Published in final edited form as:

Anal Bioanal Chem. 2013 May ; 405(13): 4569–4578. doi:10.1007/s00216-013-6727-2.

Breakfast cereal sampling study for nutritional elements

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Abstract

The National Institute of Standards and Technology (NIST) has established a Dietary Supplement Laboratory Quality Assurance Program (DSQAP) in collaboration with the National Institutes of Health Office of Dietary Supplements (NIH-ODS). The DSQAP invites laboratories twice annually to participate in interlaboratory studies where participants elect to measure concentrations of nutritional and/or toxic elements as well as active and/or marker compounds. One of these studies was designed to determine the effects of material granularity and sample processing techniques on measurement variability (precision) as well as to provide participating laboratories information on their performance relative to the NIST assigned values (bias) and to the other participants (concordance). Participants were asked to determine the mass fractions of Ca, Fe, and Zn, in mg/kg, in six breakfast cereal samples. Cereal samples consisted of three ground materials (homogenized wheat, wheat, and rice), two flake materials (wheat and rice) and a partially crushed material (a wheat/rice mixture). In general, approximately 25 % of the laboratories processed and analyzed the suite of six cereal materials with adequate to exemplary measurement precision. Over half of the laboratories (60 %) experienced measurement issues related to only a particular type of cereal matrix or for only a single element. A small number (15 %) of laboratories experienced significant sample processing or measurement problems. Future studies planned by the DSQAP may be designed to use commercial products to aid laboratories with their sampling and analytical techniques.

Keywords

Sampling; Nutritional analysis; Food; Reference materials; Nutritional elements

Introduction

Under the Nutrition Labeling and Education Act of 1990 [1], labels are required to report the Ca and Fe content of processed foods, including ready-to-eat cereals, sold in the U.S. Nutrients such as Zn, with an established reference daily intake value, may also be listed. Laboratories involved in the analysis of commercially available products need to assure that their sample processing techniques become a defined step in their analytical procedure [2, 3]. Codex Alimentarius Commission (Codex), an international organization whose aim is to protect the health of consumers, has recommended guidelines for users on sampling procedures [2]. The Codex-recommended methods of sampling are designed so that food industries use fair and valid sampling procedures. Sampling is the first step in the measurement process and may contribute to a greater level of uncertainty than the actual chemical analysis; therefore care needs to be taken on how these two components of uncertainty are combined. Sources of uncertainties that arise from initial sampling processes and from the components of the chemical analysis need to be examined separately [4, 5]. Gy [6] illustrated, in a set of experiments with sand and salt, that sampling may affect not only variability, but may also generate a bias in values even if the measurement component of the analysis is done accurately.

Most food samples by nature will not be homogeneous, with moisture content an important factor to consider [7]. Some sample matrices may be more difficult to homogenize, and samples matrices with larger particles will be more difficult to representatively sample than those with smaller particles. For example, Samuelson and Holland [8] studied sample processing techniques and the determination of nutritional elements in apples. They found that pureeing opposite sectors of an apple to a pulp would give representative results for the whole apple. Of the elements measured, Ca had a slightly greater sub-sampling error than other elements measured. However, when they measured Ca in 20 whole apples from one tree and 20 cross sectors of apples from the same tree, both resulted in not only the same Ca value, but also acceptable variability (7.3 % and 7.2 %, respectively). Therefore, using representative sectors of apples was deemed adequate for Ca analysis rather than using the whole fruit.

In 2007 the DSQAP was established at NIST, in collaboration with NIH-ODS, to enable participating laboratories to improve the accuracy and precision of their measurements for nutritional and toxic elements, active and marker compounds, and pesticides [9]. Two interlaboratory exercises are planned each year and participating laboratories may elect which of the several studies within the exercise they wish to participate in. In an effort to provide participants of the DSQAP with real life samples, the DSQAP designed the Nutritional Elements in Breakfast Cereal study to determine the effects of material granularity and sample processing techniques on measurement variability (precision), in addition to providing laboratory concordance information. Participating laboratories were asked to determine, in triplicate, the levels of Ca, Fe, and Zn in six fortified breakfast cereals (wheat flakes, rice flakes, crushed wheat/rice blend, finely ground wheat, finely ground rice, finely ground and homogenized wheat). Removing a test portion for analysis of the three ground materials was anticipated to be relatively straightforward, whereas the two flake materials were expected to require appropriate processing prior to subsampling to limit measurement variability. The wheat/rice mixture with a hand-crushed granularity was anticipated to be a more challenging material due to its inherent heterogeneity both in

content and in granularity. This study was a precursor for possible future exercises that will assess method precision using commercially available nutritional products and thus exhibiting significant heterogeneity.

