Evaluation of a Macrocombustion Method for Total Nitrogen Determination in Feedstuffs

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Kjeldahl methods for determining crude protein content evoke increasing environmental concern. The officially approved Dumas combustion method, however, allows only small sample sizes from 50 to 300 mg, requiring finely ground materials to ensure sufficiently representative sampling. The total nitrogen contents of 41 feedstuff samples containing from 0.5% to 15% N, of 5 pure chemicals, and of 3 NBS standards were analyzed on an automatic Kjeldahl analyzer (method I) and on an automatic nitrogen determiner equipped with a combustion unit and a thermal conductivity detector. The samples were either introduced to the nitrogen determiner in tin capsules as 50–300 mg of powdered materials (method II) or introduced to the nitrogen determiner directly as pressed pellets weighing about 1 g each (method III). The sample means for methods I–III were 49.0, 49.9, and 49.6 g/kg of nitrogen, the differences being statistically significant ($P < 0.05$). The repeatability of methods I and II was comparable at 1.00 and 1.24, but it was greatly reduced to 0.67 for macrocombustion method III. Accuracy, as determined with five pure chemicals and three NBS standards was also best for method III. It is recommended that this macrocombustion method be subjected to interlaboratory testing to evaluate its "official method" potential.

Combustion methods for nitrogen determination represent attractive alternatives to Kjel-Foss or classical Kjeldahl procedures, which require stringent safety and environmental protection measures.

A number of authors compared the Kjeldahl method to the Dumas combustion method for crude protein/total nitrogen determination in agricultural products (Bellomonte et al., 1987; Ebeling, 1967; Morris et al., 1968, 1969; Stitcher et al., 1969; Sweeney and Rexroad, 1987; Vondenhof and Schulte, 1979). Regarding the performance of these methods, somewhat conflicting results were propagated. Morris et al. (1968, 1969) found the Kjeldahl method to be more precise, while Bellomonte et al. (1987) predicted an equal precision for both. The other authors, cited, found the Dumas method to be superior to the Kjeldahl procedure with respect to precision and/or accuracy. The Kjeldahl and Dumas procedures, however, gained official method status by the American Association of Official Analytical Chemists (AOAC, 1984a,b).

Until now, the small sample size, ranging typically from 20 to 300 mg, represents a major drawback of the Dumas method. It requires careful sample preparation and elaborate milling techniques to overcome sample inhomogeneity. In general, it is particularly difficult to use the combustion method for the analysis of feed samples with low nitrogen or high fat content.

By introducing the samples in pelletized form into the combustion chamber, we have been able to increase sample weights to 1 g and thus to extend the Dumas method to a true macrocombustion method for nitrogen determination. The objective of this study is to compare the performance of this "macro Dumas method" to the low sample weight combustion method and to the automated Kjeldahl method, i.e., the Kjel-Foss method (AOAC 1984b).

EXPERIMENTAL SECTION

Apparatus. The automated Kjeldahl (protein/nitrogen) analyzer was a Kjel-Foss automatic instrument, Model 16210, from A/S N. Foss Electric, Hillerod, Denmark.

The Dumas (protein/nitrogen) combustion analyzer was a Leco nitrogen determiner, Model FP-228, Leco Corp., St. Joseph, MI (or Leco Instrumente GmbH, D-8011 Kirchheim, West Germany), equipped with a thermal conductivity detector. For convenience, an additional water trap consisting of a modified 6-cm Dimroth condenser (a similar device is in the meantime available from Leco Instrumente) with a water-collecting tube and a bottom outlet stopcock was installed to increase the maintenance intervals.

A manual Gressel press (a hydraulic press for pelleting samples is in the meantime available from Leco Instrumente) equipped with a simple brass pressing tool, manufactured in our workshop, served to prepare 5-mm-thick sample pellets of approximately 10-mm diameter.

