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PRACTITIONER'S REPORT

Rapid method for determination of protein content in cereals and oilseeds: validation, measurement uncertainty and comparison with the Kjeldahl method

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Abstract The objective of this research was to test suitability of the Dumas combustion method to completely substitute the Kjeldahl method in routine laboratory determination of crude protein content in cereals and oilseeds. The validation of the method demonstrated that it is able to determine crude protein content in cereals and oilseeds in an efficient and accurate manner, with a detection limit $w(\mathrm{N}) = 0.006\%$, quantification limit $w(\mathrm{N}) = 0.019\%$, repeatability precision $RSD_{\mathrm{r}} = 0.41\%$, intra-laboratory reproducibility precision $RSD_{\mathrm{R}} = 0.74\%$, trueness, expressed in terms of bias $b = 0.43\%$, and linear response between (2.36–19.2) mg N. Measurement uncertainty, expressed as relative expanded uncertainty (coverage factor $k = 2$, confidence level 95%), was calculated from validation data ($U_{\mathrm{rel}} = 2.24\%$). In order to examine the relationship between two methods, 15 cereal grain and oilseed samples were analyzed using Dumas and Kjeldahl procedure. The Kjeldahl procedure gave slightly lower $w(\mathrm{N})$ values than the Dumas procedure: $w_{\mathrm{K}}(\mathrm{N}) = 0.9905\ w_{\mathrm{D}}(\mathrm{N}) = 0.0376$ ($R^2 = 0.9996$). Relative standard deviations and results of homogeneity test obtained during analysis of complex cereal products (cereal breakfast and muesli bars) show that the Dumas combustion method may be less suitable for analysis of such samples compared to Kjeldahl method.

Keywords Dumas method · Kjeldahl method · Cereals · Oilseeds · Validation · Measurement uncertainty

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Introduction

During routine laboratory analysis, there is a demand for a fast, reliable and precise determination of nitrogen, and hence crude protein content in oilseeds, cereal grains and their products, to determine their nutritional quality. The Dumas method, or nitrogen combustion method, is an attractive alternative to the widely used Kjeldahl assay.

Main principle of the Dumas method is the conversion of all nitrogen into gaseous nitrogen oxides (NO_x) by complete combustion at 950 °C, followed by the reduction of the NO_x gasses to N_2 and quantification of N_2 by thermal conductivity. The Dumas combustion method monitors nitrogen concentration in a much quicker, safer and more reliable way than the conventional Kjeldahl method. The time required for the analysis is shortened to less than 5 min per sample, it can be semi-automated, and it avoids the use of corrosive and hazardous chemicals [1].

Several studies have compared the effectiveness of the Dumas method with that of the Kjeldahl method for various foods such as meat and meat products [2], soybean products [1] and dairy products [3]. Simonne et al. [4] have also conducted a comprehensive study of the mentioned methods for several different food products. These comparisons showed that Dumas method was suitable for replacement of the Kjeldahl method for routine laboratory analyzes. The reported differences in nitrogen content in foods measured by the Kjeldahl and Dumas methods [1–4] indicate a need to compare these techniques in analysis of cereals and oilseed samples, as well as more complex cereal products which can pose an analytical challenge due to their inhomogeneity.

Introduction of a new analytical method into the laboratory requires previous validation, as the first level of quality assurance. Method validation studies which rely on

the determination of overall method-performance parameters assess the fitness for the purpose of analytical methods. Validation of the method through interlaboratory studies is restricted to precision and trueness determination, while other important performance characteristics are not included. For that reason, single-laboratory validation and interlaboratory validation studies must be seen as two necessary and complementary stages in the validation process [5]. The extent of validation depends on the type of method. Precision, bias, linearity, and ruggedness studies should be undertaken as a basic validation requirement [5].

Estimation of measurement uncertainty has become a key requirement in a laboratory accreditation. The guide to the expression of uncertainty in measurement (GUM) published by ISO [6] establishes general rules for evaluating and expressing uncertainty for a wide range of measurements. The guide was interpreted for analytical chemistry by EURACHEM [7] which proposes the use of in-house development and validation data for estimation of measurement uncertainty.

