



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material[®] 3234

Soy Flour

This Standard Reference Material (SRM) is intended primarily for validation of methods for determining proximates, vitamins, elements, and amino acids in soy flour and similar materials. This SRM can also be used for quality assurance when assigning values to in-house reference materials. The SRM is a defatted soy flour prepared by a commercial manufacturer. A unit of SRM 3234 consists of one bottle that contains approximately 50 g of material and is sealed inside an aluminized pouch.

Certified Mass Fraction Values: The certified mass fraction values of selected elements and vitamins in SRM 3234 are provided in Tables 1 and 2, respectively. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. Analyses for value assignment were performed by NIST and collaborating laboratories. Certified values were calculated as the mean of the mean values from NIST methods and the median of the mean results provided by collaborating laboratories, where appropriate. All values were combined without weighting. The associated uncertainties are expressed at the 95 % level of confidence [2–4]. Values are reported on a dry-mass basis in mass fraction units [5].

Reference Mass Fraction Values: Reference mass fraction values are provided for sodium and carnitine (Table 3); proximates, total dietary fiber, and calories (Table 4); and amino acids (Table 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 associated uncertainties that may reflect only measurement reproducibility, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods. The reference mass fraction values were derived from results reported by NIST or collaborating laboratories. Values are reported on a dry-mass basis in mass fraction units [5].

Expiration of Certification: The certification of **SRM 3234** is valid, within the measurement uncertainty specified, until **20 August 2017**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see “Instructions for Storage and Use”). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

Coordination of the technical measurements leading to the certification of this SRM was performed by K.E. Sharpless of the NIST Chemical Sciences Division and S. Ehling of the Grocery Manufacturers Association (GMA, Washington, DC).

Analytical measurements at NIST were performed by M.M. Phillips, B.J. Porter, and L.J. Wood of the NIST Chemical Sciences Division and B. Lang of the NIST Biosystems and Biomaterials Division.

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

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Analyses for value assignment were also performed by the following laboratories participating in a GMA Food Industry Analytical Chemists Committee (FIACC) interlaboratory comparison exercise: Campbell Soup Company, Camden, NJ; Conagra Foods, Omaha, NE; Covance, Inc., Madison, WI; Del Monte Foods, Walnut Creek, CA; Eurofins Central Analytical Laboratories, Metairie, LA; Eurofins Scientific, Des Moines, IA; General Mills, Inc., Golden Valley, MN; Hormel Foods Corporation, Austin, MN; Krueger Food Laboratories, Billerica, MA; Land O'Lakes, Arden Hills, MN; Schwan Food Company, Salina, KS; Silliker, Madison, WI; The J.M. Smucker Co., Orville, OH; and The National Food Laboratory, Livermore, CA.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

NOTICE AND WARNING TO USERS

SRM 3234 IS INTENDED FOR LABORATORY USE ONLY, NOT FOR HUMAN CONSUMPTION.

INSTRUCTIONS FOR STORAGE AND USE

Storage: The SRM should be stored at controlled room temperature (20 °C to 25 °C) in the original unopened bottle. For elemental analyses, the bottle can be re-capped and test portions removed and analyzed until the material reaches its expiration date. For vitamin analyses, the bottle can be tightly re-capped and test portions removed and analyzed for at least three years after the bottle was first opened or until the material reaches its expiration date, whichever comes first.

Use: Before use, the contents of the bottle should be mixed thoroughly by rotating and/or rolling. Allow the contents to settle for one minute prior to opening to minimize the loss of fine particles. For certified values to be valid, test portions of the following masses should be used: between 0.3 g and 0.5 g for elemental analysis and between 0.5 g and 10 g for vitamin analysis. Test portions should be analyzed as received and results converted to a dry-mass basis by determining moisture content (using one of the methods described below) on a separate test portion. Results obtained in analyses should include their own estimates of uncertainty and can be compared to the certified values using procedures described in reference [6].