Experimental procedure

Study materials

Six wheat-based and rice-based fortified breakfast cereals were selected as study materials (Fig. 1). The breakfast cereals were fortified by the manufacturers and the fortification procedures were not known. The six cereals were prepared to represent a series of granularities and fortification levels, as well as a range of fiber and protein content. Table 1 provides the approximate levels of the proximates as well as the levels of Ca, Fe, and Zn in the six cereals. As shown in Table 1, the rice-based cereal was fortified with Fe but had naturally occurring levels of Ca and Zn while the wheat-based cereal was fortified with Ca, Fe, and Zn.

Cereal A (SRM 3233 Fortified Breakfast Cereal) was the only study material with established values for Ca, Fe, and Zn and therefore the only material for which the measurement results can be assessed for trueness, defined here as relative proximity to the NIST assigned values. Assigned values for elemental content of SRM 3233 were based on results provided by NIST using inductively coupled plasma optical emission spectrometry (ICP-OES) and from determinations from collaborating laboratories (Table 2). The Grocery Manufacturers Association's (GMA) Food Industry Analytical Chemists Committee (FIACC) distributed candidate SRM 3233 as part of an interlaboratory comparison exercise. The GMA laboratories were asked to use their usual methods of analysis and to make two measurements from each of two individual bottles. The collaborating laboratories used the following methods for the determination of Ca, Fe, and Zn: flame atomic absorption, direct current plasma optical emission spectrometry, ICP-OES, and inductively coupled plasma mass spectrometry. A NIST value was calculated by using the mean of the mean values from NIST methods and the median of the mean of results provided by the collaborating laboratories. At the time of this exercise, all data had not yet been compiled to establish certified values for Ca, Fe, or Zn in Cereal A [10]. Standard Reference Material 3233 Fortified Breakfast Cereal is now available and has certified, reference, and/or information mass fraction values for elements (certified for Ca, Fe, and Zn), proximates, sugars, dietary fiber, vitamins, and amino acids.

Cereals B and C were prepared from a single brand of commercially available fortified wheat flake cereal originating from two production lots (21 kg in total) that were well mixed prior to packaging. Cereals D and E were prepared from a single brand of commercially available fortified rice flake cereal originating from a single production lot (13 kg in total). For both the wheat and rice cereals, half of the production lot was packaged directly as the flake material and the remainder was ground using a Teflon disc mill. Cereal B and Cereal D were packaged as flake material; Cereal C and Cereal E were packaged as ground material (Fig. 1). Cereal F was prepared from a hand-crushed 50:50 mixture, by mass, of the wheat flake and rice flake cereals. While this material was well mixed, a visual examination of the individual packages of Cereal F suggested inhomogeneity with respect to both flake size and granularity.

Sample distribution

A letter of invitation was distributed to food and nutrition laboratories to gauge interest in a study intended to evaluate the effects of sampling techniques on measurement precision and accuracy as well as overall performance. Each laboratory was provided with six cereal

samples, in duplicate, labeled A, B, C, D, E, and F. Participating laboratories were asked to report values for triplicate measurements of Ca, Fe, and Zn in each of the six sample materials. Participants were asked to report data for all six sample materials and for all three analytes (a total of 18 values reported). However, if providing the complete data set was not feasible participants were asked to provide data for only a single element across all six materials rather than for a random selection of materials (e.g., report Fe data for the six samples, rather than report all elements for only three samples). Laboratories were also asked to provide details regarding their sample processing techniques, the amount of study material that was processed, and the amount of sub-sample that was used in the analysis.

