



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material[®] 3287

Blueberry (Fruit)

This Standard Reference Material (SRM) is intended primarily for use in validating analytical methods for the determination of organic acids and nutrients in blueberries and similar matrices. This SRM can also be used for quality assurance when assigning values to in-house control materials. A unit of SRM 3287 consists of five packets, each containing approximately 5 g of freeze-dried, powdered fruit.

The development of SRM 3287 was a collaboration between the National Institute of Standards and Technology (NIST) and the National Institutes of Health Office of Dietary Supplements (NIH-ODS).

Certified Mass Fraction Values: Certified mass fraction values of quinic acid, water-soluble vitamins, and elements are provided in Tables 1 through 3, 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]. Values were derived from the combination of results provided by NIST using two methods (organic acids); a single NIST method with confirmation by data provided by collaborating laboratories (vitamins); and the combination of NIST data and data provided by collaborating laboratories (elements). The certified values in this material are the equally weighted means of the individual sets of results (organic acid, vitamins) and the equally weighted mean of results provided by NIST and the collaborating laboratories' median (elements); the associated uncertainties are expanded uncertainties at the 95 % level of confidence [2–4]. Values are reported on a dry-mass basis in mass fraction units [5].

Reference Values: Reference mass fraction values for additional organic acids, anions, and nutrients are provided in Tables 4 through 6. Reference values for antioxidant capacity are provided in Table 7. Reference values are non-certified values that are the best estimates of the true values based on available data; however, the values do not meet the NIST criteria for certification [1] and are 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.

Expiration of Certification: The certification of **SRM 3287** is valid, within the measurement uncertainty specified, until **30 April 2015**, provided the SRM is handled and stored in accordance with instructions given in this certificate (see "Warning and 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.

Support for the development of SRM 3287 was provided in part by NIH-ODS. Technical consultation was provided by J.M. Betz (NIH-ODS).

The overall direction and coordination of the technical measurements leading to the certification of this SRM were performed by L.C. Sander, K.E. Sharpless, and S.A. Wise of the NIST Chemical Sciences Division.

Acquisition of the material was performed by K.E. Sharpless of the NIST Chemical Sciences Division.

Carlos A. Gonzalez, Chief
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Gaithersburg, MD 20899
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Certificate Revision History on Last Page

Robert L. Watters, Jr., Director
Office of Reference Materials

Analytical measurements at NIST were performed by M.M. Phillips, B.J. Porter, and L.J. Wood of the NIST Chemical Sciences Division. Results were also provided by analysts participating in an interlaboratory comparison exercise organized by the Grocery Manufacturers Association Food Industry Analytical Chemists Committee (GMA FIACC), Washington, DC: Campbell Soup Company, Camden, NJ; ConAgra Foods Analytical Laboratory, Omaha, NE; Covance, Inc., Madison, WI; Eurofins Scientific, Inc., Des Moines, IA; Eurofins Chemical Control, Cuneo, Italy; Eurofins – Strassburger and Siegel, Hanover, MD; General Mills, Inc., Minneapolis, MN; Hormel Foods Corporation, Austin, MN; Krueger Food Laboratories, Billerica, MA; McCormick & Company, Inc., Hunt Valley, MD; National Center of Food Safety and Technology, Summit-Argo, IL; Ocean Spray, Lakeville, MA; Siliker Inc., Chicago Heights, IL; The Hershey Company Technical Center, Hershey, PA; The J.M. Smucker Company, Orrville, OH; The National Food Laboratory, Livermore, CA; The Schwan Food Company, Salina, KS; and Welch's, Billerica, MA.

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

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

WARNING AND INSTRUCTIONS FOR STORAGE AND USE

Warning: For laboratory use only. Not for human consumption.

Storage: The material should be stored at controlled room temperature (20 °C to 25 °C), in an unopened packet, until needed. For elemental analyses, the packet can be opened and resealed; test portions can be removed and analyzed until the material reaches its expiration date. For other analyses, the packet can be resealed; test portions can be removed and analyzed for several weeks after the packet was first opened.

Use: Prior to removal of a test portion for analysis, the contents of a packet of material should be mixed thoroughly. For certified values to be valid, test portions of the material equal to or greater than 0.1 g for organic acid analyses, 2.5 g for vitamin analyses, and 0.5 g for element analyses should be used. The stability of organic acids, vitamins, and elements in opened packets has not been investigated. Test portions should be analyzed as received and results converted to a dry-mass basis by determining moisture content (described below) on a separate test portion.

Note: This material was packaged as a powder; however, over time the powder may become a solid mass. For hardened samples, a test portion should be removed and subdivided appropriately. The certified and reference values for composition provided in Tables 1 through 8 are valid independent of the sample consistency.

