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FEATURE ARTICLE

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Introduction

After passage of the Infant Formula Act (IFA) of 1980 [1] and the Nutrition Labeling and Education Act (NLEA) of 1990 [2], the National Institute of Standards and Technology (NIST) expanded an effort to produce food-matrix Standard Reference Materials (SRMs). The IFA set specific minimum and/or maximum allowable levels for protein, fat, 13 vitamins, 11 minerals, and linoleic acid, as well as related labeling guidelines for infant formulas sold in the United States (US). Similarly, the NLEA requires specific nutrition information to be included on labels of processed foods sold in the US. Each label must identify the amount per serving of calories, total and saturated fat, cholesterol, total carbohydrate, dietary fiber, sugars, protein, vitamins A and C, and minerals sodium, calcium, and iron. Information about the content of other vitamins and minerals may be included voluntarily, but should be reported accurately. The NLEA also requires that all health and nutrient content claims (e.g., low in saturated fat, good source of calcium) meet US Food and Drug Administration (FDA) regulations.

The FDA last conducted a non-targeted study on accuracy of food labeling in 1996. In this study, 300 food products were randomly selected and tested by a contract laboratory for compliance with mandatory labeling requirements [3]. In all, 1,976 of the 2,174 analyses (91 %) conducted were consistent with label claims (within the tolerances set by the FDA). Of those claims, results were most often consistent between laboratory and label for carbohydrates, total fat, protein, sugar, calories, saturated fat, and sodium, each with 90 % or greater consistency. Vitamin C was accurately labeled on 88 % of the products, while cholesterol, calcium, and dietary fiber analyses were only consistent with labels on 80 % of products. Labels were much less accurate for iron and vitamin A, with laboratory-to-label consistency of only 69 % and 54 %, respectively. In 2008, the Government Accountability Office (GAO) released a report detailing the accuracy of food labeling based on a targeted FDA study of 868 domestic and 783 imported food products from 2000 through 2006. [4]. Of these products, 21 % of domestic and 28 % of imported foods were found to be in violation of labeling regulations. The most common violations were incorrect or omitted nutrient information, undeclared ingredients, and incorrect formatting of nutrition labels. Penalties for violation of labeling regulations include first the issuance of warning letters, followed by enforcement actions such as seizures, injunctions, and import alerts or refusals. The ongoing inconsistencies between nutrition labels and laboratory results indicate that food manufacturers and contract laboratories are in need of improved analytical methods and reference materials for analysis of nutrients in foods.

To assist food manufacturers in quality control and the FDA in enforcing IFA and NLEA regulations, reference materials with assigned concentrations of nutrients are needed as matrix-matched controls for analytical measurements. By using matrix-matched reference materials in quality control, laboratories can improve measurement accuracy, thereby improving compliance with labeling regulations. Similarly, quality control schemes that include reference materials demonstrate to the FDA that analytical measurements are likely to be accurate. The food-matrix SRM effort at NIST has been designed to meet the needs of these user communities, and to provide a range of materials with varying fat, protein, and carbohydrate content that can serve

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as control materials for the wide variety of foods sold in the US [5–9].

Material selection

As discussed previously [10], NIST classifies food-matrix SRMs based on fat, protein, and carbohydrate content using a triangle developed by AOAC International (Fig. 1) [11, 12]. This triangle is based on the supposition that foods within each sector will have similar properties and therefore will pose similar challenges in analysis of the same nutrient. Likewise, a reference material will serve as an appropriate control material in the analysis of foods within that fat/protein/carbohydrate sector of the triangle. Selection of NIST food-matrix SRMs has been based on the distribution of foods from a typical US diet in the AOAC triangle [11, 12]. A majority of common foods are categorized in sectors 5 and 6 (e.g., fruits, vegetables, cereals, and grains), as are a majority of food-matrix SRMs. Conversely, only a small fraction of common foods are categorized in sectors 1 through 4 (higher-fat foods like meats and nuts), so fewer SRMs have been produced in those sectors. Additional materials are also prepared based on suggestions from user communities.

Material acquisition and preparation

Once a material has been identified for production as an SRM, an appropriate source (or sources) must be identified and the material must be acquired. In some cases, materials are prepared by a manufacturer as part of a single production lot and provided to NIST prepackaged. Other materials are prepared from commercially available materials that are collected or purchased in bulk and homogenized at NIST. If necessary, solid materials are ground in a blender or a disk mill and sieved. Liquid materials may be stabilized with an antioxidant (such as butylated hydroxytoluene or *tert*-butylhydroquinone) if necessary.

The final homogenized material is then packaged to maximize analyte stability. Recently, food materials have been heat-sealed inside nitrogen-flushed 4 mil polyethylene bags, which are then sealed in nitrogen-flushed aluminized plastic bags with silica gel packets, an approach which was developed to keep hygroscopic dietary supplement materials dry. Liquid materials are sealed in ampoules (either clear or opaque glass) and blanketed with argon when necessary to enhance stability. When possible, the order in which materials are packaged is carefully documented to allow future sampling and subsequent homogeneity assessment of the production lot. When necessary, materials may be irradiated after packaging to kill microbial contaminants and increase long-term stability. The final materials are then placed in long-term storage under appropriate conditions.