SOURCE, PREPARATION, AND ANALYSIS⁽¹⁾

Source and Preparation: The SRM consists of defatted soy flour. Three hundred twenty kilograms (from fourteen 50-pound bags) of soy flour were blended and bottled by High-Purity Standards (Charleston, SC). The soy flour was placed in 4-ounce amber bottles that had been flushed with nitrogen. Each bottle contains nominally 50 g of soy flour. The bottles were capped and sealed with heat-shrink tape, then individually sealed in Mylar bags. Following bottling, SRM 3234 was irradiated by Neutron Products, Inc. (Dickerson, MD) to an absorbed dose of 7 kGy to 10 kGy.

Analytical Approach for Determination of Vitamins: Value assignment of the mass fractions of the vitamins in SRM 3234 was based on results from NIST with confirmation by data provided by collaborating laboratories.

NIST Analyses for Water-Soluble Vitamins and Carnitine: Water-soluble vitamins and carnitine were measured by using liquid chromatographic (LC) methods with isotope dilution (ID) mass spectrometry (MS) or tandem mass spectrometry (MS/MS). Calibrants were prepared gravimetrically at levels intended to approximate the levels of the vitamins in the SRM. In cases where an internal standard was employed, a single solution was used for the calibrants and samples.

Thiamine, Riboflavin, Niacinamide, Niacin, Pantothenic Acid, Pyridoxine, Pyridoxamine, and Pyridoxal: Thiamine, riboflavin, niacinamide, niacin, pantothenic acid, pyridoxine, pyridoxamine, and pyridoxal were measured by LC-MS/MS in duplicate 2 g test portions taken from each of 12 bottles. Eight internal standards were added: ¹³C₃-thiamine chloride; ¹³C₄, ¹⁵N₂-riboflavin; ²H₄-niacinamide; ²H₄-niacin; calcium ¹³C₃, ¹⁵N-pantothenate; ¹³C₄-pyridoxine hydrochloride; ²H₃-pyridoxamine dihydrochloride; and ²H₃-pyridoxal hydrochloride. The analytes and internal standards were extracted into dilute acetic acid for analysis by positive-ion mode LC-MS/MS. A gradient method with an ammonium formate buffer/methanol mobile phase and a C₁₈ column were used for

⁽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.

