National Institute of Standards & Technology

Report of Investigation

Reference Material 8436

Durum Wheat Flour

A Joint Material of Agriculture Canada and NIST

Distributed by the National Institute of Standards and Technology

Reference Material (RM) 8436 is intended for use in evaluating analytical methods and instruments used for the determination of major, minor, and trace constituent elements as well as proximates, total dietary fiber, and calories in flour, flour products, and other similar food, agricultural, and biological materials. This material can also be used for quality assurance when assigning values to in-house control materials. RM 8436 consists of 50 g of dry durum wheat flour packaged in a glass bottle, sealed in an aluminum-nylon pouch.

Reference Concentration Values: Reference concentration values for major, minor, and trace constituent elements are provided in Table 1. Reference concentration values for proximates, total dietary fiber, and calories are provided in Table 2. The reference values in Tables 1 and 2 were derived from results reported in an interlaboratory comparison exercise and by four additional collaborating laboratories, respectively. Reference values are noncertified values that are the best estimates of the true values; however, the values do not meet NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods.

Information Concentration Values: Information concentration values for additional elements and one fatty acid are provided in Tables 3 and 4. These are noncertified values with no reported uncertainties as there is insufficient information to assess uncertainties. The information values are given to provide additional characterization of the material. Use of this RM to evaluate method performance for analytes other than those with reference concentration values in Tables 1 and 2 is not warranted.

Expiration of Report: The Report of Investigation of RM 8436 is valid, within the measurement uncertainty specified, until **31 August 2011**, provided the RM is handled in accordance with instructions given in this report (see "Instructions for Use"). This report is nullified if the RM is damaged, contaminated, or otherwise modified.

Maintenance of RM Value Assignment: NIST will monitor this RM over the period of its value assignment. If substantive technical changes occur that affect the value assignment before the expiration of this report, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

Statistical support was provided by M.S. Wolynetz, Statistical Research Section, Research Program Service, Agriculture Canada and L.M. Gill of the NIST Statistical Engineering Division.

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

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See Report Revision History on Page 8

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RM 8436 was prepared at Agriculture Canada under the direction of M. Ihnat, Centre for Land and Biological Resources Research (CLBRR). Coordination of the technical measurements leading to the value assignment of this RM was performed by M. Ihnat of CLBRR, Agriculture Canada, and K.E. Sharpless and S.A. Wise of the NIST Analytical Chemistry Division. Following the original analyses for elemental value assignment by the laboratories listed in Appendix A, the material was distributed by NIST to Covance Laboratories (Madison, WI), Lancaster Laboratories (Lancaster, PA), Medallion Laboratories (Minneapolis, MN), and Southern Testing and Research Laboratories (Wilson, NC) for the measurement of proximates, fatty acids, calories, and total dietary fiber.

NOTICE AND WARNING TO USERS

Storage: Until required for use, RM 8436 should be stored under refrigeration between 2 °C and 8 °C in its original bottle, tightly capped, and not exposed to intense light or ultraviolet radiation.

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

Instructions for Use: The bottle and contents should be allowed to warm up to room temperature prior to opening. Prior to each use, contents of the bottle should be well mixed by gentle shaking and rolling of the container. A recommended minimum subsample mass of 0.5 g should be taken for analysis. Moisture content should be determined on a separate subsample for conversion of analytical results to a dry-mass basis. The recommended method of drying to relate analytical results to the assigned values listed in the tables is drying for 4 h in an air oven at 85 °C. Concentrations reported in Table 1 represent total concentrations of elements in this RM. Dissolution procedures should be capable of rendering a completely dissolved sample appropriate to the method and should be designed to avoid losses of elements by volatilization or by retention on decomposition and processing containers and measuring equipment. Analytical methods should be capable of measuring total levels of elements for comparison with reference values.