The aim of this work was to evaluate the validation parameters which are not outlined in the official method [8] and to test the suitability of the Dumas combustion method to completely substitute the Kjeldahl method in routine laboratory determination of nitrogen content in cereal and oilseed samples.

During the routine laboratory analysis, more complex cereal product samples which show high degree of inhomogeneity are often encountered, and therefore, it was also necessary to assess the analytical performance of the Dumas method for analysis of this type of samples.

Materials and methods

Materials and chemicals

Six samples of whole wheat kernels and four samples of whole barley kernels were provided by the organizer of interlaboratory study, FOSS Analytical (Sweden).

Commercially available samples of cereal grains and oilseeds (five soybean, six corn, two barley and two wheat whole kernel) as well as muesli and muesli bar samples were obtained from the local market. All samples were ground to suitable fineness (to pass no. 20 sieve, Dual Manufacturing Co., Inc., Chicago, IL, USA), as defined in the official method [8]. Since the only requirement of the official method is to attain 2% of relative standard deviation for ten successive nitrogen determinations in the ground samples, it was determined that the sample mass of 150 g is adequate to be used for grinding of cereal grain and oilseed samples. With muesli bars, however, this requirement could not be achieved even with substantially larger sample size.

All chemicals used for the Kjeldahl method were of p.a. purity grade, manufactured by Merck (Darmstadt, Germany). EDTA standard with certified nitrogen content (9.57 ± 0.03)% and all chemicals used for the Dumas combustion method (sodium hydroxide, magnesium perchlorate) was supplied by Leco (St. Joseph, MI, USA).

Certified reference materials

Wheat flour 502-274, with certified nitrogen content of (2.78 ± 0.02)% (95% confidence interval) was supplied by Leco (St. Joseph, MI, USA). According to recommendation of manufacturer, wheat flour was dried 2 h at 85 °C prior to use.

Certified reference material (CRM) BCR®-563 (wheat flour, with certified protein mass fraction of 11.71 g/100 g) was used for trueness testing. This material has been certified by Community Bureau of Reference (Brussels, Belgium).

Dumas combustion method

For the nitrogen (protein) determination, TruSpec CHNS Macro (Leco, St. Joseph, MI, USA) instrument was used. It has the capability to determine carbon, hydrogen, nitrogen, and sulfur content in a variety of materials including foods, feeds, fertilizers, and soils. Optimum sample size for the protein content determination in cereals and oilseeds was 0.15–0.20 g (as recommended by the manufacturer).

According to the manufacturer's guidelines, adequately milled samples are weighed into a tin foil cup, placed into an automated sample loader, dropped into a hot furnace (950 °C), and flushed with oxygen for very rapid and complete combustion. The products of combustion are passed through a secondary furnace (Afterburner, 850 °C) for further oxidation and fine particles removal and are afterward collected in a collection vessel. Only a representative aliquot is transferred to the helium carrier flow, swept through hot copper to convert the nitrogen oxides into nitrogen, and through Lecosorb (sodium hydroxide) and Anhydrone (magnesium perchlorate) to remove carbon dioxide and water, respectively. The nitrogen is then quantitated by passing the gas through a thermal conductivity cell which emits electrical signal proportional to the nitrogen content. The final results are calculated from a calibration curve plotted using EDTA as the nitrogen calibration standard. The system is calibrated daily before analysis by determining blank and performing a drift correction.

