JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

Value Assignment of Nutrient and Aflatoxin Concentrations in Standard Reference Material 2387 Peanut Butter

KATHERINE E. SHARPLESS,* CURTIS S. PHINNEY, LAURA J. WOOD, AND JAMES H. YEN

National Institute of Standards and Technology, 100 Bureau Drive Stop 8392, Gaithersburg, Maryland 20899-8392

DANIEL W. HOWELL

National Food Processors Association, 1350 I Street Northwest, Suite 300, Washington, DC 20005

Standard Reference Material (SRM) 2387 peanut butter was recently issued, and the process used for value assignment of nutrient and aflatoxin concentrations is reported herein. Values were assigned using data provided by the National Institute of Standards and Technology (NIST) and collaborating laboratories. SRM 2387 is intended for use as a primary material for assigning values to in-house control materials and for validation of analytical methods for measurements in peanut butter and similar high-fat matrixes. SRM 2387 lies in sector 3 of AOAC International's fat−protein−carbohydrate triangle. With the addition of SRM 2387, NIST now offers materials within—or on the borders between—all sectors of the triangle. The Certificate of Analysis for SRM 2387 provides assigned values for concentrations of fatty acids, proximates, elements, and total dietary fiber, for which product labeling is required by the Nutrition Labeling and Education Act of 1990, as well as several vitamins, amino acids, and aflatoxins, for which labeling is not required. (Aflatoxin levels in peanut butter are regulated by the Food and Drug Administration.)

KEYWORDS: Certified reference material; control material; food composition; nutrition labeling; peanut butter analysis; quality assurance; Standard Reference Material

INTRODUCTION

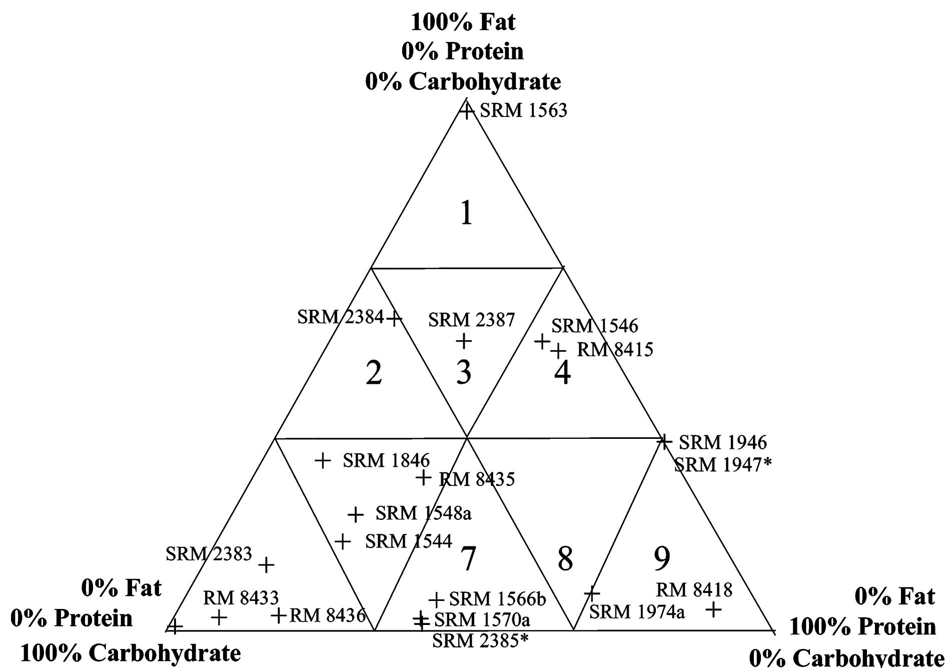
The Nutrition Labeling and Education Act (NLEA) of 1990 (*1*) has been a driving force behind the National Institute of Standards and Technology's (NIST's) introduction of food matrix standard reference materials (SRMs) with values assigned for nutrients (*2*). Prior to the initiation of this effort, NIST had many agricultural and food matrix reference materials (RMs) available, but values were assigned only for elemental compositions of these materials. Along with information on a few elements (calcium, sodium, and iron), the NLEA requires that labels on processed foods distributed in the U.S. specify the amount of total fat, saturated fat, cholesterol, total carbohydrate, dietary fiber, sugars, protein, vitamin A, and vitamin C contained in a single serving. To facilitate compliance with this law, RMs with values assigned for nutrients are needed by laboratories in the food testing and nutrition communities. Such RMs should ideally also provide measurement traceability for food exports to facilitate acceptance in many foreign markets and allow consumers to make better dietary choices because nutrition information is more accurate.