LC-MS/MS determination of thiamine, riboflavin, niacinamide, niacin, pantothenic acid, pyridoxine, pyridoxamine, and pyridoxal. The transitions m/z 266 \rightarrow m/z 123 and m/z 266 \rightarrow m/z 42 were monitored for thiamine, and the transitions m/z 269 \rightarrow m/z 123 and m/z 269 \rightarrow m/z 42 were monitored for $^{13}\text{C}_3$ -thiamine. The transitions m/z 377 \rightarrow m/z 243, m/z 377 \rightarrow m/z 198, m/z 377 \rightarrow m/z 172, and m/z 377 \rightarrow m/z 43 were monitored for riboflavin, and the transitions m/z 383 \rightarrow m/z 249, m/z 383 \rightarrow m/z 202, m/z 383 \rightarrow m/z 175, and m/z 383 \rightarrow m/z 43 were monitored for $^{13}\text{C}_4$, $^{15}\text{N}_2$ -riboflavin. The transitions m/z 123 \rightarrow m/z 80, m/z 123 \rightarrow m/z 78, and m/z 123 \rightarrow m/z 53 were monitored for niacinamide, and the transitions m/z 127 \rightarrow m/z 84, m/z 127 \rightarrow m/z 81, and m/z 127 \rightarrow m/z 56 were monitored for $^2\text{H}_4$ -niacinamide. The transitions m/z 124 \rightarrow m/z 80, m/z 124 \rightarrow m/z 78, m/z 124 \rightarrow m/z 53, and m/z 124 \rightarrow m/z 52 were monitored for niacin, and the transitions m/z 128 \rightarrow m/z 84, m/z 128 \rightarrow m/z 81, m/z 128 \rightarrow m/z 56, and m/z 128 \rightarrow m/z 53 were monitored for $^2\text{H}_4$ -niacin. The transitions m/z 220 \rightarrow m/z 90, m/z 220 \rightarrow m/z 72, m/z 220 \rightarrow m/z 43, and m/z 220 \rightarrow m/z 41 were monitored for pantothenic acid, and the transitions m/z 224 \rightarrow m/z 94, m/z 224 \rightarrow m/z 76, m/z 224 \rightarrow m/z 43, and m/z 224 \rightarrow m/z 41 were monitored for $^{13}\text{C}_3$, ^{15}N -pantothenic acid. The transitions m/z 170 \rightarrow m/z 152, m/z 170 \rightarrow m/z 134, m/z 170 \rightarrow m/z 80, and m/z 170 \rightarrow m/z 77 were monitored for pyridoxine, and the transitions m/z 174 \rightarrow m/z 156, m/z 174 \rightarrow m/z 138, m/z 174 \rightarrow m/z 83, and m/z 174 \rightarrow m/z 81 were monitored for $^{13}\text{C}_4$ -pyridoxine. The transitions m/z 169 \rightarrow m/z 152, m/z 169 \rightarrow m/z 134, and m/z 169 \rightarrow m/z 77 were monitored for pyridoxamine, and the transitions m/z 172 \rightarrow m/z 155, m/z 172 \rightarrow m/z 136, and m/z 172 \rightarrow m/z 79 were monitored for $^2\text{H}_3$ -pyridoxamine. The transitions m/z 168 \rightarrow m/z 150, m/z 168 \rightarrow m/z 94, m/z 168 \rightarrow m/z 67, and m/z 168 \rightarrow m/z 41 were monitored for pyridoxal, and the transitions m/z 171 \rightarrow m/z 153, m/z 171 \rightarrow m/z 97, m/z 171 \rightarrow m/z 70, and m/z 171 \rightarrow m/z 43 were monitored for $^2\text{H}_3$ -pyridoxal. Mass fractions were calculated for each of the transitions, and these mass fractions were then averaged for each test portion analyzed.

Choline and Carnitine: Choline and carnitine were measured in two 0.4 g test portions taken from each of 12 bottles of SRM 3234. $^2\text{H}_9$ -choline chloride and $^2\text{H}_9$ -carnitine hydrochloride were added as internal standards. The analytes and internal standards were extracted and hydrolyzed by microwave digestion into dilute hydrochloric acid for analysis by positive-ion mode LC/MS. A gradient method with an ammonium formate/acetonitrile mobile phase and a mixed-mode C_{18} column were used for LC/MS determination. Choline and $^2\text{H}_9$ -choline were measured at m/z 104 and m/z 113, respectively. Carnitine and $^2\text{H}_9$ -carnitine were measured at m/z 162 and m/z 171, respectively.

Analytical Approach for Determination of Elements: Value assignment of the mass fractions of the elements in SRM 3234 was based on the combination of measurements provided by NIST using inductively coupled plasma optical emission spectrometry (ICP-OES) and data provided by collaborating laboratories, where available.

NIST Analyses for Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn Using ICP-OES: Calcium, copper, iron, potassium, magnesium, manganese, phosphorus, and zinc were measured by ICP-OES using duplicate 0.5 g test portions taken from each of 12 bottles of SRM 3234. For sodium analyses, 1.0 g test portions were used. Samples for ICP-OES were digested in a nitric acid/hydrofluoric acid mixture using a microwave sample preparation system. Indium was added as an internal standard, and the method of standard additions was used for quantitation.

Collaborating Laboratories' Analyses: The GMA FIACC laboratories were asked to use their usual methods to make single measurements of proximates, calories, vitamins, elements, and amino acids on test portions taken from each of two bottles of SRM 3234. Because of variability among data provided by laboratories participating in an interlaboratory comparison exercise, the median of laboratory means is used, with the uncertainty estimated using the median absolute deviation (MADE) [7].