PREPARATION AND ANALYSIS[1]

Preparation: The source of material for RM 8436 was enriched durum wheat flour, containing reduced iron, ammonium chloride, and potassium bromate, obtained from Ogilvie Mills Ltd., Montreal, Quebec, Canada. All preparatory work following acquisition of the commercial product was performed at the facilities of Agriculture Canada, Ottawa [1,2]. The dry bulk powder was sterilized with cobalt-60 gamma radiation to 2.0 Mrads by Atomic Energy of Canada Ltd. Material sieving was through nylon monofilament sieve cloths supported in high-density white polyethylene holders. Pairs of sieves with openings of approximately 200 μm and 50 μm were used to yield a middle-cut fraction for use as the RM. This fraction was blended in a polymethylmethacrylate V-configuration blender and packaged into clean 150 mL brim capacity, colorless glass bottles with triseal (polyethylene)-lined white polypropylene screw caps. A total of 144 randomly selected units were used for physical and chemical characterization in the original analyses.

Homogeneity Assessment: Homogeneity testing was performed on randomly selected units for 13 elements by three laboratories [2,3]. Subsamples of 0.5 g and 2.0 g were taken from a total of four units and analyzed by M. Ihnat, Agriculture Canada, for calcium, copper, iron, potassium, magnesium, manganese, sodium, and zinc using acid digestion flame atomic absorption spectrometry [4,5]. Subsamples of 0.9 g to 6.0 g each, taken from a total of six units, were analyzed by R.W. Dabeka, Health and Welfare Canada, for cadmium, cobalt, lead, and nickel by graphite furnace atomic absorption spectrometric (GFAAS) methods following acid digestion and separation and preconcentration of the analytes using coprecipitation with ammonium pyrrolidine dithiocarbamate (cobalt, nickel, and lead) and additionally with palladium and ascorbic acid for lead; cadmium was determined directly on nitric acid digests [6-9]. Fluoride was determined by the same analyst in 0.1 g subsamples from six units by an acid-facilitated, microdiffusion-ion specific electrode method [10]. Solid sampling graphite furnace atomic absorption spectrometric determinations were carried out by M. Stoeppler and U. Bagschik, Nuclear Research Center, Jülich, Federal Republic of Germany, on a total of 40 subsamples of 0.0005 g (0.5 mg) each, from four units for copper [2,3]. In addition, the analytical results obtained from a large number of analysts (Appendix A) participating in the interlaboratory characterization campaign were assessed to provide homogeneity estimates for other elements [2,3].

[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.

No statistically significant heterogeneity was found for aluminum, barium, bromine, cadmium, calcium, chlorine, chromium, cobalt, copper, iodine, iron, lead, magnesium, manganese, mercury, molybdenum, nickel, nitrogen, phosphorus, potassium, rubidium, selenium, sodium, strontium, sulfur, vanadium, and zinc in sample sizes ranging from 0.1 g to 2 g, depending on the sample size typically required by the analytical technique. Data for all analytes (including the proximates, fatty acids, and total dietary fiber) have been treated as though they are homogeneous, although the homogeneity of other analytes has not been investigated.

Value Assignment: Chemical analyses to establish reference concentrations of elements were conducted in an interlaboratory comparison exercise involving Agriculture Canada and selected analysts in other laboratories (Appendix A) using analytical methods listed in Table 5. Analyses were performed by each participant on duplicate subsamples from randomly selected (typically four) units of material; subsample sizes and methods were left to the discretion of the analyst. Subsample sizes ranged from 0.001 g to 6 g, typically 0.9 g. Elemental determinations were performed on the material "as received," with conversion of results to a dry-mass basis using moisture values determined on separate 2 g subsamples by the drying procedure specified in the "Instructions for Use" section of this report.

Following the original elemental determinations, NIST distributed RM 8436 to four laboratories (Appendix B) for measurement of proximates, fatty acids, calories, and total dietary fiber. Each laboratory analyzed one portion from each of three bottles of RM 8436 using their routine methods (Table 6). Determinations were performed on the material "as received," with conversion of results to a dry-mass basis using moisture values determined on separate subsamples taken from each of the three bottles. Standard Reference Material (SRM) 1846 Infant Formula was analyzed for quality assurance.

