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**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION**

Amending the Method of Calculating
Protein Content in Human Food

Docket No. 00P-1680

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Submitted by

Protein Technologies International, Inc.

00P-1680

CP 1

Mailing Address
P. O. Box 88940 - 59747/1 - #1077971 v1
St. Louis, MO 63188 USA

Shipping Address
824 Gratiot Street
St. Louis, MO 63102

Overnight Address
1034 Danforth Drive
St. Louis, MO 63102

Telephone (314) 982-1010
Toll Free (800) 325-7108



A DuPont Business



Dockets Management Branch
Food and Drug Administration
12420 Parklawn Drive
Room 1-23
Rockville, Maryland 20857

Citizen's Petition

Protein Technologies International, Inc. (PTI), is the largest manufacturer of isolated soy protein (ISP) in the United States, supplying ISP to manufacturers of a wide range of value-added conventional food and dietary supplement products. Under the Nutrition Labeling and Education Act, these food manufacturers must provide nutrition information to consumers, including information about the protein content of their packaged foods. Current regulations rely on a single complex method for calculating protein content in food that involves the use of toxic chemicals because it was the only method espoused by the AOAC International (formerly the Association of Official Analytical Chemists) ten years ago when the regulations were first proposed. Since then, AOAC has adopted another method of measuring protein. PTI submits this petition to request that the Food and Drug Administration (FDA) amend 21 C.F.R. § 101.9(c)(7) to provide for the use of any method of calculating protein content in food endorsed by the AOAC International.

I. ACTION REQUESTED

Petitioners hereby respectfully request that FDA modify the reference to the method of calculating protein content, found at 21 C.F.R. § 101.9(c)(7), *i.e.*, "the appropriate method of analysis as given in the *Official Methods of Analysis* of the AOAC International (formerly the Association of Official Analytical Chemists), 15th ed. (1990)," to read "the appropriate method of analysis as given the *Official Methods of Analysis* of the AOAC International, 17th ed. (2000)."

II. STATEMENT OF GROUNDS

In many instances throughout its regulations, FDA relies on the AOAC International's designated methodologies for calculating the content of numerous substances for compliance purposes, including the nutrient content of foods. AOAC International standards are recognized among the scientific

Mailing Address
P. O. Box 88940
St. Louis, MO 63188 USA

Shipping Address
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Overnight Address
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Telephone (314) 982-1010
Toll Free (800) 325-7108

community for their reliability. Analytical methods proposed for inclusion in the *Official Methods of Analysis* are subjected to eight or more laboratory collaborative studies conducted according to internationally recognized standards. The results receive rigorous scientific review. Once a method is adopted by AOAC, it is published in the *Journal of AOAC International* and compiled in the *Official Methods of Analysis*, which is updated annually and is currently in its 17th edition.

At the time FDA promulgated its regulations establishing the method for determining protein content for purposes of nutrition labeling, FDA relied upon the then current AOAC methodology for measuring protein content as set forth in the 15th edition of the *Official Methods of Analysis*. 1/ FDA should continue to rely on the most current AOAC recommendations regarding nutrient calculations. The agency should not be forced to rely on outdated methods of analysis or be limited to those methods recognized at the time the regulations were drafted. Therefore, FDA should amend its regulations to provide for the most efficient and effective methods as recognized by the AOAC.

A. Method Required Under Current Regulations

Current regulations direct manufacturers to calculate the protein content of foods using any method approved by the AOAC's *Official Methods of Analysis*, 15th Edition. The only method approved for use for human food in the 15th edition is a method referred to as the Kjeldahl method. This method involves use of a mercury catalyst, a dangerous chemical.

The use of mercury is potentially hazardous. Mercury is corrosive; its salts are toxic and humans can be harmed through ingestion or inhalation. Individuals required to conduct nutritional testing using this dangerous chemical are unnecessarily exposed to health risks. In addition, mercury disposal is complicated and very expensive. For example, landfill disposal of mercury is prohibited in the United States, so waste must be sent to an approved recycler/reclamation firm for disposal.

The Kjeldahl method also requires long analysis times. Because of the long wait for results and the use of dangerous chemicals, most laboratories have abandoned the Kjeldahl method.

1/ 21 C.F.R. § 101.9(c)(7).

B. Other Methods: The Combustion Method

The more recent editions *Official Methods of Analysis* have allowed for an alternative method, the Combustion method, also known as the Dumas method, to measure protein levels in some human foods. The accuracy and reliability of the Combustion method has been widely adopted by responsible laboratories and is supported by scientific studies. These scientific studies demonstrate that the Combustion method may be used in lieu of the Kjeldahl method with no loss of accuracy or reliability. We have attached key studies that demonstrate the comparability of these two methods. ^{2/} Yet, FDA continues to recognize only one method. Since another safer and reliable method exists that has been accepted by the scientific community, FDA should permit its use for measuring protein content. Petitioners, therefore, request that FDA amend its regulations to permit the use of all methods for measuring protein content specified in the most recent edition of the *Official Methods of Analysis* of the AOAC International, 17th ed. (2000).

III. ENVIRONMENTAL IMPACT

The action requested by the petition is not expected to have a significant effect on the quality of the human environment and is subject to categorical exclusion under 21 C.F.R. § 25.30(i) because it is a technical change in the regulations.

^{2/} See Attachment A: Wiles et. al., *Routine Analysis of Proteins by Kjeldahl and Dumas Methods: Review and Interlaboratory Study Using Dairy Products*, 81 Journal of AOAC International No. 3, at 620 (1998); Berner and Brown, *Protein Nitrogen Combustion Method Collaborative Study I. Comparison with Smalley Total Kjeldahl Nitrogen and Combustion Results*, 71 JAOCS No. 11, at 1291 (1994); Bicsak, *Comparison of Kjeldahl Method for Determination of Crude Protein in Cereal Grain and Oilseeds with Generic Combustion Method: Collaborative Study*, 76 Journal of AOAC International No. 4 at 780 (1993); King-Brink & Sebranek, *Combustion Method for Determination of Crude Protein in Meat and Meat Products: Collaborative Studies*, 76 Journal of AOAC International, No. 4, at 787 (1993); Tate, *Determination of Nitrogen in Fertilizer by Combustion: Collaborative Studies*, 77 Journal of AOAC International, No. 4 at 829 (1994).

IV. ECONOMIC IMPACT

An economic impact statement under 21 C.F.R. § 10.30(b) is not required at this time.

* * * * *

The undersigned certifies that, to the best of their knowledge, this petition includes all information and views on which the petition relies, and that it includes representative data and information known to the petitioner which are unfavorable to the petition.

Respectfully submitted,
Protein Technologies International, Inc.

Katherine Harris

Katherine Harris
Vice President and General Counsel

APPENDIX A

Routine Analysis of Proteins by Kjeldahl and Dumas Methods: Review and Interlaboratory Study Using Dairy Products

PETER G. WILES and IAN K. GRAY

New Zealand Dairy Research Institute, Private Bag 11 029, Palmerston North, New Zealand

ROGER C. KISSLING

New Zealand Dairy Board, PO Box 417, Wellington, New Zealand

Collaborators: C. Delahanty; J. Evers; K. Greenwood; K. Grimshaw; M. Hibbert; K. Kelly; H. Luckin; K. McC Morris; M. Petersen; P. Ross; M. Valli

The Kjeldahl and Dumas (combustion) methods were compared in 11 laboratories analyzing samples of milk, skim milk powder, whole milk powder, whey protein concentrate, infant formula, casein, caseinate, 2 reference compounds (glycine and EDTA), and a secondary reference skim milk powder. The comparison was conducted by using international standards where applicable. Overall means were 8.818 g N/100 g by the Kjeldahl method and 8.810 g N/100 g by the Dumas method. No evidence was found for a consistent bias between methods that may be of concern in the trading of dairy produce. A review of more than 10 related trials revealed a lack of consensus in the bias between the 2 methods, suggesting that differences in methodology and sources of systematic error may be contributors. For samples containing >2 g N/100 g, the Dumas relative repeatability and reproducibility standard deviations were consistently about 0.35 and 0.75%, respectively, whereas the corresponding Kjeldahl values declined generally with N content and were significantly larger. The Dumas precision characteristics may be due to the dominance of Leco analyzers in this trials, and in most other recent trials, rather than an inherent method attribute. Protein determination methods for dairy products need to be reviewed and updated. The Dumas method needs Codex Alimentarius status as a recognized test method.

