National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1544

Fatty Acids and Cholesterol in a Frozen Diet Composite

This Standard Reference Material (SRM) is intended primarily for verifying the accuracy of methods used for the determination of fatty acids and cholesterol in food materials. A unit of SRM 1544 consists of four bottles, each containing approximately 15 g of a frozen composite food material that has been thoroughly ground and blended.

Certified Concentration Values: The certified values for cholesterol and six fatty acids are listed in Table 1. All concentration values are expressed as mass fractions [1] on a wet mass basis.

The certified value for cholesterol is the mean of results from the NIST definitive method for cholesterol [2]. Each certified value for the fatty acids is the equally weighted mean of results from NIST and three collaborating laboratories, Hazleton Laboratories, Madison, WI; Lancaster Laboratories, Lancaster, PA; and Medallion Laboratories, Minneapolis, MN. Each uncertainty, computed according to the CIPM method as described in the ISO Guide [3], is an expanded uncertainty at the 95 % level of confidence which includes uncertainty due to both measurement processes as well as material variability. Each certified value and expanded uncertainty define a range of values within which the true concentration is expected to lie for at least 95 % of the samples.

Noncertified Values: Noncertified values for six proximates are listed in Table 2. These results are the equally weighted means of measurements from the three collaborating laboratories using AOAC official methodologies. The associated uncertainties are expanded uncertainties at the 95 % level of confidence [3].

Information Values: Information values for three nutrient elements and eight additional fatty acids based upon results from two or more of the collaborating laboratories are listed in Table 3. The mean values reported are for those instances when there was agreement between two or more laboratories.

This project was initiated by W.R. Wolf of the U.S. Department of Agriculture (USDA) and the Technical Division on Reference Materials, AOAC International. The overall direction and coordination of the technical measurements leading to the certification of this SRM were performed by M.J. Welch of the NIST Analytical Chemistry Division.

Analytical measurements performed at NIST were by P. Ellerbe, R. Fischer, and L.T. Sniegoski of the NIST Analytical Chemistry Division. Measurements by the collaborating laboratories were performed under contract to the U.S. Department of Agriculture (USDA).

Preparation of this material was provided by the Beltsville Human Nutrition Research Center (BHNRC), USDA, under the direction of W.R. Wolf and J. Holden.

Statistical consultation was provided by S.B. Schiller of the NIST Statistical Engineering Division.

The technical and support aspects involved in the certification and issuance of this SRM were coordinated through the Standard Reference Materials Program by J.C. Colbert and G.V. Iyengar.

Gaithersburg, MD 20899
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NOTICE AND WARNING TO USERS

Warning: SRM 1544 is for laboratory use only. It is not intended for human consumption and could contain unhealthy levels of bacteria if not handled properly.

Storage: Prior to thawing, SRM 1544 should be stored in the dark at temperatures at or below -20 °C.

Expiration of Certification: This certification is valid for one year from the date of shipment from NIST. Should any of the values change before the expiration of the certification, purchasers will be notified by NIST. Return of the attached registration form will facilitate notification.

Instructions for Use: Each vial to be analyzed should be allowed to thaw and warm to room temperature. The contents should then be thoroughly mixed to ensure homogeneous distribution of the lipid materials in the matrix. Sample sizes of less than 1 g are not recommended since the chances of taking a nonrepresentative sample increase with decreasing sample size. Once the material is thawed, samples should be taken and processed immediately. Certified concentrations may not be valid for refrozen material or for material stored at refrigerator or room temperatures for more than a few hours.

Source of the Material: The individual foods chosen and the quantities of each used were designed to provide a composite material with levels of many nutrients in the range found in typical U.S. diets. The foods selected for the composite were combined, ground, and blended at the Food Analysis Laboratory Coordinating Center at Virginia Polytechnic Institute and State University, Blacksburg, VA. The composite material was then reblended and bottled at the Food Composition Laboratory, BHNRC, ARS, USDA, Beltsville, MD. During the bottling process, the mixture was stirred constantly and the temperature maintained in a narrow range to reduce separation of the lipid components.