Categories of assigned values

A certified reference material (CRM) is a well-characterized homogenous substance that is provided with a certificate of analysis (COA) including one or more certified values, associated uncertainty, and a statement of metrological traceability [13]. A CRM issued by NIST that meets additional NIST-specific criteria is a Standard Reference Material, or SRM [14]. A COA from NIST contains a plethora of information about the material, including the intended use, an expiration date, and recommendations for proper storage and handling (Fig. 2). Analytical methods used for characterization and quantitative values with uncertainties for analytes of interest are also included in the COA (Fig. 3). Certified values may be assigned by using a single primary

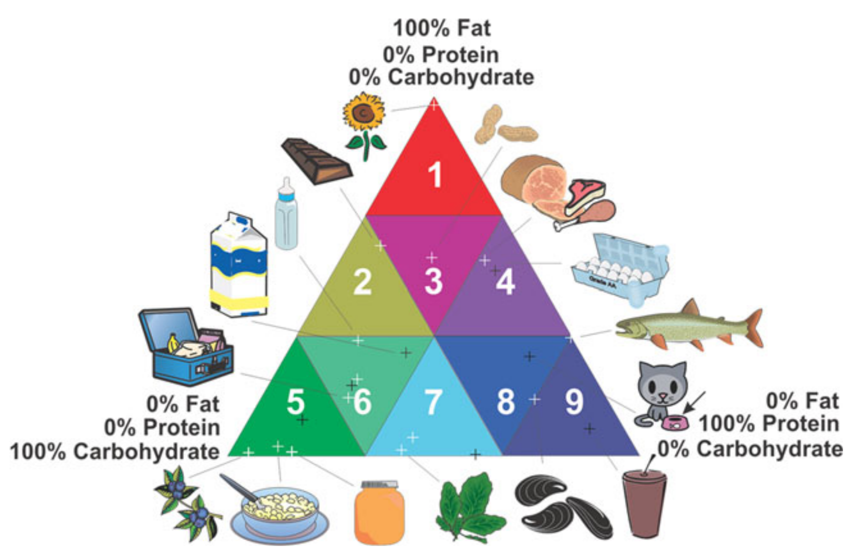
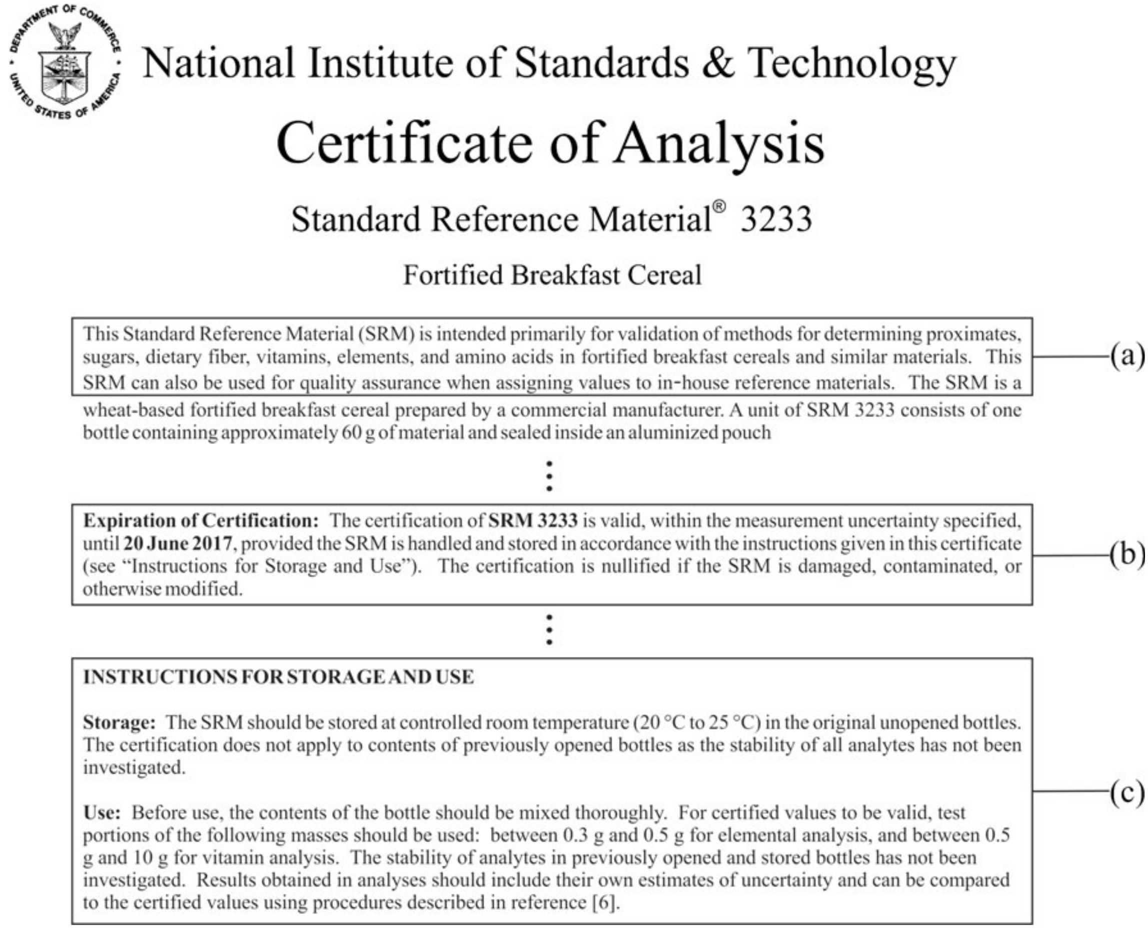


Fig. 1 The AOAC fat/protein/carbohydrate food composition triangle with NIST food-matrix SRM locations. Locations of materials in preparation are shown with black symbols


National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 3233

Fortified Breakfast Cereal

This Standard Reference Material (SRM) is intended primarily for validation of methods for determining proximates, sugars, dietary fiber, vitamins, elements, and amino acids in fortified breakfast cereals and similar materials. This SRM can also be used for quality assurance when assigning values to in-house reference materials. The SRM is a wheat-based fortified breakfast cereal prepared by a commercial manufacturer. A unit of SRM 3233 consists of one bottle containing approximately 60 g of material and sealed inside an aluminized pouch (a)

⋮

Expiration of Certification: The certification of **SRM 3233** is valid, within the measurement uncertainty specified, until **20 June 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. (b)

⋮

INSTRUCTIONS FOR STORAGE AND USE

Storage: The SRM should be stored at controlled room temperature (20 °C to 25 °C) in the original unopened bottles. The certification does not apply to contents of previously opened bottles as the stability of all analytes has not been investigated.