The traditional (manual) macro Kjeldahl method is the international reference method for determining total nitrogen (TN) or crude protein. However, with very little time to analyze large numbers of samples, commercial laboratories no longer use manual methods routinely. The generic Kjeldahl method has been automated to various degrees in various pro-

prietary systems (e.g., systems based on block digestion, as well as Kjelfoss instruments). Such systems are used widely even though throughput, still is limited and safety concerns are significant. Instruments based on the Dumas (combustion) method are an increasingly attractive alternative.

More than 10 comparisons of the Kjeldahl and Dumas methods for determining TN have been done (1-16), several of which were conducted as multilaboratory trials (1, 3, 5, 8, 10, 13, 16). The range of samples analyzed in these comparisons is considerable: cereals, grains, oil seeds, meats, brewing substances, a variety of plant material, blood serum protein, prepared foods, and dairy products, most notably milk, skim milk powder (SMP), and cheese. H. Frister (17) conducted a major trial involving some 30 laboratories analyzing samples of milk and SMP. Also recently, M. King-Brink and J.G. Sebranek (18) reported preliminary findings from a comparison trial devoted to dairy products. In most cases, the findings to date show that the Dumas technique gives generally higher TN values than does the Kjeldahl method. However, the differences reported vary markedly, and little consensus has emerged in the way of an agreed relationship between the methods. A summary of the salient features of these trials is given in Table 1.

We report results of a comparison trial conducted in 1995 involving commercial laboratories using a variety of instruments (based on the generic Kjeldahl and Dumas methods), to analyze a variety of dairy products covering a TN range of 0.5-14.5 g N/100 g. We have examined the precision characteristics of these N determination methods in the course of routine laboratory operations. We compare our results with those of previous trials and discuss the possible causes of the variable bias in various trials.

Experimental

The trial design was based on 2 international standards: International Dairy Federation IDF 135B (19) and International

Table 1. Summary of previous comparisons of Dumas and Kjeldahl methods

Study (reference)	Samples (year)	Ratio of Dumas mean/Kjeldahl mean in arbitrary units (Relative bias, %)	Comments
Ebeling (13)	Animal feedstuffs (1988)	Pooled mean 5.72/5.77 (-0.8)	Ten laboratories using Coleman 29A N analyzers. Acetanilide used as reference. Seven feed samples in duplicate.
Griepink et al. (1)	Skim milk powder (1983)	5.88/5.83 (0.9)	Collaborative European trial to set European Community reference milk protein standard. (Used multiple replicates.) Kjeldahl assays performed with Cu catalyst.
Sweeney and Flexroad (16)	Animal feedstuffs (1987)	Pooled mean 8.61/8.58 (0.35)	Single Leco instrument. Used lysine-HCl, tryptophan, and EDTA as reference agents. Cu Kjeldahl catalyst used.
Bellomonte et al. (2)	Infant formulas and a variety of food commodities (1987)	(2-3)	Single instrument (Carlo Erba Dumas analyzer) comparison. Kjeldahl assays performed with Cu catalyst.
Sweeney (10)	Animal feedstuffs (1989)	Fine: 47.65/47.41 (0.5) Coarse: 31.82/31.50 (1.0)	Used closely matched pairs. Collaborative U.S. trial. Kjeldahl assays used Hg catalyst. All 9 Dumas instruments were Leco.
Hansen (14)	Animal feedstuffs (1989)	Kjelfoss (pooled mean, 2.6) Kjeltec (pooled mean, 4.4)	Single Leco instrument. Examined effect of nitrate as source of bias. Kjelfoss used Hg catalyst, and block digester used Se catalyst.
Smith (12)	220 diverse samples in duplicate (1991)	In 11 categories, category means ranged from 4.05/3.98 (1.8) to 3.99/4.01 (-0.5)	Single instrument, in-house comparison of Foss-Heraeus Macro N with Kjelfoss (using Hg catalyst). Nine reference materials used in Macro N.
King-Brink and Sebranek (8)	Meat products (1993)	15.75/15.69 (1.0)	Used closely matched pairs. Kjeldahl assays performed with Hg catalyst. U.S. comparison.
Blondel and Vlan (4)	Blood proteins (1993)	4% albumin: 40.58/39.73 (2.1) 20% albumin: 19.77/20.12 (-1.8)	Single laboratory using Leco FP-428 and Tecator Kjeltec instruments. Used Tecator Kjelab S5 catalyst. Reference materials not reported.
Biscak (3)	Grains, cereals, and oil seeds (1993)	Pooled mean 25.23/25.18 (0.2) (Nicotinic acid results excluded but lysine-HCl included)	Used closely matched pairs. U.S. comparison with 9 Dumas instruments in 7 laboratories and 3 Kjeldahl instruments (using Hg catalyst).
Buckee (5)	Malt, barley, and beer (1994)	Barley: 1.428/1.415 (0.9) Malt: 1.720/1.666 (2.0)	UK comparison with 20 Dumas and a mixture of 17 Kjeldahl/Kjelfoss instruments. (Used ISO 5725 but no reference compounds reported.)
Dawn and Declercq (6)	Oil seeds (1994)	(Sunflower seed, 1.8) (Soybean, 3.7) (Bias depended on seed type)	In-house multi-instrument comparison. (Used nicotinic acid, glycine, and ammonium p-toluene sulfonate as reference compounds.) Kjeldahl assays used Cu/N catalyst.
Jakob et al. (7)	Liquid milk (1995)	0.563/0.528 (6.7)	Single laboratory and Dumas instrument (Leco FP-428) comparison.
King-Brink and Sebranek (18)	Dairy products (1995)	Positive and increasing with high TN contents	Used closely matched pairs. Comparison U.S. trial using mainly Leco analyzers. Kjeldahl assays performed with Cu catalyst.
Frister (17)	Dairy products (1996)	Milk: 0.558/0.544 (2.6) Skim milk powder: 5.76/5.83 (2.3)	Comparison using 30 laboratories. Followed ISO 5725. Tyrosine used as digestion recovery check. Kjeldahl catalyst not reported.
Sachen and Thiex (16)	Cellulosic samples (1997)	(Hay, -3 to 7)	Three laboratories using Leco instruments. Bias depended on sample porosity and occluded air. Kjeldahl catalyst not reported.

Table 2. Dairy samples used in comparison

Product	Nominal TN content, g N/100 g
Whole milk (UHT treated)	0.5
Infant formula (powder)	2.0
Whole milk powder	4.5
Skim milk powder	5.7
Secondary reference skim milk powder	6.4 ^a
Whey protein concentrate (70% protein)	12.0
Casein (10% moisture)	13.7
Sodium caseinate	14.5

^a Based on a previous comparison trial using 9 laboratories and a total of 83 assays, the wet basis mean Kjeldahl TN content was 6.407 g N/100 g, with a repeatability standard deviation of 0.018 g N/100 g and a reproducibility standard deviation of 0.032 g N/100 g (21).