ANALYTICAL METHODS

Fatty Acids: Certification of the concentrations of the fatty acids in this SRM was based on results from analyses using gas chromatography/mass spectrometry (GC/MS) at NIST and gas chromatography in the collaborating laboratories. The NIST analyses involved spiking a known aliquot of the wet material (typically 1 g) with known quantities of deuterium-labeled internal standards that were analogs of four of the analytes (C12:0, C16:0, C18:0, and C18:1). The samples were then treated with 10 mL of a 1 mol/L solution of NaOH in methanol and refluxed at 60 °C for 30 min. Approximately 2 mL of 6 mol/L HCl was added to neutralize the sample, followed by 5 mL of pH 4 buffer and 0.5 g to 1 g of NaCl (not accurately measured). The solution was extracted three times with 10 mL portions of hexane. One milliliter of the combined hexane extract was transferred to a small tube, dried over sodium sulfate, and treated with 50 μL of 1,1-dimethoxytrimethylamine to form the methyl esters of the fatty acids. Calibration standards consisting of known masses of pure fatty acids and the same deuterated standards added to the samples were prepared and derivatized.

The GC/MS analyses were performed using a standard quadrupole mass spectrometer with a 30 m fused silica capillary column (5 % phenyl substituted methylsiloxane) interfaced directly to the ion source. The mass spectrometer was operated in the selected ion monitoring mode and was set up to measure the molecular ion of each analyte and internal standard as they eluted from the GC column. Calibration curves were constructed based upon the ion intensity ratios measured for the analytes and their corresponding internal standards. For the C14:0 analyte, the response of the pure C14:0 material was ratioed to the deuterated internal standards for C12:0 and C16:0 and the results were averaged. For the C18:2 analyte, the pure standard was ratioed to the deuterated standards for C16:0, C18:0, and C18:1 and the results were averaged. Three sets of six samples each were prepared for analysis.

The three collaborating laboratories each performed single analyses on each of two vials of the material, using a GC method that has been thoroughly tested [4]. Two of the laboratories reported results for a number of fatty acids in addition to those certified, while the third laboratory had results for only one additional fatty acid. Where there was reasonable agreement between two or more laboratories for these additional acids, the mean values are reported as information values in Table 3.

Cholesterol: Cholesterol determinations were performed using the NIST isotope dilution mass spectrometric definitive method for serum cholesterol modified for food materials [2,5]. Three sets of samples were analyzed with each set consisting of two samples from each of three vials.

Proximates: Determinations of the proximates were performed by the three collaborating laboratories. Protein was determined by a modification of the Kjeldahl nitrogen method with a factor of 6.25 used to convert nitrogen to protein [6]. Moisture was determined by weight loss in a vacuum oven [7]. Total fat was determined by an acid hydrolysis method [8]. Ash was determined by weighing the residue left after ignition in a furnace [9]. Total carbohydrate was calculated by subtracting the sum of the percentages of protein, moisture, total fat, and ash from 100 [10]. Similarly, calories were calculated based upon the generally accepted factors of 4, 9, and 4 for calorie equivalents of protein, fat, and carbohydrate, respectively [10].

Table 1. Certified Mass Fractions for Cholesterol and Specific Fatty Acids

Analyte	Common Name	Mass Fraction (in g/kg)
Cholest-5-en-3β-ol	Cholesterol	0.1483 ± 0.0094
Dodecanoic Acid (C12:0)	Lauric Acid	1.31 ± 0.12
Tetradecanoic Acid (C14:0)	Myristic Acid	1.01 ± 0.10
Hexadecanoic Acid (C16:0)	Palmitic Acid	5.77 ± 0.52
Octadecanoic Acid (C18:0)	Stearic Acid	2.00 ± 0.22
(Z)-9-Octadecenoic Acid (C18:1)	Oleic Acid	11.64 ± 0.94
(Z,Z)-9,12-Octadecadienoic Acid (C18:2)	Linoleic Acid	6.56 ± 0.62

Each uncertainty, computed according to the CIPM method as described in the ISO Guide [3], is an expanded uncertainty at the 95 % level of confidence which includes uncertainty due to both measurement processes as well as material variability. Each certified value and expanded uncertainty define a range of values within which the true concentration is expected to lie for at least 95 % of the samples.