Materials. The samples analyzed consisted of 41 feeds or feed ingredients, the three NBS standard reference materials bovine liver (SRM-1577a), citrus leaves (SRM-1572), and tomato leaves (SRM-1573), and the 5 pure chemicals acetanilide, EDTA, L-glutamic acid, L-lysine monohydrochloride, and nicotinic acid. The 41 agricultural samples represented great product variety and covered a nitrogen range from 0.5% to 14%, which is equivalent to a crude protein range from 3.1% to 87.5% (using the protein conversion factor $f = 6.25$). They were chosen from external samples arriving for nutritive value testing at our official Swiss laboratory for feedstuffs analysis. The NBS standards were purchased directly from the National Bureau of Standards, US Department of Commerce, Gaithersburg, MD. The pure chemicals and other reagents, all of them of analytical grade, were obtained from Merck Corp.

Sample Preparation. All feedstuff samples were dried at 60 °C and then quantitatively ground in a Brabender cutting mill to pass a 1-mm sieve. Pure chemicals were dried at 105 °C and then ground in a mortar, while NBS standard reference materials were only dried. The samples were carefully mixed and stored in tightly closed glass bottles. Sample weights for all three methods were taken on a Mettler AE200 analytical balance at close time intervals to prevent errors from environmental humidity changes. Sample pellets were weighed immediately after preparation. Pellets that tended to flake were stabilized by sprinkling a few drops of a 2% solution of polyethylene in ethyl acetate onto the pellet surface. After a few minutes, the solvent had evaporated, yielding a nonsticky, film-protected pellet. This treatment, which does not interfere in any way with the determination itself, is also favorably used in connection with the autosampler device.

Determinations. The nitrogen content of all samples was determined in duplicate by the following three methods: method I, automatic Kjel-Foss procedure using a mercury oxide catalyst according to the AOAC prescription (AOAC, 1984b); method II, combustion method proposed by the instrument manufacturer, Leco Corp., using low sample weights (details of this method are given by Sweeney and Rexroad (1987)); method III, macrocom-

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Table I. Comparison of Kjel-Foss and Two Combustion Methods for Total Nitrogen Determination[a]

product	method			difference		
	I[b]	II[c]	III[d]	I–II	I–III	II–III
straw 1	5.6	7.1	5.3	−1.5	0.3	1.8
straw 2	6.2	6.1	6.5	0.1	−0.3	−0.4
corn silage 1	11.2	12.1	11.4	−0.9	−0.2	0.7
porc soup 1	11.8	12.0	11.7	−0.2	0.1	0.3
hay 1	12.6	12.7	12.9	−0.1	−0.3	−0.2
corn silage 2	13.1	14.5	13.1	−1.4	0.0	1.4
corn grain 1	13.4	14.0	13.5	−0.6	−0.1	0.5
corn grain 2	13.9	14.3	13.8	−0.4	0.1	0.5
barley 1	15.6	15.7	14.6	−0.1	1.0	1.1
barley 2	16.9	18.1	17.2	−1.2	−0.3	0.9
oats 1	17.4	18.0	17.2	−0.6	0.2	0.8
grass silage 1	17.3	18.9	17.8	−1.6	−0.5	1.1
oats 2	18.1	18.7	17.9	−0.6	0.2	0.8
triticale 1	18.3	18.7	18.0	−0.4	0.3	0.7
wheat 1	19.5	20.0	19.7	−0.5	−0.2	0.3
hay 2	19.8	21.4	20.7	−1.6	−0.9	0.7
dried grass 1	22.2	22.8	22.0	−0.6	0.2	0.8
grass silage 2	24.0	24.5	24.9	−0.5	−0.9	−0.4
cow premix 1	26.6	28.2	26.9	−1.6	−0.3	1.3
NBS citrus leaves	27.5	29.7	28.2	−2.2	−0.7	1.5
dried grass 2	34.0	33.9	34.7	0.1	−0.7	−0.8
porc soup 2	34.1	33.6	34.2	0.5	−0.1	−0.6
coconut meal	34.8	36.9	36.1	−2.1	−1.3	0.8

[a] N results in grams per kilogram (nitrogen range: 5–40 g/kg). [b] Kjel-Foss. [c] Low-weight combustion. [d] Macrocombustion.

