National Institute of Standards & Technology

# Certificate of Analysis

## Standard Reference Material® 2384

### Baking Chocolate

This Standard Reference Material (SRM) is intended primarily for use in validating methods for determining proximates, fatty acids, calories, and elements in baking chocolate and similar matrices. This SRM can also be used for quality assurance when assigning values to in-house reference materials. The SRM is baking chocolate prepared from 100 % cocoa beans by a manufacturer of baking chocolate and consists of a single production lot. A unit of SRM 2384 consists of five 91 g (3.2 oz) individually wrapped bars of baking chocolate.

**Certified Mass Fraction Values:** Certified mass fraction values for elements and fat in SRM 2384 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 taken into account [1]. Analyses for value assignment were performed by NIST and collaborating laboratories. Certified mass fraction values were calculated as the mean of the mean values from NIST methods and the median or the mean of the mean measurements made by collaborating laboratories, where appropriate. The associated uncertainties are expressed at an approximately 95 % level of confidence [2,3]. Values are reported on an as-received (not dry-mass) basis in mass fraction units [4].

**Reference Mass Fraction Values:** Reference mass fraction values for sodium, fatty acids, proximates, calories, and total dietary fiber are provided in Tables 3 through 5. 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 associated 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. Reference mass fraction values were derived from results reported by NIST and collaborating laboratories. Values are reported on an as-received (not dry-mass) basis in mass fraction units [4].

**Expiration of Certification:** The certification of **SRM 2384** is valid, within the measurement uncertainty specified, until **31 December 2029**, provided the SRM is handled and stored in accordance with instructions given in this certificate (see "Instructions for Storage and Use"). This 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 this 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 H.B. Chin, I-P. Ho, and D.W. Howell of the Grocery Manufacturers Association (GMA, Washington, DC).

Analytical measurements at NIST were performed by K.D. Chieh, K.E. Murphy, B.C. Nelson, C.S. Phinney, B.J. Porter, K.E. Sharpless, J.R. Sieber, and L.J. Wood of the Chemical Sciences Division.

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

Carlos A. Gonzalez, Chief
Chemical Sciences Division

Gaithersburg, MD 20899
Certificate Issue Date: 15 January 2020
*Certificate Revision History on Last Page*

Steven J. Choquette, Director
Office of Reference Materials

Analyses for value assignment were also performed by the following laboratories participating in a GMA Food Industry Analytical Chemists Committee (FIACC) interlaboratory comparison exercise: Covance, Inc. (Madison, WI); General Mills, Inc. (Minneapolis, MN); Hormel Foods Corporation (Austin, MN); Kraft Foods (Glenview, IL); Nabisco, Inc. (East Hanover, NJ); Nestlé USA (Dublin, OH); Novartis Nutrition Corporation (St. Louis Park, MN); Pillsbury (St. Paul, MN); Ralston Purina Company (St. Louis, MO); U.S. Department of Agriculture, Food Composition Laboratory (Beltsville, MD); and Woodson-Tenent Laboratories (Memphis, TN). Data for extractable fat was also provided by Dionex Corporation (Salt Lake City, UT).

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

**NOTICE TO USERS**

SRM 2384 IS INTENDED FOR LABORATORY USE ONLY, NOT FOR HUMAN CONSUMPTION.

**INSTRUCTIONS FOR STORAGE AND USE**

**Storage:** Until required for use, the baking chocolate should be stored under refrigeration at approximately 4 °C.

**Use:** Test portions for analysis may be melted or grated as described in AOAC Official Method 970.20 [5]. The following masses used for NIST analyses should be used as the minimum sample size to ensure valid results: 1 g for fat and fatty acids and 0.5 g for elements.

**SOURCE, PREPARATION, AND ANALYSIS**[(1)]

**Source and Preparation:** SRM 2384 is baking chocolate prepared from 100 % cocoa beans and taken from a single production lot.

**NIST Analyses for Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn:** Mass fractions of calcium, copper, iron, potassium, magnesium, manganese, sodium, phosphorus, and zinc were measured by inductively-coupled plasma optical emission spectroscopy (ICP-OES) in six bars of chocolate on two or more days. A quarter of each bar was melted in individual beakers and single or duplicate 0.5 g portions were taken from each bar and digested in a microwave sample preparation system using nitric acid. Digests were transferred to plastic bottles and diluted with the appropriate volume of 1.5 % (volume fraction) nitric acid. Quantitation was based on the method of standard additions using SRM 3100 series single element standard solutions. The ICP-OES result for sodium was confirmed using X-ray fluorescence (XRF) spectroscopy.

