National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2383a

Baby Food Composite

This Standard Reference Material (SRM) is intended primarily for validation of methods for determining proximates, calories, sugars, carotenoids, and elements in food matrices. This SRM can also be used for quality assurance when assigning values to in-house control materials. The baby food composite is a mixture of fruits, vegetables, macaroni, rice flour, and milk powder. A unit of SRM 2383a consists of four jars, each containing approximately 70 g of material.

Certified Mass Fraction Values: Certified mass fraction values for carotenoids and elements in SRM 2383a, reported on an as-received basis, are provided in Tables 1 and 2. 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 unweighted mean of the mean values from NIST methods and the median of the mean 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 2383a, reported on an as-received basis, are provided in Tables 3 through 6. 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 an uncertainty 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.

Information Mass Fraction Values: Information mass fraction values for several elements in SRM 2383a, reported on an as-received basis, are provided in Table 7. A NIST information value is a value that may be of interest to the SRM user, but insufficient information is available to assess the uncertainty associated with the value, and therefore no uncertainty is provided [1]. Information values may not be used to establish metrological traceability.

Expiration of Certification: The certification of **SRM 2383a** is valid, within the measurement uncertainty specified, until **01 January 2029**, provided the SRM is handled and stored in accordance with 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, and S. Ehling of the Grocery Manufacturers Association (GMA, Washington, DC).

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Certificate Revision History on Last Page

Steven J. Choquette, Director
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Analyses at NIST were performed by C. Boonyakong, C. Bryan, W.C. Davis, R. Oflaz, D.J. O'Kelly, T.O. Okumu, Y. Nuevo Ordóñez, R.L. Paul, J.B. Thomas, B.E. Tomlin, and L.J. Wood of the NIST Chemical Sciences Division.

Analysts at the following laboratories performed measurements that contributed to the value assignment of nutrients in SRM 2383a Baby Food Composite as part of the GMA Food Industry Analytical Chemists Committee (FIACC) interlaboratory comparison exercise and as part of an interlaboratory comparison exercise coordinated by NIST: Campbell Soup Company (Camden, NJ); ConAgra Foods Analytical Laboratory (Omaha, NE); Covance Laboratories (Madison, WI); Del Monte Foods (Walnut Creek, CA); Eurofins Central Analytical Laboratories (Metairie, LA); Eurofins Nutritional Analysis Center, Inc. (Des Moines, IA); Eurofins Strasburger & Siegel (Hanover, MD); Eurofins Steins (Vejen, Denmark); General Mills, Inc./Medallion Laboratories (Minneapolis, MN); Hormel Foods Corporation (Austin, MN); Krueger Food Labs (Billerica, MA); Land O' Lakes (Arden Hills, MN); Merieux NutriSciences (Crete, IL); Nestlé QA Center (Dublin, OH); Silliker, Inc. (Chicago Heights, IL); Silliker JR Laboratories (Burnbury, BC, Canada); The National Food Laboratory (Livermore, CA); and The Schwan Food Company (Salina, KS).

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 2383a IS INTENDED FOR RESEARCH USE, NOT FOR HUMAN CONSUMPTION.

INSTRUCTIONS FOR STORAGE AND USE

Storage: The SRM should be stored under refrigeration at a temperature between 2 °C and 8 °C, in the dark, and in the original sealed jars. The certification does not apply to contents of previously opened jars that have been stored for more than one day as the stability of all analytes beyond this period has not been investigated.

Use: Before use, the contents of the jar should be mixed thoroughly. The following masses used for NIST analyses should be used as the minimum sample size to ensure valid results: 1.5 g for carotenoids; 1 g to 3.5 g for elements (see Analytical Approach for Determination of Elements below). Results obtained in analyses should include their own estimates of uncertainty and can be compared to the certified and reference values using procedures described in reference 5.

SOURCE, PREPARATION, AND ANALYSIS[(1)]

Source and Preparation: SRM 2383a is a mixture of the following ingredients (in order of decreasing mass): water, orange juice concentrate, corn, rice flour, papaya puree, spinach, macaroni, carrots, tomato paste, and non-fat milk powder. This material is different from its predecessor, SRM 2383, in that infant formula was not added to the material. Therefore, it contains no preformed vitamin A nor other constituents that would be present in a fortified food product.

Analytical Approach for Determination of Carotenoids: Value assignment of the mass fractions of the carotenoids in SRM 2383a was based on the combination of measurements from two analytical methods at NIST and results obtained by collaborating laboratories, where available. NIST provided measurements by using liquid chromatography methods with absorbance detection (LC-absorbance) and with mass spectrometric detection (LC-MS).