Use: Before use, the contents of the bottle should be mixed thoroughly. 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. The stability of analytes in previously opened and stored bottles has not been investigated. 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]. (c)

Fig. 2 Selected portions of the Certificate of Analysis for SRM 3233 Fortified Breakfast Cereal, demonstrating sections describing (**a**) intended use, (**b**) expiration, and (**c**) storage and handling. The complete certificate is available at http://www.nist.gov/srm

method at NIST with confirmation by other methods, by using two or more independent critically-evaluated methods at NIST, or by using one NIST method combined with other methods by external collaborating laboratories [14]. All of these approaches are utilized for certification of nutrients in food-matrix SRMs. In some cases, the measurement of a nutrient does not meet the NIST criteria for assignment of a certified value, one in which NIST has the highest confidence in its accuracy and potential sources of bias have been investigated or taken into account. When this occurs, data from external laboratories or from a single NIST method can be used to assign reference or information values. Reference values are the best estimate of the true value where sources of bias have not been fully investigated, and are typically less precise than certified values. Information values are provided when insufficient information is available to assess the uncertainty, but the value may be of interest to the user. In the past, NIST also released certificates in which reference and information values are grouped together as "non-certified" values, but that terminology has been replaced. Certificates of Analysis for all NIST SRMs can be found at http://www.nist.gov/srm.

Available food SRMs

Plant and fish oils

Sector 1 of the AOAC food composition triangle contains foods with approximately 66 % to 100 % fat content, and the peak of the triangle corresponds to fats and oils. The most common analytes of interest in these materials are fatty acids, with which some foods are fortified. The possible health benefits of fatty acids have also made plant and fish oils one of the most common dietary supplements. NIST has developed a variety of oil SRMs that can be used as control materials for foods with very high fat content [15, 16]. The first food oil material, SRM 1563 Cholesterol and Fat-Soluble Vitamins in Coconut Oil (Natural and Fortified), was released in 1987. The material was prepared from natural coconut oil with added butylated hydroxytoluene as an antioxidant. The batch was heated and subdivided, and the fortified oil was prepared by addition of cholesterol and fat-soluble vitamin solutions prepared in toluene. The COA for SRM 1563 provided certified values for cholesterol, vitamin D, and vitamin E in the fortified material, as well

Fig. 3 Selected portions of the Certificate of Analysis for SRM 3233 Fortified Breakfast Cereal, demonstrating sections describing (**d**) analytical methods and (**e**) certified values. The complete certificate is available at http://www.nist.gov/srm

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 3233

Fortified Breakfast Cereal

⋮

SOURCE, PREPARATION, AND ANALYSIS[1]

Source and Preparation: The SRM is a fortified breakfast cereal, prepared by a commercial manufacturer. Two hundred kilograms (450 lbs) of fortified breakfast cereal was received as flakes in a single large box. The contents of the box were ground to 180 µm (80 mesh), blended, and bottled by High-Purity Standards (Charleston, SC). The cereal powder was placed in 4 oz amber bottles that had been flushed with nitrogen. Each bottle contains 60 g of cereal powder. The bottles were capped and sealed with heat-shrink tape, then individually sealed in Mylar bags. Following bottling, SRM 3233 was irradiated by Neutron Products, Inc. (Dickerson, MD) to an absorbed dose of 9.0 kGy to 11.5 kGy.

Analytical Approach for Determination of Vitamins: Value assignment of the mass fractions of the vitamins in SRM 3233 was based on the combination of results provided from various analytical methods at NIST, USDA, and collaborating laboratories.

NIST Analyses for Fat-Soluble Vitamins: Vitamin A (as retinyl palmitate) and vitamin E (as α-tocopheryl acetate) were measured at NIST using liquid chromatography with mass spectrometric detection (LC/MS). Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the fat-soluble vitamins in the SRM. Internal standards were employed; a single solution was used for the calibrants and samples.

Retinyl Palmitate and α-Tocopheryl Acetate: Duplicate 10 g test portions of powder from each of 12 bottles were accurately weighed into 50 mL polyethylene centrifuge tubes, and internal standard solutions containing tocol and retinyl palmitate-d_4 were added. Analytes were extracted into hexane by sonication and mixing/rotation for 60 min three times. Three additional extractions were performed using sonication in ethyl acetate and 60 min of mixing/rotation three times. The supernatants for the individual test portions were combined and were evaporated to approximately 25 mL under nitrogen. The extracts were washed with water, the organic phase was evaporated to dryness, and the residue was reconstituted in ethanol. Separations were performed on a C_{18} column with an isocratic mobile phase of 60 % methanol and 40 % acetonitrile containing 5 mmol/L ammonium acetate. Retinyl palmitate, retinyl palmitate-d_4, tocol, and α-tocopheryl acetate were monitored at m/z 269, 273, 388, and 473, respectively. Retinyl palmitate was not homogeneously distributed in SRM 3233, with values ranging between 2 µg/g and 12 µg/g, and a value for retinol could not be assigned. (d)