It is important to match the matrix of an RM to that of the test material being analyzed; therefore, food matrix RMs representative of a wide variety of foods are necessary. To demonstrate the applicability of an analytical method to a wide variety of food matrixes, AOAC International's Task Force on Methods for Nutrition Labeling developed a triangle partitioned into sectors in which foods are placed based on their protein, fat, and carbohydrate content. AOAC International anticipated that one or two foods in a given sector would be representative of other foods in that sector and therefore would be useful for method assessment. Similarly, one or two RMs in a given sector should be useful for quality assurance for analyses involving the other foods in the sector (*3*, *4*). The position of SRM 2387 in this triangle is shown in **Figure 1**, along with the locations of other food matrix RMs available from NIST. (To obtain Certificates of Analysis for SRM 2387 as well as the other materials, visit http://www.nist/gov/srm, and enter the SRM number.) SRM 2387 was developed to fill the void that previously existed in sector 3. Peanut butter was selected over other possible candidates in this sector (e.g., pasteurized processed cheese sauce) because it presented the opportunity to assign values for analytes such as the aflatoxins.

SRM 2387 is intended primarily for validation of analytical methods for the measurement of proximates, fatty acids, amino acids, vitamins, elements, aflatoxins, etc., for which certified or reference values are provided, in peanut butter or foods of similar composition. The material may also be used as a primary

* To whom correspondence should be addressed. Tel: 301-975-3121. Fax: 301-977-0685. E-mail: katherine.sharpless@nist.gov.

10.1021/jf034637o CCC: $25.00 © 2003 American Chemical Society
Published on Web 10/03/2003



SRM 1544 Fatty Acids and Cholesterol in a Frozen Diet Composite
SRM 1546 Meat Homogenate
SRM 1548a Typical Diet
SRM 1563 Cholesterol and Fat-Soluble Vitamins in Coconut Oil
SRM 1566b Oyster Tissue
SRM 1570a Trace Elements in Spinach Leaves
SRM 1846 Infant Formula
SRM 1946 Lake Superior Fish Tissue
SRM 1947 Lake Michigan Fish Tissue*
SRM 1974a Mussel Tissue
SRM 2383 Baby Food Composite
SRM 2384 Baking Chocolate
SRM 2385 Spinach*
SRM 2387 Peanut Butter
RM 8415 Whole Egg Powder
RM 8418 Wheat Gluten
RM 8432 Corn Starch
RM 8433 Corn Bran
RM 8435 Whole Milk Powder
RM 8436 Durum Wheat Flour

*In preparation; expected to lie in sector indicated

Figure 1. Location of SRM 2387 in the fat–protein–carbohydrate triangle, as well as the locations of other food matrix RMs from NIST for which fat, protein, and carbohydrate concentrations are assigned.

material in the value assignment of in-house control materials. In general, because of their cost and limited supply, natural matrix SRMs are not intended for routine daily use as quality control materials.

NIST recently reevaluated 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 (*5*). 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 (*5*). NIST reference values represent a best estimate of the true value where all known or suspected sources of bias have not been fully investigated; 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) (*5*). Reference values may be assigned if no NIST data are available or if sources of bias in NIST measurements have not been fully resolved. Although none are provided for SRM 2387, NIST information values may be provided for analytes that may be of interest to the SRM or RM user but for which insufficient information is available to assign the uncertainty associated with the value (and therefore, typically, no uncertainty is reported) (*5*).

NIST has used several modes for assignment of analyte concentrations in SRM 2387, two of which involve the use of data provided by collaborating laboratories—as part of an interlaboratory comparison exercise and in combination with NIST-generated data. 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.

MATERIALS AND METHODS

Preparation of SRM 2387. SRM 2387 is creamy peanut butter containing roasted peanuts, sugar, partially hydrogenated vegetable oils (48% rapeseed, 40% cottonseed, and 12% soybean oil), and salt and was prepared for NIST as part of a larger production run. Raw, shelled Florunner (primarily) peanuts were received from several suppliers and were roasted. The skins were removed from the roasted peanuts, and

discolored peanuts were discarded. The roasted peanuts were then ground, and the remaining ingredients were added. After it was mixed, the peanut butter was further ground to a fine particle size, air was removed, and the peanut butter was cooled and packed within 30 min in 3300 colorless polyethyl tetraethylene jars with white screw caps and foil liners. The peanut butter was frozen (−20 °C) upon receipt by NIST to enhance long-term stability.

NIST Analyses for Fat. One set of three samples of peanut butter was prepared for gravimetric analysis of fat. One gram portions of peanut butter were mixed with diatomaceous earth. The mixture was briefly chilled at 4 °C to improve handling. The fat was extracted from the mixture by pressurized fluid extraction (PFE) using hexane:acetone (4:1 volume fraction). Extracts were evaporated under nitrogen and then dried at 100 °C to constant mass.

NIST Analyses for Fatty Acids. Twelve fatty acids were measured in two sets of six samples of peanut butter prepared on two different days. The fat was extracted from approximately 1 g samples of peanut butter by PFE using a mixture of hexane:acetone (4:1 volume fraction). A two step process employing methanolic sodium hydroxide and boron trifluoride was used to convert the fatty acids to their methyl esters. Fatty acid methyl esters (FAMEs) were extracted into hexane and analyzed by gas chromatography (GC) with flame ionization detection. Methyl nonadecanoate (C19:0 FAME) was used as an internal standard.

