Analyses for Al, Cl, Cr, Co, Fe, Mg, Mn, Mo, Na, Se, V, and Zn Using INAA: Mass fractions of chromium, cobalt, iron, molybdenum, 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, standards, and controls were packaged individually in clean polyethylene bags. Samples, standards, and controls were irradiated together for 4 h; irradiation capsules were then inverted 180 degrees, and materials were irradiated another 4 h. For the determination of chromium, cobalt, iron, molybdenum, selenium and zinc, samples were allowed to radioactively decay from a few days to several months prior to counting on a high purity germanium detector. Mass fractions of aluminum, chlorine, magnesium, manganese, sodium, and vanadium were measured by INAA in duplicate test portions of approximately 0.2 g taken from each of six packets of SRM 1845a. Samples, standards, and control materials were individually irradiated together with one flux monitor foil for 60 s in pneumatic tube RT-2. For the determination of aluminum, chlorine, magnesium, manganese, sodium, and vanadium, samples were 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 together 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 separated from the matrix as described in reference 8. The 559 keV line from decay of ^{76}As was used for quantitation. The 239 keV line from decay of ^{77}As was evaluated for yield determination.

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 various analytical methods at NIST and collaborating laboratories.

NIST Analyses for Thiamine, Riboflavin, Niacinamide, Pantothenic Acid, Pyridoxamine, and Pyridoxal: Mass fractions of thiamine, riboflavin, niacinamide, pantothenic acid, pyridoxamine, and pyridoxal were measured by ID-LC-MS/MS in duplicate 2 g test portions taken from each of 10 packets. The analytes and internal standards were extracted into dilute acetic acid for analysis by positive-ion mode ID-LC-MS/MS. A gradient method with an ammonium formate buffer/methanol mobile phase and a C_{18} column were used for ID-LC-MS/MS determination of the vitamins. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the vitamins in the SRM. A single internal standard solution was used for the calibrants and samples.

Table 1. ID-LC-MS/MS Transitions Monitored for Vitamins

Compound	Precursor Ion (*m/z*)	→ Product Ion (*m/z*)	Internal Standard	IS Precursor Ion (*m/z*)	→ IS Product Ion (*m/z*)
Thiamine	266	42	$^{13}C_3$-Thiamine	269	42
		123			123
Riboflavin	377	43	$^{13}C_4$,$^{15}N_2$-Riboflavin	383	43
		172			175
		198			202
		243			249
Niacinamide	123	53	$^{2}H_4$-Niacinamide	127	56
		78			81
		80			84
Pantothenic Acid	220	41	$^{13}C_3$,^{15}N-Pantothenic Acid	224	41
		43			43
		72			76
		90			94
Pyridoxamine	169	77	$^{2}H_3$-Pyridoxamine	172	79
		134			136
		152			155
Pyridoxal	168	41	$^{2}H_3$-Pyridoxal	171	43
		67			70
		94			97
		150			153

NIST Analyses for Tocopherols Using LC/Absorbance: Duplicate 1 g test portions of powder from each of six packets were accurately weighed into 50 mL polyethylene centrifuge tubes. An aliquot of an ethanolic tocol internal standard solution was added. The sample was suspended in approximately 30 mL of hexane:petroleum ether (50:50 volume fractions) by vortex mixing for 1 min. Analytes were extracted from the sample using an ultrasonic water bath for 1 h, followed by overnight mixing by rotation. The hexane:petroleum ether layer was removed and additional hexane added prior to extraction using an ultrasonic water bath for 2 h. Two additional extractions were performed and the supernatants from the individual extractions were combined and evaporated to dryness under nitrogen. The residues were resuspended in approximately 0.5 mL of ethyl acetate, to which 50 µL ethanol containing 30 mg/L butylated hydroxytoluene (BHT) was added. Separations were performed on a C_{18} column with an isocratic mobile phase of methanol:water (97:3 volume fraction). The separation was monitored using an absorbance detector at 298 nm for α-tocopherol and γ-tocopherol.

NIST Analyses for Tocopherols Using LC/Fluorescence: Duplicate 1 g to 2 g test portions of powder from each of 10 packets were accurately weighed into 50 mL polyethylene centrifuge tubes. An aliquot of an ethanolic tocol internal standard solution was added. The sample was suspended in approximately 5 mL of water and an aliquot of dipotassium oxalate solution was added. The analytes were then extracted from the suspended sample into a mixture of ethanol:*tert*-butylmethylether:petroleum ether (10:5:7 volume fractions) by rotational agitation for 15 min. Five additional extractions were performed using 15 min of mixing/rotation. The supernatants for the individual extractions were combined, washed with water twice, and were evaporated to dryness under nitrogen. The residues were resuspended in approximately 2 mL of ethanol:ethyl acetate (50:50 volume fraction). Separations were performed on a C_{30} column with an isocratic mobile phase of methanol:water (90:10). The separation was monitored using a fluorescence detector at an excitation wavelength of 295 nm and an emission wavelength of 330 nm for α-tocopherol and γ-tocopherol.