Table 1. Reference Concentration Values of Constituent Elements

Major Constituents	Mass Fraction (%)[a]	Methods[b]
Nitrogen[c]	2.709 ± 0.059	I01, J01, J02
Potassium	0.318 ± 0.014	A01, B01, B02, D01, E01
Phosphorus	0.290 ± 0.022	B02, B03, F01, F02, M01
Sulfur	0.193 ± 0.028	B02, B03, F04, J03, J04
Magnesium	0.107 ± 0.008	A01, A03, B02, D01

Minor and Trace Constituents	Mass Fraction (mg/kg)[a]	Methods[b]
Chlorine	680 ± 90	D01, F02, K02, K03
Calcium	278 ± 26	A01, B02, B03, D01, E01
Iron	41.5 ± 4.0	A01, A03, B02, B03, E01, E02
Zinc	22.2 ± 1.7	A01, A03, B02, B03, D01, E01, H01
Manganese	16.0 ± 1.0	A01, A03, A05, B02, B03, D01, E01, E02
Sodium	16.0 ± 6.1	A01, B02, B03, D01
Aluminum	11.7 ± 4.7	A05, B02, B03, D01
Bromine	6.6 ± 1.1	D01, E01
Copper	4.30 ± 0.69	A01, A03, A05, A06, B02, B03, C03, C06, D01, E01, 01
Barium	2.11 ± 0.47	B02, B03, C03, D01
Rubidium	2.0 ± 0.4	D01, E01
Selenium	1.23 ± 0.09	C01, C04, G01
Strontium	1.19 ± 0.09	B02, B03, C03
Molybdenum	0.70 ± 0.12	B02, C03, C06, D03, F01, H06
Nickel	0.17 ± 0.08	A05, A16, C03, H01
Cadmium	0.11 ± 0.05	A04, A05, A06, C03, H01
Lead	0.023 ± 0.006	A04, A16, C01, C03
Chromium	0.023 ± 0.009	A06, A12, C05
Vanadium	0.021 ± 0.006	B02, D01, D03
Cobalt	0.008 ± 0.004	A16, H01
Iodine	0.006 ± 0.004	D03, D06, F01
Mercury	0.0004 ± 0.0002	A10, A15, D03

[a] Reference values, expressed as mass fractions, are based on the dry material, dried according to instructions in this report and are equally weighted means of results from at least two, but typically several, different analytical methods applied by analysts in different laboratories. Uncertainties are imprecision estimates expressed either as a 95 % confidence interval or occasionally (cobalt, iodine, mercury) as an interval based on the entire range of accepted results for a single future determination, based on a sample mass of at least 0.5 g. These uncertainties, based on among-method, among-laboratory, among-unit and within-unit estimates of variances, include measures of analytical method and laboratory imprecisions and biases and material inhomogeneity. **NOTE:** NIST has replaced the previously used term "best estimate" with "reference value."

[b] Analytical method codes and descriptions are provided in Table 5.

[c] Nitrogen results have been updated to include results from four additional laboratories (Appendix B). Each reference concentration value, expressed as a mass fraction on a dry-mass basis, is a weighted mean of the two group means from the laboratories shown in Appendices A and B; results were weighted at 75 % and 25 %, respectively, based on the number of laboratories that provided data in the two studies. The uncertainty in the reference values is expressed as an expanded uncertainty, U, at the 95 % level of confidence, and is calculated according to the method described in the ISO and NIST Guides [11]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte.

Table 2. Reference Concentration Values of Proximates, Total Dietary Fiber, Selected Fatty Acids (as Triglycerides), and Calories

	Mass Fraction, as received (%)[a]	Mass Fraction, dry-mass basis (%)[a]
Moisture	10.1 ± 1.3	0 (by definition)
Solids	89.9 ± 1.3	100 (by definition)
Protein[b]	13.91 ± 0.32	15.48 ± 0.50
Carbohydrate	72.2 ± 1.3	80.34 ± 0.40
Fat	2.53 ± 0.24	2.82 ± 0.25
Ash	1.226 ± 0.087	1.364 ± 0.087
Total Dietary Fiber	4.14 ± 0.63	4.61 ± 0.73
Hexadecanoic Acid (C16:0) (Palmitic Acid)	0.55 ± 0.12	0.61 ± 0.13
Octadecanoic Acid (C18:0) (Stearic Acid)	0.031 ± 0.011	0.034 ± 0.012
(Z) - 9 - Octadecenoic Acid (18:1) (Oleic Acid)	0.40 ± 0.12	0.45 ± 0.13
(Z,Z) - 9,12 - Octadecadienoic Acid (C18:2) (Linoleic Acid)	1.32 ± 0.47	1.47 ± 0.52
(Z,Z,Z) - 9,12,15 - Octadecatrienoic Acid (C18:3) (Linolenic Acid)	0.068 ± 0.026	0.076 ± 0.029
Calories[c]	(367.3 ± 5.6) kcal/100 g	(408.6 ± 1.1) kcal/100 g