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Certified Mass Fraction Values for Selected Vitamins: Each certified mass fraction value is the mean from the combination of the mean results from each set of analyses by NIST, the median of the mean of results provided by collaborating laboratories, and the mean result provided by the material manufacturer, where available. 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 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 3233

	Mass Fraction (mg/kg)		Coverage Factor, k
Thiamine (Vitamin B_1)[a,b,c,e]	60.2	9.4	2.00
Riboflavin (Vitamin B_2)[b,d,e]	76	2	2.00
Niacinamide[c,e]	799	27	2.00
Total Vitamin B_3 as Niacinamide[b,e,g]	822	39	2.00
Pantothenic Acid[b,c,e]	540	40	2.00
Pyridoxine[c,e]	78.0	4.7	2.00
Total Vitamin B_6 as Pydridoxine[b,e,h]	81.9	9.0	2.00
Folic Acid[b,e,f]	15.1	1.2	2.00
Total α-Tocopherol (Vitamin E)[b,c,i]	1350	220	2.00

(e)

[a] Reported as thiamine ion (265.36 g/mol), not chloride or chloride hydrochloride
[b] Collaborating laboratories
[c] NIST ID-LC/MS
[d] NIST LC/MS
[e] USDA
[f] NIST ID-LC/MS/MS
[g] Measured as the sum of niacinamide and niacin, which was mathematically converted to niacinamide by multiplication by the ratio of the relative molecular masses.
[h] Measured as the sum of pyridoxine and pyridoxal, which was mathematically converted to pyridoxine by multiplication by the ratio of the relative molecular masses
[i] α-Tocopherol was added to SRM 3233 as RRR-α-tocopheryl acetate. This certified value is expressed as α-tocopherol equivalents and includes "naturally occurring" α-tocopherol as well as the α-tocopherol acetate that was added.

as reference values for fat and 6 fatty acids in both materials. Information values were also provided for cholesterol and vitamins A, D, and E in the natural oil, vitamin A in the fortified oil, and 4 fatty acids in both oils. SRM 1563 was discontinued in 2010 due to instability of a number of the vitamins of interest.

SRM 1588 Organics in Cod Liver Oil, prepared from cod liver oil fortified with six chlorinated contaminants, was released in 1989 with a certified value for α-tocopherol in addition to certified and non-certified values for numerous organic contaminants, including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and chlorinated pesticides. The replacement material, SRM 1588a, was the same material released in 1998 with a reference value for α-tocopherol in addition to certified, reference, and information values for numerous organic contaminants. Subsequently, SRM 1588b, again the same original material, was released in 2006 with certified values for 15 fatty acids and reference values for 5 fatty acids and α-tocopherol. Certified, reference, and information values for PCB and PBDE congeners as well as chlorinated pesticides were also provided on the COA. A new material, SRM 1588c Organics in Fish Oil, is composed of menhaden oil (*Brevoortia tyrannus*) collected in 2003 and 2004. The material was released in 2011 with certified values for 44 PCBs, 8 chlorinated pesticides, 7 PBDEs, and 18 fatty acids.

SRM 3274 Botanical Oils Containing Omega-3 and Omega-6 Fatty Acids contains a suite of commercial botanical oils (borage [*Borago officinalis*], evening primrose [*Oenothera biennis*], flax [*Linium usitatissimum*], and perilla [*Perilla frutescens*]) and was released in 2009 with certified values for 22 fatty acids. SRM 3276 Carrot Extract in Oil was purchased commercially and combined with butylated hydroxytoluene as an antioxidant to extend the shelf life of the material. The material was released in 2007, and the current COA contains certified values for 12 fatty acids and 2 tocopherols [17]. SRM 3278 Tocopherols in Edible Oils was prepared from soybean, canola, safflower, and sunflower oils to yield a 1:1 ratio of α-tocopherol and γ-tocopherol. The material was released in 2009 with certified values for four tocopherols [18]. Each of these SRMs has a distinct profile of fatty acids and vitamins resulting from each unique oil source. The collective provides a suite of materials designed for use as control materials in the analysis of food oils and other matrices in sector 1 of the food composition triangle.

Baking chocolate

The second row of the AOAC food triangle (sectors 2 through 4) contains foods with high fat content (approximately 50 %), but also with significant proportions of protein and carbohydrates. For example, commercial baking chocolate has a normalized fat/protein/carbohydrate profile of approximately 55/15/30, which corresponds to sector 2 on the AOAC food composition triangle. SRM 2384 Baking Chocolate was prepared from 100 % cocoa beans and was released in 2002 with certified values for fat and 11 fatty acids, as well as caffeine, theobromine, calcium, iron, and catechins [19, 20]. Reference values are also provided for proximates and calories, vitamins, theophylline, procyanidins, acrylamide, and additional elements. This SRM is an appropriate control material for samples in sector 2 of the AOAC food composition triangle, such as avocados and black olives.

Peanut butter

Sector 3 also contains foods with approximately 50 % fat, but a more equal distribution of protein and carbohydrates than foods in sector 2. Commercial peanut butter is categorized into sector 3, with a normalized fat/protein/carbohydrate profile of approximately 55/25/20. SRM 2387 Peanut Butter was prepared by a commercial manufacturer of peanut butter from roasted peanuts ground with sugar, partially hydrogenated vegetable oils, and salt. The material was released in 2003 with certified values for fat and 12 fatty acids, 9 elements, and 4 tocopherols [21]. Reference values are also provided for proximates, calories, 18 amino acids, 4 vitamins, and aflatoxin and acrylamide contaminants. This SRM is an appropriate control material for samples in sector 3 of the AOAC food composition triangle, including other nuts and nut products.