The relative variability of triplicate measurements for Ca, Fe, and Zn in each of the six materials was assessed and compared with the sample processing information provided by each laboratory. Comparison with the sample processing information is important because the various matrices in this suite of cereal samples may respond differently to differing sample preparation techniques (e.g., mechanical grinding, hand-crushing), influencing an individual laboratory's ability to provide reproducible results for each material.

Results and discussion

Comparison to candidate SRM 3233

Twenty laboratories participated in the study. Figure 2a through c show the NIST target range, the consensus median, and the consensus variability for Ca, Fe, and Zn. The NIST target range is the expanded uncertainty (U) surrounding the NIST certified values for each element [11]. This target range is considered to contain the true value with 95 % confidence [12]. The consensus median is the median from all of the participating laboratories, and the consensus variability is the $\pm 2\sigma$ confidence interval surrounding the median values for each element [11]. Each laboratory's mean value ($\pm 1\sigma$) is plotted in Fig. 2a through c for comparison to the NIST target range and to the consensus median. Participants can use their estimates of uncertainty when comparing their values to that of the NIST assigned values [13].

As shown in Fig. 2a, 10 out of 19 participating laboratories (53 %) are within the expanded uncertainty (U) for Ca. In Fig. 2b, Fe results show 11 out of 19 participating laboratories (58 %) are within the expanded uncertainty (U), and, in Fig. 2c, Zn results show 8 out of 20 participating laboratories (40 %) within the expanded uncertainty (U). The uncertainty associated with Fe (5 %) for the NIST assigned value was twice that of Ca (2.5 %) and Zn (2.5 %), allowing participants a greater opportunity to fall within the target range for Fe.

Sample processing

A summary of the techniques used by each laboratory for processing the flake and hand-crushed cereals C, E, and F is provided in Table 3. Almost half (45 %) of the laboratories used a mortar and pestle, while 40 % of the participating laboratories used a metal mechanical grinder. There were only three laboratories (15 %) that used other techniques: hand crushing, a hammer, and a non-metal mechanical grinder. The majority (90 %) of laboratories processed the study material *before* subsampling for triplicate analyses. Two laboratories processed the materials *after* subsampling. The amount of material processed ranged from 1 g to 60 g (two packages of sample material processed together). The range of subsample size used for triplicate analysis ranged from 0.2 g to 10 g, with a median value of 0.5 g. Approximately 25 % of the laboratories appeared to have no trouble processing and analyzing the suite of six cereal materials. Another 60 % of the laboratories experienced measurement issues related to only a particular type of cereal matrix (e.g., wheat vs. rice or the more challenging wheat/rice sample) or for only a select element (e.g., Fe vs. Ca or Zn).

A small number ($\approx 15\%$) of laboratories experienced significant sample processing and/or measurement problems.

Variability

In addition to obtaining the correct value for a sample, laboratories must also do so with a certain degree of precision. An overview of the variability of the participating laboratories' performance for all 18 analyte/sample pairs is summarized in Table 4. Table 4 illustrates that a majority of laboratories analyzed triplicate samples with reasonable precision. The relative standard deviation (RSD) is the standard deviation divided by the absolute value of the mean and is often given as a percent. For each cereal/nutrient pair the median RSD is under 5% indicating that half of the participating laboratories were able to analyze the samples with a 5% RSD or less. Some laboratories experienced greater difficulties, with RSDs of 10% or greater, and with some as high as 45%. Some of the greatest variability was observed for measurement of Ca in the rice-based cereals (D, E, and F). Cereals D and E were not fortified with Ca and had a higher protein content than the wheat-based cereal. Most laboratories sampled and analyzed Fe with the same degree of precision across the six materials. Some laboratories had difficulty with Fe in all of the sample matrices, perhaps because Fe contamination is more likely to occur than with either Ca or Zn. This contamination can occur during the sample processing step or during the chemical analysis. The variability of Zn measurement was similar to that of Ca measurement. Both ground wheat sample materials gave participating laboratories little difficulty, with variability less than 6.5%.