**NIST Analyses for Cd and Pb:** Mass fractions of cadmium and lead were measured by isotope dilution inductively-coupled plasma mass spectrometry (ID ICP-MS) in six bars of chocolate. Two 5 g squares of each bar were melted in individual beakers and duplicate 1.0 g portions were spiked with isotopically enriched $^{206}$Pb and $^{111}$Cd and digested in a microwave sample preparation system using nitric acid. Digests were transferred to plastic bottles and diluted with the appropriate volume of 2 % (volume fraction) nitric acid. Lead was measured by ICP-MS in standard mode, whereas cadmium was measured in collision cell/kinetic energy discrimination mode. Quantification was based on calibration by reverse isotope dilution ICP-MS using primary standard solutions prepared from SRM 746 *Cadmium-Vapor Pressure*, SRM 3108 *Cadmium (Cd) Standard Solution*, SRM 3128 *Lead (Pb) Standard Solution*, and from Pb metal (Johnson Matthey, 99.999+ % pure).

**NIST Analyses for Fat:** The mass fraction of fat was measured by pressurized-fluid extraction in two sets of seven samples of chocolate. One-gram portions of grated chocolate were extracted into petroleum ether. Extracts were evaporated under nitrogen and then dried at 100 °C to constant mass, per AOAC Official Method 963.15, Fat in Cacao Products [5].

**NIST Analyses for Fatty Acids:** Mass fractions of fatty acids were measured by gas chromatography with flame ionization detection (GC-FID) in three sets of five samples of chocolate over a three-day period. Using pressurized-fluid extraction, fat was extracted into petroleum ether from approximately 1 g samples of grated chocolate. Methyl nonadecanoate (C19:0 fatty acid methyl ester [FAME]) was used as an internal standard. A two-step process employing methanolic sodium hydroxide and boron trifluoride was used to convert the fatty acids to their methyl esters. FAMEs were extracted into hexane and samples were analyzed by GC-FID. Calibrants were

[(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.

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 were based on average response factors for the calibrants.

**Analyses by Collaborating Laboratories:** Data from additional sources were used for value assignment, including an interlaboratory comparison exercise organized by the GMA FIACC. Not every laboratory measured every analyte, and some data were excluded as outliers. The GMA FIACC laboratories were asked to use AOAC methods or their equivalent, to make single measurements from each of two bars, and to report the analytical method that was used.

**Homogeneity Assessment:** The homogeneity of elements and fatty acids was assessed at NIST using the methods described above. A small but statistically significant heterogeneity was found for cadmium, calcium, lead, and manganese, and an inhomogeneity component has been included in the expanded uncertainties for these analytes. All other analytes, including those for which homogeneity was not assessed, have been treated as homogeneous.

**Value Assignment:** The collaborating laboratories reported individual results for two to eight analyses for a given analyte. The mean of each laboratory's results was then determined. For calculation of assigned values for analytes that were measured only by the collaborating laboratories, the mean of the collaborating laboratory means was determined. For analytes that were also measured by NIST, the median or mean of the individual collaborating laboratory means and the mean of the individual sets of NIST data were averaged, as appropriate. For analytes that were only measured by NIST, the mean of the individual sets of NIST data were averaged, as appropriate.

**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 or mean 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, the certified value should be treated as a normally distributed random variable with mean $x$ and standard deviation $U_{95\%}(x)/2$ [2-4,6,7]. The uncertainties for cadmium, calcium, lead, and manganese also incorporate an additional uncertainty component for possible inhomogeneity. The measurands are the total mass fractions of elements in baking chocolate 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.