NIST Analyses for Elements. Calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc were measured in eight jars of peanut butter. Two 0.5 g portions were taken from each jar and digested in a nitric, perchloric, and hydrofluoric acid mixture. Because of the high fat content, the samples were predigested on a hotplate before digestion 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: Zn, 99%; Mg, 102%; P, 98%; K, 99%; Cu, 103%; Mn, 98%, Fe, 101%; Na, 103%; and Ca, 98%.

NIST Analyses for Tocopherols. δ-Tocopherol, γ- (plus β-) tocopherol, and α-tocopherol were measured in test portions taken from six jars of peanut butter over a 7 day period. (The peanut butter 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 5−7 g were 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 analyzed by liquid chromatography (LC) on a C_{18} column; analytes were eluted using a gradient of acetonitrile, methanol, and ethyl acetate (*6*). A programmable UV/visible absorbance detector set to 450 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.

Analyses by Collaborating Laboratories. Data from two additional sources were used for certification of this material: an interlaboratory comparison exercise organized by the NFPA Food Industry Analytical Chemists Subcommittee (FIACS) with 13 laboratories participating and four laboratories participating in an exercise in which only aflatoxins were measured. All laboratories are identified in **Table 1**. Not every laboratory measured every analyte. The NFPA FIACS laboratories were asked to use AOAC methods or their equivalent, to make single measurements from each of two jars and to report the analytical method that was used. The laboratories that measured only the aflatoxins were asked to use their usual methods to make single measurements of aflatoxins in each of three jars. Methods reported by the laboratories are provided below, in the tables in which assigned values are reported.

Homogeneity Assessment. The homogeneity of calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, zinc, fatty acids, and tocopherols was assessed at NIST using the methods described above. Because of a possible between-jar heterogeneity of ≤1% for some analytes (calcium, 0.6%; manganese, 0.4%; individual fatty acids and fat as the sum of fatty acids, 1%) in a variance components analysis, an inhomogeneity component of 1% has been included in the expanded uncertainty for all analytes.

Table 1. Laboratories that Performed Measurements Contributing to Value Assignment of SRM 2387

Laboratory	Location
Beech-Nut Nutrition Corporation	Canajoharie, NY
Campbell Soup Company	Camden, NJ
Covance, Inc.	Madison, WI
Eurofins/Woodson-Tenent Labs	Memphis, TN
Food and Drug Administration	Atlanta, GA[a]
General Mills, Inc.	Golden Valley, MN
Hormel Foods Corporation	Austin, MN
Kraft Foods, Inc.	Glenview, IL
Kraft Foods, Inc./Nabisco	East Hanover, NJ
Krueger Food Laboratories, Inc.	Cambridge, MA
Neogen Corporation	Lansing, MI[a]
Nestlé Food Corporation	Dublin, OH
Nestlé Purina Pet Care	St. Louis, MO
Novartis Nutrition Technical Center	St. Louis Park, MN
Trilogy Analytical Laboratory	Washington, MO[a]
U.S. Department of Agriculture, Agricultural Marketing Service	Blakely, GA[a]
U.S. Department of Agriculture, Food Composition Laboratory	Beltsville, MD

[a] Not an NFPA FIACS laboratory; measured aflatoxins only.

Value Assignment. The NFPA FIACS laboratories reported values for 2−12 analyses. The laboratories measuring only aflatoxins reported values for 3−9 analyses. The mean for each laboratory was determined from these values, and a mean of laboratory means was calculated. In cases where NIST also made measurements (i.e., fat, individual fatty acids, tocopherols, and elements), the mean of means was averaged with the NIST mean to obtain the certified value. In cases where NIST did not make measurements, the mean of laboratory means became the assigned (reference) value. Data provided by collaborating laboratories were rejected as outliers on an individual analyte basis if the laboratory's mean result was greater than three standard errors from the interlaboratory mean.

The uncertainty in the assigned values is expressed as an expanded uncertainty, U, calculated according to the method described in the ISO Guide (*7*). The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, 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 (estimated using the Welch−Satterwaite procedure) and 95% confidence for each analyte (*7*). Because the between-laboratory uncertainty generally exceeds the within-laboratory uncertainty (see, for example, **Figures 3** and **4**, discussed below), the between-laboratory standard error (reproducibility) estimates both within- (repeatability) and between-laboratory uncertainty components (*8*). The standard uncertainty is the root sum of squares of the variation between labs (estimated via the standard error of lab means) and the 1% inhomogeneity component. For clarity, a detailed statistical analysis for protein in SRM 2387 is provided in **Table 2**.

RESULTS AND DISCUSSION

The certified concentration values of fat, selected fatty acids, elements, and tocopherols in SRM 2387 are provided in **Tables 3** and **4**; these values are based on a combination of data provided by NIST and collaborating laboratories. Reference concentration values for additional proximates, fatty acids, amino acids, calories, total dietary fiber, vitamins, and aflatoxins, based on data provided by collaborating laboratories, are provided in **Tables 5−9**. Relative expanded uncertainties for the proximates range from 2% for solids and protein to 7% for carbohydrates. Relative expanded uncertainties for the vitamins range from 4% for niacin to 20% for vitamin B_1 hydrochloride.