Because this study was primarily designed to compare material granularity and sample processing techniques, additional statistical analyses for assignment of variability between- and within-laboratory were conducted to determine each individual laboratory's ability to provide reproducible results for each material. These analyses were performed to assess the consistency of the data across the multiple sample processing approaches. The *h*- and *k*-consistency estimators, described in the ASTM E691 protocol for the determination of precision of a test method [14], were used to explore the between- and within-laboratory components of variance for Ca, Fe, and Zn in all six materials. The *h*-consistency statistic is an indicator of how one laboratory's average result for a particular material compares with the average of that of the other laboratories in the study. The *k*-consistency statistic is an indicator of how one laboratory's repeatability for particular material compares with the pooled repeatability over all the laboratories in the study, where the pooled repeatability is the square-root of the average of the variance of the triplicate measurements from each participant [14]. The triplicate data for Ca, Fe, and Zn for all six materials were first analyzed with respect to between-laboratory (using the *h*-consistency statistic) and within-laboratory (using the *k*-consistency statistic) components of variance using the established ASTM E691 protocol for the determination of precision of a test method [15].

Examples of *h* and *k* plots for the determination of Zn in the ground and flake wheat cereals (A, B, and C) are shown in Fig. 3. In the top figure, laboratories considered to be significantly different relative to the other laboratories (between-laboratory variance) will approach or fall above or below the critical value of $|2.56|$ (5% significance level). Because the variability of values determined for Cereal C, in comparison to Cereals A and B, are more dependent on the completeness of sample processing, a greater between-laboratory variance (*h*-consistency statistic) was expected for laboratories that had not adequately processed all samples. The data given in the top figure indicate that most laboratories have similar *h*-consistency statistics for the three wheat materials, implying that all samples were adequately processed and that the method of processing the material is not contributing significantly to the overall measurement variability for Zn.

In the bottom figure, the k-consistency statistics of laboratories that approach or fall above the critical value of 2.21 (5 % significance level) indicate significantly greater within-laboratory variability for these measurements. However, while small k values are indicative of small variability, very small k values (near zero) may also indicate measurement problems such as measurement insensitivity [16, 17]. This may be an important tool when looking into reasons a laboratory may have very small within-laboratory variability but still not be close to a true or consensus value. For laboratories that had not adequately processed all samples, a greater within-laboratory variance (k-consistency statistic) would be expected for Cereal C (wheat flake) in comparison to Cereals A and B (wheat ground and homogenized and wheat ground, respectively). The data given in the bottom figure indicate, however, that most laboratories have similar k-consistency statistics for the three wheat materials, reinforcing the h-consistency conclusion (top figure) that most samples were adequately processed and that the method of processing the material is not contributing significantly to the overall measurement variability for Zn.

Eighteen h- and k-consistency plots similar to Fig. 3 were generated (one for each element and material combination). A summary of the data that were outside the critical range (5 % significance level) are provided in Table 5. Table 5 illustrates which laboratories are outliers with respect to the h and k indicators for the three elements across the six materials investigated. When several h indicators show up in one sample material, for example Zn in Cereal F, these can indicate sample matrix heterogeneity. The few h indicators randomly scattered across materials and elements can indicate anything from improper sample handling or preparation to an error in the laboratories' data processing. The clusters of k indicators around labs and materials are indicative of problems within a laboratory, either with sample preparation prior to analysis or with the analysis itself.

If outliers are observed (h-consistency statistics) for both ground and flake materials, this may be an indication of a potential calibration, digestion, or dilution error. When this is the case, technical guidelines can be recommended for good laboratory practices. Complete digestions are required to ensure that all elements are in solution when analyzing food and dietary supplements. Complete dissolution is required in order to obtain results within the 95 % confidence interval about the reference value. Leachate-type digestions will not produce complete digestions for food or dietary supplements, and results for these types of digestions were found to be below the NIST target values.

Calibration standards and appropriate Certified Reference Materials (CRMs), or traceable in-house controls, should be used with each analysis. Calibration standards must be obtained from a reliable source, validated as fit for purpose, and used to determine linearity and measurement range of the instrument. Working sample solution concentrations must fall within the measurement range of the instrument, and CRMs as well as procedural reagent blanks should be prepared and carried through the entire experiment to validate the measurement process as a whole.