Table 1. Certified Mass Fraction Values for Elements in SRM 2384

| | Mass Fraction (mg/kg) |
|---|---|
| Cadmium (Cd)[a] | 0.0734 ± 0.0077 |
| Calcium (Ca)[b,c] | 840 ± 74 |
| Copper (Cu)[b,c] | 23.9 ± 1.0 |
| Iron (Fe)[b,c] | 132 ± 11 |
| Lead (Pb)[a] | 0.0357 ± 0.0046 |
| Magnesium (Mg)[b,c] | 2610 ± 120 |
| Manganese (Mn)[b,c] | 20.8 ± 1.3 |
| Phosphorus (P)[b,c] | 3330 ± 210 |
| Potassium (K)[b,c] | 8650 ± 400 |
| Zinc (Zn)[b,c] | 37.6 ± 1.9 |

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

**Certified Mass Fraction Value for Fat:** The certified mass fraction value is the combined mean from the mean of results from analyses by NIST and the mean of results provided by collaborating laboratories. The value is 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 measurand is the total mass fraction of extractable fat in baking chocolate as listed in Table 2 on an as-received basis. Metrological traceability is to the measurement processes and standards used by NIST and collaborating laboratories.

Table 2. Certified Mass Fraction Value for Total Extractable Fat in SRM 2384

| | Mass Fraction (g/100 g) |
|---|---|
| Total Fat (extractable) | 51.4 ± 1.1 |

**Reference Mass Fraction Value for Sodium:** The reference mass fraction value for sodium is the mean of results provided by NIST using ICP-OES. The value is 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 total mass fraction of sodium in baking chocolate as listed in Table 3 on an as-received basis as determined by ICP-OES. Metrological traceability is to the SI measurement unit for chemical mass fraction, expressed as milligrams per kilogram, as realized by the method used.

Table 3. Reference Mass Fraction Value for Sodium in SRM 2384

| | Mass Fraction (mg/kg) |
|---|---|
| Sodium (Na) | 49 ± 17 |

**Reference Mass Fraction Values for Fatty Acids as Free Fatty Acids:** Each reference mass fraction value is the combined mean from the mean of results from analyses by NIST and the mean of results provided by collaborating laboratories, as available. 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 fatty acids in baking chocolate as listed in Table 4 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 4. Reference Mass Fraction Values for Fatty Acids as Free Fatty Acids in SRM 2384

| | Common Name | Mass Fraction (g/100 g) |
|---|---|---|
| Dodecanoic Acid (C12:0)[a] | Lauric Acid | 0.021 ± 0.004 |
| Tetradecanoic Acid (C14:0)[a,b] | Myristic Acid | 0.076 ± 0.005 |
| Pentadecanoic Acid (C15:0)[a] | | 0.017 ± 0.003 |
| Hexadecanoic Acid (C16:0)[a,b] | Palmitic Acid | 12.44 ± 0.26 |
| (Z)-9-Hexadecenoic Acid (C16:1 n-7)[a,b] | Palmitoleic Acid | 0.127 ± 0.007 |
| Heptadecanoic Acid (C17:0)[a] | Margaric Acid | 0.110 ± 0.006 |
| Octadecanoic Acid (C18:0)[a,b] | Stearic Acid | 17.24 ± 0.38 |
| (Z)-11-Octadecenoic Acid (C18:1 n-7)[a,b] | Vaccenic Acid | 0.172 ± 0.017 |
| (Z,Z)-9,12-Octadecadienoic Acid (C18:2 n-6)[a,b] | Linoleic Acid | 1.458 ± 0.046 |
| (Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3 n-3)[a,b] | α-Linolenic Acid | 0.093 ± 0.006 |
| Eicosanoic Acid (C20:0)[a,b] | Arachidic Acid | 0.501 ± 0.012 |
| 9-Eicosenoic Acid (C20:1)[a] | Gadoleic Acid | 0.022 ± 0.004 |
| Docosanoic Acid (C22:0)[a,b] | Behenic Acid | 0.088 ± 0.006 |
| Tetracosanoic Acid (C24:0)[a,b] | Lignoceric Acid | 0.048 ± 0.002 |
| Total Fat (as the sum of fatty acids as triglycerides) | | 50.3 ± 1.1 |

[a] Collaborating laboratories
[b] NIST GC-FID

**Reference Mass Fraction Values for Proximates and Calories:** Each reference mass fraction value is the mean 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]. For proximates and fiber, the measurands are the mass fractions in baking chocolate as listed in Table 5 on an as-received basis as determined by the methods indicated. For calories, the measurand is the caloric content in baking chocolate as listed in Table 5 on an as-received basis as determined by the methods indicated. Metrological traceability is to the measurement processes and standards used by collaborating laboratories.