Table 2. Data for Protein (Mass Fraction, in %) in SRM 2387 Peanut Butter and the Calculation of Its Assigned Value and Associated Uncertainty[a]

lab	protein (%)	mean
1	22.11, 22.17	22.14
2	22.23, 22.21	22.22
3	23.04, 22.87	22.95
4	22.45, 22.01	22.23
5	22.82, 22.77	22.80
6	22.22	22.22
7	21.10, 21.10	21.10
8	22.10, 21.90	22.00
9	22.17, 22.60	22.39
10	22.39, 22.28	22.33
11	22.36, 21.77	22.07
mean of laboratory means		22.22
SD of the mean		0.14
degrees of freedom (among laboratory)		10
1% inhomogeneity component (maximum)		0.22
degrees of freedom (homogeneity)		18
effective total degrees of freedom		27.4
coverage factor (*k*)		2.05
standard uncertainty		0.26
expanded uncertainty		0.54
final assigned value		22.2 ± 0.5

[a] The same raw data—the means and SDs for protein reported by the individual laboratories—are plotted in **Figure 4** for graphical comparison.

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

	mass fraction (%)
fat (extractable)	51.6 ± 1.4
fat (sum of fatty acids)	49.8 ± 1.9
saturated fat	10.4 ± 0.2
monounsaturated fat	24.4 ± 0.9
polyunsaturated fat	13.2 ± 0.4

	mass fraction (%) as the triglyceride	mass fraction (%) as the fatty acid
tetradecanoic acid (C14:0) (myristic acid)	0.025 ± 0.002	0.024 ± 0.002
hexadecanoic acid (C16:0) (palmitic acid)	5.18 ± 0.15	4.94 ± 0.15
(*Z*)-9-hexadecenoic acid (C16:1 n-7) (palmitoleic acid)	0.046 ± 0.011	0.044 ± 0.010
octadecanoic acid (C18:0) (stearic acid)	2.23 ± 0.08	2.13 ± 0.08
(*Z*)-9-octadecenoic acid (C18:1 n-9) (oleic acid)	24.43 ± 0.94	23.38 ± 0.90
(*Z*)-11-octadecenoic acid (C18:1 n-7) (vaccenic acid)	0.266 ± 0.017	0.255 ± 0.016
(*Z*,*Z*)-9,12-octadecadienoic acid (C18:2 n-6) (linoleic acid)	13.75 ± 0.43	13.15 ± 0.41
(*Z*,*Z*,*Z*)-9,12,15-octadecatrienoic acid (C18:3 n-3) (linolenic acid)	0.031 ± 0.001	0.030 ± 0.001
eicosanoic acid (C20:0) (arachidic acid)	0.739 ± 0.030	0.710 ± 0.029
(*Z*)-11-eicosenoic acid (C20:1 n-9) (gondoic acid)	0.669 ± 0.032	0.643 ± 0.031
docosanoic acid (C22:0) (behenic acid)	1.88 ± 0.08	1.81 ± 0.08
tetracosanoic acid (C24:0) (lignoceric acid)	0.808 ± 0.045	0.781 ± 0.044

	method
extractable fat	acid digestion, ether extraction (2) chloroform/methanol extraction (1) soxhlet ether extraction (1) PFE (NIST)
fatty acids	hydrolysis followed by GC (11) PFE (hexane/acetone) followed by GC (NIST)

[a] Fat as the sum of the fatty acids represents the sum of quantified individual fatty acid peaks (for which both certified and reference values are provided) as the triglycerides. The certified values for saturated, monounsaturated, and polyunsaturated fats are sums of the assigned values (certified and reference) for the individual fatty acids (as the fatty acids) in each group. The number of laboratories using a particular analytical method is provided in parentheses.

Table 4. Certified Concentration Values for Elements and Tocopherols and the Methods Used for Their Determination[a]

	mass fraction (mg/kg)	method
calcium	411 ± 18	flame atomic absorption spectrometry (1) direct current plasma atomic emission spectrometry (1) ICP-OES (10 + NIST)
copper	4.93 ± 0.15	flame atomic absorption spectrometry (2) direct current plasma atomic emission spectrometry (1) ICP-OES (8 + NIST)
iron	16.4 ± 0.8	flame atomic absorption spectrometry (1) direct current plasma atomic emission spectrometry (1) ICP-OES (10 + NIST)
magnesium	1680 ± 70	flame atomic absorption spectrometry (1) direct current plasma atomic emission spectrometry (1) ICP-OES (10 + NIST)
manganese	16.0 ± 0.6	flame atomic absorption spectrometry (1) direct current plasma atomic emission spectrometry (1) ICP-OES (9 + NIST)
phosphorus	3378 ± 92	absorption spectrophotometry (3) ICP-OES (9 + NIST)
potassium	6070 ± 200	flame atomic absorption spectrometry (1) direct current plasma atomic emission spectrometry (1) ICP-OES (9 + NIST)
sodium	4890 ± 140	flame atomic absorption spectrometry (1) direct current plasma atomic emission spectrometry (1) ICP-OES (9 + NIST)
zinc	26.3 ± 1.1	flame atomic absorption spectrometry (1) direct current plasma atomic emission spectrometry (1) ICP-OES (10 + NIST)
δ-tocopherol	10 ± 3	saponification–RPLC–fluorescence detection (1 + NIST) saponification–NPLC–absorbance detection (1) saponification–NPLC–fluorescence detection (3)
γ- + β-tocopherol	100 ± 19	saponification–RPLC–fluorescence detection (1 + NIST) saponification–NPLC–absorbance detection (1) saponification–NPLC–fluorescence detection (3)
α-tocopherol	108 ± 11	saponification–reversed phase LC (RPLC)–fluorescence detection (3 + NIST) saponification–normal phase LC (NPLC)–absorbance detection (1) saponification–NPLC–fluorescence detection (3)