Consensus plots using DerSimonian-Laird (DSL) means and multiples of Horn-Horn-Duncan (HHD) standard uncertainty lines were generated in order to display the participating laboratories' data in relation to the consensus values (Fig. 4) [18, 19]. The DSL location estimator, widely used in biostatistics, is a computational simple weighted-mean that includes a between-participant component of variance in the weighting function. This limits the influence that very precise but potentially biased results have on the consensus estimate. The HHD approach has been strongly recommended as providing realistic estimates for the uncertainty of the DSL and similar weighted-means estimators [17]. In Fig. 4, consensus plots for Zn in the ground and flake wheat cereals (B and C, respectively) using (DSL) means and multiples of (HHD) standard uncertainty lines are shown. The DSL

weighted consensus mean is a method often used in biostatistical literature to determine interlaboratory consensus values, accounting for both the within- and between-laboratory variance components [18]. Horn-Horn-Duncan (HHD) is a related method used to estimate consensus uncertainty of the mean [19]. The examples in Fig. 4 show the relationship of each laboratory's three individual Zn values to the consensus mean. The spread of the three data points illustrates the variability within a laboratory for a given element and material. Eighteen DSL consensus plots were prepared for each element and material combination.

Study results

Using this data, some initial comparisons of the sampling procedures used by each laboratory in Table 2 can be made. From this set of data, it is not readily apparent that the sample processing *method* grossly affects analytical outcome. From Table 5 and Fig. 3, it does appear that *when* the processing was employed (grinding before or after sub-sampling for analysis) and the *amount* of sample used for grinding can influence the between- and within-laboratory variability. In Fig. 3, two laboratories (E510 and E531) have results outside of the critical range for h- and k-consistency statistics. Both of these laboratories reported processing the sample material after subsampling for analysis. Laboratory number E538 is also outside of the critical range for both hand k-consistency statistics when measuring Ca and Fe (Table 5). This laboratory reported using the smallest amount of sample of any of the participants when processing material for subsampling.

Between-laboratory differences (h-consistency statistic) observed in flake materials may be an indication of material inhomogeneity potentially caused by improper sample processing, sampling a very small amount, or processing the sample after it has been subsampled for analysis. The majority of laboratories (70 %) indicated that the entire bottle or package was used for grinding prior to subsampling. Using the entire package is desirable, as partitioning the original sample into relatively small subsamples prior to grinding and homogenization may lead to replicate subsamples that differ in granularity and nutrient element content. When outliers are observed due to variability within a laboratory's measurements (k-consistency statistic), methodological sources of error such as improper sample processing, improper calibration, or incomplete digestions are all possible sources of error.

Increased variability may also be a product of material heterogeneity, as in Cereal F. Results for the Ca, Fe, and Zn levels in Cereal F may be more variable among the participating laboratories with some laboratories reporting much higher (or lower) nutrient levels for Cereal F depending on whether the mixture contained more of the wheat flake or more of the rice flake. Cereal F is a practical example of a material that laboratories may be required to evaluate, in which values from replicate bottles or packages do not agree as a result of material inhomogeneity. When analyzing what would be considered a real sample, a representative sample must be obtained [8, 20]. The mass of the processed sample may need to be increased to give a representation of the entire product or appropriately random samples need to be taken at intervals to assure chemical homogeneity between samples [5–7]. In this cereal study, between-laboratory results tended to be more variable for Cereal F. However, within-laboratory results for triplicate measurements of Cereal F should not be affected if appropriate sample processing and subsampling techniques are employed [3].

Conclusion

From the data collected, it appears that the optimal sample preparation procedure is to process (i.e., grind, crush) the entire sample received (bottle or package) first and then subsample for analysis *after* processing the sample. Participating laboratories that processed samples in their entirety produced a more homogeneous material for subsampling.