Meat and eggs

Foods in sector 4 of the AOAC triangle also contain approximately 50 % fat, but have higher protein content than foods in sectors 2 and 3. Meat and egg products are typically categorized into sector 4, with normalized fat/protein/carbohydrate profiles of approximately 50/45/5. The first meat SRM produced by NIST was SRM 1546 Meat Homogenate, which is a mixture of pork, mechanically-separated chicken, ham, salt, sucrose, water, and spices. SRM 1546 was prepared by a commercial process that included cooking, grinding, blending, and sieving prior to canning under sterile conditions. The material was released in 1999 and currently has certified values for 7 fatty acids, cholesterol, and 6 elements. Reference values are also provided for an additional 6 fatty acids and 5 elements, proximates, calories, 6 vitamins, sucrose, and 18 amino acids [22]. SRM 1546a is currently being developed as a renewal material to replenish depleted stock of SRM 1546. Assigned values for proximates, elements, vitamins, fatty acids, cholesterol, and amino acids will be provided on the COA for SRM 1546a. These SRMs are intended as control materials for meat products and similar samples in sector 4 of the AOAC food triangle.

An additional meat material based on bovine liver, a low fat animal tissue (15 % fat), has also been developed. Prior to the introduction of food-matrix SRMs with values assigned for organic nutrients, NIST had a long history of providing food-matrix materials with values assigned for elements. Bovine liver is one of these early materials and would be classified in sector 8 of the food triangle, providing variety for users analyzing lower fat meats. SRM 1577 Bovine Liver was prepared from blended, frozen, and lyophilized bovine livers and was released in 1972 with certified values for 16 elements and non-certified values for an additional 13 elements. The replacement material, SRM 1577a, was released in 1982 with certified values for 23 elements and non-certified values for an additional 5 elements. The subsequent material, SRM 1577b, was released in 1991 with certified values for 18 elements and non-certified values for an additional 8 elements. The current iteration, SRM 1577c, was released in 2009 with certified values for 20 elements, reference values for 8 elements, and information values for 2 elements [23, 24]. These materials are intended primarily for use in evaluating the accuracy of analytical methods for elements in low-fat animal tissues and other related biological materials in sector 8 of the food composition triangle.

In 1989, NIST made available SRM 1845 Cholesterol in Whole Egg Powder consisting of dried and sterilized Grade A (Canada) chicken eggs with an added anticaking agent. In a joint effort with Agriculture Canada, a portion of this same material was packaged as RM 8415 Whole Egg Powder, with reference values for proximates, 27 elements, 9 vitamins, 11 fatty acids, and an expiration date of 2011 [25]. Both of these materials are no longer available. A more encompassing material, SRM 1845a Whole Egg Powder, is under development as a replacement for SRM 1845 and RM 8415. Certified values for proximates, elements, vitamins, fatty acids, cholesterol, and amino acids will be provided on the COA for SRM 1845a. These SRMs are intended as control materials for eggs, egg products, and similar samples in sector 4 of the AOAC food composition triangle. NIST also offers RM 8445 Spray-Dried Whole Egg for Allergen Detection (without added stabilizers) with a reference concentration for total protein for use in the evaluation of allergen test kits.

Dairy

Foods that have a lower proportion of fat (10 % to 30 %) are categorized into the bottom sectors (5 through 9) of the AOAC food composition triangle. Dairy products comprise one group of such foods, with an approximate fat content of 30 % and a location in sector 6 of the food composition triangle. NIST has a number of milk-based SRMs available, which began with the 1984 release of SRM 1549 Non-fat Milk Powder, a spray-dried, skimmed milk material. This SRM actually contained 1 % fat, and the COA reported certified values for 17 elements and non-certified values for 11 elements, ascorbic acid, and lactose. NIST then released RM 8435 Whole Milk Powder in 1993 as a joint effort with Agriculture Canada. This RM reported reference values for proximates, 19 elements, 6 vitamins, and 11 fatty acids, and information values for an additional 10 elements, 5 fatty acids, and 5 vitamins [25]. Following the discontinuation of RM 8435 in 2009, NIST began developing SRM 1549a Whole Milk Powder as a replacement for SRM 1549 and RM 8435. Values will be assigned for proximates, vitamins, elements, fatty acids, cholesterol, and amino acids in this material. SRM 1549a will be intended for use as a control material for milk, milk products, and similar matrices in sector 6 of the AOAC food composition triangle.

