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Value Assignment of Nutrient Concentrations in Standard Reference Material 2384 Baking Chocolate

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Standard Reference Material (SRM) Baking Chocolate was recently issued, and the process used for value assignment of nutrient concentrations is reported herein. SRM 2384 is intended for use as a primary control material for assigning values to in-house control materials and for validation of analytical methods for the measurement of fatty acids, proximates, vitamins, and elements in chocolate and similar high-fat matrices. The Certificate of Analysis for SRM 2384 provides assigned values for concentrations of fatty acids, proximates, vitamins, elements, and total dietary fiber, for which product labeling is required by the Nutrition Labeling and Education Act of 1990, as well as for catechins, caffeine, theobromine, and theophylline. These assigned values were based on measurements by NIST and/or collaborating laboratories.

KEYWORDS: Chocolate analysis; certified reference material; control material; food composition; nutrition labeling; quality assurance; Standard Reference Material

INTRODUCTION

The Nutrition Labeling and Education Act (NLEA) of 1990 (*1*) has been a driving force behind the National Institute of Standards and Technology's (NIST's) introduction of food-matrix Standard Reference Materials (SRMs) with values assigned for nutrients. The NLEA requires that labels on processed foods distributed in the United States specify the amount of total fat, saturated fat, cholesterol, total carbohydrate, dietary fiber, sugars, protein, vitamin A, vitamin C, sodium, calcium, and iron contained in a single serving. The manufacturer may also choose to provide information about any other vitamin, mineral, or nutrient to assist the consumer in maintaining a healthy diet. To facilitate compliance with this law, well-characterized reference materials (RMs) are needed by laboratories in the food testing and nutrition communities. Such RMs also provide measurement traceability for food exports to facilitate acceptance in many foreign markets, and they improve the accuracy of nutrition information that is provided to assist consumers in making sound dietary choices.

In 1996, in a study of nutrition labeling accuracy sponsored by the U.S. Food and Drug Administration (FDA), nutrient information on 300 product labels was compared to results obtained by a contract laboratory (*2*). The criterion for "accuracy" was a 20% difference, in the direction causing no nutritional harm, between experimentally determined and labeled values. Labeling accuracy ranged from better than 90% for analytes such as total fat and sodium to as low as 54% for vitamin A. A lack of labeling accuracy may be attributed to a lack of reliable methods of analysis, as well as to a lack of food-matrix RMs for assessing the reliability and accuracy of analytical methods. In 1996, the most recent year in which label accuracy was assessed by FDA, few food-matrix RMs existed with values assigned for nutrients other than minerals and trace elements. Since 1996, NIST has introduced several food-matrix Standard Reference Materials (SRMs) characterized for nutrient concentrations (*3*).

AOAC International's Task Force on Methods for Nutrition Labeling has proposed a triangle partitioned into sectors in which foods are placed based on their protein, fat, and carbohydrate content. AOAC International anticipates that one or two reference materials in a given sector will be representative of other foods in that sector and therefore will be useful for method assessment and quality assurance for analyses involving those other foods (*4*, *5*). The position of SRM 2384 in this triangle is shown in **Figure 1**, along with the locations of other food-matrix reference materials available from NIST. SRM 2384 is the only SRM available in sector 2 of the triangle; a material in this sector was the top priority of a NIST workshop held in 1999 to identify reference materials needs of the food industry.