Kjeldahl method

A Tecator Digestion System (Hilleroed, Denmark) and a Tecator Distillation unit, Kjeltec 1003, were used for the analysis. The sample size used in the Kjeldahl procedure was around 1.00 g. Samples were weighed and transferred into Kjeldahl digestion flask containing 7.00–10.0 g of catalyst (prepared by mixing 9 g of K_2SO_4 and 1 g of $CuSO_4 \times 5H_2O$) and 25 mL of concentrated H_2SO_4. After 2.5 h of digestion in a unit with electrical heat and fume removal and cooling to room temperature, 80 mL of NaOH base (mass fraction $w = 33\%$) was added to each flask. By distillation, ammonium hydroxide was trapped as ammonium borate in a boric acid solution (mass concentration $\gamma = 40$ g L^{-1}). Total nitrogen was determined by titration with standardized HCL to a mixed indicator endpoint (1 mg mL^{-1} bromocresol green and 1 mg mL^{-1} methyl red in ethanol of volume concentration $\sigma = 950$ mL L^{-1}).

Method validation

For determination of repeatability precision wheat flour sample (Leco, St. Joseph, MI, USA) was successively analyzed 12 times (three different sample weights in four replicates). Intra-laboratory reproducibility precision was determined by analyzing the same sample in 76 replicates during 2-month period by three different analysts. As a part of interlaboratory study, wheat and barley whole kernel samples were analyzed. The z-score, as performance criteria for the participating laboratories, is calculated by dividing the difference between the laboratory mean and the best estimate of the true value by the standard deviation of the method. Three different sample weights of certified reference material, analyzed in three replicates, were used for trueness determination. Range of linearity was evaluated by checking the linear regression coefficient (R^2) of the calibration curve. The linearity of the calibration curve was considered acceptable when $R^2 > 0.995$. For determination of limit of detection (LOD) and limit of quantification (LOQ), 10 blank samples (empty tin foil cup) were analyzed and standard deviation was calculated ($S_{r(\text{blank})}$).

Measurement uncertainty

The measurement uncertainty estimation was based on the general principles outlined in the EURACHEM/CITAC guide [9].

Statistical analysis

Statistical software STATISTICA (StatSoft, Inc., 2008, Tulsa, OK, USA) was used for statistical analysis of the data. Statistical analysis was carried out using the ANOVA test. In addition, when a statistically significant difference ($p < 0.05$) was found using the ANOVA test, the Tukey honestly significant difference (HSD) multiple comparison was employed. The outlier testing was performed using the Grubbs test ($p = 0.05$).

Results and discussion

Validation parameters of Dumas combustion method

Extensive in-house validation studies were performed in order to test the characteristics of new equipment (TruSpec CHNS, Leco, St. Joseph, MI, USA) and to determine other important performance characteristics which are not specified in the official method (AOAC Official Method 992.23). The following method-performance parameters were determined: repeatability and intra-laboratory reproducibility precision, interlaboratory reproducibility precision, trueness, linearity, LOD, and LOQ.

Repeatability and intra-laboratory reproducibility precision, expressed as standard deviation and relative standard deviation, are shown in the Table 1. The obtained results were in accordance with performance characteristics required by the official method.

For all 10 samples analyzed as a part of interlaboratory study the absolute values of z-score was below 2, which corresponds to good laboratory performance.

If CRM is used, trueness can be expressed in terms of bias or z-score [10]:

$$z = (X_{\text{found}} - X_{\text{certified}}) \Bigg/ \sqrt{\frac{S^2_{\text{found}}}{n_{\text{found}}} + \frac{S^2_{\text{certified}}}{n_{\text{certified}}}}$$

X is the mean determined value [mass fraction of nitrogen (%)]; n is the number of measurements for which standard deviation (S) was calculated.

Certified reference material (BCR®-563) had protein mass fraction of 11.71 g/100 g, and uncertainty (taken as the half-width of the 95% confidence interval of the mean value) of 0.13 g/100 g, for seven accepted sets of data. For nine successive analysis of CRM (three different sample weights in three replicates), the following results were obtained: protein mass fraction of (11.76 ± 0.07) g/100 g, bias $b = 0.43\%$, and z-score = 1.63. Since $|z| < 2$, it can be concluded that applied method has a satisfactory trueness.