In addition to bovine milk, NIST has developed SRMs based on human milk for contaminant testing. Human milk has a fat/protein/carbohydrate profile similar to that of bovine milk, and is also classified in sector 6 of the AOAC food composition triangle. SRM 1953 Organic Contaminants in Non-Fortified Human Milk and SRM 1954 Organic Contaminants in Fortified Human Milk are designed for use in the determination of contaminants in human milk and have certified values for PCB and PBDE congeners as well as chlorinated pesticides. Released in 2009, these materials were prepared from pooled samples of human milk (total of 100 L) and also have reference values assigned for 9 elements. The most common substitute for human milk is milk-based infant formula, which can consist of a powdered material prepared from fortified liquid bovine milk. In 1996, NIST released SRM 1846 Infant Formula, prepared from a spray-dried formula base combined with a dry-blend vitamin premix. Certified values were provided on the COA for 6 vitamins and iodine [26]. The COA also included reference values for proximates, calories, 9 additional vitamins, 9 elements, and 11 fatty acids, and information values for an additional 2 elements and 7 fatty acids. In 2009, SRM 1846 was replaced by SRM 1849 Infant/Adult Nutritional Formula, prepared from a base liquid containing all constituents that was conventionally heat processed, homogenized, and then spray-dried. The COA for SRM 1849 contained certified values for 16 fatty acids, 13 elements, and 14 vitamins, and reference values for proximates, cholesterol, lactose, calories, 6 fatty acids, 4 vitamins, selenium, 19 amino acids, and 4 nucleotides [27]. The popularity of SRM 1849 led to a rapid depletion of the stock, and SRM 1849a was released in 2011 as a replacement. The certificate for SRM 1849a includes certified values for 16 fatty acids, cholesterol, 13 elements, and 13 vitamins, and reference values for proximates, lactose, calories, 5 fatty acids, 6 fatty acid groups, 3 vitamins, 18 amino acids, and 4 nucleotides. These materials are intended for use in the validation of methods for determining the aforementioned compounds in human breast milk and substitutes such as infant formula, as well as adult nutritional formulas and similar materials in sector 6 of the food composition triangle.

Composite diets

Also in sector 6 of the food composition triangle are mixed diet materials that are often utilized in nutrition and food research. These composite diets are prepared from prescribed quantities of food items that represent a menu cycle spanning multiple days and have a composition profile of approximately 20/20/60 fat/protein/carbohydrate. SRM 1548 Total Diet was a freeze-dried, pulverized, sieved, and radiation sterilized homogenate released in 1990 with certified values for proximates, calories, and 14 elements and non-certified values for an additional 10 elements. The replacement material, SRM 1548a Typical Diet, was released in 1998 with certified values for 20 elements and reference values for proximates, calories, and 4 additional elements. Information values are also included for 7 additional elements. A similar frozen material, SRM 1544 Fatty Acids and Cholesterol in a Frozen Diet Composite, was released in 1996 with certified values for cholesterol and 6 fatty acids. The COA also lists non-certified values for proximates, calories, 3 elements, and 8 additional fatty acids. These materials are intended for use as control materials in the analysis of mixed diets and similar food matrices in sector 6 of the food composition triangle.

Fish and shellfish

Many fish and shellfish are classified into sectors 8 and 9 of the food composition triangle, with approximately 60 % protein. Fish such as trout contain about 35 % fat and fall into sector 9 close to the border of sector 8. Shellfish such as oysters and mussels have much lower fat content (15 %) and fall nearer to the bottom of the triangle in sectors 8 and 9. To address reference material needs for fish and shellfish, NIST has developed a number of SRMs, many of which target the environmental arena with certified values for PCBs, PBDEs, polycyclic aromatic hydrocarbons (PAHs), and pesticides. Nutrient information is included on COAs for some of these SRMs, such as SRM 1946 Lake Superior Fish Tissue and SRM 1947 Lake Michigan Fish Tissue, both frozen fish tissue homogenates prepared from fillets of adult lake trout (*Salvelinus namaycush*) collected from Lake Superior and Lake Michigan, respectively, in 1997. SRM 1946 was released in 2003 and the COA contains certified values for 13 fatty acids and 4 elements, as well as reference values for proximates, 12 additional fatty acids, and 9 additional elements. SRM 1947 was released in 2007 and the COA contains certified values for 9 elements and reference values for proximates, calories, and 16 fatty acids. These materials are designed primarily for use as control materials in the analysis of fish tissue and similar matrices in sectors 8 and 9 of the food triangle.

A number of freeze-dried shellfish SRMs have also been developed at NIST for use as control materials. The first material, SRM 1566 Oyster Tissue, was released by NIST in 1979 with certified values for 20 elements and non-certified values for 10 additional elements. The replacement material, SRM 1566a, was released in 1989 with certified values for 25 elements and non-certified values for 16 additional elements. The third in the series, SRM 1566b, was released in 2001 with certified values for 23 elements and reference values for proximates and 8 additional elements. A number of frozen mussel homogenates have also been developed at NIST with certified values for PAHs, PCBs, and pesticides. SRM 1974 Organics in Mussel Tissue (*Mytilus edulis*) was released in 1990 and included non-certified values for 34 elements. The replacement material, SRM 1974a, was released in 1995 with a certified value for mercury and reference values for 32 additional elements. The subsequent release, SRM 1974b, was released in 2003 with a certified value for mercury and reference values for 11 additional elements. SRM 1974c was released in 2012 with values for PAHs, PCBs, PBDEs, and chlorinated pesticides, and addition of values for nutrients and total arsenic is planned. In 2009, SRM 2974 Organics in Freeze-Dried Mussel Tissue (*Mytilus edulis*) was released as a freeze-dried preparation of the same material used for SRM 1974a. The COA for SRM 2974 contained a certified value for mercury and reference values for 32 additional elements. This material was replaced in 2010 by SRM 2974a, the COA for which also listed a certified value for mercury and reference values for 32 additional elements. This large suite of materials is best suited for use as control materials in the analysis of marine bivalve tissues, foods, or similar materials in sector 8 of the food composition triangle.