Samples

The dairy products used in the trial are listed in Table 2.

Two analytical-grade reference compounds were also used: glycine, minimum assay, 99.7% (theoretically 18.67 g N/100 g), and ethylenediaminetetraacetic acid (EDTA), minimum assay, 99.0% (theoretically 9.58 g N/100 g). Both were AnalaR grade (BDH Laboratory Supplies, Poole, United Kingdom). Samples of reference compounds were supplied from containers sharing common batch numbers and were subsampled into 50 mL screw-topped plastic sample containers.

The secondary reference powder was a commercial SMP that had been analyzed in an earlier comparison trial to determine its mean Kjeldahl TN content (6.407 g N/100 g wet basis; 21).

Sample Preparation

For all the dairy powder samples, except the infant formula and secondary reference SMP, a single 25 kg bag of each was obtained from a commercial factory. The infant formula was obtained from a retail outlet.

Ultra high temperature (UHT) treated commercial cartonized whole milk (Anchor Blue Top) was used. All the cartons used were taken from the same production run (February 22, 1995), and homogeneity among cartons was confirmed by sampling at random and analyzing prior to use in the trial.

To ensure homogeneity, all powder samples, except the secondary reference SMP, were blended (Quadblend blender, Unitech Industries, Auckland, New Zealand) for 30 min. Immediately after blending, samples were transferred to an air-conditioned room where samples of ca. 30 g were placed in foil-laminated sachets that were immediately heat-sealed.

Analysis Procedures

Laboratories set up and operated their (macro) Kjeldahl block digestion and distillation equipment according to manufacturers' specifications. Block digestion was performed with $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ catalyst. Digestion times were in the range 60–150 min. The 3 Kjelfoss instruments used 0.75 g N_2O with a digestion time of 6 min. For the milk sample, Kjeldahl assays were performed with a 5 g sample.

The Dumas instruments also were set up and calibrated according to manufacturers' instructions. Six laboratories with Leco instruments used a furnace temperature of 950°C; one lab used 800°C. The Leco instruments were operated with an oxygen profile of either medium-high-high or high-high-high. The Foss-Heraeus instrument was operated with a furnace temperature of 1020°C.

The laboratories were requested to verify the performance/calibration of their instruments according to their accredited standard procedures prior to analysis of trial samples.

On receipt of a set of samples, laboratories were asked to perform 6 replicate assays of each material on each instrument. Kjeldahl assays were conducted according to IDF methods (22–24). Dumas assays were conducted according to methods specified by instrument manufacturers. Participants also were asked to perform 6 replicate moisture determinations of the dairy powders. Where possible, moisture determinations were performed concurrently with the TN assays, using the 102°C oven-drying reference method (25).

If a laboratory was unable to complete the series of assays in one day, it was directed to repeat the glycine and EDTA reference assays and to analyze the dairy products in the following sequence to avoid the possibility that all low-protein samples were analyzed on one day and all high-protein samples were analyzed on the other, with the possibility of interday bias influencing the results: day 1: Glycine, EDTA, whole milk, SMP, whole milk powder (WMP), casein; day 2: Glycine, EDTA, infant formula, caseinate, whey protein concentrate (WPC), secondary reference powder.

Finally, the New Zealand Dairy Research Institute performed nitrate and nitrite assays on each of the dairy products according to the method of the U.S. Environmental Protection Agency (26).

Instruments

Table 3 summarizes the instruments of participating laboratories.

Statistical Analysis: Screening for Outliers

The process for determining outliers involved the following steps.

(1) Results were screened for outlying laboratories by the Cochran test (testing for the homogeneity of intralaboratory variances). This continued until no more than 2/9 of the laboratories had been discarded (19). A significance level of 1% was used.

(2) For laboratories identified in (1), Grubb's single and double tests were applied (also at the 1% level) to determine whether the outliers of any of the outlying laboratories were due to isolated (i.e., 1 or 2) outlying values (20). The outlier analysis deviated from IDF 135B (19) and followed ISO 5725-2 (20) because IDF 135B requires rejection of all observations from an outlying laboratory, not just a single discrepant value that might have produced an outlier. There were some obvious examples where single discrepant values inflated the variability used in the outlier test.

(3) For laboratory means (i.e., for a particular product), the Grubb's single and double tests were used to determine whether

Table 3. Laboratories participating in comparison and their instruments

Laboratory	Kjeldahl (Model) (Catalyst)	Dumas (Model)
NZDG, Ta Awamutu	No suitable equipment	Leco (FP-428)
NZDG, Hautapu	Kjelfoss (16210) (Hg)	Leco (FP-428)
NZDG, Waitoa	No suitable equipment	Leco (FP 428)
Tui Milk Products Ltd., Longburn	Buchi (435 digestion unit; 323 distillation unit) (Cu)	No suitable equipment
Tui Milk Products Ltd., Pahiatua	Gerhardt (Vapodest 6) (Cu)	Leco (FP-428)
Northland Cooperative Dairy Co. Ltd., Kauri	Gerhardt (Vapodest) (Cu)	Leco (FP-428)
Kwi Dairies Ltd., Hawera	Buchi (435 digestion unit; 425 distillation unit) (Cu)	Foss-Heraeus (Macro N)
MAF Quality Management, Auckland	Kjelfoss (16210) (Hg)	Leco (FP-428)
Grayson Laboratories Ltd., Auckland	Gerhardt (Vapodest 2) (Cu)	Leco (FP-428)
New Zealand Dairy Research Institute	Kjelfoss (16210) (Hg)	Leco (FP-428)
Agriculture Victoria, Kyabram Dairy Centre, Victoria	Buchi (435 digestion unit; 323 distillation unit) (Cu)	Leco (FP-428)

any laboratory was biased with respect to the majority (20). Such means otherwise would inflate the between-laboratory variance and therefore the reproducibility.

Outlier tests were applied with the assumption of an equal number of observations in each cell (i.e., for each product-laboratory combination). When a laboratory submitted only one result, say a moisture determination, the result was treated as if it was the mean of several observations. Although this treatment allowed use of Grubb's outlier test, there would have been only a small increase in the chance of falsely identifying an outlier.

Values not identified by the above procedures but suspected of being discrepant (e.g., yielding a protein content greater than the possible value, as with sum of protein and moisture and expected mineral content significantly exceeding 100%) also were removed.

Laboratories were notified of their outliers (significant at $P = 1\%$) and stragglers (significant at $P = 5\%$ but not at $P = 1\%$; 20) and were asked to check and confirm their records. Upon confirmation that there were no transcription, calculation, or instrument errors, stragglers were retained but outliers were removed from the final data set.

Calculation of Precision Characteristics

Estimates of repeatability and reproducibility standard deviations (RSD_r and RSD_R , respectively) were calculated according to the procedure given in ISO 5725-2 (20) by using one-way analysis of variance (ANOVA) to extract relevant variance components.

A test of whether a significant bias exists between methods was performed with the laboratories as a separate effect, by using a generalized linear model procedure. This procedure allowed a 2-way ANOVA to be performed on data sets that were not fully balanced. Strictly speaking, this procedure assumed homogeneity of variances across both methods and all laboratories in the trial. However, the finding of no significant difference between methods for most of the samples was expected to still apply.

Results

We use coded laboratory numbers to refer to results of specific laboratories to preserve anonymity. Six of the 9 laboratories using the Kjeldahl assay and 7 of the 10 laboratories using the Dumas assay conducted analyses over consecutive days.