An important consideration when faced with known sample inhomogeneity is determination of an appropriate sample size for a sample preparation procedure prior to sample analysis. Sample size is limited for many analyses, often dependent on instrumentation. Sample size may be as little as 0.25 g to 1 g. However, in order for a manufacturer to follow the labeling requirements of the Nutrition Labeling and Education Act [1], the laboratory must use a representative sample in order to get “accurate results”. If their sample should be granola bars, they would not be able to cut off a 1 g piece and get a representative sample. One bar would most likely not constitute a representative sample and one box of six granola bars may not even constitute a representative sample. In order to properly assess mass fraction values for nutrients an appropriate and homogeneous material would need to be prepared prior to analytical analysis by an appropriate sample preparation procedure. From this a small sample for analysis can be taken which would be more representative of the whole product. Laboratories must determine on a case-by-case basis the amount of material which needs to be processed in order to obtain a representative sample for evaluation.

Acknowledgments

We thank the members of GMA's Food Industry Analytical Chemists Committee and the collaborating laboratories for assisting in the characterization of SRM 3233; we recognize and appreciate the time and effort they contributed in assisting in value assignment. We thank David L. Duewer for his editorial advice and suggestions.

Funding sources

The on-going DSQAP is a collaborative effort with NIH-ODS, and we acknowledge NIH-ODS for their partial support of these activities.

Certain commercial equipment, instruments, materials, or entities are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply the materials or equipment identified are necessarily the best available for the purpose.

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Fig. 1. Overview of the preparation and relative matrix differences for the six cereals (a–f) used in the sampling study

Fig. 2.

a. The NIST certified mass fraction for Ca in SRM 3233 Fortified Breakfast Cereal is 36910 mg/kg $\pm$ 920 mg/kg, $k=2.00$. The uncertainty surrounding the mean is an expanded uncertainty (U) covering the measurand with approximately 95 % confidence. **b.** The NIST certified mass fraction for Fe in SRM 3233 Fortified Breakfast Cereal is 766 mg/kg $\pm$ 36 mg/kg, $k=2.00$. The uncertainty surrounding the mean is an expanded uncertainty (U) covering the measurand with approximately 95 % confidence. **c.** The NIST certified mass fraction for Zn in SRM 3233 Fortified Breakfast Cereal is 628 mg/kg $\pm$ 16 mg/kg, $k=2.00$. The uncertainty surrounding the mean is an expanded uncertainty (U) covering the measurand with approximately 95 % confidence.

Fig. 3.

Plots represent both the h-consistency statistics (between-laboratory) and k-consistency statistics (within-laboratory) for Zn in wheat cereals A, B, and C (wheat ground and homogenized, wheat ground, and wheat flake, respectively). The 5 % significance levels (critical values) are denoted by a dashed horizontal line for each plot

Fig. 4. Consensus plots for Zn in Cereal B (wheat ground) and Cereal C (wheat flake). The DerSimonian-Laird (DSL) consensus means are represented by the dark solid lines with $\pm 1, 2, 3, 4, 5, 6$ Horn-Horn-Duncan (HHD) standard uncertainty lines. The individual data are represented as symbols (X) displayed on top of each corresponding box plot summary for each individual laboratory

Table 1

Approximate nutrient levels for the six cereals used in the sampling study (nutrient levels for Cereals B-F were according to package labels)

Nutrient	Cereal A: wheat ground and homogenized (SRM 3233)	Cereal B: wheat ground	Cereal C: wheat flake	Cereal D: rice ground	Cereal E: rice flake	Cereal F: wheat/rice crushed
Fiber (mg/g)	100	100	100	33	33	67
Protein (mg/g)	67	67	67	200	200	133
Carbohydrates (mg/g)	770	770	770	770	770	770
Fe (μ g/g)	750	800	800	500	500	650
Ca (μ g/g)	36,500	35,000	35,000	200	200	17,500
Zn (μ g/g)	600	700	700	20	20	350

Table 2

Grocery Manufacturers Association's (GMA) Food Industry Analytical Chemists Committee (FIACC) laboratories reporting data for value assignment of candidate SRM 3233 fortified breakfast cereal. (Note that these laboratories were not the participants in the sampling study being reported here)