NIST Analyses for Choline and Carnitine: Mass fractions of choline and carnitine were measured in duplicate 0.1 g test portions taken from each of 10 packets of SRM 1845a. $^{2}H_{9}$-choline chloride and $^{2}H_{9}$-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 C_{18} column were used for LC/MS determination. Choline and $^{2}H_{9}$-choline were measured at *m/z* 104 and *m/z* 113, respectively. Carnitine and $^{2}H_{9}$-carnitine were measured at *m/z* 162 and *m/z* 171, respectively.

Collaborating Laboratories' Analyses: The GMA FIACC laboratories were asked to use their usual methods to make single measurements of proximates, calories, vitamins, elements, 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].

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, riboflavin, γ-tocopherol, 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: 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 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 collaborating 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 collaborating laboratory means, as appropriate. For fatty acids, the calculation of assigned values is the mean of the means of two NIST results with confirmation provided by collaborating laboratories.

Certified Mass Fraction Values for Fatty Acids as Triglycerides: Each certified mass fraction value is the combined mean from the mean of NIST GC/MS data and the mean of NIST GC/FID data, with confirmation using results provided by collaborating laboratories. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c incorporates the observed difference between the results from the methods and their respective uncertainties consistent with the ISO/JCGM Guide and k is a coverage factor corresponding to approximately 95 % confidence [2–4].

Certified Mass Fraction Value for Cholesterol: The certified mass fraction for cholesterol is the mean of results obtained by NIST using ID-GC/MS. The uncertainty provided with the value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c represents the combined uncertainty consistent with the ISO/JCGM Guide and k is a coverage factor corresponding to approximately 95 % confidence [2]. The uncertainty for cholesterol 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.

Table 2. Certified Mass Fraction Values for Fatty Acids (as Triglycerides) and Cholesterol in SRM 1845a

	Common Name	Mass Fraction (%)			Coverage Factor, *k*
Tetradecanoic Acid (C14:0)	Myristic Acid	0.1155	±	0.0051	2.00
(Z)-9-Tetradecenoic Acid (C14:1)	Myristoleic Acid	0.0195	±	0.0008	2.00
Hexadecanoic Acid (C16:0)	Palmitic Acid	8.63	±	0.27	2.00
(Z)-9-Hexadecenoic Acid (C16:1 n-7)	Palmitoleic Acid	0.872	±	0.025	2.00
Octadecanoic Acid (C18:0)	Stearic Acid	2.93	±	0.10	2.00
(Z)-9-Octadecenoic Acid (C18:1 n-9)	Oleic Acid	11.5	±	1.5	2.00
(Z)-11-Octadecenoic Acid (C18:1 n-7)	Vaccenic Acid	0.556	±	0.016	2.00
(Z,Z)-9,12-Octadecadienoic Acid (C18:2 n-6)	Linoleic Acid	5.67	±	0.12	2.00
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3 n-3)	α-Linolenic Acid	0.1718	±	0.0049	2.00
(Z,Z,Z)-6,9,12-Octadecatrienoic Acid (C18:3 n-6)	γ-Linolenic Acid	0.0473	±	0.0019	2.00
(Z,Z,Z,Z)-5,8,11,14-Eicosatetratrienoic Acid (C20:4 n-6)	Arachidonic Acid	0.670	±	0.018	2.00
(Z,Z,Z,Z,Z)-7,10,13,16,19-Docosapentaenoic Acid (C22:5 n-3)	DPA	0.0210	±	0.0007	2.00
(Z,Z,Z,Z,Z,Z)-4,7,10,13,16,19-Docosahexaenoic Acid (C22:6 n-3)	DHA	0.1767	±	0.0080	2.00
		Mass Fraction (mg/g)			Coverage Factor, *k*
Cholesterol		17.67	±	0.29	2.00

Certified Mass Fraction Values for Selected Elements: Each certified mass fraction value is the combined mean from the mean of results from analyses by NIST and the median of the mean of results provided by collaborating laboratories, where appropriate. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c incorporates the observed difference between the results from the methods and their respective uncertainties consistent with the ISO/JCGM Guide and k is a coverage factor corresponding to approximately 95 % confidence [2-4]. The uncertainties for calcium, manganese, phosphorus, and zinc also incorporate an additional component for possible inhomogeneity.