Linsinger [11] gave another approach of comparing the measurement result with the certified value. This approach takes into account the measurement result, the certified value, and their respective uncertainties, unlike expressing the trueness in term of z-score in which only standard

Table 1 Results of repeatability and intra-laboratory reproducibility precision obtained using the Dumas combustion method and comparison with criteria defined in the official method

Precision	Obtained values from n determinations of protein mass fraction			Values of protein mass fraction outlined in AOAC official method 992.23	
	S_r (%)	RSD_r (%)	n	S_r (%)	RSD_r (%)
Repeatability	0.06	0.41	12	0.15	0.99
Intra-laboratory reproducibility	0.12	0.74	76	0.27	1.74

* All parameters refer to crude protein mass fractions (5.7 times the values w(N))

errors are included. Following this approach, the uncertainties of measurement results (0.02 g/100 g) and certified value (0.05 g/100 g) of the BCR®-563 are subsequently combined and the expanded uncertainty (0.12 g/100 g) is compared to the absolute difference between mean measured value and certified value (0.05 g/100 g). Since the calculated absolute difference is lower than expanded uncertainty, there is no significant difference between the measurement result and the certified value.

However, the usage of CRM in a validation procedure does not necessarily guarantee trueness of the results over a long period of time. Use of wheat flour with certified nitrogen content as a control sample was the way to monitor trueness, as an important aspect of internal quality control. Nitrogen content of wheat flour was determined on a daily basis, in two replicates, during 2-month period ($n = 76$). For average value of w(N) determined (2.77 ± 0.02)% and certified value of w(N) (2.78 ± 0.02)%, z-test was performed. Since z-score was −1.99 ($|z| < 2$), it can be concluded that trueness was satisfactory over a long period of time. These results were also statistically evaluated using analysis of variance (ANOVA) in order to test day-to-day variations. After excluding results of 4th and 16th day as outliers (using Grubbs test), there was no significant difference between days.

Calibration curve, made with EDTA as a calibration standard, constructed from five points (each point was measured four times) had regression coefficient $R^2 = 0.9990$ and covered a wide range of nitrogen masses (2.36–19.2 mg N).

Standard deviation of ten successively analyzed blank samples was used to calculate limit of detection and limit of quantification. LOD was expressed as the mass fraction of nitrogen corresponding to $0 + 3\ S_{r(blank)}$ (w(N) = 0.006%). LOQ was expressed as the mass fraction of nitrogen corresponding to $0 + 10\ S_{r(blank)}$ (w(N) = 0.02%).

Calculation of measurement uncertainty

Different approaches exist for the estimation of overall measurement uncertainty. The most used, well-known traditional approach is based on identifying, quantifying, and combining all individual contributions to uncertainty [6]. However, because of its complexity, significant time effort and costs, this methodology has never found widespread application. Data from method validation studies can give all or nearly all the information required to evaluate the uncertainty [12, 13]. Since the Dumas method procedure is very simple and almost completely automated, there are not a lot of sources of uncertainty compared to the Kjeldahl procedure. All the uncertainty components (homogeneity of samples, weighing, and calibration) were included in the precision and bias study.

Precision study

Analysis of sample duplicates enables testing a wide variety of sample types and analyte concentrations. Relative standard uncertainty of sample duplicates (u_{dup}) was calculated using the following equation:

$$u_{dup} = S/\sqrt{n}$$

where S is the standard deviation of the relative percentage difference of duplicate samples, n is 2 for duplicate analyzes.

Results of nitrogen content in 47 samples of different cereal grains, oilseeds and their products was used for calculation of $u_{dup} = 1.41\%/\sqrt{2} = 0.997\%$. Since the data were collected over a 6-month period, estimated uncertainty includes natural variations of all factors affecting the results.

Bias study

Measurements of certified reference material provide information about combined effects of many potential sources of uncertainty. When the bias is found to be insignificant, the uncertainty associated with the bias is simply the combination of the standard uncertainty of CRM value and the standard deviation associated with the bias [9].