PREPARATION AND ANALYSIS⁽¹⁾

Material Acquisition and Preparation: The material for production of SRM 3287 was a combination (approximately 50/50) of Tifblue and Rubel (highbush and rabbiteye varieties) acquired from the U.S. Highbush Blueberry Council (Folsom, CA), which provided freeze-dried powdered blueberries (40 mesh) in nitrogen-flushed cans. The material was shipped to High-Purity Standards (Charleston, SC), where it was blended, aliquoted, and heat-sealed inside nitrogen-flushed 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel each. Following packaging, SRM 3287 was irradiated (Neutron Products, Inc., Dickerson, MD) to an absorbed dose of 7.2 kGy to 8.4 kGy.

Analytical Approach for Determination of Organic Acids: Value assignment of the concentrations of the organic acids in SRM 3287 was based on the combination of measurements from two different methods: isotope dilution liquid chromatography with mass spectrometric detection (ID-LC/MS) and ion chromatography with conductivity detection (IC-CD). Duplicate test portions of approximately 0.1 g were taken from each of six packets for analysis using each of the methods, and internal standards were added. Organic acids were extracted into water, and the solutions from four such successive extractions were combined.

⁽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.

For analysis by ID-LC/MS, an organic acid column was held at 40 °C. An aqueous mobile phase containing 0.5 % (volume fraction) formic acid was used under isocratic conditions at a flow rate of 0.5 mL/min. The mass spectrometer was operated in negative ion mode, with atmospheric pressure electrospray ionization (AP-ESI). Each organic acid was matched with a ¹³C- or ²H-labeled internal standard, and quantitation was based on response factors calculated from the relative peak areas and concentrations [6].

For analysis by IC-CD, a hydroxide-selective anion exchange column was held at 30 °C, and a flow rate of 1.5 mL/min was used for the separation. Ultrapure water was used for generation of a hydroxide gradient, and a current of 186 mA was applied for suppression of the background conductivity from the hydroxide mobile phase. Quantitation was based on relative peak areas with trifluoroacetic acid (TFA) as an internal standard [6].

Analytical Approach for Determination of Free Water-Soluble Vitamins: Value assignment of the concentrations of free water-soluble vitamins in SRM 3287 was based on ID-LC/MS at NIST with confirmation from data provided by one collaborating laboratory. Duplicate test portions of approximately 2.5 g were taken from each of six packets for analysis, and internal standards were added. Vitamins were extracted into water that contained acetic acid, and the solutions from four such successive extractions were combined.

For analysis by ID-LC/MS, a C₁₈ column with a gradient consisting of a mobile phase of methanol and 20 mmol/L ammonium formate in water was used with a flow rate of 0.8 mL/min. The mass spectrometer was operated with electrospray ionization in the positive ion mode. Each vitamin was matched with a ¹³C-, ¹⁵N-, or ²H-labeled internal standard, and quantitation was based on response factors calculated from the relative peak areas and concentrations [6].

Analytical Approach for Determination of Elements: NIST and collaborating laboratories' data were combined to provide certified values [7]. For NIST analyses, two 0.5 g test portions from each of six packets of SRM 3287 were analyzed for calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc by using inductively coupled plasma optical emission spectrometry (ICP-OES). Test portions were digested in sealed vessels with nitric and hydrofluoric acids using a microwave digestion system. Quantitation was based on the method of standard additions using calibration solutions prepared from the SRM 3100 Series of single-element standard solutions.

Collaborating Laboratories' Analyses: The GMA FIACC laboratories were asked to use their usual methods to make single measurements on test portions taken from each of two packets of SRM 3287 for measurement of nutrients and test portions taken from each of three packets for measurement of antioxidants. The collaborating laboratories' data were combined with NIST data for calculation of certified values for elements. Collaborating laboratories' data alone were used to assign reference values for proximates, sugars, amino acids, and antioxidant capacity. Three antioxidant methods were employed: oxygen radical absorbance capacity (ORAC), Folin-Ciocalteu's reagent (Folin-C), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) as the reagent.

Determination of Moisture: Moisture content of SRM 3287 was determined at NIST (see "Warning and 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 28 d; and (3) drying for 1 h in a forced-air oven at 80 °C. Unweighted results obtained using all three techniques were averaged to determine a conversion factor of (0.9859 ± 0.0065) 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 (0.32 %) 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.

Homogeneity Assessment: The homogeneity of organic acid and element contents was assessed at NIST by using the methods described above. An analysis of variance did not show inhomogeneity for the test portions analyzed (see "Warning and Instructions for Storage and Use").

Value Assignment: The equally weighted means of the mean results from each set of data available were used to calculate the assigned values. The results of the GMA FIACC data are the medians of the collaborating laboratories means.

Certified Mass Fraction Values: The certified mass fraction value for quinic acid is the equally weighted mean of results provided by ID-LC/MS and IC-CD. Certified mass fraction values for free water-soluble vitamins are the mean of results provided by ID-LC/MS at NIST with confirmation by GMA FIACC data. Certified mass fraction values for elements are the equally weighted means of results provided by NIST and the medians GMA FIACC laboratories' means. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence; it expresses both the observed difference between the results

from the methods and their respective uncertainties, incorporating an uncertainty component for moisture correction, consistent with the JCGM Guide and its Supplement 1 [2–4]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the effects of the combined components of uncertainty, and k is a coverage factor corresponding to approximately 95 % confidence for each analyte.