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

	Mass Fraction (mg/kg)			Coverage Factor, k
Barium (Ba)[a,b]	1.458	±	0.026	2.00
Calcium (Ca)[a,c]	2369	±	91	2.00
Copper (Cu)[a,c]	2.40	±	0.11	2.00
Iron (Fe)[a,c,d]	83.0	±	2.3	2.00
Magnesium (Mg)[a,c]	406	±	12	2.00
Manganese (Mn)[a,c,d]	1.15	±	0.11	2.00
Phosphorus (P)[a,c]	8640	±	610	2.00
Potassium (K)[a,c]	4110	±	400	2.00
Selenium (Se)[b,d]	1.554	±	0.054	2.00
Sodium (Na)[a,c,d]	4576	±	93	2.00
Strontium (Sr)[a,b]	1.6962	±	0.0060	2.00
Zinc (Zn)[a,c,d]	55.9	±	2.3	2.00

[a] NIST ICP-OES
[b] NIST ICP-MS
[c] Collaborating laboratories
[d] NIST INAA

Certified Mass Fraction Values for Selected Vitamins: Each certified 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. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c incorporates the observed difference between the results from the methods and their respective uncertainties consistent with the ISO/JCGM Guide and k is a coverage factor corresponding to approximately 95 % confidence [2–4]. The uncertainty for riboflavin incorporates an additional component for possible inhomogeneity. The certified values for these vitamins represent the free (unbound) forms.

Table 4. Certified Mass Fraction Values for Selected Vitamins in SRM 1845a

	Mass Fraction (mg/kg)			Coverage Factor, k
Riboflavin (Vitamin B_2)[a,b]	16.51	±	0.58	2.00
Niacinamide (Vitamin B_3)[a]	1.421	±	0.014	2.09
Pantothenic Acid (Vitamin B_5)[a,b]	64.6	±	9.6	2.00
Pyridoxal Hydrocholoride (Vitamin B_6)[a]	0.1619	±	0.0063	2.09
Pyridoxamine Dihydrochloride (Vitamin B_6)[a]	0.0676	±	0.0036	2.09
Total Vitamin B_6 as Pyridoxal[a,c]	0.2190	±	0.0072	2.09

[a] NIST ID-LC/MS/MS
[b] Collaborating laboratories
[c] Total vitamin B_6 is the sum of pyridoxal and pyridoxamine, which was mathematically converted to pyridoxal by multiplying its mass fraction by the ratio of the relative molecular masses of pyridoxal and pyridoxamine.

Reference Mass Fraction Values for Selected Elements: Each reference mass fraction value is the mean result of NIST analyses or the mean from the combination of the mean results from multiple analyses by NIST, where appropriate. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c represents the combined uncertainty consistent with the ISO/JCGM Guide and k is a coverage factor corresponding to approximately 95 % confidence [2]. For values based on more than one data source, the combined uncertainty incorporates the observed difference between the results from the methods and their respective uncertainties, consistent with the ISO/JCGM Guide [2–4]. The uncertainty for these elements incorporates any observed uncertainty due to a blank effect that may be present. The uncertainties for molybdenum and vanadium also incorporate an additional component for possible inhomogeneity.

Table 5. Reference Mass Fraction Values for Selected Elements in SRM 1845a

	Mass Fraction (mg/kg)			Coverage Factor, *k*
Aluminium (Al)[a]	973	±	11	2.07
Arsenic (As)[b]	0.00635	±	0.00028	2.00
Chlorine (Cl)[a]	5699	±	57	2.05
Chromium (Cr)[c]	0.112	±	0.011	2.06
Cobalt (Co)[a,c]	0.0089	±	0.0018	2.00
Iodine (I)[c]	3.03	±	0.10	2.00
Molybdenum (Mo)[c]	0.418	±	0.012	2.00
Nickel (Ni)[c]	0.0753	±	0.0044	2.06
Vanadium (V)[c]	0.0461	±	0.0022	2.00

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

Reference Mass Fraction Values for Selected Vitamins, 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. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c represents the combined uncertainty consistent with the ISO/JCGM Guide and k is a coverage factor corresponding to approximately 95 % confidence [2]. For values based on more than one data source, the combined uncertainty incorporates the observed difference between the results from the methods and their respective uncertainties, consistent with the ISO/JCGM Guide [2–4]. The uncertainty for γ-tocopherol also incorporates an additional component for possible inhomogeneity.