Screening of Outliers

A level of significance of $P = 1\%$ was used with the Cochran and Grubb's tests to reject outliers and $P = 5\%$ was used to identify stragglers. Generally, stragglers were retained unless some other attribute provided additional basis for rejection. These significance levels are similar to those adopted by Griepink et al. (1), but slightly different significance criteria have been adopted by other researchers. Frister (17), for example, used Grubb's test at the $P = 5\%$ significance level to reject discrepant laboratories. Sweeney (10) also used $P = 5\%$ as the significance level to detect sample \times laboratory interaction effects. A difference in significance level to accept or reject outliers can affect estimates of repeatability (r) and reproducibility (R) and mean values depending on the distribution of the results. We believe our basis for rejecting outliers was conservative.

In addition to outliers identified by sequential use of the Cochran and Grubb's tests, some values were rejected on technical grounds. Laboratory 9 presented Kjeldahl caseinate TN values that averaged 15.1 g N/100 g. Conversion of this TN value to a protein basis ($\times 6.38$) gives a protein content of 96.3 g/100 g. However, the pooled mean moisture content of the sample was 4.65 g/100 g, and laboratory 9's moisture value was consistent with this pooled mean. So even before mineral and minor components of the sample are considered, the composition already exceeds 100%. Therefore, laboratory 9's TN results were rejected on the grounds of being, at best, suspect. The same was true for Kjeldahl casein results submitted by laboratory 9.

Kjeldahl results from laboratory 5 revealed a single outlier or straggler for several samples analyzed. Discussion with the laboratory staff revealed that one Kjelfoss flask had been faulty. Results from this flask were rejected. Further scrutiny of this laboratory's results showed that another sample not highlighted

by statistical screening was also affected. Values for this sample also were excluded.

Scrutiny of SMP results of laboratory 2 revealed that inadvertent substitution of the SMP sample by the secondary reference SMP had occurred. Accordingly, these SMP results were excluded.

Laboratories 1 (Kjelfoss), 8 (Kjelfoss), and 10 (Buchi) reported Kjeldahl TN recoveries from EDTA in the range 85.6–96.6%, substantially less than the means from other laboratories, which averaged >99.5%. In a previous trial we conducted (21), where we also used EDTA as a reference standard, we found that some laboratories (notably those using Kjelfoss instruments) experienced difficulty in obtaining thorough digestion of this material. On the basis of this experience, we excluded these EDTA results from calculation of the overall EDTA mean.

Overall, about 8% of the TN results (including the reference materials) were excluded for the various reasons noted above.

With the exception of the second-day assays of reference compounds, all final method comparisons were made with a data pool of at least 44 results for each method. With the exception of EDTA, each pool contained contributions from at least 8 laboratories.

After removal of outliers, the overall pooled mean for Kjeldahl assays of the dairy products was 7.374 g N/100 g. With inclusion of the 2 reference compounds, it was 8.818 g N/100 g. Corresponding values for the Dumas assays were 7.346 and 8.810 g N/100 g. Ratios of the Dumas means to the Kjeldahl means were 0.9963 and 0.9992, respectively. These results can be compared with the corresponding raw data means, before removal of outliers, of 8.806 g N/100 g (Kjeldahl) and 8.817 g N/100 g (Dumas), giving a Dumas/Kjeldahl ratio of 1.0012. The pooled means (both with and without outliers), standard deviations (exclusive of outliers), and numbers of accepted assays for individual samples are shown in Table 4. Results of a 2-way ANOVA comparison of methods (by laboratory and method) also are shown in Table 4. Method differences were significant only for milk, infant formula, WMP and WPC. Estimates of Kjeldahl and Dumas RSD, and RSD_R are shown in Table 4 and plotted in Figure 1.

Nitrate and nitrite concentrations in the dairy samples are shown in Table 5. Calculated recoveries of N from reference materials are shown in Table 6.

Comparison of Results with Those of Other Trials

Differences between results of Dumas and Kjeldahl methods are plotted in Figure 2. We chose to plot separately the mean values for EDTA and glycine obtained on days 1 and 2, as this provided an insight into the likely day-to-day variations that were experienced in instrument calibration/performance. For comparison, in Figure 3, we plotted the main results of previous studies on a similar basis.

It is evident that our findings differ significantly from most previously published work in 2 important respects. We find no significant evidence for a positive bias toward the Dumas method; neither do we find a tendency for the bias to increase with protein concentration. We also note that this trial covers a

Table 4. Summary of results

Sample	Kjeldahl mean, g N/100 g (No. of accepted observations) (Mean inclusive of outliers)	Dumas mean, g N/100 g (No. of accepted observations) (Mean inclusive of outliers)	2-way ANOVA comparison of method means: level of significance (P), %	Estimates of relative standard deviations, % (Kjeldahl, RSD _D , and RSD _R ; Dumas, RSD _D , and RSD _R)	F-ratio comparison of estimates of method standard deviations: level of significance (P) ^a , %	Mean moisture content (standard deviation, g/100 g) (No. of accepted observations)
Milk	0.553 (53) [0.552]	0.537 (53) [0.498]	<0.01	0.65 1.9 2.0 7.3	<0.001 <0.001	NA ^b
Infant Formula	1.905 (53) [1.899]	1.887 (54) [1.930]	<0.01	1.7 4.1 0.48 1.72	<0.001 <0.001	2.54 [0.08] (38)
WMP	4.387 (53) [4.381]	4.359 (60) [4.359]	3.6	1.47 2.52 0.93 0.82	<0.001 <0.001	2.88 [0.17] (36)
SMP	5.787 (47) [5.844]	5.773 (54) [5.760]	32	0.74 2.29 0.49 0.87	1.9 <0.001	3.67 [0.13] (35)
Secondary Reference SMP	6.411 (47) [6.394]	6.425 (47) [6.454]	13	0.84 1.8 0.33 0.72	<0.001 <0.001	3.12 [0.04] (30)
EDTA (day 1 + day 2)	9.601 (59) [9.303]	9.590 (88) [9.592]	91	0.85 1.56 0.30 0.52	<0.001 <0.001	NA
WPC	12.611 (51) [12.570]	12.575 (59) [12.575]	1.5	0.59 1.56 0.31 0.74	0.02 <0.001	4.02 [0.22] (42)
Casein	13.847 (44) [13.932]	13.863 (59) [13.869]	80	0.34 1.16 0.30 0.64	27 <0.001	9.88 [0.11] (45)
Caseinate	14.483 (45) [14.526]	14.483 (59) [14.477]	20	0.59 1.33 0.35 0.62	0.37 <0.001	4.65 [0.14] (41)
Glycine (day 1 + day 2)	18.611 (88) [18.664]	18.630 (83) [18.653]	71	0.40 0.81 0.33 0.73	7.0 19.3	NA

^a P values were calculated from the lesser tail area, that is, the lesser of the 2 areas that is less than or greater than the observed statistic. This accommodates situations where $t(\text{Kjeldahl})$ and $t(\text{Dumas}) < t(\text{Kjeldahl})$ etc.

^b NA, not applicable.