Determination of Moisture: Moisture content of SRM 3234 was determined at NIST (see "Instructions for Storage and Use") by (1) freeze-drying to constant mass over 7 d; (2) drying over magnesium perchlorate in a desiccator at room temperature for 21 d; and (3) drying for 2 h in a forced-air oven at 90 °C. Unweighted results obtained using all three techniques were averaged to determine a conversion factor of (93.87 ± 0.49) gram dry mass per gram as-received mass, which was used to convert data from an as-received to a dry-mass basis; the uncertainty shown on this value is an expanded uncertainty. An uncertainty component for the conversion factor (0.26 %) obtained from the moisture measurements is incorporated in the uncertainties of the certified and reference values, reported on a dry-mass basis, that are provided in this certificate. Note that this moisture correction value differs from the reference value assigned for solids, which was determined by the GMA laboratories.

Homogeneity Assessment: The homogeneity of vitamins and elements was assessed at NIST using the methods and test portion sizes described above. Analysis of variance did not show statistically significant heterogeneity. Other analytes have been treated as though they are homogeneously distributed in the material although only the homogeneity of vitamins and elements was assessed.

Value Assignment: The collaborating laboratories reported the individual results for each of their analyses for a given analyte. The mean of each laboratory's results was then determined. For calculation of assigned values for analytes that were measured only by the collaborating laboratories, the median of the laboratory means was used. For analytes that were also measured by NIST, 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 Selected Elements: Each certified mass fraction value is the mean from the combination of the mean of results from analyses by NIST using ICP-OES and the median of the mean of results provided by collaborating laboratories. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c incorporates the observed difference between the results from the methods and their respective uncertainties and an uncertainty component for moisture correction, consistent with the ISO/JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2–4].

Table 1. Certified Mass Fraction Values (Dry-Mass Basis) for Selected Elements in SRM 3234

	Mass Fraction (mg/kg)			Coverage Factor k
Calcium	3191	±	56	2.00
Copper	15.34	±	0.26	2.00
Iron	80.3	±	2.7	2.00
Magnesium	3487	±	60	2.00
Manganese	36.78	±	0.88	2.00
Phosphorus	8080	±	210	2.00
Potassium	25010	±	560	2.00
Zinc	48.9	±	1.1	2.00

Certified Mass Fraction Values for Selected Vitamins: Each certified mass fraction value is the mean from the analyses by NIST; these results were confirmed by data provided by collaborating laboratories. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c represents the combined uncertainty, incorporating an uncertainty component for moisture correction, consistent with the ISO/JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2–4].

Table 2. Certified Mass Fraction Values (Dry-Mass Basis) for Selected Vitamins in SRM 3234

	Mass Fraction (mg/kg)			Coverage Factor k
Thiamine (Vitamin B ₁) ^(a,b)	8.60	±	0.20	2.06
Riboflavin (Vitamin B ₂) ^(b)	3.363	±	0.041	2.03
Niacin ^(b)	4.59	±	0.17	2.07
Niacinamide ^(b)	11.49	±	0.13	2.03
Total Vitamin B ₃ as Niacinamide ^(b,c)	16.04	±	0.24	2.05
Pantothenic Acid ^(b)	11.45	±	0.12	2.02
Pyridoxal Hydrochloride ^(b)	1.72	±	0.10	2.00
Pyridoxamine Dihydrochloride ^(b)	1.277	±	0.056	2.00
Pyridoxine Hydrochloride ^(b)	0.734	±	0.089	2.05
Total Vitamin B ₆ as Pyridoxine Hydrochloride ^(b,d)	3.56	±	0.20	2.00
Choline ^(e)	2799	±	18	2.00

^(a) Reported as thiamine ion (relative molecular mass of 265.36 g/mol), not chloride or chloride hydrochloride.

^(b) NIST ID-LC/MS/MS

^(c) NIST measured niacinamide and niacin individually, and niacin was mathematically converted to niacinamide by multiplication by the ratio of the relative molecular masses.

^(d) NIST measured pyridoxal, pyridoxamine, and pyridoxine individually, and pyridoxal and pyridoxamine were mathematically converted to pyridoxine by multiplication by the ratio of the relative molecular masses.