NIST Analyses for Carotenoids Using LC-Absorbance: Lutein, zeaxanthin, and β-carotene were measured by LC-absorbance in duplicate 1.5 g test portions taken from each of ten jars. An aliquot of internal standard, *trans*-β-apo-10'-carotenal oxime in ethanol containing butylated hydroxytoluene (BHT) as an antioxidant, was added to each sample. The sample was suspended in approximately 6 mL of stabilized tetrahydrofuran:methanol (50:50, volume fraction) by ultrasonic mixing for 1 min. The sample was saponified by addition of methanolic potassium hydroxide and heated at 40 °C for 1 h with constant stirring. The pH of the sample was adjusted to approximately pH 7 using glacial acetic acid and the analytes extracted into at least three 15-mL portions of hexane:petroleum ether (50:50 volume fraction) following addition of 10 mL of aqueous sodium chloride solution. Hexane:petroleum ether

[(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.

phases were combined and washed with water prior to evaporation under a stream of nitrogen. The residue was redissolved in 0.5 mL of ethyl acetate and injected for LC-absorbance analysis using a C18 column. Carotenoids were separated using a gradient method consisting of acetonitrile/methanol/ethyl acetate each containing 0.05 % triethylamine. The methanol phase also contained 0.5 mol/L ammonium acetate. Elution of carotenoids was monitored using absorbance at 450 nm. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the carotenoids in the SRM, and concentrations were assigned based on spectrophotometric purity evaluation. A single internal standard solution was used for the calibrants and samples.

NIST Analyses for Carotenoids Using LC-MS: Lutein, zeaxanthin, and β-carotene were measured by LC-MS in duplicate 2.5 g test portions taken from each of eight jars. Aliquots of two internal standards, *trans*-β-apo-10'-carotenal oxime and d_6-β-carotene, each in ethanol containing butylated hydroxytoluene (BHT) as an antioxidant, were added to each sample. Approximately 0.5 g of calcium carbonate was added and the sample was suspended in approximately 100 mL of hexane:petroleum ether (50:50, volume fraction) by vortex mixing for 1 min. The mixture was vacuum filtered and the filtrate rinsed with tetrahydrofuran:methanol (50:50, volume fraction) until all orange color was removed from the solids. The solids were then combined with an aqueous sodium chloride solution and the analytes extracted into diethyl ether:petroleum ether (50:50, volume fraction). The organic fraction was washed with water prior to evaporation to 0.5 mL under a stream of nitrogen. The residue was redissolved in 3.5 mL of ethanol. The ethanol extract was saponified for 30 min at room temperature by addition of methanolic potassium hydroxide containing pyrogallol. The pH of the sample was adjusted to approximately pH 7 using ascorbic acid and the analytes extracted into diethyl ether:petroleum ether (50:50, volume fraction). The organic phase was washed with water prior to evaporation under a stream of nitrogen. The residue was redissolved in 1 mL of ethanol and injected for LC-MS analysis using a C30 column. Carotenoids were separated using a gradient method consisting of water and acetone. Elution of carotenoids was monitored using atmospheric pressure photoionization and mass spectrometric detection in selected ion monitoring (SIM) mode using the following ions: *m/z* 551 for lutein; *m/z* 569 for zeaxanthin; *m/z* 417 for *trans*-β-apo-10'-carotenal oxime; *m/z* 537.4 for β-carotene; *m/z* 543.4 for d_6-β-carotene. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the carotenoids in the SRM, and concentrations were assigned based on spectrophotometric purity evaluation. A single internal standard solution was used for the calibrants and samples.

Analytical Approach for Determination of Elements: Value assignment of the mass fractions of the elements in SRM 2383a was based on the combination of measurements from at least two different analytical methods at NIST or a single NIST result and results obtained by collaborating laboratories, where available. 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, Ni, P, Na, Sr, Sn, and Zn by ICP-OES and/or ICP-MS: Mass fractions of calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc were determined by ICP-OES from duplicate 3.5 g test portions taken from each of ten jars of SRM 2383a. Samples were digested in a nitric acid/hydrofluoric acid mixture in open beakers on a hotplate. For the determination of mass fractions of barium and strontium by ICP-OES and the determination of barium, chromium, cobalt, molybdenum, nickel, strontium, and tin by ICP-MS, duplicate 1.5 g test portions were taken from each of ten jars of SRM 2383a and were digested in a nitric acid/hydrofluoric acid mixture in closed vessels in a microwave oven. For the determination of iodine by ICP-MS, single 1 g test portions were taken from six jars of SRM 2383a and from four other jars several months later. Samples were digested in aqueous tetramethylammonium hydroxide in closed vessels in a microwave oven. Quantitation was based on the method of standard additions using the SRM 3100 series single element standard solutions.