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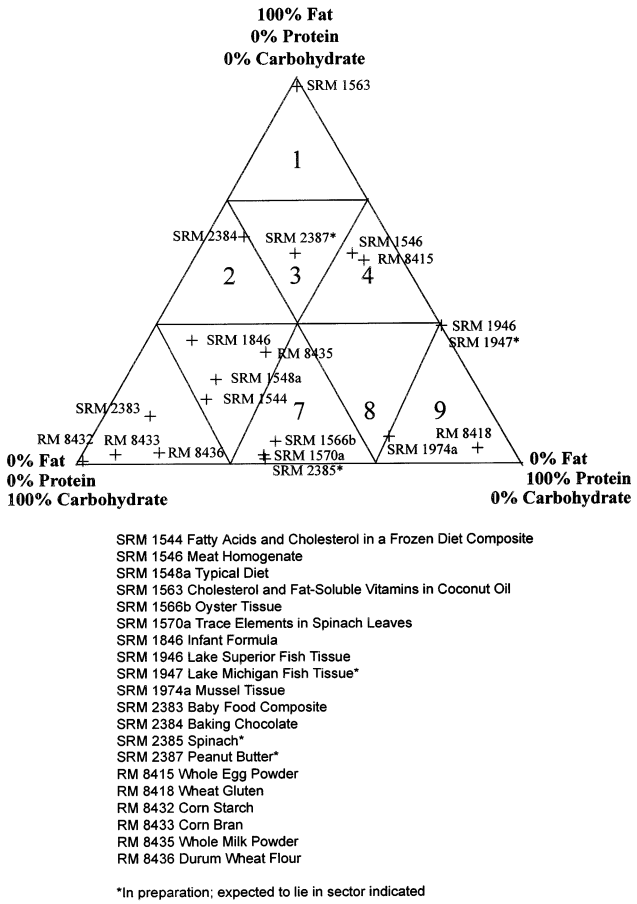

Figure 1. Location of SRM 2384 in the fat–protein–carbohydrate triangle, as well as the locations of other food-matrix reference materials from NIST for which fat, protein, and carbohydrate concentrations are assigned.

NIST recently re-evaluated the process of assigning values to its SRMs for chemical measurements. This process resulted in the identification of three categories of assigned values—certified, reference, and information values—and seven value-assignment modes (*6*). A NIST certified value is a value in which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been fully investigated or accounted for by NIST (*6*). NIST analysts must provide data for a value to be certified. If NIST data are not available for a particular analyte, a reference or information value can be assigned. NIST reference values represent a best estimate of the true value where all known or suspected sources of bias have not been fully investigated by NIST; reference values have associated uncertainties that may not include all sources of uncertainty and may represent only a measure of the precision of the measurement method(s) (*6*). NIST information values are provided for analytes that may be of interest to the SRM or RM user, but for which insufficient information is available to NIST to assign the uncertainty associated with the value (and therefore, typically, no uncertainty is reported) (*6*).

NIST has used several modes for assignment of analyte concentrations in SRM 2384, two of which involve the use of data provided by collaborating laboratories, in combination with NIST-generated data and alone. The use of such data has enabled NIST to provide assigned values for many analytes that NIST does not have the resources or analytical expertise to measure.

SRM 2384 is intended primarily for validation of analytical methods for the measurement of proximates, fatty acids, vitamins, elements, etc., for which certified or reference values are provided, in baking chocolate or foods of similar composition. The material may also be used as a primary control material in the value assignment of in-house control materials; i.e., the SRM or RM can be analyzed at the same time as the in-house control material to establish accuracy in the characterization of the (less expensive and more readily available) in-house control material. In general, due to their cost and limited supply, natural-matrix SRMs are not intended for routine daily use as quality control materials.

MATERIALS AND METHODS

Preparation of SRM 2384. SRM 2384 is baking chocolate prepared from 100% cocoa and taken from a single production lot. The 91-g (3.2-oz) bars of chocolate, which are wrapped in paper-lined foil, are packed in zip-lock polyethylene bags in lots of five, constituting one SRM sales unit. The chocolate is stored under refrigeration at 4 °C. No run-order information was available for this material.

NIST Analyses for Fat. Two sets of seven samples of chocolate were prepared for analysis by pressurized fluid extraction. One-gram portions of grated chocolate were extracted into petroleum ether. Extracts were evaporated under nitrogen and then dried at 100 °C to constant weight, per AOAC Official Method 963.15, Fat in Cacao Products (*7*). Additional details are provided in ref (*8*).

NIST Analyses for Fatty Acids. Fatty acids were measured in three sets of five samples of chocolate over a 3-day period. Using pressurized-fluid extraction, fat was extracted into petroleum ether from approximately 1-g samples of grated chocolate. Methyl nonadecanoate 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). FAMEs were extracted into hexane and injected into a gas chromatograph employing flame ionization detection. Additional details are provided in ref (*8*).