[a] Each reference concentration value, expressed as a mass fraction on an as-received or dry-mass basis, is an equally weighted mean of results from the laboratories shown in Appendix B. The uncertainty in the reference values is expressed as an expanded uncertainty, U, at the 95 % level of confidence and is calculated according to the method described in the ISO Guide [11]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. Analytical methodology information is provided in Table 6.

[b] The protein concentration was calculated from the nitrogen values reported by the laboratories shown in Appendix B using a conversion factor of 5.7; subsequent calculations of carbohydrates and calories were also based on these protein concentrations. The nitrogen values reported by the laboratories shown in Appendix B were combined with the original data for calculation of the reference value for nitrogen provided in Table 1.

[c] The value for calories is the mean of the individual caloric calculations. If the mean proximate values are used for calculation, with caloric equivalents of 9, 4, and 4 for fat, protein, and carbohydrate, respectively, the mean caloric content is 367.2 kcal/100 g and 408.6 kcal/100 g on an as-received and dry-mass basis, respectively.

Table 3. Information Concentration Values of Constituent Elements

	Mass Fraction (mg/kg)[a]	Methods[b]
Arsenic	0.03	A07, A08
Fluorine	0.1	H04
Titanium	5	B02, C08, E01, E02

[a] These analytical values, on a dry-mass basis, are estimates given strictly for information only as they are based on results of limited determinations or only one method; no uncertainties are provided.
[b] Analytical method codes and descriptions are provided in Table 5.

Table 4. Information Concentration Values for an Additional Fatty Acid (as the Triglyceride)

	Mass Fraction, as received (%)[a]	Mass Fraction, dry-mass basis (%)[a]
9 - Eiconsenoic (C20:1) (Gadoleic Acid)	0.017	0.019

[a] These information values, reported on an as-received or dry-mass basis, are the equally weighted means of results reported by the laboratories shown in Appendix B. These values are based on results from determinations by three or four of the laboratories and are included to provide additional characterization of the material; no uncertainties are provided. Analytical methodology information is provided in Table 6.

Table 5. Analytical Methods Used by Collaborating Laboratories (Appendix A) to Determine Reference and Information Concentration Values of Elements[a]

Analytical Method	Code	Elements Determined
Acid digestion flame atomic absorption spectrometry	A01	Ca, Cu, Fe, K, Mg, Mn, Na, Zn
Dry ashing flame atomic absorption spectrometry	A03	Cu, Fe, Mg, Mn, Zn
Acid digestion electrothermal atomic absorption spectrometry	A04	Cd, Pb
Closed vessel acid digestion electrothermal atomic absorption spectrometry	A05	Al, Cd, Cu, Mn, Ni
Dry ashing electrothermal atomic absorption spectrometry	A06	Cd, Cr, Cu
Acid digestion hydride generation atomic absorption spectrometry	A07	(As)
Dry ashing hydride generation atomic absorption spectrometry	A08	(As)
Closed vessel acid digestion cold vapor atomic absorption spectrometry with preconcentration	A10	Hg
Dry ashing digestion electro-thermal atomic absorption	A12	Cr