[a] The number of laboratories using a particular analytical method is provided in parentheses.

Relative expanded uncertainties for the elements are in the 3−5% range.

Outliers in the data provided by collaborating laboratories were excluded in several cases. For the proximate determinations, one laboratory's protein data were excluded because the mean was more than three standard errors from the mean of the interlaboratory comparison exercise. Some individual fatty acid, element, and vitamin data from collaborating laboratories were excluded for the same reason. Of the 552 individual pieces of data submitted by the collaborating laboratories for proximates, individual fatty acids, fat groups, and total dietary fiber, 36 were considered to be outliers—a rejection rate of 7%, mostly attributable to the rejection of 24 results for individual fatty acids. The rejection rate for element data was 26 out of 291 (9%)—12 rejections were attributed to one laboratory—and 14 of 99 pieces (14%) of reported vitamin data were rejected. Although methodological information was provided by collaborating laboratories, it is generally not possible to link outliers to method failures because insufficient detail was requested. For example, eight laboratories measured total dietary fiber using an enzymatic digestion followed by gravimetry, and data provided by two laboratories were rejected. One mean was significantly higher than the interlaboratory mean, and the other was significantly lower; the variability on the measurements was 2−4 times higher than that of the other laboratories.

Certified values are provided for fat as the sum of fatty acids as triglycerides and as extractable fat; relative expanded uncertainties are 4 and 3%, respectively. (NIST offers three other

Table 5. Reference Concentration Values for Proximates and Caloric Contentand Methods Used for Their Determination[a]

	Mass Fraction (%)
solids	99.2 ± 2.1
ash	3.10 ± 0.10
protein	22.2 ± 0.5
carbohydrate (by difference)	25.0 ± 1.8
total dietary fiber	5.57 ± 0.42
	Calories (kcal/100 g)
caloric content[b]	629 ± 15

solids	moisture determined by mass loss after oven drying: forced air oven (3) and vacuum oven (8)
ash	mass loss after ignition in muffle furnace (11)
nitrogen	Kjeldahl (7) thermal conductivity (1) pyrolysis, GC (1) pyrolysis, conductivity (1) Dumas combustion (1)
protein	calculated; a factor of 5.46 was used to calculate protein from nitrogen results
carbohydrate	calculated; solids − (protein + fat as the sum of fatty acids + ash)
total dietary fiber	enzymatic gravimetry (8)
calories	calculated; 9 (fat as the sum of fatty acids) + 4 (protein) + 4 (carbohydrate)

[a] The certified values for fat are provided in **Table 3**. The number of laboratories using a particular method is provided in parentheses. [b] 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 637 kcal/100 g.

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

	mass fraction (%)	
	as the triglyceride	as the fatty acid
heptadecanoic acid (C17:0) (margaric acid)	0.050 ± 0.001	0.048 ± 0.001
heptadecenoic acid (C17:1)	0.035 ± 0.006	0.033 ± 0.006
eicosadienoic acid (C20:2)	0.017 ± 0.007	0.016 ± 0.007
(*Z,Z,Z,Z*)-5,8,11,14-eicosatetraenoic acid (C20:4 n-6) (arachidonic acid)	0.025 ± 0.016	0.024 ± 0.015
(*Z*)-13-docosenoic acid (C22:1 n-9) (erucic acid)	0.056 ± 0.012	0.054 ± 0.012

[a] The 11 collaborating laboratories used hydrolysis followed by GC for measurement of these fatty acids.

Table 7. Reference Concentration Values for Amino Acids[a]

	mass fraction (%)		mass fraction (%)
alanine	0.93 ± 0.10	lysine	0.78 ± 0.08
arginine	2.65 ± 0.31	methionine	0.21 ± 0.04
aspartic acid	2.83 ± 0.19	phenylalanine	1.21 ± 0.08
cystine	0.27 ± 0.01	proline	0.96 ± 0.08
glutamic acid	4.69 ± 0.26	serine	1.16 ± 0.09
glycine	1.41 ± 0.12	threonine	0.54 ± 0.08
histidine	0.55 ± 0.06	tryptophan	0.21 ± 0.06
isoleucine	0.77 ± 0.07	tyrosine	0.81 ± 0.14
leucine	1.56 ± 0.09	valine	0.94 ± 0.09

[a] The five laboratories that reported results used analytical methods involving hydrolysis, derivatization, and LC.