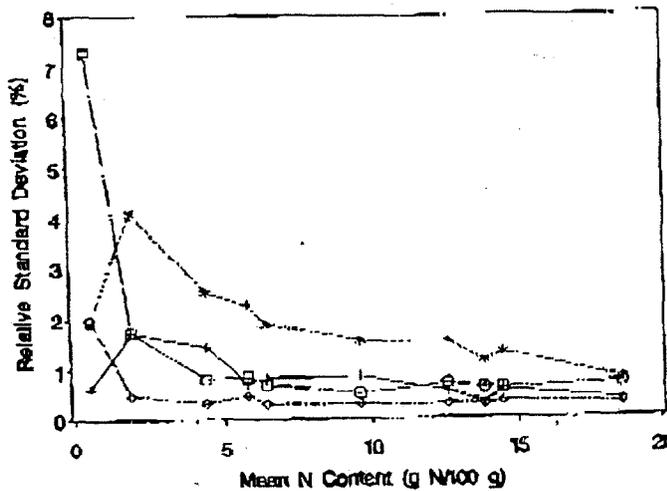


Figure 1. Comparison of Kjeldahl and Dumas precision characteristics: relative repeatability and reproducibility standard deviations. Key: +, Kjeldahl repeatability; *, Kjeldahl reproducibility; o, Dumas repeatability; □, Dumas reproducibility.

range of TN values (0.5–18.6 g N/100 g)—and protein contents ranging from 3.5 g/100 g (whole milk) to 92.4 g/100 g (caseinate)—that is broader than in most previous comparisons. The overall picture suggests a lack of agreement among the various trials of a consistent trend in the relationship between the methods, implying that some variables are not being controlled adequately during these comparison trials.

Discussion

Repeatability and Reproducibility

Figure 1 shows the behavior of the estimates of RSD_r and RSD_R for both methods. For the Kjeldahl method analyzing milk, RSD_r (0.65%) and RSD_R (1.9%) values are considerably greater than those determined by Barbano et al. (27; 0.385 and 0.504%, respectively) but close to those reported by Grappin and Horwitz (28; 0.51 and 1.02%, respectively). The earlier reported values relate strictly to milk and were obtained under formal collaborative trial conditions. On the other hand, our samples were analyzed routinely in commercial laboratories and were not accorded any special treatment.

The possible effect of widening the scope of the Kjeldahl method from milk to other dairy samples is suggested by the increase in RSD_R values for infant formula, WMP, and SMP.

Table 5. Nitrate and nitrite content of samples

Sample	Nitrate, $\mu\text{g/g}$	Nitrite, $\mu\text{g/g}$
WMP	4.7	<1*
SMP	4.9	<1
WPC	31.5	<1
Casein	12.2	4.1
Sodium caseinate	4.9	<1
Infant formula	13.5	<1

* Detectable limit, 1 $\mu\text{g/g}$.

Table 6. Details of reference compound performance in this work after removal of outliers

Reference compound (TN, g N/100 g)	Mean Kjeldahl digestion N recovery, %	Mean Dumas N recovery, %
	[No. of observations retained (day 1 + day 2)]	[No. of observations retained (day 1 + day 2)]
EDTA (9.58)	100.2 (52) ^a	100.1 (88)
Glycine (19.67)	99.7 (66)	98.8 (83)
Secondary reference SMP (6.407, Kjeldahl wet basis)	100.1 (47)	100.3 (47)

* Laboratories 1, 6 (both Klattross), and 10 (Bucht) were removed from the set used to calculate these values.

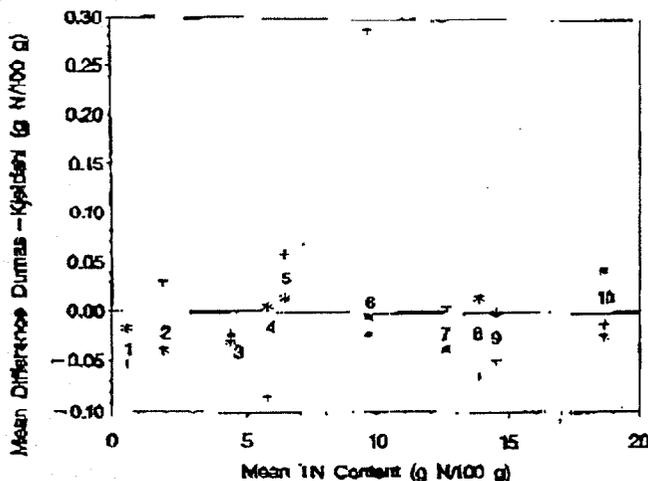


Figure 2. Difference between Dumas and Kjeldahl methods (this work). Key: +, all data including outliers; *, data excluding outliers. Samples: 1, milk; 2, infant formula; 3, WMP; 4, SMP; 5, secondary reference SMP; 6, EDTA (days 1 and 2); 7, WPC; 8, casein; 9, caseinate; 10, glycine (days 1 and 2).

The relative precision of the Kjeldahl method tended to improve as the TN concentration increased. In contrast, the Dumas method gave almost uniform RSD, and RSD_R values (about 0.35 and 0.75%, respectively) for all samples with >2 g N/100 g. They were typically less than half the corresponding Kjeldahl values, and the differences in variability were significant in many instances (*F* ratios shown in Table 4). The Dumas RSD, and RSD_R values compare very favorably with those reported by Sweeney (10; 0.59 and 1.1%, respectively), although Sweeney's samples were animal feedstuffs that would have been less homogeneous than our dairy samples. The superior performance of the Dumas method is also consistent with results of other studies (1, 13, 17).

The substantially higher RSD_i and RSD_R values from the Dumas method for milk may have been due to use of Leco equipment that was configured for sampling of solid materials rather than handling of liquid samples. In addition, sample size may not have been optimal for low TN levels, because the precision improved dramatically as the TN level increased to about 4 g N/100 g and was essentially uniform thereafter.

In this trial, and essentially in all published comparisons in the past 10 years, Dumas analyses have been performed predominantly with Leco equipment. The extent to which precision characteristics depend on the proprietary brand of instrument rather than being a generic property of the Dumas method is unclear. As development of instruments continues, further improvements in accuracy will be achieved (e.g., see Sachse & Thiex, reference 16, regarding recent Leco improvements). The performance of any standard method therefore needs to be re-examined periodically.

Differences in N Detection by the 2 Methods

In theory, the 2 methods may not measure the same level of protein in a sample. The Kjeldahl method measures TN from

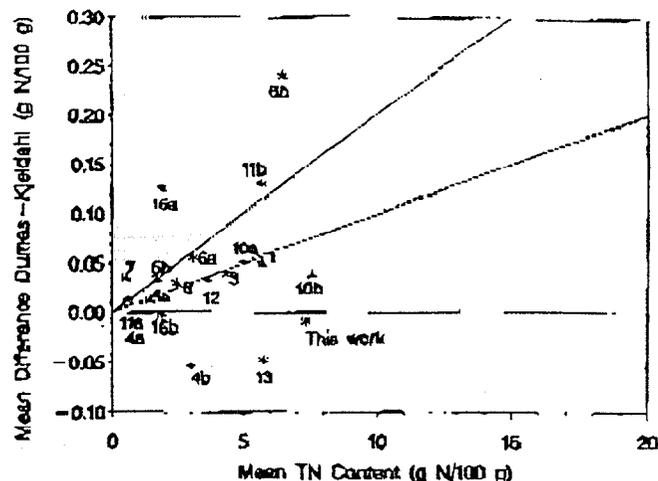


Figure 3. Comparison of mean biases reported in other trials. The rays from the origin correspond to increasing relative biases between the methods of 0, 1, and 2%, respectively. Legend: 1, SMP (1); 3, cereals (3); 4a and 4b, blood albumin (4 and 20% solutions); 4); 5a and 5b, barley and malt (5); 6a and 6b, sunflower seed and soybeans (6); 7, milk (7); 8, meats (8); 10a and 10b, animal feeds (1 mm and 0.5 mm grind; 10); 11a and 11b, milk and SMP (17); 12, dairy products (18); 13, animal feeds (13); 16a and 16b, hay without and with sample pelletization (16); this work, dairy products and reference compounds.

all organic molecules in a sample that can be converted to ammonia. Inorganic N, of which the principal source in dairy products would be nitrate (NO₃) and nitrite (NO₂), is assumed not to be measured by the Kjeldahl method. However, all forms of N—including both organic and inorganic plus dissolved N and occluded atmospheric N—are expected to be measured by the Dumas method. Nitrates and nitrites are the most common forms of inorganic N that the Dumas method is likely to detect in foods and feedstuffs. Many plant materials, preserved meats, and some types of cheese can contain levels of either NO₃ or NO₂ that could elevate TN values by the Dumas method relative to the Kjeldahl method. Nitrate levels as high as 3000 µg NO₃/g (wet basis), which corresponds to about 0.07% N (wet basis), have been found in some vegetables (29). An inorganic N level of this order should result in a significant systematic difference between methods. Dried samples, or assays expressed on a dry basis, would result in even greater absolute differences between methods.