Table 2. Noncertified Mass Fractions for Proximates

Proximate	Mass Fraction (in %)
Protein	5.28 ± 0.30
Moisture	73.12 ± 0.79
Total Fat	3.68 ± 0.48
Ash	0.97 ± 0.22
Carbohydrate	16.9 ± 1.2
Calories	1221 cal/kg ± 67 cal/kg

Each uncertainty, computed according to the CIPM method as described in the ISO Guide [3], is an expanded uncertainty at the 95 % level of confidence which includes uncertainty due to within- and between-lab variability as well as material variability. Each value and expanded uncertainty define a range of values within which the concentration, as measured using the AOAC Official Methods, is expected to lie for at least 95 % of the samples. NOTE: the mean result for calories is the mean of caloric calculations from the laboratories. If the mean proximate values below are used with the caloric equivalents of 4, 9, and 4 for protein, fat, and carbohydrate, respectively, the caloric mean is 1218.

Table 3. Information Values (for Fatty Acids and Nutrients) as Mass Fractions

Fatty Acids		Nutrients	
Fatty Acid	Mass Fraction (in g/kg)	Nutrient	Mass Fraction (in g/kg)
Caprylic (C8:0)	0.3	Calcium	0.52
Capric (C10.0)	0.3	Potassium	1.5
Palmitoleic (C16:1)	0.4	Sodium	1.7
Linolenic (C18:3)	0.6		
Arachidic (C20:0)	0.1		
Eicosenoic (C20:1)	0.1		
Arachidonic (C20:4)	0.1		
Behenic (C22:0)	0.1		

The methods used for the fatty acids and the nutrients are given in references [4] and [11], respectively.

REFERENCES

[1] Taylor, B.N., "Guide for the Use of the International System of Units (SI)", NIST Special Publication 811, 1995 Ed., (April 1995).

[2] Ellerbe, P., Meiselman, S., Sniegoski, L.T., Welch, M.J., and White, V.E., *Anal. Chem.*, 61, 1718-23, (1989).

[3] *Guide to the Expression of Uncertainty in Measurement*, ISBN 92-67-10188-9, 1st Ed., ISO, Geneva, Switzerland, (1993). See also: Taylor, B.N. and Kuyatt, C.E., "Guidelines for Evaluation and Expressing the Uncertainty of NIST Measurement Results", NIST Technical Note 1297, (1994).

[4] *Official and Tentative Methods of the American Oil Chemists Society*, Method CE 1-62, AOCS, Champaign, IL, (1981) modified.

[5] Hydrolysis Procedure Used: Method 4.235, *Official Methods of Analysis*, 13th Ed., AOAC, Gaithersburg, MD (1980).

[6] *Official Methods of Analysis*, 15th Ed., Method 955.04C, 979.09, AOAC, Gaithersburg, MD, (1990) modified.

[7] *Official Methods of Analysis*, 15th Ed., Method 926.08, 925.09, AOAC, Gaithersburg, MD, (1990) modified.

[8] *Official Methods of Analysis*, 15th Ed., Method 922.06, 954.02, AOAC, Gaithersburg, MD, (1990) modified.

[9] *Official Methods of Analysis*, 15th Ed., Method 923.03, AOAC, Gaithersburg, MD, (1990) modified.

[10] *Methods of Analysis for Nutrition Labeling*, Sullivan, D.M. and Carpenter, D.E., eds, AOAC INTERNATIONAL, Gaithersburg, MD, p. 106, (1993).

[11] Official Methods of Analysis 15th Ed., Method 984.27 (calcium) and Method 985.35 (sodium and potassium), AOAC, Gaithersburg, MD, (1990) modified.