Table 8. Reference Concentration Values for Selected Vitamins and Analytical Methods Used for Their Determination[a]

	mass fraction (mg/kg)	analytical method
niacin	142 ± 6	microbiological (7)
pantothenic acid	10.8 ± 3.2	microbiological (6)
vitamin B_1 hydrochloride	0.84 ± 0.17	digestion–fluorescence detection (4) extraction–RPLC–fluorescence detection (1) extraction–ion pair chromatography–fluorescence detection (1)
vitamin B_6	4.66 ± 0.62	LC–fluorescence detection (1) microbiological (5)

[a] The number of laboratories using a particular method is provided in parentheses.

Table 9. Reference Concentration Values for Aflatoxins[a]

	mass fraction (ng/g)
aflatoxin B1	4.2 ± 0.9
aflatoxin B2	0.7 ± 0.3
total aflatoxins[b]	5.0 ± 0.5

[a] These analyses were performed by laboratories using LC with fluorescence detection (five laboratories), TLC (one laboratory), and ELISA (one laboratory). [b] The reference value for total aflatoxins is the mean of the laboratory means of the sum of aflatoxins Bl and B2.

high-fat SRMs: SRM 1563 Coconut Oil (in sector 1 of the AOAC triangle), SRM 2384 Baking Chocolate (sector 2), and SRM 1546 Meat Homogenate (sector 4). Comparative details will be provided in reference (*9*).) Certified values are also provided for many of the individual fatty acids and for saturated, monounsaturated, and polyunsaturated fats; relative expanded uncertainties are generally 5% or less. **Figure 2** shows a comparison of the levels of individual fatty acids in SRM 2387. Despite its high fat content, researchers at Penn State and the University of Rochester Medical Center (in a project supported by The Peanut Institute) have found that peanut butter may reduce the risk of cardiovascular disease because many of its fatty acids are unsaturated (*10*).

NLEA requires that information about the concentrations of three elements be provided on nutrition labels: calcium, iron, and sodium. At low levels, the measurement of these and other elements can be difficult. The results for iron reported by NIST and collaborating laboratories, along with the certified value for iron in SRM 2387, are plotted in **Figure 3**. Iron data from three laboratories were discarded as outliers (greater than three standard errors from the mean of the interlaboratory comparison). Some laboratories may be overestimating the iron concentration in samples in which it occurs at low levels, while others may be underestimating it. This highlights the importance of and the need for food matrix RMs with values assigned for low-level elements and further emphasizes the importance of quality assurance or interlaboratory comparison programs that enable a laboratory to identify analytical problems or weaknesses.

Peanut butter is a high-protein food. The protein results reported by participating laboratories and the reference value for protein in SRM 2387 are plotted in **Figure 4**. One laboratory's data were excluded, with a mean greater than three standard errors lower than the interlaboratory comparison mean. (The same data, with the outlier excluded, are provided in **Table 2**, which shows how the uncertainty was calculated.) Because of its high concentration of protein, it is also appropriate to

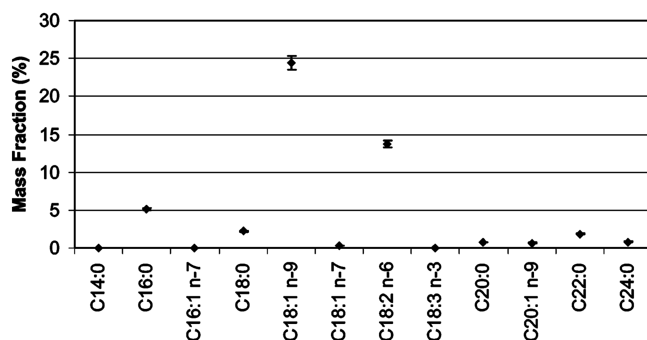


Figure 2. Comparison of certified values and their expanded uncertainties for fatty acid concentrations (as triglycerides) in SRM 2387.

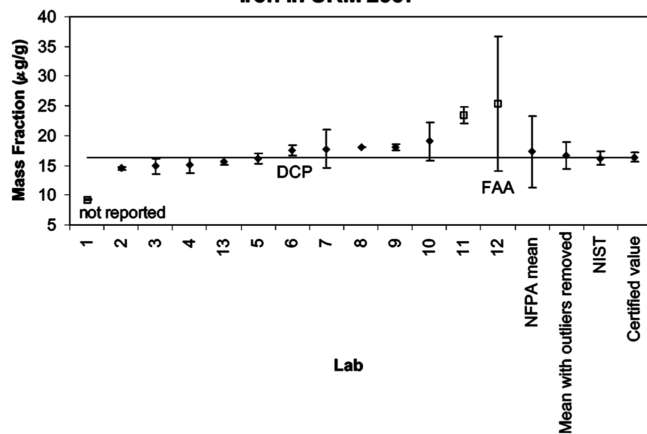


Figure 3. Comparison of the certified value and its expended uncertainty and the means and two SDs (approximating a 95% confidence interval) for individual laboratory data for iron. Data from three laboratories, shown as hollow squares, were discarded as outliers. The mean for the interlaboratory comparison is shown, with and without the outliers included. Analytical methods used are provided in the figure below the data point obtained using that particular method; unlabeled data were obtained using ICP-OES.