Table II. Comparison of Kjel-Foss and Two Combustion Methods for Total Nitrogen Determination[a]

product	method			difference		
	I[b]	II[c]	III[d]	I–II	I–III	II–III
milk powder	42.7	43.2	44.1	−0.5	−1.4	−0.9
bone meal 1	43.4	44.4	42.1	−1.0	1.3	1.3
NBS tomato leaves	44.8	52.3	50.7	−7.5	−5.3	1.6
rapeseed 1	54.7	57.0	57.2	−2.3	−2.5	−0.2
protein conc 1	57.9	60.9	60.0	−3.0	−2.1	0.9
protein conc 2	62.8	62.6	63.2	0.2	−0.4	−0.6
bone meal 2	63.7	63.3	63.7	0.4	0.0	−0.4
soybean meal 1	64.4	64.3	65.4	0.1	−1.0	−1.1
yeast 1	67.2	68.3	69.6	−1.1	−2.4	−1.3
soybean meal 2	67.9	69.9	69.9	−2.0	−2.0	0.0
peanut meal	82.4	84.0	85.0	−1.6	−2.6	−1.0
meat meal 1	93.7	92.4	93.6	1.3	0.1	−1.2
meat meal 2	96.8	99.2	99.6	−2.4	−2.8	−0.4
fish meal 1	100.0	99.3	99.9	0.7	0.1	−0.6
NBS bovine liver	105.3	106.5	107.1	−1.2	−1.8	−0.6
fish meal 2	117.0	117.6	119.7	−0.6	−2.7	−2.1
wheat gluten 1	117.6	117.5	117.1	0.1	0.5	0.4
wheat gluten 2	134.0	135.3	134.0	−1.3	0.0	1.3
greaves 1	138.9	140.8	139.5	−1.9	−0.6	1.3
greaves 2	139.5	140.6	141.8	−1.1	−2.3	−1.2

[a] N results in grams per kilogram (nitrogen range: 40–140 g/kg). [b] Kjel-Foss. [c] Low-weight combustion. [d] Macrocombustion.

bustion method introducing 1-g pelletized samples directly into the combustion chamber. All other analytical conditions were identical with those of method II.

The nitrogen determination of the pure chemicals and the NBS standards was repeated 4 times to judge the accuracy of the three methods, and precision was further evaluated by analyzing the samples of lysine, barley, soybean, and greaves, each 10 times.

RESULTS AND DISCUSSION

The average total nitrogen results (in grams/kilogram) obtained with the three methods tested are presented in Tables I and II. In general, the results of the three methods agree well, yielding overall means of 49.0 for the Kjel-Foss procedure (method I), 49.9 for the low-weight combustion procedure (method II), and 49.6 for the macrocombustion procedure (method III). These small differences are nevertheless statistically significant (at $P = 0.05$) (statistical treatments: analysis of variance and Duncan test for comparing method mean differences).

The largest discrepancy between the total nitrogen results of the Kjel-Foss and the combustion methods occurs in the tomato leaves sample. This NBS standard is suspected to contain significant amounts of nonprotein nitrogen. The observed differences might therefore mainly be due to this nonprotein nitrogen, which is only partially determined by the Kjel-Foss procedure, while most of it is recovered in the combustion methods.