Campbell Soup Co., Camden, NJ
ConAgra Foods Analytical Laboratory, Omaha, NE
Covance, Inc., Madison, WI
Del Monte Foods, Walnut Creek, CA
Eurofins Central Analytical Laboratories, Metairie, LA
Eurofins Scientific, Des Moines, IA
General Mills, Inc., Golden Valley, MN
Hormel Foods Corp., Austin, MN
Krueger Food Laboratories, Billerica, MA
Land O'Lakes, Arden Hills, MN
The National Food Laboratory, Livermore, CA
Schwan Food Co., Salina, KS
Silliker, Madison, WI
The J.M. Smucker's Co., Orrville, OH

Table 3

Sample preparation details for the three textured (non-ground) cereals (C, E, F)

Lab code	Sample processing method	When ^a	Subsample amount (g) used for analyses	Cereal C: wheat flake processed (g)	Cereal E: rice flake processed (g)	Cereal F: wheat/rice crushed processed (g)
E510	metal mechanical grinder	after	0.2	60	30	30
E509	metal mechanical grinder	before	10	all ^b	all ^b	all ^b
E511	metal mechanical grinder	before	2.2	all ^b	all ^b	all ^b
E520	metal mechanical grinder	before	n/a	30	30	30
E522	metal mechanical grinder	before	0.5	30	40	30
E526	metal mechanical grinder	before	0.5	all ^b	all ^b	n/a
E529	metal mechanical grinder	before	3–8	30	30	30
E540	metal mechanical grinder	before	0.5	all ^b	all ^b	n/a
E531	mortar & pestle	after	0.25	5	5	5
E530	mortar & pestle	before	0.5	5	5	5
E533	mortar & pestle	before	2–2.5	n/a	n/a	n/a
E534	mortar & pestle	before	0.5	all ^b	all ^b	all ^b
E537	mortar & pestle	before	0.5	30	30	15
E538	mortar & pestle	before	n/a	1	1	n/a
E539	mortar & pestle	before	0.25	10	10	10
E541	mortar & pestle	before	1	30	30	30
E543	mortar & pestle	before	0.5	30	30	30
E502	non-metal mechanical grinder	before	2	60	60	30
E513	hammer	before	1	all ^b	all ^b	all ^b
E515	hand	before	0.5	n/a	n/a	n/a

^a Stage when processing of the sample was performed, i.e., before or after subsampling for analysis

^b All of the original sample was reported to be used for processing (i.e., grinding, crushing, etc.) before taking subsamples for analysis. This includes labs that used all material from one package or combined all material from both packages

Table 4

Overview of variability for each cereal/nutrient pair

	Median $\pm$ 2 σ mg/kg	N	Median % RSD	Range % RSD
Ca in Cereals A - F				
A	37248 $\pm$ 2128	19	1.3	0.2 – 8.5
B	34617 $\pm$ 966	18	1.0	0.4 – 7.0
C	34974 $\pm$ 1309	19	2.0	0.2 – 5.6
D	197 $\pm$ 55	18	4.3	0.5 – 25.2
E	216 $\pm$ 53	17	3.8	1.4 – 45.3
F	17700 $\pm$ 2533	19	4.4	0.8 – 20.3
Fe in Cereals A - F				
A	746 $\pm$ 80	19	1.8	0.8 – 16.5
B	787 $\pm$ 97	19	2.5	0.2 – 19.2
C	819 $\pm$ 61	19	1.9	0.3 – 19.6
D	510 $\pm$ 41	19	2.4	1.3 – 12.3
E	500 $\pm$ 67	18	3.0	0.9 – 12.2
F	654 $\pm$ 100	19	2.3	0.9 – 19.9
Zn in Cereals A - F				
A	608 $\pm$ 58	20	1.0	0.1 – 6.1
B	677 $\pm$ 69	20	1.0	0.2 – 6.4
C	692 $\pm$ 63	20	1.7	0.3 – 12.7
D	20 $\pm$ 3	16	3.4	0.4 – 24.4
E	20 $\pm$ 3	18	3.2	0.4 – 12.5
F	358 $\pm$ 78	20	4.1	0.8 – 15.4

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