assign values for individual amino acid concentrations in this material. This is NIST's first food matrix SRM with values assigned for amino acids. A comparison of the concentrations of the amino acids is provided in **Figure 5**. The U.S. Department of Agriculture has been including amino acid values in their nutrient databases for several years (*11*), and until now, NIST had no SRMs available with values assigned for amino acids to provide quality assurance for these measurements. (Interestingly, the values for all nutrients in SRM 2387, including the amino acids, are in good agreement with the values provided in the USDA's database for NDB No. 16098, Peanut butter, smooth style, with salt. The SRM is represenative of peanut butter that is manufactured for consumption as food, which is not surprising, since the SRM was manufactured as part of a larger production run of peanut butter intended for sale as food. However, the SRM is not intended for human consumption.)

Peanuts are one of the eight most common allergenic foods. Enzyme-linked immunosorbent assay (ELISA) test kits are typically used to screen for the presence of peanut proteins—as well as other allergenic proteins—in foods. A suspension of SRM 2387 has been used to spike several types of food to evaluate the performance of this type of test kit (*12*), as well as to demonstrate the feasibility of benchmarking such an evaluation

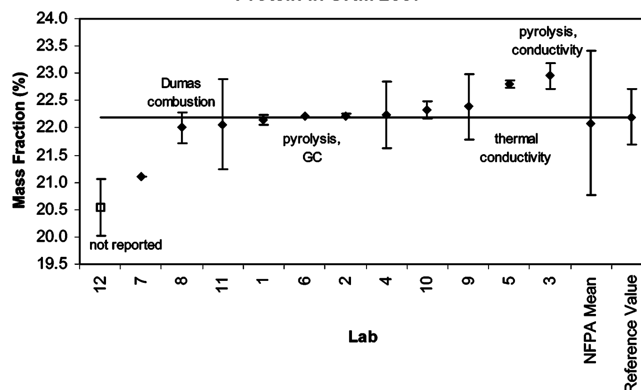


Figure 4. Comparison of the reference value and its expanded uncertainty and the means and two SDs (approximating a 95% confidence interval) for individual laboratory data for protein. Data from one laboratory, shown as a hollow square, were discarded as outliers. The mean for the interlaboratory comparison is shown, with the outlying data included. Protein was calculated from nitrogen. Analytical methods used for nitrogen determination are provided in the figure above or below the data point obtained using that particular method; unlabeled data were obtained using the Kjeldahl method.

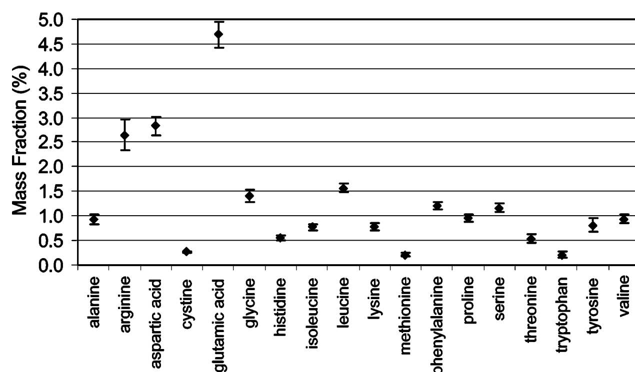


Figure 5. Comparison of reference values and their expanded uncertainties for amino acid concentrations in SRM 2387.

against a material known to contain solely—rather than traces of—the proteins of interest.

Levels of aflatoxins in peanut butter are regulated at the 20 ng/g level. At 5.0 ng/g, the levels present in SRM 2387 are well below this limit. A comparison of the results reported by collaborating laboratories, with the different methods of analysis indicated, and the reference value for total aflatoxins in the SRM is provided in **Figure 6**. ELISA test kits are frequently used to detect aflatoxins. Note that the one laboratory that used such a test kit was unable to detect aflatoxins in SRM 2387 and therefore reported a value that was less than their limit of detection of 5 ng/g. Because of the perhaps challenging low level in SRM 2387, an analyst who successfully measures aflatoxins in the SRM will have a degree of confidence that he or she is not overlooking aflatoxins in other materials being analyzed.