Hansen (14) reported up to 1.14% N as NO₃ in plant samples used to examine method bias. Even at such high levels of NO₃, no correlation with method bias was found. Sweeney and Rexroad (15) reported that one of their samples contained 0.19% N as NO₃ but it accounted only partly for the anomalous method bias found with this sample. These findings suggest

that the fate of NO_3^- (or any inorganic N) in both methods needs further examination and, until resolved, contributes to method bias uncertainty.

With the exception of Sweeney and Rexroad (15), Hansen (14), and Sachen and Thiex (16), none of the authors of published comparisons (1-13) reported levels of NO_3^- or NO_2^- in their samples. Where NO_3^- or NO_2^- levels are normal in dairy products, this systematic error between the 2 methods would be insignificant. For example, a milk containing $5 \mu\text{g NO}_3^-/\text{g}$ would give rise to a N concentration of $1.12 \mu\text{g/g}$, which is equivalent to 0.0007% as milk protein. Only when a product is contaminated significantly with NO_3^- or NO_2^- at levels greater than $150 \mu\text{g/g}$ (in total of the 2 anions) would the difference between methods become significant ($>0.005\%$ N Dumas - Kjeldahl). The NO_3^- and NO_2^- concentrations in samples used in this trial are shown in Table 5. All are substantially less than $100 \mu\text{g/g}$; thus, negligible bias resulted from this potential source of error.

Reference Compounds

International Standard IDF 20A:1986 (22) provides a choice of reference compounds (tryptophan, phenacetin, and lysine-HCl) to verify digestion of organic N in the Kjeldahl method. This standard has been superseded by IDF 20B:1993 (23), which offers a choice of only tryptophan and lysine-HCl. This change brings IDF 20 into line with the recovery verification procedure specified in AOAC Official Method 991.20 (30). Despite these requirements, laboratories in this trial used a variety of compounds to verify recovery performance of their Kjeldahl instruments, including tryptophan (3 labs), acetanilide (2 labs) and lysine-HCl, nicotinic acid, tris(hydroxymethyl) amino methane, glycine, and a secondary reference WMP (one lab each). Irrespective of the compounds used, in all cases, laboratories achieved the required $>98\%$ N recovery threshold specified in the standards (22, 23, 30). In addition, one laboratory used ammonium sulfate to verify distillation performance.

At the time of the trial, no standard method was available for operation of Dumas instruments for analysis of dairy products. Only after completion of the trial did a draft provisional standard become available from the IDF (31). EDTA (independent of the EDTA supplied in the trial) was used as a primary reference compound to calibrate the Leco instruments according to manufacturer's recommendations.

Use of Reference Compounds To Verify Method Efficacy

Use of reference compounds in method comparison trials differs widely. Some trials note the use of reference compounds, but present no N recovery results for either the Kjeldahl method (4, 6, 12, 14) or both methods (5, 16). We are inclined to place less weight on the results obtained in such cases. Other trials reported results from using a variety of Kjeldahl recovery or digestion verification compounds and reference materials. Bellomonte et al. (2) used acetanilide and alropine; Sweeney & Rexroad (15) used lysine-HCl, tryptophan,

as did King-Brink and Sebranek in both of their trials (8, 18). Biscak (3) used nicotinic acid, lysine-HCl, and tryptophan at some laboratories in their Dumas instruments; Daun and Declercq (6) used nicotinic acid, glycine, and ammonium *p*-toluene sulfonate; and Frister (17) used tyrosine. Hansen (14) used nicotinic acid followed by 8 reference amino acids to calibrate the Leco. Ammonium sulfate, nicotinic acid, and acetanilide were used to confirm performance of the Kjelfoss, and the Kjeltec analyzer was calibrated with ammonium sulfate. Ebeling (13) used acetanilide. Sachen and Thiex (16) standardized their Leco with uric acid (subsequently replaced with urea) and checked calibration with lysine-HCl and a reference peach leaf sample. Smith (12) used aspartic acid to calibrate the Foss-Heraeus Macro N instrument and tested performance with 9 reference compounds. The diversity of calibration procedures and reference agents suggests that greater use of, and standardization in the use of, reference and recovery verification aids would facilitate comparison of different trials.

Lysine-HCl and tryptophan are specified as alternative digestion validation compounds in IDF 20B:1993 (23) and AOAC 991.20 (30). However, recent unpublished results by Dutch researchers suggest that when used in the block digestion procedure, these 2 compounds may not be equivalent as digestion verification agents (32). The Dutch results (Table 7) suggest that lysine-HCl is less easily digested than tryptophan even at a block temperature of 425°C . (Further details of digestion conditions were not reported, and only one result with tryptophan exceeded comfortably the 98% recovery threshold. The difficulty with obtaining satisfactory N recovery from lysine-HCl when used in block digestors using Cu catalyst is corroborated by data published by Tecator (33) for their Kjeltec autosampler system. According to the data, even after 60 min digestion, mean N recovery from 6 replicates is only 90.0% compared with essentially 100% with a Hg catalyst and 30 min digestion. The data also show N recoveries of $<93\%$ for lysine HCl with Se and Ti catalysts and 60 min digestion (the longest time reported). Because block digestion is currently the most commonly used version of the Kjeldahl method in commercial laboratories, the reported difficulties in recovering N from lysine-HCl highlight concerns regarding the equivalence of reference compounds and the interpretation of recovery results.

Table 7. Reported N recoveries with tryptophan and lysine-HCl in block digesters^a

Block digester temperature, $^\circ\text{C}$	Digestion recovery, %	
	Tryptophan	Lysine-HCl
410	97.4	91.8
410	97.8	89.6
410	98.1	87.1
425	99.2	89.9
425	97.4	90.5
425	98.2	90.7

^a Permission to publish these results was given by RIKILT-DLO, Wageningen, The Netherlands, and we are grateful for their cooperation.

Both Kjeldahl standards (IDF 20B:1993 Parts 1 and 2, and AOAC 991.20) require >98% N recovery for sample digestion (23, 30) during method verification. N recovery in any Kjeldahl instrument that just satisfies this limit implies the potential for a negative bias of up to 2%, relative to the true N content of the reference sample. If a Dumas instrument in the same laboratory is calibrated to attain 100% N recovery, then an apparent relative bias between the 2 instruments of up to 2% would manifest itself. The upper ray in Figure 3 represents a 2% relative bias between the methods. Several results in other trials are very close to this bias level (data points 4a, 5h, 6a, 11a), a few exceed it (data points 6b, 7, 11b, 16a), but most are well within this limit (data points 1, 3, 4b, 5a, 8, 10a, 10b, 12, 16b; mostly consistent with a relative bias of about 1% and shown by the middle ray in Figure 3). This distribution suggests that a number of Kjeldahl instruments in some of these trials were operating with barely adequate digestion or N recovery. This conclusion reinforces the need to report the N recovery from a standard set of reference compounds to facilitate interpretation of dissimilar results.