Corn

The remaining food materials developed at NIST have very low fat content (less than 10 %) but a varying distribution of protein and carbohydrates to address additional challenges in food analysis. To serve as control materials for foods with nearly 100 % carbohydrate content, which are in sector 5 of the food triangle, RM 8432 Corn Starch and RM 8433 Corn Bran were released in 1993 as a collaboration with Agriculture Canada. RM 8432 was purchased from a commercial manufacturer of corn starch and was radiation sterilized and sieved post-acquisition. The report of investigation listed reference concentrations for proximates, calories, and 14 elements. Information values were also provided for 5 fatty acids and an additional 10 elements. RM 8433 was purchased from a commercial manufacturer, then radiation sterilized and sieved post-acquisition. The report of investigation listed reference concentrations for proximates, calories, and 26 elements. Information values were also provided for fat, 8 fatty acids and an additional 4 elements. Although these materials are no longer available from NIST, they were part of a past effort to develop reference materials for use in validating analytical methods for determination of nutrients in corn and similar matrices in sector 5 of the food composition triangle.

Berries

A suite of *Vaccinium* berry materials has also been developed, containing materials designed for use in both the food and dietary supplement industries. The food materials include SRM 3281 Cranberry (Fruit), SRM 3282 Low-Calorie Cranberry Juice Cocktail, and SRM 3287 Blueberry (Fruit), all of which were released in 2010. SRM 3281 was prepared from frozen, freeze-dried, and ground cranberries, and the COA contains reference values for 9 elements [28]. SRM 3282 was prepared from commercially available products, and the COA contains certified values for 6 elements and reference values for 2 additional elements [28]. SRM 3287 was prepared from frozen, freeze-dried, and ground blueberries, and the COA contains certified values for 8 elements and 4 vitamins, and reference values for proximates, calories, sodium, and 16 amino acids [28]. All of the COAs for berry materials also list certified and/or reference values for organic acids as well as reference values for antioxidant content and sugars. In the future, NIST plans to add values for anthocyanins and/or anthocyanidins, the primary antioxidants in *Vaccinium* berries. These berry SRMs are intended for use in validating analytical methods for determination of nutrients in berries and similar matrices in sector 5 of the food composition triangle.

Fortified breakfast cereal

Also in sector 5 of the food composition triangle is a new material, SRM 3233 Fortified Breakfast Cereal, with a normalized fat/protein/carbohydrate profile of 2/8/90. Cereal flakes were ground and homogenized for use as a control material in the analysis of fortified cereals and similar materials. SRM 3233 was released in 2012 with certified values for 12 elements and 9 vitamins. Reference values are also provided for proximates, calories, 3 additional elements, 4 additional vitamins, 5 fatty acids, and 17 amino acids. This material is important for the food industry because the vitamin and mineral content is fortified, and therefore the concentrations of these compounds are high and the vitamin forms are predictable. This material is also notable because method-specific dietary fiber values are assigned.

Baby food

For food samples with low fat content (less than 5 %) but slightly higher protein content (10 % to 15 %), SRM 2383 Baby Food Composite was released in 1997. The baby food was prepared by a commercial baby food manufacturer from orange juice, infant formula, corn, rice flour, creamed spinach, carrots, papaya juice, tomato paste, beef, macaroni, wheat flour, non-fat milk, Romano cheese, soya protein, onion powder, green pepper, celery oil, and oregano oil. The COA for SRM 2383 reports certified values for 9 vitamins and carotenoids, as well as reference values for an additional 19 vitamins and carotenoids, proximates, calories, cholesterol, 11 fatty acids, and 10 elements [29, 30]. Information values are provided for an additional 10 fatty acids, 5 vitamins, 3 elements, and sugars. After the stock of this material was depleted, a replacement material, SRM 2383a, was prepared with a slightly different composition and therefore slightly different innate nutrient levels. SRM 2383a was prepared from water, orange juice concentrate, corn, rice flour, papaya puree, spinach, macaroni, carrots, tomato paste, and non-fat milk powder. The material was released in 2012 with certified values for 9 water-soluble vitamins and 12 elements, as well as reference values for proximates, calories, and an additional 2 water-soluble vitamins and 3 elements. Addition of values for fat-soluble vitamins and carotenoids is also planned. These materials are intended for use in validation of methods for determination of proximates, calories, vitamins, and elements in food matrices with fat/protein/carbohydrate profiles in sector 5 of the food composition triangle.

Flours

A suite of flour materials has also been developed for analysis of food samples with low fat content (less than 5 %). The suite contains a variety of commercial flours with varying protein levels, including rice flour (7 % protein), wheat flour (15 % protein), defatted soy flour (60 % protein), and wheat gluten (85 % protein). SRM 1567 Wheat Flour and SRM 1568 Rice Flour were released in 1978 with certified and non-certified values for elements. Updated materials were released in 1987 and 1988, respectively. SRM 1567a was certified for concentrations of 14 elements, and concentrations of 12 additional elements were provided as non-certified values. The COA for SRM 1568a lists certified values for 17 elements and non-certified values for 10 additional elements. SRMs 1567b and 1568b are currently being characterized, and values for arsenic species (including organoarsenics) will be assigned in SRM 1568b. As a joint project with Agriculture Canada, RM 8418 Wheat Gluten and RM 8436 Durum Wheat Flour were purchased from commercial manufacturers, radiation sterilized, and sieved before being released in 1993. RM 8418 was provided with reference values for proximates, calories, 24 elements, and 6 fatty acids. Information values were also provided for dietary fiber, 8 additional elements, and 7 additional fatty acids. RM 8436 was provided with reference values for proximates, calories, 27 elements, and 5 fatty acids. Information values were also provided for 3 additional elements and 1 additional fatty acid. These materials are no longer available from NIST. SRM 3234 Soy Flour was released in 2012 with a more extensive nutrient profile, including certified values for 8 elements and 7 vitamins, as well as reference values for proximates, calories, sodium, carnitine, 6 fatty acids, and 18 amino acids. Addition of values for isoflavones, common marker compounds in soy products, is also planned. These materials are intended to serve as control materials in the analysis of flours and other agriculturally related food products in sectors 5 (rice and wheat flours) and 7 (soy flour) of the food composition triangle.