Table 5. Reference Mass Fraction Values for Proximates and Calories in SRM 2384

| | Mass Fraction (g/100 g) |
|---|---|
| Solids | 98.37 ± 0.35 |
| Ash | 2.78 ± 0.11 |
| Protein[(a)] | 13.18 ± 0.46 |
| Carbohydrates | 32.4 ± 1.9 |
| Total Dietary Fiber | 14.5 ± 3.0 |
| | **Energy (kcal per 100 g)** |
| Calories[(b)] | 631.0 ± 9.3 |

[(a)] A factor of 6.25 was used to convert nitrogen results to protein.

[(b)] The reference value for calories is the median of 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 635 kcal per 100 grams.

## REFERENCES


[1] May, W.; Parris, R.; Beck II, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136; U.S. Government Printing Office: Washington, DC (2000); available at https://www.nist.gov/system/files/documents/srm/SP260-136.PDF (accessed Jan 2020).

[2] JCGM 100:2008; *Evaluation of Measurement Data - Guide to the Expression of Uncertainty in Measurement*; (GUM 1995 with Minor Corrections), Joint Committee for Guides in Metrology (JCGM) (2008); available at https://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Jan 2020); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297, U.S. Government Printing Office: Washington, DC (1994); available at https://www.nist.gov/pml/nist-technical-note-1297 (accessed Jan 2020).

[3] Levenson, M.S.; Banks, D.L.; Eberhardt, K.R.; Gill, L.M.; Guthrie, W.F.; Liu, H.-k.; Vangel, M.G.; Yen, J.H.; Zhang, N.F.; *An Approach to Combining Results from Multiple Methods Motivated by the ISO GUM*; J. Res. Natl. Inst. Stand. Technol., Vol. 105, pp. 571–579 (2000).

[4] Thompson, A; Taylor, B.N.; *Guide for the Use of the International System of Units (SI)*; NIST Special Publication 811; U.S. Government Printing Office: Washington, DC (2008); available at https://www.nist.gov/pml/pubs/sp811/index.cfm (accessed Jan 2020).

[5] AOAC International; *Official Methods of Analysis of AOAC International*; 17th ed.; Horwitz, W., Ed.; Gaithersburg, MD (2000).

[6] JCGM 101:2008; *Evaluation of Measurement Data – Supplement 1 to the "Guide to the Expression of Uncertainty in Measurement" - Propagation of Distributions using a Monte Carlo Method*; JCGM (2008); available at https://www.bipm.org/utils/common/documents/jcgm/JCGM_101_2008_E.pdf (accessed Jan 2020).

[7] Efron, B.; Tibshirani, R.J.; *An Introduction to the Bootstrap*; Chapman & Hall: London (1993).


**Certificate Revision History**: **15 January 2020** (Change of expiration date; removal of certified values for oleic acid, caffeine, theobromine, catechin, epicatechin, and catechin monomers based on observed instability; removal of reference values for theophylline and total procyanidins based on observed instability; removal of reference values for thiamine, riboflavin, niacinamide, niacin, total vitamin $B_3$, pantothenic acid, pyridoxamine, pyridoxine, and total vitamin $B_6$ based on NIST's decision to no longer support these measurement capabilities in this matrix; certified values for fatty acids and total fat as the sum of fatty acids downgraded to reference values to properly reflect traceability and moved from Table 2 to Table 4; correction of reference value for lignoceric acid based on a rounding error; editorial changes); **05 February 2015** (Addition of certified values for Cd and Pb; update of reference values for Na, riboflavin and niacin; addition of reference values for vitamins; change of reference values for elements (Cu, Mg, Mn, P, K, and Zn) to certified values; removal of reference values for tocopherols; editorial changes); **25 September 2014** (Extension of certification period; removed acrylamide reference value in Table 6; editorial changes); **19 July 2013** (Removed specific rotation notation from catechin and epicatechin; editorial changes) **27 January 2009** (Change of expiration date); **23 January 2007** (Editorial changes); **28 September 2004** (This revision reflections the addition of a reference value for acrylamide); **04 June 2003** (Correction in Table 1 certified values for (+)-catechin, (–)-epicatechin, catechin monomers; correction in reference values for theophylline and total procyanidins; correction text of Appendix C-Other Analytes; units for caffeine and theobromine given in mg/kg (were g/kg)); **22 March 2002** (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: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at https://www.nist.gov/srm.*