Role of Reference Compounds in Interpretation of Published Trial Results

In some trials, reference compounds performed 2 roles. They were used to verify performance (or trueness) of the Kjeldahl system (as required by the standards), and in some cases, the Kjeldahl performance verification results were included as part of the basis to compare the 2 methods by performing a corresponding set of assays in the Dumas instruments on the same materials. This at first sight appears desirable, but care is required. Some important questions can arise. If the N recovery verification criterion for the Kjeldahl method is not met—especially when 2 reference compounds are used and an instrument fails to meet the criterion for both—should Kjeldahl results for other samples from this instrument be used in the overall method comparison? Or should the rest of that instrument's results be rejected? It is apparent from published results of trials that either approach can be taken.

One view is that the validity of all sample assays from an instrument is potentially suspect, if the recovery criterion is not met. Total rejection of all results from the instrument not fulfilling the method recovery verification criterion would be justified as a precaution, and we will call this view the precautionary approach.

The other view is that even if the Kjeldahl recovery verification criterion is not met results of ensuing assays could still be valid if it is assumed that reference compounds are appreciably harder to digest than the samples analyzed. Thus the reference compounds are treated as a worst case. Despite our reservations regarding the robustness of this approach, we will call it the optimistic assumption. However, if the assumption is not valid (i.e., samples in fact are not adequately digested) and the Dumas performance is satisfactory, a method bias becomes a certainty.

The precautionary approach itself is not inherently robust. If recovery verification is performed with an agent that is easily digested (e.g. glycine) and the recovery criterion is met, it does not follow necessarily that ensuing samples for routine or trial analysis would be adequately digested. In this case, the credi-

bility of results may be no greater than if the optimistic assumption had been adopted in conjunction with difficult-to-digest recovery verification compounds. However, by following the standard method (i.e., by using tryptophan or lysine-HCl), it is assumed that the specified recovery verification compounds in the standard have been selected for their robustness relating to the range of materials included for analysis that is prescribed in the scope of the standard method. (Despite the reported differences noted between the digestibility of lysine-HCl and tryptophan [Table 7], either may be adequate for recovery verification depending on the method's scope.) Thus the precautionary approach is most secure when it is adopted with strict adherence to the method verification procedures laid down in the relevant standard method.

A more subtle aspect of the optimistic assumption is important when the same amino acids used as recovery verification agents occur in the protein substrates included in the trial (e.g., lysine-HCl, tryptophan, tyrosine, and glycine). The problem with this assumption and the use of these compounds is that, if there is incomplete digestion of the reference amino acid at the recovery verification stage, then as constituents of the proteins in the trial samples, there is the possibility of incomplete digestion in the samples as well. Consequently, the Kjeldahl results could vary between samples as the amino acid profiles vary and the results would have a propensity to be biased below the Dumas results. An inconsistent bias could appear.

Results of Sweeney (10), Biscak (3), and King Brink and Sebranek (8, 18) and our own unpublished results show that Kjeldahl instruments have greater difficulty in recovering N from nicotinic acid than from either lysine-HCl or tryptophan. Sweeney (10) noted that 5 of 9 laboratories obtained low Kjeldahl recoveries from nicotinic acid and that 2 of the 5 also obtained low recoveries from lysine-HCl. Accordingly, these 2 laboratories had their Kjeldahl results rejected from further analysis in Sweeney's trial. This appears to be an example of the precautionary approach, and we have no reservations regarding the overall results.

Biscak (3) used a combined approach in which results from 3 Kjeldahl instruments were compared with results from 9 Dumas instruments. The results for both nicotinic acid and lysine-HCl were included directly in the method comparison. However, because of low recoveries reported for nicotinic acid, 2 overall mean biases were quoted: 0.25% protein (Dumas - Kjeldahl) with the nicotinic acid results included and 0.05% protein (Dumas - Kjeldahl) with the nicotinic acid results removed. We think the limited number of Kjeldahl laboratories, together with the dual role of the recovery compounds (displaying mixed Kjeldahl efficacy), is cause for some concern. In this trial, statements of the overall bias with and without inclusion of nicotinic acid results suggest that elements of both the optimistic assumption and the precautionary approach have been adopted, with the readers able to weigh their inferences accordingly.

King-Brink and Sebranek (8) seem to have adopted a less rigorous approach. Six of 12 Kjeldahl instruments failed to achieve 98% recovery verification with nicotinic acid and 5 of the same 6 failed to achieve recovery verification for lysine-HCl. Despite application of Grubb's single and double tests and

Cochran's test to the full set of results from the Kjeldahl instruments, no outliers were detected. Results from the 5 laboratories that failed to achieve the method recovery verification criterion for either reference compound appear to have been retained and used in their overall method comparison. The validity of this approach seems to hinge on the optimistic assumption. Incorporation of results from instruments that were unable to demonstrate satisfactory recovery performance (almost half the instruments) raises doubt in our minds regarding the overall method comparison. In trials that encountered difficulties with the optimistic assumption approach, variable bias would be the likely outcome.

We have largely adopted the precautionary approach and have avoided merging of recovery verification results with the main body of results by insisting that instrument calibration and performance verification be performed independently and successfully in accordance with each laboratory's accredited procedures before analysis of trial samples. It might be argued that the use of EDTA as a primary calibration agent for Dumas (Leco) instruments and as a reference compound in the trial represents a degree of blurring of trial assay results with instrument calibration. Sweeney and Rexroad (15) also used EDTA as a reference agent for both their Dumas and Kjeldahl instruments. Any difficulty experienced by 3 Kjeldahl instruments digesting EDTA was not an attribute of general digestion deficiencies because of prior achievement of the N recovery verification criterion with reference compounds already noted. In previous use of EDTA as a recovery reference compound (21), we had observed that some Kjeldahl instruments (notably Kjeldahl instruments) experienced difficulty recovering N from it; however, in this earlier work, these instruments were not associated with an inability to recover N from SMP samples. With this foreknowledge and with use of 2 other reference materials (glycine and secondary reference SMP), we consider the approach to be robust (see Table 6 for reference recoveries attained).

Effect of Blanks

Sachen and Thiex (16) investigated the effect of atmospheric blanks associated with occluded air in fluffy, porous, and fibrous samples on method bias. Various amounts of occluded air were found to be responsible for much of the method bias. Once the sample was introduced into the Leco analyzer in the form of a dense pellet, the size of the blank could be reduced and controlled, and method bias was reduced to insignificant levels (see data points 16a and 16b in Figure 3). However, no Kjeldahl reference recovery results were stated, and the TN levels investigated were limited to a range of about 1.5–3 g N/100 g. They recommended that the Dumas method be modified to use powdered cellulose to check the instrument blank and that low-density samples be introduced into the analyzer in pellet form. There appears to be a need to use these techniques in a full collaborative trial that includes a wide range of sample densities and TN levels, and with a selection of reference compounds.

Barbano et al. (27) conducted a thorough collaborative trial that optimized the block digestion procedure for testing milk samples (from which followed AOAC Method 991.20). During their trial, Barbano et al. noted that "it was requested that

2 blanks instead of 1 blank be run by each laboratory in the collaborative study, because 1 erroneous blank value would bias all the results from a laboratory." This approach appears more sound, and in future collaborative trials, it is suggested that results of blanks for each method be reported alongside other performance verification results.