NIST Analysis of Calcium, Iron, and Sodium. Calcium, iron, and sodium were measured in five bars of chocolate. Five whole bars were melted in individual beakers, and two 0.5-g portions were taken from each bar and digested in nitric acid in a microwave oven. Digests were transferred to plastic bottles and diluted with the appropriate volume of 1.5% (volume fraction) nitric acid. To correct for matrix effects caused by differences between samples and calibrants, the method of standard additions was used; spikes were added to one aliquot prepared from each 0.5-g test portion. Four measurements using inductively coupled plasma optical emission spectrometry (ICP-OES) were made and averaged for each sample and each spiked solution. Results were corrected for spike recoveries. The ICP-OES result for sodium was confirmed using X-ray fluorescence (XRF) spectrometry and the method of standard additions. Known amounts of sodium sulfonate solution were added to four samples from a single bar of chocolate, and mineral oil was added to match oil content among the samples, which were then melted, mixed, and weighed into liquid cells. The mineral oil also served to minimize outgassing during measurements. The $K-L_{2,3}$ X-ray lines of sodium were measured in a helium environment at 4-kW X-ray power. Spectral interference from Zn was corrected by treating it as a blank subtraction.

NIST Analysis of Tocopherols. δ-Tocopherol, γ- (plus β-) tocopherol, and α-tocopherol were measured by liquid chromatography (LC) in single squares taken from each of eight bars of chocolate over a 5-day period. The baking chocolate may contain β-tocopherol, but the chromatographic system described below is incapable of resolving β- and γ-tocopherol; the instrument was calibrated using only γ-tocopherol. Samples of approximately 5−6 g were melted and then saponified using potassium hydroxide. Analytes were extracted into a mixture of diethyl ether and hexane, which was subsequently evaporated, and the analytes were redissolved in a mixture of ethanol and ethyl acetate. Samples were injected onto a C_{18} column, and analytes were eluted using a gradient of acetonitrile, methanol, and ethyl acetate. Additional methodological detail pertaining to the LC method is provided in ref (*9*). Both a programmable UV/visible absorbance detector [set to 292 nm for measurement of the tocopherols and 422 nm for measurement of *trans*-β-apo-10′-carotenal oxime (the internal standard)] and a fluorescence detector (excitation wavelength of 295 nm, emission wavelength of 335 nm) were used for quantitation of the tocopherols.

Table 1. Laboratories That Performed Measurements Contributing to Value Assignment of SRM 2384

Covance Laboratories, Madison, WI
Dionex Corp.,[a] Salt Lake City, UT
European Commission—DG Joint Research Centre,[a] Ispra, Italy
General Mills, Inc., Minneapolis, MN
Hormel Foods Corp., Austin, MN
Antioxidant Research Group,[a] King's College, London, UK
Kraft Foods, Glenview, IL
M & M/Mars, Inc.,[a] Hackettstown, NJ
Nabisco, Inc., East Hanover, NJ
Nestlé USA, Dublin, OH
Novartis Nutrition Corp., St. Louis Park, MN
Pillsbury, St. Paul, MN
Ralston Purina Co., St. Louis, MO
U.S. Department of Agriculture, Food Composition Laboratory, Beltsville, MD
U.S. Department of Agriculture,[a] Little Rock, AR
Woodson-Tenent Laboratories, Memphis, TN

[a] Not an NFPA FIACS laboratory.

NIST Analysis of Caffeine, Theobromine, and Theophylline. Caffeine, theobromine, and theophylline were measured by LC in single 1-g test portions taken from each of eight bars of chocolate over an 8-day period. The chocolate was melted, an internal standard (β-hydroxyethyltheophylline) was added, and fat was removed from the sample via four successive extractions into hexane. The defatted chocolate was dried under a stream of nitrogen. Water was added, and the sample was placed in an ultrasonicating bath and then centrifuged. The supernatant was filtered twice. Samples were injected onto a C_{18} column, and analytes were eluted using an isocratic mixture of acetonitrile, water, and acetic acid. Absorbance was measured at 274 nm. Additional details are provided in ref (*10*).