Table 1. Certified Mass Fraction Value for Quinic Acid

	Mass Fraction (mg/g)	k
Quinic Acid	25.53 ± 0.73	2.00

Table 2. Certified Mass Fraction Values for Free Water-Soluble Vitamins

	Mass Fraction (mg/kg)	k
Free Thiamin	1.679 ± 0.030	2.13
Free Niacin ^(a)	2.864 ± 0.090	2.18
Free Pantothenic Acid	3.36 ± 0.19	2.19
Free Pyridoxine ^(b)	1.263 ± 0.020	2.11

^(a) Measured as niacinamide and converted to niacin by multiplication by the ratio of the relative molecular masses.

^(b) Measured as the sum of pyridoxine and pyridoxal, which was converted to pyridoxine by multiplication by the ratio of the relative molecular masses.

Table 3. Certified Mass Fraction Values for Elements

	Mass Fraction (mg/kg)	k
Calcium	323 ± 16	2.00
Copper	2.22 ± 0.16	2.00
Iron	12.20 ± 0.74	2.00
Magnesium	313.7 ± 7.2	2.00
Manganese	8.47 ± 0.59	2.00
Phosphorus	671 ± 21	2.00
Potassium	4490 ± 220	2.00
Zinc	6.49 ± 0.61	2.00

Reference Mass Fraction Values: Reference mass fraction values for organic acids and anions are the means of results provided by IC-CD except for shikimic acid, which was determined by ID-LC/MS. Reference values for proximates, sugars, total dietary fiber, calories, amino acids, and antioxidant capacity are the medians of the means provided by the collaborating laboratories. The reference value for sodium was determined at NIST using ICP-OES. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence [2]. The uncertainty incorporates within-method uncertainty and a component related to moisture correction. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the effects of the combined components of uncertainty, and k is a coverage factor corresponding to approximately 95 % confidence for each analyte.

Table 4. Reference Mass Fraction Values for Organic Acids and Anions

	Mass Fraction (mg/g)	k
Galacturonic Acid	0.130 ± 0.006	2.19
Glycolic Acid	0.171 ± 0.005	2.17
Isocitric Acid	0.225 ± 0.008	2.18
Oxalic Acid	0.089 ± 0.006	2.19
Phosphate	0.777 ± 0.044	2.19
Shikimic Acid	0.438 ± 0.015	2.18
Sulfate	1.17 ± 0.26	2.20

Table 5. Reference Values for Proximates, Sugars, Total Dietary Fiber, Sodium, and Calories

	Mass Fraction (%)			<i>k</i>
Solids	98.59	±	0.65	2.04
Ash	1.126	±	0.084	2.26
Fat	1.40	±	0.37	2.36
Protein	3.43	±	0.30	2.26
Carbohydrate	91.92	±	0.83	2.05
Total Sugars	60.4	±	3.3	2.35
Fructose	30.5	±	1.5	2.34
Glucose	30.5	±	1.4	2.34
Total Dietary Fiber	18.4	±	1.3	2.43
	Mass Fraction (mg/kg)			<i>k</i>
Sodium	16.39	±	0.74	2.19
	Energy (kcal/100 g)			
Calories ^(a)	392	±	10	2.36

^(a) 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 394 kcal/100 g.

Table 6. Reference Mass Fraction Values for Amino Acids

	Mass Fraction (%)			<i>k</i>
Alanine	0.167	±	0.095	3.18
Arginine	0.342	±	0.037	3.15
Aspartic Acid	0.279	±	0.087	3.18
Cysteine	0.056	±	0.023	4.29
Glutamic Acid	0.402	±	0.079	3.17
Glycine	0.165	±	0.006	2.99
Isoleucine	0.110	±	0.034	3.18
Leucine	0.211	±	0.022	3.15
Lysine	0.149	±	0.022	3.17
Methionine	0.061	±	0.007	3.15
Phenylalanine	0.134	±	0.012	3.14
Proline	0.121	±	0.019	3.17
Serine	0.141	±	0.019	3.16
Threonine	0.121	±	0.015	3.16
Tyrosine	0.088	±	0.020	3.17
Valine	0.147	±	0.060	3.18

Table 7. Reference Values for Antioxidant Capacity

Method	Result	Units	<i>k</i>
ORAC	360 ± 41	micromoles Trolox ^(a) equivalents per gram	2.57
Folin-C	31.8 ± 5.4	milligrams gallic acid per gram	2.36
DPPH	416 ± 128	micromoles Trolox ^(a) equivalents per gram	4.30

^(a)Trolox Equivalents: (S)-(-)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid equivalents.

REFERENCES

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Certificate Revision History: 27 March 2013 (Removed certified mass fraction values for citric and malic acid based on stability tests; updated storage and use information; editorial changes); 22 December 2011 (Certified water-soluble vitamins values added; addition of reference values for antioxidant capacity; editorial changes); 14 September 2010 (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>.