Results of nitrogen content of certified reference material (wheat flour, 502-274, Leco, St. Joseph, MI, USA) used

in method validation, obtained during 2-month period, were the basis for estimation of relative standard uncertainty of bias (u_b). A significance test was used in order to determine weather mean value of nitrogen content from 76 replicates is significantly different from certified value. The independent one-sample t-test was performed using the following equation:

$$t = |\bar{x} - \mu_0| / \left(S_R / \sqrt{n}\right)$$

where S_R is the standard deviation of test results, n is the number of replicates, $\bar{x}$ is the mean determined value, and μ_0 is the certified value. The degrees of freedom used in this test is $n - 1$.

Since the calculated t value ($t = 4.36$) was greater than critical value for 75 degrees of freedom at 95% confidence ($t_{crit} \cong 2$), the value of bias had to be included in the uncertainty estimation.

Relative standard uncertainty of bias (u_b) was calculated using the following equation:

$$u_b = \sqrt{u_{Cref}^2 + b^2 + \left(RSD_R / \sqrt{n}\right)^2}$$

where u_{Cref} is relative standard uncertainty of CRM, b (bias) is a difference between obtained and certified value expressed in percentages, and RSD_R is relative standard deviation of n replicates.

Confidence interval of $w(N) = 0.02\%$ for CRM was first divided by 2 to convert to standard uncertainty, and then converted to relative standard uncertainty: $u_{Cref} = 0.01/2.78 = 0.0036 = 0.36\%$. From the results of bias ($b = 0.36\%$) and $RSD_R = 0.74\%$ (for 76 replicates), relative standard uncertainty of bias ($u_b = 0.51\%$) was calculated.

Combined uncertainty ($u_{combined}$) is calculated as the squares root of the sum of squares of individual uncertainties:

$$u_{combined} = \sqrt{u_{dup}^2 + u_b^2} = 1.12\%$$

Relative expanded uncertainty is obtained by multiplying the combined uncertainty with coverage factor of 2, giving $U_{rel} = k\, u_{combined} = 2 \times 1.12\% = 2.24\%$.

Comparison between Kjeldahl and Dumas methods

For comparison of the results of two methods, 15 samples of cereal grains and oilseeds were analyzed. Even though values for the Dumas and the Kjeldahl methods were highly correlated ($R^2 = 0.9990$, Fig. 1), the Dumas method gave significantly higher values (ANOVA and Tukey's post hoc test was performed) for most of the samples analyzed (Table 2). The obtained results are comparable with those observed by Jung et al. [1], who reported high correlation between the compared methods ($R^2 = 0.9997$)

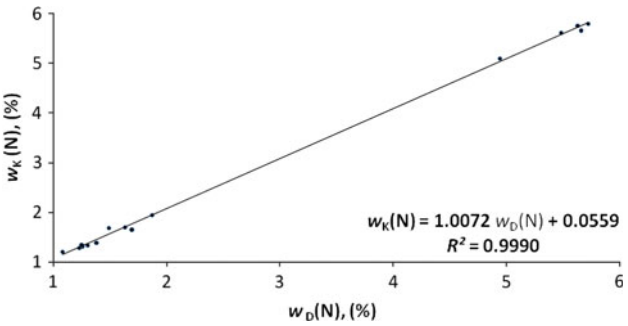

Fig. 1 Relationship between the nitrogen mass fractions $w_K(N)$ and $w_D(N)$ obtained by the Kjeldahl and the Dumas method, respectively, for cereal grain and oilseed samples (R^2 denotes linear regression coefficient)

during analysis of soybean products. Results for the ratio between the mean values of the two methods for soybean samples ($w_K(N)/w_D(N) = 0.98 \pm 0.01$) were in accordance with the results of the above-mentioned authors ($w_K(N)/w_D(N) = 0.97$), as well as those observed by Simonne et al. (1.00 ± 0.00) [4]. The standard deviations calculated for the Dumas method were lower than those of the Kjeldahl method (Table 2), indicating better precision of the combustion method for this type of samples. The obtained results of $w_K(N)/w_D(N)$ for several cereal samples (0.96 ± 0.04) are in accordance with those observed by Simonne et al. (0.95 ± 0.05) [4].