NIST Analysis of Catechins. (+)-Catechin and (−)-epicatechin were measured by LC/mass spectrometry (MS) in single 250-mg test portions taken from eight bars of chocolate on a single day. Approximately 1 g of chocolate was combined with an internal standard solution (tryptophan methyl ester hydrochloride), and the chocolate was melted. Fat was removed from the sample via three successive extractions into hexane. The defatted chocolate was dried under a stream of nitrogen. The dried powder was stirred, and approximately 250 mg was removed. Catechins from this aliquot were extracted into two portions of methanol via ultrasonication. The supernatants were filtered and combined. The extract was diluted with water. Samples were injected onto a C_{18} column, and analytes were eluted using a gradient of water and acetonitrile, both of which contained trifluoroacetic acid. Analytes were measured by atmospheric pressure chemical ionization (APCI) mass spectrometry using selected ion monitoring of *m*/*z* 291 for the catechins and *m*/*z* 219 for the internal standard. Additional details are provided in ref (*11*).

Collaborating Laboratories' Analyses. SRM 2384 was analyzed by 11 laboratories (**Table 1**) that participated in a National Food Processors Association (NFPA) Food Industry Analytical Chemists Subcommittee (FIACS) interlaboratory comparison exercise, and several additional laboratories measuring "specialty" analytes (**Table 1**). NFPA FIACS laboratories were asked to analyze test portions from two bars of SRM 2384 using AOAC or equivalent methods. Not every laboratory reported results for every analyte. SRM 1546 (Meat Homogenate) was analyzed for quality control (*12*). The other laboratories made measurements on test samples taken from four bars of chocolate using their usual methods. In-house control materials were used when available.

Value Assignment. NIST analysts measured fat, fatty acids, calcium, iron, sodium, caffeine, theobromine, theophylline, δ-, γ- + β-, and α-tocopherol, (+)-catechin, and (−)-epicatechin in SRM 2384. For these analytes, the NIST value was averaged with the mean of the collaborating laboratories' means to calculate the assigned values. For the analytes that were not measured by NIST, the equally weighted means of the collaborating laboratories' means were used for value assignment. Some fatty acid data from collaborating laboratories were excluded (1) if the laboratory's result for a particular fatty acid disagreed with the majority of the other laboratories' results and if that laboratory's mean result was more than three standard errors from the mean of the interlaboratory comparison exercise or (2) if the standard deviation of that laboratory's results for a particular fatty acid was an order of magnitude larger than any other laboratory's standard deviation for that fatty acid.

Table 2. Data for Protein (Mass Fraction, in %) in SRM 2384 Baking Chocolate and the Calculation of Its Assigned Value and Associated Uncertainty[a]

lab	protein	mean
1	14.25	14.34
	14.44	
2	13.12	12.96
	12.79	
3	13.48	13.44
	13.39	
4	12.9	13.3
	13.5	
	13.2	
	13.5	
5	13.3	13.2
	13.2	
6	13.3	13.2
	13.1	
7	12.22	12.18
	12.13	
10	13.15	13.16
	13.17	
11	13.9	13.75
	13.6	
mean (of means)		13.18
standard error		0.205
degrees of freedom		9
coverage factor (*k*)		2.26
uncertainty		0.046
final assigned value		13.18 ± 0.46

[a] The same raw data—the individual data points, means, and standard deviations for fat reported by the individual laboratories—are plotted in **Figure 2** for graphical comparison.

The uncertainty in the assigned values is expressed as an expanded uncertainty, *U*, at the 95% level of confidence and is calculated according to the method described in the ISO *Guide to the Expression of Uncertainty in Measurement* (*13*). Because the between-laboratory uncertainty generally exceeds the within-laboratory uncertainty (see, for example, **Figures 2**−**5**, discussed below), the between-laboratory standard error (reproducibility) estimates both within- (repeatability) and between-laboratory uncertainty components (*14*). The expanded uncertainty is calculated as $U = ku_c$, where u_c represents, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, *k*, is determined from the Student's *t* distribution corresponding to the appropriate associated degrees of freedom and 95% confidence for each analyte. For clarity, a detailed statistical analysis for protein in SRM 2384 is provided in **Table 2**.

A small but statistically significant heterogeneity was found for calcium, and an inhomogeneity component has been included in the expanded uncertainty for this analyte. All other analytes, including those for which homogeneity was not assessed, have been treated as homogeneous.