spectrometry		
Acid digestion cold vapor atomic absorption spectrometry with preconcentration	A15	Hg
Acid digestion coprecipitation electrothermal atomic absorption spectrometry	A16	Co, Ni, Pb
Acid digestion atomic emission spectrometry	B01	K
Acid digestion inductively coupled plasma atomic emission spectrometry	B02	Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, P, S, Sr, (Ti), V, Zn
Closed vessel acid digestion inductively coupled plasma atomic emission spectrometry	B03	Al, Ba, Ca, Cu, Fe, Mn, Na, P, S, Sr, Zn
Acid digestion isotope dilution mass spectrometry	C01	Pb, Se
Closed vessel acid digestion isotope dilution inductively coupled plasma mass spectrometry	C03	Ba, Cd, Cu, Mo, Ni, Pb, Sr
Acid digestion dry ashing hydride generation isotope dilution inductively coupled plasma mass spectrometry	C04	Se
Dry ashing acid digestion isotope dilution mass spectrometry	C05	Cr
Acid digestion isotope dilution inductively coupled plasma mass spectrometry	C06	Cu, Mo
Acid digestion inductively coupled plasma mass spectrometry	C08	(Ti)
Instrumental neutron activation analysis	D01	Al, Ba, Br, Ca, Cl, Cu, Fe, K, Mg, Mn, Na, Rb, V, Zn
Neutron activation analysis with radiochemical separation	D03	Hg, I, Mo, V
Preconcentration neutron activation analysis	D06	I
Particle induced X-ray emission spectrometry	E01	Br, Ca, Cu, Fe, K, Mn, Rb, (Ti), Zn
X-ray fluorescence	E02	Fe, Mn, (Ti)
Acid digestion light absorption spectrometry	F01	I, Mo, P

Dry ashing light absorption spectrometry	F02	Cl, P
Combustion light absorption spectrometry	F04	S
Acid digestion fluorometry	G01	Se
Closed vessel acid digestion anodic stripping voltametry	H01	Cd, Co, Cu, Ni, Zn
Extraction ion selective electrode	H04	(F)
Dry ashing catalytic adsorption polarography	H06	Mo
Kjeldahl method for nitrogen-volumetry	I01	N[(b)]
Combustion elemental analysis-thermal conductivity	J01	N[(b)]
Combustion elemental analysis with chromatographic separation-thermal conductivity	J02	N[(b)]
Combustion elemental analysis-infrared spectrometry	J03	S
Combustion elemental analysis-fluorometry	J04	S
Dry ashing volumetry	K02	Cl
Combustion volumetry	K03	Cl
Acid digestion gravimetry	M01	P

[(a)] Letter codes refer to classes of similar methods; number codes refer to specific variants. Elements in parentheses have only information values in this RM. **NOTE:** NIST has replaced the previously used term "best estimate" with "reference value".
[(b)] See Table 6 for additional information.

Table 6. Methods Used by Collaborating Laboratories (Appendix B) for the Determination of Proximates, Fatty Acids, Calories, and Total Dietary Fiber

Ash	mass loss after ignition in a muffle furnace
Calories	calculated; [(9 × fat) + (4 × protein) + (4 × carbohydrate)]
Carbohydrate	calculated; [solids – (protein + fat + ash)]
Fat	sum of individual fatty acids
Fatty acids	hydrolysis followed by gas chromatography
Moisture	mass loss after drying in a vacuum oven (2 laboratories); mass loss after drying in a forced-air oven (2 laboratories)
Nitrogen	Dumas (1 laboratory); modified Dumas (1 laboratory); Kjeldahl (2 laboratories). Note that in the original elemental determinations, laboratories provided results by Kjeldahl, combustion - thermal conductivity, and combustion - chromatographic separation - thermal conductivity
Protein	calculated from nitrogen using a factor of 5.7
Solids	calculated; (sample mass – moisture)
Total dietary fiber	enzymatic digestion followed by gravimetry

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Users of this RM should ensure that the report in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-6776; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.

APPENDIX A

Collaborating Analysts for Elemental Determinations

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APPENDIX B

Collaborating Laboratories for Proximate, Fatty Acid, Total Dietary Fiber, and Calorie Determinations

Covance Laboratories, Madison, WI, USA.
Lancaster Laboratories, Lancaster, PA, USA.
Medallion Laboratories, Minneappolis, MN, USA.
Southern Testing and Research Laboratories, Wilson, NC, USA.