^(e) NIST ID-LC/MS

Reference Mass Fraction Values for Sodium and Carnitine: Each reference mass fraction value is the mean result of a NIST analysis using a single method. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c represents the combined uncertainty, incorporating an uncertainty component for moisture correction, consistent with the ISO/JCGM Guide, and k is a coverage factor corresponding to approximately 95 % confidence [2].

Table 3. Reference Mass Fraction Values (Dry-Mass Basis) for Sodium and Carnitine in SRM 3234

	Mass Fraction (mg/kg)	Coverage Factor k
Sodium ^(a)	2.52 ± 0.45	2.11
Carnitine ^(b)	1.98 ± 0.11	2.08

^(a) NIST ICP-OES

^(b) NIST ID-LC/MS

Reference Values for Proximates, Fatty Acids, Total Dietary Fiber, and Calories: Each reference mass fraction value is the median of the mean results provided by collaborating laboratories. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c represents the combined uncertainty, incorporating an uncertainty component for moisture correction, consistent with the ISO/JCGM Guide, and k is a coverage factor corresponding to approximately 95 % confidence [2–4].

Table 4. Reference Values (Dry-Mass Basis) for Proximates, Fatty Acids, Total Dietary Fiber, and Calories in SRM 3234

	Mass Fraction (%)	Coverage Factor k
Solids	98.91 ± 0.67	2.00
Ash	6.77 ± 0.14	2.13
Protein ^(a)	53.37 ± 0.36	2.00
Fat (as the sum of fatty acids as triglycerides)	1.49 ± 0.12	2.23
Hexadecanoic Acid (C16:0) (Palmitic Acid)	0.353 ± 0.040	2.26
Octadecanoic Acid (C18:0) (Stearic Acid)	0.095 ± 0.012	2.26
(Z)-9-Octadecenoic Acid (C18:1 n-9) (Oleic Acid)	0.142 ± 0.023	2.31
(Z)-11-Octadecenoic Acid (C18:1 n-7) (Vaccenic Acid)	0.026 ± 0.006	2.78
(Z,Z)-9,12-Octadecadienoic Acid (C18:2 n-6) (Linoleic Acid)	0.671 ± 0.060	2.26
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3 n-3) (α -Linolenic Acid)	0.0695 ± 0.0085	2.26
Carbohydrates	37.14 ± 0.69	2.16
Total Dietary Fiber	18.19 ± 0.37	2.23
	Energy (kcal per 100 g)	Coverage Factor k
Calories ^(b)	377.7 ± 3.7	2.09

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

^(b) The reference value for calories is the median of lab mean caloric calculations from the interlaboratory comparison exercise. If the mean proximate values above are used for calculation, with caloric equivalents of 9, 4, and 4 for fat (as the sum of fatty acids), protein, and carbohydrate, respectively, the mean caloric content is 375.5 kcal/100 g.

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

Table 5. Reference Mass Fraction Values (Dry-Mass Basis) for Amino Acids in SRM 3234

	Mass Fraction (%)			Coverage Factor k
Alanine	2.28	±	0.16	2.57
Arginine	3.72	±	0.31	2.57
Aspartic Acid	6.0	±	1.2	2.57
Cysteine	0.74	±	0.15	3.18
Glutamic Acid	10.2	±	1.4	2.57
Glycine	2.22	±	0.15	2.57
Histidine	1.222	±	0.089	2.57
Isoleucine	2.31	±	0.23	2.57
Leucine	4.03	±	0.42	2.57
Lysine	3.20	±	0.25	2.57
Methionine	0.69	±	0.13	2.57
Phenylalanine	2.45	±	0.13	2.57
Proline	2.71	±	0.23	2.57
Serine	2.69	±	0.32	2.57
Threonine	2.02	±	0.11	2.57
Tryptophan	0.66	±	0.14	3.18
Tyrosine	1.76	±	0.43	2.57
Valine	2.45	±	0.41	2.57

REFERENCES

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Certificate Revision History: 08 July 2013 (Addition of certified values for forms of vitamin B₆; editorial changes); 28 September 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: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at <http://www.nist.gov/srm>.