RESULTS AND DISCUSSION

Certified values for fat, selected fatty acids, calcium, iron, caffeine, theobromine, and catechins in SRM 2384 are provided in **Tables 3** and **4**. Reference values for additional proximates, fatty acids, and elements, calories, total dietary fiber, and other analytes are provided in **Tables 5**−**8**. Analytical methodology is provided within each of these tables. This is the first reference material available from NIST for which values are assigned for

Table 3. Certified Concentration Values for Fat and Selected Fatty Acids and Methods Used for Their Determination

	mass fraction (%)
fat (extractable)	51.4 ± 1.1
fat (sum of fatty acids)[a]	50.3 ± 1.1

	mass fraction (%) as the triglyceride	mass fraction (%) as the fatty acid
tetradecanoic acid (C14:0) (myristic acid)	0.080 ± 0.005	0.076 ± 0.005
hexadecanoic acid (C16:0) (palmitic acid)	13.06 ± 0.27	12.44 ± 0.26
(*Z*)-9-hexadecenoic acid (C16:1) (palmitoleic acid)	0.133 ± 0.007	0.127 ± 0.007
octadecanoic acid (C18:0) (stearic acid)	18.01 ± 0.40	17.24 ± 0.38
(*Z*)-9-octadecenoic acid (C18:1) (oleic acid)	16.44 ± 0.36	15.73 ± 0.35
(*Z*)-11-octadecenoic acid (C18:1) (vaccenic acid)	0.180 ± 0.018	0.172 ± 0.017
(*Z*,*Z*)-9,12-octadecadienoic acid (C18:2) (linoleic acid)	1.524 ± 0.048	1.458 ± 0.046
(*Z*,*Z*,*Z*)-9,12,15-octadecatrienoic acid (C18:3) (linolenic acid)	0.097 ± 0.006	0.093 ± 0.006
eicosanoic acid (C20:0) (arachidic acid)	0.521 ± 0.013	0.501 ± 0.012
docosanoic acid (C22:0) (behenic acid)	0.091 ± 0.006	0.088 ± 0.006
tetracosanoic acid (C24:0) (lignoceric acid)	0.050 ± 0.002	0.050 ± 0.002

extractable fat[b]	acid digestion, ether extraction (8) alkali pretreatment, diethyl ether/petroleum ether extraction (1) Soxhlet extraction (1) pressurized-fluid extraction (NIST)
fatty acids	transesterification followed by gas chromatography (10 + NIST)

[a] Fat as the sum of the fatty acids represents the sum of quantified individual fatty acid peaks for which certified (**Table 3**) and reference values (**Table 6**) are provided). [b] The number of laboratories using a particular method is provided in parentheses.

caffeine, theophylline, theobromine, (+)-catechin, and (−)-epicatechin. Relative expanded uncertainties are 5% for caffeine, 2% for theophylline, 10% for theobromine, 21% for (+)-catechin, and 20% for (−)-epicatechin. The measurement of these analytes in SRM 2384 is discussed in detail in refs (*10*) and (*11*).

Relative expanded uncertainties for the proximates range from 0.4% for solids to 5.8% for carbohydrates. Certified values are provided for fat as the sum of fatty acids and as extractable fat; relative expanded uncertainties are 2% in both cases. Certified values are also provided for many of the individual fatty acids; relative expanded uncertainties are generally 5% or less. Baking chocolate contains a low amount of protein relative to its fat and carbohydrate content. The protein results reported by participating laboratories are plotted in **Figure 2**, along with the reference value, which has a 3% relative expanded uncertainty. (The same data are provided in **Table 2**, which shows how the uncertainty was calculated.)

Relative expanded uncertainties for the vitamins range from 13% for vitamin B_2 to 24% for α-tocopherol. Results reported by participants measuring α-tocopherol are provided in **Figure 3**. One laboratory reported a value of 55 mg/kg, which is approximately an order of magnitude higher than the assigned value. This difference could not be traced to a decimal or calculation error. This data point was not used in value assignment and is not plotted in **Figure 3**. Although NIST

Table 4. Certified Concentration Values for Selected Additional Analytes and Methods Used for Their Determination

	mass fraction (g/kg)
caffeine	1.06 ± 0.05
theobromine	11.6 ± 1.1

	mass fraction (mg/kg)
calcium	840 ± 74
iron	132 ± 11
(+)-catechin	0.245 ± 0.051
(−)-epicatechin	1.22 ± 0.24
catechin monomers	1.49 ± 0.22

caffeine and theobromine[a]	extraction, LC with absorption detection (6 + NIST)
calcium	FAAS (1) direct current plasma AES (1) ICP-OES (9 + NIST)
iron	FAAS (2) direct current plasma AES (1) ICP-OES (8 + NIST)
(+)-catechin	extraction–RPLC–MS (1 + NIST)
(−)-epicatechin	extraction–NPLC–fluorescence detection (1) extraction–RPLC–absorbance detection (2)
catechin monomers	sum of (+)-catechin and (−)-epicatechin: extraction–NPLC–absorbance detection (1) mathematical summation (4 + NIST)