NIST Analyses for Co, Cr, Se, and Zn by INAA: For the determination of the mass fractions of chromium, cobalt, selenium, and zinc by INAA, material in ten jars of SRM 2383a was freeze dried and pulverized, and individual disks were prepared from 0.2 g test portions taken from the resulting powder. Standards were prepared from compounds of known purity. Samples, standards, and controls were packaged individually in clean polyethylene bags and irradiated individually at 20 MW for 8 h. Nuclides were counted for 8 h after decays of more than 120 d for chromium and 180 d for cobalt, selenium, and zinc. The following gamma-ray energies were used in the INAA analyses: 1173 keV and 1332 keV for the determination of cobalt, 320 keV for chromium, 136 keV and 265 keV for selenium, and 1115 keV for zinc.

NIST Analyses for As by RNAA: For the determination of the mass fraction of arsenic by RNAA, material in ten jars of SRM 2383a was freeze dried and pulverized, and individual disks were prepared from 0.2 g test portions taken from the resulting powder. Standards were prepared from SRM 3103a Arsenic (As) Standard Solution. Samples, standards, and controls were packaged individually in clean polyethylene bags and irradiated individually at 20 MW for 8 h. Samples were combined with ^{77}As prior to chemical separation. Samples were dissolved in a mixture of nitric

and perchloric acids, and As was separated from the matrix as described in reference 6. 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.

Collaborating Laboratories' Analyses: The GMA FIACC laboratories were asked to use their usual methods to make single measurements on test portions taken from each of two jars of SRM 2383a. The laboratories participating in the NIST exercise were asked to make duplicate measurements on each of two days. Because of the variability among data provided by laboratories participating in an interlaboratory comparison exercise, the median of the means is used. Collaborating laboratories' data alone were used to assign reference values for proximates, sugars, and amino acids.

Homogeneity Assessment: The homogeneity of carotenoids and elements was assessed at NIST using the methods and test portion sizes described above; analysis of variance did not show statistically significant heterogeneity. All analytes have been treated as though they are homogeneously distributed in the material although the homogeneity of the other analytes that were measured by collaborating laboratories (e.g., proximates) was not assessed.

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 collaborating laboratories, the median of the laboratory means was used. For carotenoids and elements that were measured by NIST and collaborating laboratories, the median of the individual collaborating laboratory means and the mean of the individual sets of NIST data were averaged, as appropriate.

Certified Mass Fraction Values for Carotenoids: Each certified mass fraction value, reported on an as-received basis, is the mean from the combination of means of NIST data sets and the median of the mean results provided by collaborating laboratories. 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, treat the certified value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [2-4]. The measurand is the total mass fraction of each carotenoid in baby food composite 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 kilogram through the molar absorptivity of the compound.

Table 1. Certified Mass Fraction Values for Carotenoids in SRM 2383a

Analyte	Mass Fraction (mg/kg)
Total Lutein[a,b]	0.89 ± 0.20
Total Zeaxanthin[a,b,c]	0.67 ± 0.11

[a] Collaborating laboratories
[b] NIST LC-absorbance
[c] NIST LC-MS

Certified Mass Fraction Values for Elements: Each certified mass fraction value, reported on an as-received basis, is the mean from the combination of means of NIST data sets and the median of the mean 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, treat the certified value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [2-4]. The measurand is the total mass fraction of each element in baby food composite 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 2383a

Analyte	Mass Fraction (mg/kg)		
Barium (Ba)[a,b]	0.278	±	0.020
Calcium (Ca)[a,c]	342.6	±	5.0
Cobalt (Co)[b,d]	0.048	±	0.005
Copper (Cu)[a,c]	0.758	±	0.082
Iron (Fe)[a,c]	4.42	±	0.51
Magnesium (Mg)[a,c]	212.2	±	4.0
Manganese (Mn)[a,c]	0.963	±	0.064
Phosphorus (P)[a,c]	453	±	11
Potassium (K)[a,c]	2910	±	220
Sodium (Na)[a,c]	195	±	29
Strontium (Sr)[a,b]	4.445	±	0.047
Zinc (Zn)[a,c,d]	2.22	±	0.18

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

Reference Mass Fraction Values for *trans*-β-Carotene and Tocopherol: Each reference mass fraction value, reported on an as-received basis, is the mean from the combination of means of NIST data sets and the median of the mean results provided by collaborating laboratories, where appropriate. 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 measurand is the mass fraction for each analyte listed in Table 3 on an as-received basis as determined by the methods indicated. Metrological traceability is to the measurement processes and standards used by NIST and collaborating laboratories.

Table 3. Reference Mass Fraction Values for Additional Analytes in SRM 2383a

Analyte	Mass Fraction (mg/kg)		
Total α-Tocopherol[a]	5.4	±	1.4
trans-β-Carotene[a,b,c]	3.1	±	1.1

[a] Collaborating laboratories
[b] NIST LC-absorbance
[c] NIST ID-LC-MS

Reference Mass Fraction Values for Elements: Each reference mass fraction value, reported on an as-received basis, is the mean of the results of a single NIST data set. 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 measurand is the mass fraction for each element listed in Table 4 as determined by the methods indicated. Metrological traceability is to the SI measurement unit for chemical mass fraction, expressed as milligrams per kilogram or micrograms per kilogram, as realized by the methods used.