During routine laboratory analysis, complex cereal product samples (i.e., cereal breakfast and muesli bars) are often encountered, and they can be a source of inconsistent analytical results due to their inhomogeneity. Suitability of the Dumas method for analysis of these kinds of samples was evaluated and compared with the Kjeldahl method. For this kind of samples, mean values of nitrogen content for the two methods were not highly correlated ($R^2 = 0.8626$, Fig. 2) as it was the case with cereal and oilseed kernel samples.

Unusually high repeatability standard deviations (S_r) and relative standard deviations (RSD_r) of nitrogen mass fraction (Table 3) obtained by the Dumas method can be explained by an inadequate homogeneity of the samples. Homogeneity was estimated by calculating the homogeneity factor according to equation [14]:

$$H = RSD_r\, m^{1/2}$$

where H is homogeneity factor (% $g^{1/2}$), RSD_r is relative standard deviation of nitrogen mass fraction (%) and m is sample mass (g). Homogeneity factors below 10% $g^{1/2}$, determined in small sample masses, are considered sufficient for the homogeneity of the materials [14]. The obtained results show higher RSD_r values and higher H-factors for the Dumas method when compared to

Table 2 Comparison of the Kjeldahl and the Dumas method results (mean values of nitrogen mass fraction, repeatability standard deviations and repeatability relative standard deviations) for cereal grain and oilseed samples

Sample	w_K(N) (%)	S_r (%)	RSD_r (%)	w_D(N) (%)	S_r (%)	RSD_r (%)	w_D(N) − w_K(N) (%)
Soybean-sample 1	4.940	0.035	0.70	5.092	0.028	0.55	0.152
Soybean-sample 2	5.480*	0.069	1.26	5.617*	0.043	0.76	0.137
Soybean-sample 3	5.627	0.046	0.82	5.757	0.016	0.27	0.131
Soybean-sample 4	5.657	0.012	0.20	5.790	0.009	0.16	0.003
Soybean-sample 5	5.720*	0.052	0.91	5.792*	0.016	0.28	0.074
Corn-sample 1	1.260*	0.035	2.75	1.312*	0.008	0.64	0.052
Corn-sample 2	1.230	0.017	1.41	1.277	0.019	1.52	0.047
Corn-sample 3	1.303	0.012	0.89	1.332	0.010	0.79	0.029
Corn-sample 4	1.380*	0.017	1.26	1.383*	0.003	0.21	0.003
Corn-sample 5	1.083	0.058	5.33	1.208	0.004	0.32	0.124
Corn-sample 6	1.250	0.052	4.16	1.351	0.010	0.75	0.101
Barley-sample 1	1.490	0.046	3.10	1.684	0.011	0.65	0.194
Barley-sample 2	1.693	0.012	0.68	1.651	0.014	0.88	−0.043
Wheat-sample 1	1.870	0.035	1.85	1.939	0.004	0.21	0.069
Wheat-sample 2	1.633	0.023	1.41	1.696	0.013	0.76	0.062

* All samples were analyzed in three replicates

Mean nitrogen contents for the Kjeldahl and Dumas methods in the same row marked with * are not significantly different ($p < 0.05$)

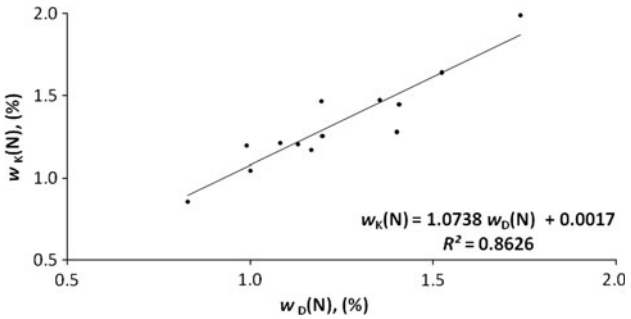

Fig. 2 Relationship between the nitrogen mass fractions w_K(N) and w_D(N) obtained by the Kjeldahl and the Dumas method, respectively, for cereal breakfast and cereal bar samples (R^2 denotes linear regression coefficient)

Kjeldahl method, making it less suitable for analysis of complex cereal samples.