Repeatability. The repeatability (r) was calculated with the repeatability standard deviation $s(r)$ obtained from double determinations of all 44 agricultural and NBS standard samples according to ISO international standard guidelines (1981): $r = 2.83\, s(r)$ (r represents the upper limit of the absolute difference between two single test results obtained on identical test materials under repeatability conditions, for a specified probability of 95%). Expressed in grams/kilogram, the repeatability (r) of the total nitrogen determination attained values of 1.00, 1.24, and 0.67 for methods I–III, respectively. Clearly, macrocombustion method III yielded the highest precision, the r being almost 2 times smaller than the corresponding value of low-weight combustion method II.

To a large degree, the lower performance of method II may be due to sample inhomogeneity, which becomes a more crucial factor at sample weights on the order of 0.1 g. The data of Table III, showing the standard deviations (s) and variation coefficients obtained from 10 repetitive analyses of four selected samples, further support this finding. For L-lysine, a pure, homogeneous substance, the standard deviation decreased, i.e., precision improved from Kjel-Foss to the combustion procedures, but s was identical for the latter two methods, while for the three feedstuffs precision turned out to be the best for macrocombustion method III.

Accuracy. The average total nitrogen contents estimated from carrying out four analysis repetitions on five pure chemicals and three NBS standard reference materials are listed in Table IV for each method tested together with the respective theoretical—or NBS—values. These data reveal the outstanding agreement between the results of method III and the corresponding reference value. The accuracy of this macrocombustion method is, indeed, remarkable and superior to the other two methods tested.

The data of Table IV also confirm that the Kjel-Foss results generally tend to be low, even if the figures for EDTA, a substance known to be poorly digested by this

Table III. Precision of the Kjel-Foss Procedure (Method I) and Two LECO Combustion Procedures (Methods II and III) for Total Nitrogen Determination

product	N content, g/kg	s,[a] g/kg			variation coeff, %		
		I	II	III	I	II	III
barley	15.5	0.28	0.15	0.13	1.8	0.93	0.84
soybean	64.5	0.14	0.15	0.13	0.22	0.24	0.21
greaves	140.2	0.19	0.16	0.11	0.13	0.12	0.08
L-lysine	153.3	0.16	0.11	0.11	0.10	0.07	0.07

[a] s estimated from 10 analysis repetitions.

Table IV. Accuracy of Kjel-Foss and Two Combustion Methods for Determining Total Nitrogen Content of Five Pure Chemicals[a]

compd	method I[b]	method II[c]	method III[d]	theor N content or NBS value
NBS std 1572 (citrus leaves)	27.5	29.7	28.2	28.6
NBS std 1573 (tomato leaves)	44.8	52.3	50.7	50.0
NBS std 1577a (bovine liver)	105.3	106.5	107.1	107.0
L-glutamic acid	93.8	96.1	95.6	95.4
EDTA	79.7	95.9	96.1	95.9
acetanilide	101.7	102.5	103.6	103.6
nicotinic acid	110.7	112.1	113.2	113.7
L-Lys·HCl	153.2	152.4	154.0	153.3

[a] Average N results in grams per kilogram (n = 4). [b] Kjel-Foss. [c] Low-weight combustion. [d] Macrocombustion.

procedure, are omitted. These results are consistent with well-documented experimental evidence that Kjel-Foss nitrogen or, for that reason, Kjeldahl nitrogen is but rarely equivalent to total nitrogen content.

CONCLUSIONS

This study indicates that the performance of the combustion method for total nitrogen determination can be improved by pelletizing the samples. The sample size may thereby be increased 10-fold to reach 1 g. Accuracy and repeatability of the proposed new macrocombustion procedure are very good and prove to be superior to the Kjel-Foss and the low-weight Leco combustion method proposed by the instrument manufacturer. Depending on the sample composition, significant differences between nitrogen results from the Kjel-Foss and combustion procedures may however occur, which need to be taken into account adequately.

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Registry No. N_2, 7727-37-9; EDTA, 60-00-4; L-glutamic acid, 56-86-0; acetanilide, 103-84-4; nicotinic acid, 59-67-6; L-lysine hydrochloride, 56-87-1.

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