Table 4. Reference Mass Fractions for Elements in SRM 2383a

Analyte	Mass Fraction (mg/kg)
Iodine (I)[(a)]	0.0737 ± 0.0083
Tin (Sn)[(a)]	0.361 ± 0.019
	Mass Fraction (µg/kg)
Selenium (Se)[(b)]	0.028 ± 0.001

[(a)] NIST ICP-MS
[(b)] NIST INAA

Reference Values for Proximates, Sugars, and Calories: Each reference mass fraction value, reported on an as-received basis, is the median of the mean values 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 sugars, the measurands are the mass fractions of each analyte listed in Table 5, as determined by the methods indicated. For calories, the measurand is the caloric content listed in Table 5, as determined by the method indicated. Metrological traceability is to the measurement processes and standards used by collaborating laboratories.

Table 5. Reference Mass Fraction Values for Proximates, Sugars, and Calories in SRM 2383a

Analyte	Mass Fraction (g/100 g)
Solids	22.45 ± 0.68
Ash	0.705 ± 0.021
Protein	1.96 ± 0.06
Carbohydrates	19.41 ± 0.70
Total Fat (as Sum of Fatty Acids as Triglycerides)	0.29 ± 0.20
Polyunsaturated Fatty Acids (as Free Fatty Acids)	0.122 ± 0.077
Saturated Fatty Acids (as Free Fatty Acids)	0.072 ± 0.035
Total Sugars	12.05 ± 0.50
Sucrose	3.57 ± 0.12
Glucose	3.80 ± 0.11
Fructose	3.96 ± 0.09
Lactose	0.50 ± 0.10
	Energy (kcal per 100 g)
Calories[(a)]	89.0 ± 3.5

[(a)] The reference value for calories is the median of lab mean caloric calculations from the interlaboratory comparison exercise. If the mean proximate values are used for calculation, with caloric equivalents of 9, 4, and 4 for fat (total), protein, and carbohydrate, respectively, the mean caloric content is 88.1 kcal/100 g.

Reference Mass Fraction Values for Amino Acids: Each reference mass fraction value, reported on an as-received basis, is the median of the mean results provided by collaborating laboratories, using either LC-fluorescence or LC-absorbance. 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 at a confidence level of approximately 95 %. The measurand is the mass fraction for each amino acid listed in Table 6 as determined by the method indicated. Metrological traceability is to the measurement processes and standards used by collaborating laboratories.

Table 6. Reference Mass Fraction Values for Amino Acids in SRM 2383a

	Mass Fraction (g/100 g)
Alanine	0.0734 ± 0.0084
Arginine	0.126 ± 0.022
Aspartic Acid	0.1993 ± 0.0063
Cystine	0.0188 ± 0.0068
Histidine	0.0545 ± 0.0038
Phenylalanine	0.0236 ± 0.0073
Tyrosine	0.0452 ± 0.0054
Valine	0.070 ± 0.012

Information Mass Fraction Values for Elements: Each information mass fraction value (except for chromium) is the mean result of a NIST analysis using a single method. The chromium value is the mean from two sets of NIST results. No uncertainty is provided because there is insufficient information available for its assessment.

Table 7. Information Mass Fraction Values for Elements in SRM 2383a

Analyte	Mass Fraction (mg/kg)
Chromium (Cr)[a,b]	0.2
Molybdenum (Mo)[b]	0.06
Nickel (Ni)[b]	0.2
Arsenic (As)[c]	0.003

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

REFERENCES

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[5] Sharpless, K.E.; Duewer, D.L.; *Standard Reference Materials for Analysis of Dietary Supplements*; J. AOAC Int., Vol. 91, pp. 1298–1302 (2008).

[6] Paul, R.L.; *Determination of Arsenic in Food and Dietary Supplement Standard Reference Materials by Neutron Activation Analysis*; Anal. Chem., Vol. 83, pp. 152-156 (2011).

Certificate Revision History: 28 August 2019 [Addition of reference values for total fat (as the sum of fatty acids as triglycerides), polyunsaturated fatty acids, saturated fatty acids, amino acids, and α-tocopherol; correction of units for selenium value from mg/kg to µg/kg; removal of values for water-soluble vitamins, total lycopene, *trans*-lycopene, α-carotene, β-carotene, 9-*cis*-β-carotene, β-cryptoxanthin, and total dietary fiber; editorial changes]; 21 July 2015 (Addition of values for carotenoids; clarification and addition of footnotes for water-soluble vitamins; editorial changes); 18 October 2012 (Original certificate date).

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at https://www.nist.gov/srm.