Muesli and cereal breakfast samples, although prepared in a same manner as the other cereal samples, were characterized by a wide inhomogeneity because they consist of relatively big, hard, and fat pieces of raw materials. One problem associated with a high fat content in muesli samples could be agglomeration of a sample during homogenization, and even an increase of particles [12]. Variations of nitrogen content in small sample amounts used for Dumas method are probably a result of an uneven distribution of components of which the sample consists. Therefore, it could be beneficial to optimize the homogenization process for this kind of samples in order to improve sample homogeneity. For this kind of samples, the measurement uncertainty should also be estimated.

Conclusion

Based on validation parameters, it can be concluded that the Dumas combustion method (performed by using TruSpec CHNS, Leco, St. Joseph, MI, USA) gives satisfactory results for determination of nitrogen in cereals and oilseeds. Repeatability and reproducibility standard deviation values for analyzes of these products are even lower than required by the official AOAC method. Analysis of certified reference material showed satisfactory trueness and precision over a longer period of time, confirming the overall stability of the method.

Determination of the nitrogen content in certain complex cereal products (muesli bars and cereal breakfast) using Dumas combustion method, however, can represent an analytical challenge due to the small sample size required for the analysis. Therefore, it is essential that uniform and representative samples are analyzed. Assessing the performance of Dumas combustion method has demonstrated possible difficulties in obtaining a desired homogeneity for this type of samples during routine laboratory analyzes. Relative standard deviation and homogeneity factor values are showing that the Dumas combustion method may be less suitable for analysis of such samples compared to the Kjeldahl method.

Table 3 Comparison of the Kjeldahl and the Dumas method results (mean values of nitrogen mass fraction, repeatability standard deviations and repeatability relative standard deviations) for cereal breakfast and cereal bar samples

Sample	w_K(N) (%)	S_r (%)	RSD_r (%)	w_D(N) (%)	S_r (%)	RSD_r (%)	w_D(N) − w_K(N) (%)
Corn flakes-sample 1	1.131*	0.012	1.02	1.203*	0.047	3.90	0.072
Corn flakes-sample 2	1.167*	0.015	1.31	1.169*	0.026	2.20	0.002
Corn flakes-sample 3	1.407*	0.012	0.82	1.446*	0.025	1.76	0.039
Corn flakes-sample 4	1.001	0.001	0.12	1.043	0.007	0.67	0.043
Corn flakes-sample 5	1.400	0.001	0.07	1.278	0.060	4.66	−0.122
Corn flakes-sample 6	1.197	0.006	0.51	1.253	0.015	1.19	0.056
Muesli-sample 1	1.195	0.005	0.42	1.466	0.024	1.65	0.271
Muesli-sample 2	1.523	0.015	1.00	1.641	0.043	2.65	0.118
Muesli-sample 3	1.738	0.008	0.44	1.988	0.072	3.62	0.250
Muesli-sample 4	1.082	0.004	0.35	1.209	0.044	3.66	0.127
Muesli-sample 5	1.353	0.025	1.86	1.472	0.093	6.30	0.119
Muesli bars-sample 1	0.830*	0.020	2.41	0.855*	0.137	16	0.025
Muesli bars-sample 2	0.990	0.036	3.64	1.195	0.058	4.89	0.205

* All samples were analyzed in three replicates

Mean nitrogen contents for the Kjeldahl and Dumas methods in the same row marked with * are not significantly different ($p < 0.05$)

High values for relative standard deviation were the most important limiting factor during validation of the Dumas method for analysis of complex cereal products, and attention should be given to the additional method optimization when analyzing samples with an increased inhomogeneity.

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