[a] The number of laboratories using a particular method is provided in parentheses. FAAS, flame atomic absorption spectrometry; AES, atomic emission spectrometry; ICP-OES, inductively coupled plasma optical emission spectrometry; RPLC, reverse-phase liquid chromatography; NPLC, normal-phase liquid chromatography.

Table 5. Reference Concentration Values for Proximates and Caloric Content and Methods Used for Their Determination

	mass fraction (%)
solids	98.37 ± 0.35
ash	2.78 ± 0.11
protein	13.18 ± 0.46
carbohydrate (by difference)	32.4 ± 1.9
total dietary fiber	14.5 ± 3.0
calories[a]	(631.0 ± 9.3) kcal/100 g

solids[b]	moisture determined by mass loss after oven-drying: forced-air oven (5) vacuum oven (5) convection oven (1)
ash	mass loss after ignition in muffle furnace (10)
nitrogen	Kjeldahl (5) thermal conductivity (2) pyrolysis, gas chromatography (1) combustion (2)
protein	calculated; a factor of 6.25 was used to calculate protein from nitrogen results
carbohydrate	calculated; [solids − (protein + fat + ash)]
total dietary fiber	enzymatic–gravimetry (6)
calories	calculated; [9(fat) + 4(protein) + 4(carbohydrate)]

[a] The value for caloric content is the mean of individual caloric calculations from the laboratories listed in **Table 1**. If the proximate values above are used for calculation, with caloric equivalents of 9, 4, and 4 for fat (as the sum of the fatty acids), protein, and carbohydrate, respectively, the mean caloric content is 635 kcal/100 g. [b] The number of laboratories using a particular method is provided in parentheses.

Table 6. Reference Concentration Values for Fatty Acids and Methods Used for Their Determination[a]

	mass fraction (%) as the triglyceride	mass fraction (%) as the fatty acid
dodecanoic acid (C12:0) (lauric acid)	0.022 ± 0.004	0.021 ± 0.004
pentadecanoic acid (C15:0)	0.018 ± 0.003	0.017 ± 0.003
heptadecanoic acid (C17:0) (margaric acid)	0.115 ± 0.006	0.110 ± 0.006
(*Z*)-9-eicosenoic acid (C20:1) (gadoleic acid)	0.023 ± 0.004	0.022 ± 0.004

[a] The 10 collaborating laboratories and NIST used transesterification followed by gas chromatography for measurement of these fatty acids.

Table 7. Reference Concentration Values for Elements and Methods Used for Their Determination

	mass fraction (mg/kg)
copper	23.2 ± 1.2
magnesium	2570 ± 150
manganese	20.3 ± 1.3
phosphorus	3330 ± 210
potassium	8200 ± 500
sodium[a]	40 ± 2
zinc	36.6 ± 1.7

copper[b]	FAAS (2)
	direct current plasma AES (1)
	ICP-OES (8)
magnesium	FAAS (1)
	direct current plasma AES (1)
	ICP-OES (9)
manganese	FAAS (2)
	direct current plasma AES (1)
	ICP-OES (7)
phosphorus	absorption spectrophotometry (1)
	FAAS (1)
	ICP-OES (9)
potassium	FAAS (2)
	direct current plasma AES (1)
	ICP-OES (8)
sodium	ICP-OES (NIST)
zinc	FAAS (2)
	direct current plasma AES (1)
	ICP-OES (8)

[a] The reference concentration value for sodium, expressed as a mass fraction on an as-received basis, is the mean result of analyses performed by NIST using one analytical technique. The expanded uncertainty, U, is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined standard uncertainty calculated according to the ISO Guide (*13*). The coverage factor, k, is determined from the Student's t distribution corresponding to the appropriate associated degrees of freedom and 95% confidence for each analyte. [b] The number of laboratories using a particular method is provided in parentheses. (See **Table 4** footnote for abbreviations.)

provided data for α-tocopherol, the assigned value is categorized as a reference value because of the greater-than-expected differences between the collaborating laboratories' data.