With the introduction of SRM 2387, RMs in all nine sectors of the AOAC triangle are available from NIST to address NLEA concerns (see **Figure 1**). SRM 2387 is the first RM available from NIST for which values are assigned for amino acids and for aflatoxins. SRM 2387 and the others in the series of food

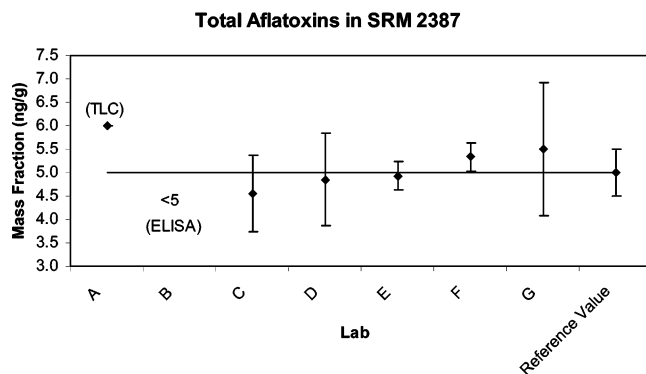


Figure 6. Comparison of the reference value and its expanded uncertainty and the means and two SDs (approximating a 95% confidence interval) for individual laboratory data for total aflatoxins. The assigned value was generated by laboratories using LC with fluorescence detection; semi-quantitative information was provided by thin-layer chromatography (TLC) and ELISA.

RMs will help support measurement accuracy and traceability for laboratories performing measurements in the food and nutrition communities. Values are assigned for some "specialized" analytes in food matrix RMs that are currently available (e.g., aflatoxins in SRM 2387 and caffeine and catechins in SRM 2384), and it may be possible to focus future materials on additional specialized analytes for which nutrition labeling is not required but which are needed for other purposes by the food and nutrition communities.

ACKNOWLEDGMENT

We thank the members of the NFPA's Food Industry Analytical Chemists Subcommittee and the other collaborating laboratories (**Table 1**) for analyzing SRM 2387; we recognize and appreciate the amount of time and effort the collaborating laboratories contributed in assisting in value assignment of this SRM. We also thank Mark Cronise (NIST Standard Reference Materials Group [SRMG]) for his assistance in distributing samples in the NFPA interlaboratory comparison exercise and Jennifer Colbert and Bruce MacDonald (NIST SRMG) for support in this project. We thank the company that manufactured this material for us—both for the production itself and for providing information about the finished product.

LITERATURE CITED

(1) Nutrition Labeling and Education Act of 1990. Public Law 101-535 (H. R. 3562); November 8, 1990.

(2) Sharpless, K. E.; Colbert, J. C.; Greenberg, R. R.; Schantz, M. M.; Welch, M. J. Recent Developments in Food-Matrix Reference Materials at NIST. *Fresenius J. Anal. Chem.* **2001**, *370*, 275.

(3) Wolf, W. R. In *Methods of Analysis for Nutrition Labeling*; Sullivan, D. M., Carpenter, D. E., Eds.; AOAC International: Arlington, VA, 1993; pp 111−122.

(4) Wolf, W. R.; Andrews, K. W. A System for Defining Reference Materials Applicable to All Food Matrices. *Fresenius J. Anal. Chem.* **1995**, *352*, 73−76.

(5) May, W.; Parris, R.; Beck, 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.

(6) Epler, K. S.; Ziegler, R. G.; Craft, N. E. Liquid Chromatographic Method for the Determination of Carotenoids, Retinoids, and Tocopherols in Human Serum and in Food. *J. Chromatogr., B: Biomed. Sci. Appl.* **1993**, *619*, 37−48.

(7) *Guide to the Expression of Uncertainty in Measurement*, 1st ed.; ISO: Geneva, Switzerland, 1993; 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 http://www.physics.nist.gov/Pubs/guidelines/contents.html).

(8) *Accuracy (Trueness and Precision) of Measurement Results, Part 2: Basic Method for Repeatability and Reproducibility of a Standard Measurement Method*; ISO 5725; ISO: Geneva, Switzerland, 1997; see also http://www.itl.nist.gov/div898/handbook.

(9) Phinney, C. S.; Leigh, S. D.; Schantz, M. M.; Sharpless, K. E.; Welch, M. J.; Yen, J. H. Value Assignment of Fatty Acid Concentrations in Food-Matrix Standard Reference Materials. Manuscript in preparation.

(10) Kris-Etherton, P. M.; Pearson, T. A.; Wan, Y.; Hargrove, R. L.; Moriarty, K.; Fishell, V.; Etherton, T. D. High-monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations. *Am. J. Clin. Nutr.* **1999**, *70*, 1009−1015.

(11) http://www.nal.usda.gov/fnic/foodcomp.

(12) Trucksess, M. W.; Brewer, V. A.; Williams, K. M.; Westphal, C. D.; Falci, K. J.; Heeres, J. T. Preparation of a Peanut Butter Suspension for the Determination of Peanuts Using Enzyme-Linked Immunoassay Kits. *J. AOAC Int.*, in press.

Received for review June 16, 2003. Revised manuscript received August 13, 2003. Accepted September 2, 2003. Certain commercial products are identified to specify adequately the experimental procedure. Such identification does not imply endorsement or recommendation by the NIST nor does it imply that the materials identified are necessarily the best available for the purpose.

JF034637O