Spinach

For more variety in the analysis of plant materials, SRMs have been prepared from spinach leaves. The first material, SRM 1570 Trace Elements in Spinach, was prepared from lyophilized spinach leaves and was released in 1976. The COA for SRM 1570 contained certified values for 16 elements, and the concentrations of 11 additional elements were provided as noncertified values. The replacement material, SRM 1570a, was released in 1994 with certified values for 18 elements and reference values for proximates and 5 additional elements. Information values were also provided on the COA for 3 additional elements, additional proximates, calories, and 10 fatty acids. An additional spinach material, SRM 2385 Slurried Spinach, was released in 2003 as a wet, pureed material in contrast to the dried spinach leaves. The COA for SRM 2385 contains certified values for 7 elements and 2 carotenoids and reference values for proximates, calories, 2 elements, 2 vitamins, and *trans*-β-carotene. These SRMs are intended primarily for use in validation of methods for determination of elements in botanical materials, agricultural food products, and materials of similar matrix in sector 7 of the food composition triangle.

Food materials in preparation

Soy milk

In addition to the soy flour material described previously, the development of a soy milk SRM is underway. The material was prepared from commercially available soy milk that was homogenized and repackaged at NIST. SRM 3235 Soy Milk will have certified values for tocopherols and isoflavones. This material will be intended for use as a control material for the determination of tocopherols in soy products, milks, and related food matrices in sector 6 of the food composition triangle.

Kelp

Kelp is a food commonly consumed in Asian cultures and is of increasing popularity in Western foods and dietary supplements for being a unique natural source of iodine and vitamin K. NIST is preparing SRM 3232 Kelp from dried and blended kelp leaves and will have assigned values for iodine and other elements, vitamin K, and arsenic species. This material will be useful as a control material for determination of elements and vitamin K in plant-derived food products in sector 5 of the food composition triangle.

Protein drink mix

Protein powders are commonly used by athletes interested in increasing muscle mass. These powders are mixed with water, milk, or juice and often consumed immediately before or after exercising. SRM 3252 Protein Drink Mix was prepared from commercially available chocolate-flavored protein powders and will be characterized for content of elements, vitamins, fatty acids, amino acids, and caffeine. Reference values will also be assigned for proximates. This 90 % protein material will fill an important void in sector 9 of the food composition triangle, and will be intended as a control material for analysis of powdered supplements and other high-protein matrices.

Pet food

Similar to food for human consumption, pet food content is also regulated at the federal and state levels, with standards set by the Association of American Feed Control Officials (AAFCO). A reference material for pet food would be useful for the pet food industry, but also for laboratories looking for a control material within sector 8 of the food composition triangle. SRM 3290 Dry Cat Food was prepared from commercially-available products and will be certified for the content of elements, fatty acids, amino acids, and vitamins. In addition, reference values will be assigned for proximates.

Table salt

Another food product that cannot be classified on the food composition triangle is table salt. Table salt is the main source of iodine in the US diet, and the FDA recommends addition of iodine (as iodide) at a mass fraction of at least 45 mg/kg to table salts sold in the US. SRM 3530 Iodized Table Salt (Iodide) is under development at NIST as a quality assurance tool for this industry. Similarly, SRM 3531 Iodized Table Salt (Iodate) is under development to support quality assurance in European, Australian, and Asian markets, where table salts are supplemented with iodate. Certified values for iodine (as iodide or iodate) as well as additives or contaminants will be determined in each of the materials.

Material use

The SRMs developed at NIST are valuable analytical method-development tools for optimization of separation, detection, and extraction/sample preparation conditions. By comparing laboratory results with certified or reference values found on the COA, SRMs can be used to validate method parameters such as accuracy, repeatability, selectivity, and scope of applicability. By including a blinded SRM in an analytical sample stream, SRMs can also be used for quality control/quality assurance. Additionally, by simultaneous analysis of an in-house control material and a NIST SRM over time, a laboratory can demonstrate metrological traceability. Matrix-based SRMs should not be used for calibration, however, because extraction from the natural matrix complicates the accurate determination of analyte concentration. In addition, relative uncertainties on compounds in natural-matrix materials are often in the percent range, compared to fractions of a percent in a solution prepared from a crystalline material, which will significantly increase the uncertainty of the overall determination.

An appropriate SRM for use in food analysis should be selected using the AOAC food composition triangle (Fig. 1) and information about the fat/protein/carbohydrate content of the samples to be analyzed. Ideally, the matrices of the SRM and sample should be closely matched to increase the likelihood of comparable analyte recovery. Additionally, the analyte of interest should have an assigned value in the SRM within the concentration range expected in the sample, which will ensure comparable recovery challenges and linearity in calibration. By following these guidelines, a laboratory can have confidence in all portions of a method when determined results (including the laboratory uncertainty) are within the acceptable range for the SRM (determined from the certified or reference value and its uncertainty).

Food-matrix SRMs can be used to demonstrate that analytical methods for nutrient determination are both appropriate and effective. As such, these SRMs should be used regularly as an important tool in quality control/quality assurance protocols. The data from SRM measurements can be used as evidence for regulatory agencies and customers as well as in standard operating procedures for system suitability requirements. In addition to measuring nutrients, some SRMs can be used in methods for determining contaminants such as toxic elements or pesticides, which may also be relevant to regulatory agencies. As the NIST food material effort continues to grow, a wider range of food-matrix SRMs will be added to the catalog.

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