Relative expanded uncertainties for the elements are in the 5−9% range. Results for iron and sodium are provided in **Figures 4** and **5**, respectively. None of the collaborating laboratories' data were used for value assignment of the sodium concentration. NIST data obtained using ICP-OES were used to generate the reference value, which was confirmed by XRF. The chocolate contains a low level of sodium, and it is possible that contamination caused some of the participating laboratories to report erroneously high values. The inhomogeneity (2.4%)

Table 8. Reference Concentration Values for Selected Vitamins and Other Analytes and Methods Used for Their Determination

	mass fraction (mg/kg)
δ-tocopherol[a]	3.42 ± 0.47
γ-tocopherol[a,b]	108.2 ± 1.9
α-tocopherol	7.2 ± 1.7
vitamin B_2	1.21 ± 0.16
niacin	12.1 ± 2.0
theophylline	0.151 ± 0.003
total procyanidins[c]	10.3 ± 1.1

α-tocopherol[d]	saponification–RPLC–absorbance detection (3 + NIST)
	saponification–RPLC–fluorescence detection (NIST)
	saponification–NPLC–absorbance detection (1)
	saponification–NPLC–fluorescence detection (2)
	enzymatic digestion–RPLC–absorbance detection (1)
	enzymatic digestion–NPLC–absorbance detection (1)
δ-tocopherol	saponification–RPLC–fluorescence detection (NIST)
γ-tocopherol	saponification–RPLC–absorbance detection (NIST)
	saponification–RPLC–fluorescence detection (NIST)
total vitamin B_2	microbiological (1)
	digestion–fluorescence detection (2)
	extraction–RPLC–fluorescence detection (3)
niacin	microbiological (6)
theophylline	extraction, LC with absorption detection (NIST)
total procyanidins	extraction–NPLC–absorbance detection (1)
	extraction–NPLC–mass spectrometry (1)

[a] Reference values expressed as mass fractions for δ-tocopherol, γ-tocopherol, and theophylline are the means of results obtained by NIST using one analytical technique. The expanded uncertainty, U, is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined standard uncertainty calculated according to the ISO Guide (*13*). The coverage factor, k, is determined from the Student's t distribution corresponding to the appropriate associated degrees of freedom and 95% confidence for each analyte. [b] May include β-tocopherol. [c] "Total procyanidins" represents the sum of (+)-catechin, (−)-epicatechin, and the dimer through the decamer. [d] The number of laboratories using a particular method is provided in parentheses. (See **Table 4** footnote for abbreviations.)

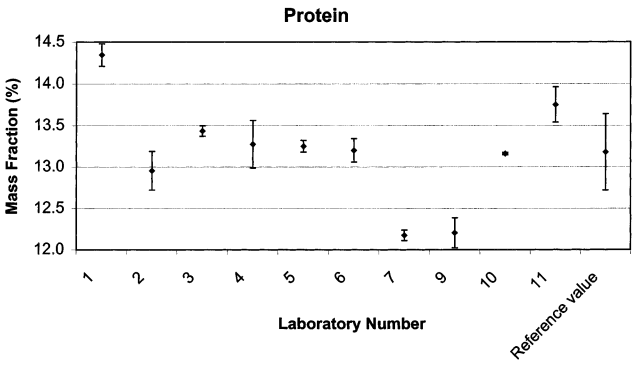

Figure 2. Comparison of the reference value and its expanded uncertainty and the means and standard deviations for individual laboratory data for protein.

observed in the NIST calcium data is shown in **Figure 6**. Duplicate samples were prepared from each of five bars, and each of these duplicates was measured twice. (A single sample was prepared from bar 5.) Note that for bars 1, 2, and 5, the within-bar variability is less than the overall variability of the

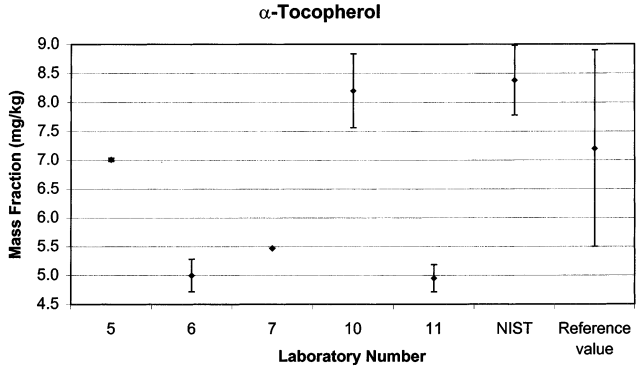

Figure 3. Comparison of the reference value and its expanded uncertainty and the means and standard deviations for individual laboratory data for α-tocopherol.