Table 6. Reference Mass Fraction Values for Selected Vitamins, Choline, and Carnitine in SRM 1845a

	Mass Fraction (mg/kg)			Coverage Factor, *k*
Thiamine (Vitamin B_1)[a,b]	1.87	±	0.68	2.00
Total Vitamin B_6 by Microbiological Assay[b]	4.2	±	1.2	3.18
Total Folate by Microbiological Assay[b]	1.300	±	0.069	4.30
Total Biotin by Microbiological Assay[b]	0.75	±	0.31	12.71
Retinol[b,c]	0.78	±	0.18	3.18
α-Tocopherol[b,c,d,e]	35.9	±	9.0	2.00
γ-Tocopherol[b,c,d,e]	15.0	±	3.0	2.00
Choline[b,f]	16400	±	3800	2.00
Carnitine[f]	6.15	±	0.57	2.09

[a] NIST ID-LC/MS/MS
[b] Collaborating laboratories
[c] The reference value is metrologically traceable to the SI through the molar absorptivity of the compound.
[d] NIST LC/Absorbance
[e] NIST LC/Fluorescence
[f] NIST ID-LC/MS

Reference Values for Proximates, Fatty Acids, and Calories: Each reference mass fraction value is the mean result from analyses by NIST or the median of the mean of results provided by collaborating laboratories. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c represents the combined uncertainty consistent with the ISO/JCGM Guide and k is a coverage factor corresponding to approximately 95 % confidence [2].

Table 7. Reference Values for Proximates, Fatty Acids, and Calories in SRM 1845a

	Common Name	Mass Fraction (%)	Coverage Factor, *k*
Solids[a]		95.87 ± 0.37	2.18
Ash[a]		5.498 ± 0.078	2.18
Nitrogen[a]		6.933 ± 0.072	2.16
Protein[a,b]		43.32 ± 0.47	2.18
Carbohydrates[a]		4.6 ± 1.8	2.20
Fat (as the sum of fatty acids as triglycerides)[a]		43.4 ± 1.4	2.18
Pentadecanoic Acid (C15:0)[a]		0.0300 ± 0.0014	2.31
Heptadecanoic Acid (C17:0)[a]	Margaric Acid	0.100 ± 0.010	2.26
Heptadecenoic Acid (C17:1)[a]	Margaroleic Acid	0.059 ± 0.014	2.36
(E)-9-Octadecenoic Acid (C18:1 n-9)[c]	Elaidic Acid	0.0055 ± 0.0017	2.26
(E)-11-Octadecenoic Acid (C18:1 n-7)[c]	Transvaccenic Acid	0.0129 ± 0.0041	4.30
Eicosanoic Acid (C20:0)[c]	Arachidic Acid	0.0049 ± 0.0021	2.31
(Z)-9-Eicosenoic Acid (C20:1 n-7)[a]	Gadoleic Acid	0.118 ± 0.013	2.45
(Z)-11-Eicosenoic Acid (C20:1 n-9)[c]	Gondoic Acid	0.045 ± 0.021	2.16
(Z)-11,14-Eicosadienoic Acid (C20:2 n-6)[a]	Eicosadienoic Acid	0.102 ± 0.031	2.26
(Z,Z,Z)-8,11,14-Eicosatrienoic Acid (C20:3 n-6)[a]	Dihomo-γ-linolenic Acid	0.112 ± 0.041	2.57
Tetracosanoic Acid (C24:0)[a]	Lignoceric Acid	0.0095 ± 0.0015	3.18
(Z)-15-Tetracosenoic Acid (C24:1 n-9)[c]	Nervonic Acid	0.0616 ± 0.0018	2.11

	Energy (kcal per 100 g)	Coverage Factor, *k*
Calories[d]	581.2 ± 5.3	2.20

[a] Collaborating laboratories
[b] A factor of 6.25 was used to convert nitrogen results to protein.
[c] NIST GC/FID
[d] The reference value for calories is the median of 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), protein, and carbohydrate, respectively, the mean caloric content is 582.3 kcal per 100 grams.

Reference Mass Fraction Values for Amino Acids: Each reference mass fraction value is the median of the mean results provided by collaborating laboratories. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c represents the combined uncertainty consistent with the ISO/JCGM Guide and k is a coverage factor corresponding to approximately 95 % confidence [2].

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

	Mass Fraction (%)			Coverage Factor, *k*
Alanine	2.42	±	0.45	2.78
Arginine	3.0	±	1.1	2.78
Aspartic Acid	4.37	±	0.20	3.18
Cysteine	1.02	±	0.65	4.30
Glutamic Acid	5.39	±	0.49	2.78
Glycine	1.40	±	0.12	2.78
Histidine	0.95	±	0.14	2.78
Isoleucine	2.13	±	0.36	2.78
Leucine	3.53	±	0.65	2.78
Lysine	3.02	±	0.74	2.78
Methionine	1.37	±	0.18	3.18
Phenylalanine	2.20	±	0.22	3.18
Proline	1.63	±	0.62	2.78
Serine	3.35	±	0.52	3.18
Threonine	2.01	±	0.26	2.78
Tryptophan	0.63	±	0.03	4.30
Tyrosine	1.69	±	0.40	3.18
Valine	2.43	±	0.89	3.18

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