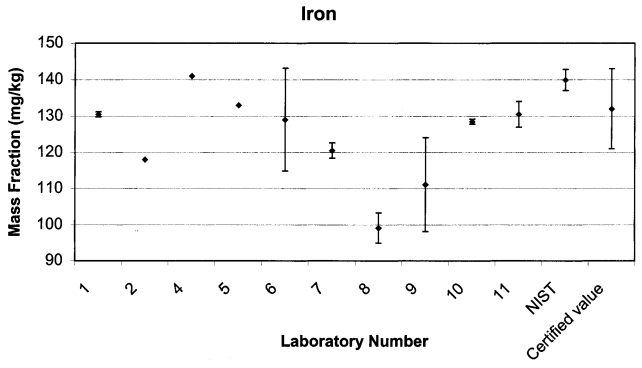

Figure 4. Comparison of the certified value and its expanded uncertainty and the means and standard deviations for individual laboratory data for iron.

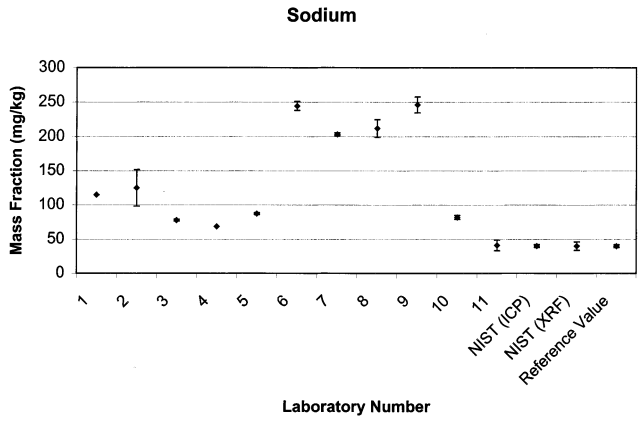

Figure 5. Comparison of the reference value and its expanded uncertainty and the means and standard deviations for individual laboratory data for sodium.

measurement. Because sodium and iron, the other two elements measured by NIST, were found to be homogeneous, the inhomogeneity factor was not applied to other analytes; it was only included in the expanded uncertainty on the certified value for calcium.

With the introduction of SRM 2384 and a material currently being developed (SRM 2387 Peanut Butter), reference materials in all nine sectors of the AOAC triangle will be available from NIST to address NLEA concerns. SRM 2384 and the others in the series of food reference materials will help support measurement accuracy and traceability for laboratories performing measurements in the food and nutrition communities. Although values are assigned for some "specialized" analytes in the

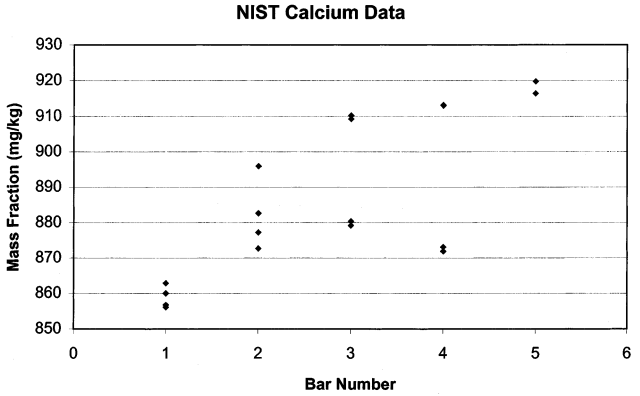

Figure 6. NIST data for calcium in SRM 2384.

materials that are currently available (e.g., catechins and caffeine in SRM 2384), it may be possible to focus on such specialized analytes in future materials.

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