Interpretation of Methods

McKenzie (34) noted particularly the need to ensure the correct digestion temperature when using the block digestion procedure, stating that "... despite the strong recommendation, indeed the mandatory need, to measure the temperature of the digestion mixture, many workers even in the 1990s still measure the temperature of the digestion block." Recovery of N from reference materials by the block digestion procedure noted earlier by Dutch researchers (32) gives an illustration of the temperature effect (Table 7). Barbano et al. (27) noted that differences in line voltage can result in differences in block temperature. Differences that appear between Parts 2 and 3 of IDF 20B:1993 (23) further add to the potential for differences in digestion. Part 2 states: "After the digest clears (clear with light blue-green color) continue digestion at 410°–430°C for at least 1 h. During this period the sulfuric acid must be boiling." In contrast, digestion conditions are more vague in Part 3 than in Part 2, with the method stating: "Transfer the digestion tube to the digestion block, set at the temperature specified by the manufacturer ... Digest the sample for the period specified by the manufacturer of the block—normally 40 min ..." No mention is made in Part 3 of any need for the mixture to clear or for the mixture to boil. There is a need for greater consistency and clarity in defining digestion conditions.

The trial followed principally IDF 20B:1993 Part 2: Macro Block-Digestion Method (23) for the Kjeldahl assays. This method is validated for determination of the protein content of milk. However, the procedure described in Part 1 (traditional method) and Part 3 (semimicro rapid routine method using block digestion) of this standard states: "The procedure described in Part 1 of this International Standard may, with slight modification, be used for the determination of the nitrogen content of a range of milk products." Products including milk powder (skimmed, whole, or fat filled), whey powder, whey protein concentrate, ice cream, cheese, and cream are listed in the annex to Parts 1 and 3. Casein and caseinate are not included. In contrast, Part 2, as well as AOAC 991.20 (30), has no annex for a modified procedure that extends the method to any substances other than milk. The AOAC methods for determining protein content of dried milks (Method 930.29; 35) and the N content of cheese (Method 920.123; 36) each specify a sample size and without further details prescribe that Method 991.20 be followed. No reference is cited in either method justifying the linkage of the analysis of cheese or milk powder to that of milk. We also note that the operation of Kjeldahl instruments does not fall strictly within the scope of any of these standards.

Our concern is how the phrase "with slight modification" might be interpreted and what its consequences are in method comparison trials or routine analysis of dairy products. Sample type and size, moisture content, and fat content can all affect the

efficacy of Kjeldahl digestion. The modified procedures given in the annexes of IDF 20B provide adjustments to the method for sample size, adjustments to some extent for water content, and specific adjustments for fat content that are absent in AOAC Methods 930.29 and 920.123. With the exception of milk and WMP, we avoided dairy products with high moisture and fat levels in the trial.

The standard method for determination of protein content of casein and caseinates is specified in IDF 92:1979 (24). Despite use of block digesters in this trial and perhaps by others to analyze these products, IDF 92 has not been updated to encompass their use. Thus when block digestion is used to analyze casein or caseinate, the procedure is in limbo as it does not fall strictly within the scope of either IDF 20B or IDF 92. With new processing technology being commercialized to produce a rapidly expanding array of dairy products that blurs the distinction between milk powders, caseins, WPCs, and dairy-based nutritional products, a review and update of these standards seems necessary.

The block digestion method never has been subjected to a collaborative trial to verify the Kjeldahl method for analysis of dairy products other than milk when used with a copper catalyst (27, 28). Given the very diverse array of samples used in trials published to date (including dairy products), and given the critical requirements of the Kjeldahl method that must be followed precisely to achieve method performance recovery in milk, it is not surprising that laboratories using samples and method variations that have not been subject to detailed validation may obtain dissimilar results. Such variations may contribute to the varying bias between the Kjeldahl and Dumas methods, as revealed in Figure 3.

The differences in scope and technique, which are revealed by close examination of the Kjeldahl method standards, will have an effect, which is unclear, on this comparison and others involving dairy products. However, we acknowledge that the Dumas method has yet to be subjected to the same degree of scrutiny as the Kjeldahl method to ensure that its performance is validated properly for the range of samples analyzed.

Commercial Considerations

Protein analysis is required for a very wide range of animal and human nutrition products. Contractual specifications in many commercial transactions, as well as the need to meet specified protein levels for the purposes of commodity classification and determination of tariffs, duties, and quotas in foodstuffs subject to international trade, demand that the protein content must exceed a minimum value. Demonstration of compliance with a specified protein threshold by using the Dumas method, to an agreed level of statistical confidence, will depend on the bias associated with the instrument and its precision characteristics, relative to the internationally recognized Kjeldahl method. The situation is more complex when batch inhomogeneity is taken into account.

With the current emphasis on liberalizing international trade, countries are increasingly having to adopt international covenants relating to agreed quality systems and standard testing methods. For protein determination, the Kjeldahl method is specified for a variety of foodstuffs in FAO/WHO Codex Ali-

mentarius standards (37) as the reference method. Despite these requirements, and as noted earlier, the traditional macro (manual) Kjeldahl method is seldom performed in modern commercial laboratories because the numbers of samples for analysis are too large and laboratory health and safety regulations are becoming more severe. Although the semiautomated block digestion Kjeldahl procedure is used widely (and also the Kjeldahl instruments), more and more laboratories are using Dumas instruments. The number of published comparisons in the last 10 years suggests that resolution of the Dumas-Kjeldahl bias question and their respective precision characteristics is a complex technical and statistical task. Adoption of the Dumas technique as an official AOAC or IDF method for analysis of dairy products, although essential, would not completely resolve the issue because the question of bias and, with it, doubt over specification compliance could still arise. According to the Dumas method Codex Alimentarius Type III recognition (38; i.e., as an alternative approved method that may be used for control, inspection, or regulatory purposes) for N determination in a wide range of foodstuffs would reduce greatly the uncertainty surrounding the unofficial use of Dumas analyses.

Given sufficient observations, a statistical comparison between 2 methods will almost inevitably reveal a significant bias. However, from a commercial perspective, such differences might be quite immaterial. Therefore, once all the relevant variables affecting the methods have been adequately controlled, further comparisons will not reveal any more useful information. Instead a decision is required on what level of bias (uncertainty) can be accepted by the parties involved. Examination of the results in Figures 2 and 3 suggests that a relative bias of under 1% or ± 0.05 g N/100 g (whichever is the more severe criterion) appears to be achievable and could be treated as negligible, given that any bias is arguably as much a failing of the Kjeldahl method as the Dumas method. There is the possibility, with the use of appropriate laboratory quality control procedures and with further improvements in instrument design and operator training, that these limits could be reduced and perhaps halved.

Conclusions

A comparison of protein analysis instruments in 11 laboratories analyzing 8 dairy products and 2 pure reference compounds finds no evidence for a significant generic difference between the 2 methods that would be of concern to most dairy produce traders who face contractual obligations based on crude protein content. This result applies across the range of TN levels in the samples (0.5–18.6 g N/100 g). Comparison of these results with those of previous studies suggests that a consensus on the relationship between the 2 methods has yet to emerge.

For samples containing >2 g N/100 g, the Dumas RSD_r and RSD_x estimates were independent of protein concentration and were consistently about 0.35 and 0.75%, respectively. The corresponding Kjeldahl values tended to decline as the protein concentration increased, were less consistent as a result, and were at least twice as large.

Differences between the methods revealed in trials to date may be due as much to poor Kjeldahl N recovery verification and differences in Kjeldahl methodology as to inconsistent performance of the Dumas method.

In future trials, nitrate and nitrite levels in samples used for analysis should be reported.

Instrument calibration and performance verification should be conducted and satisfied before samples (including reference compounds) are analyzed. The choice between lysine-HCl or tryptophan to verify Kjeldahl digestion efficacy should be made with some care. At least 2 reference compounds (the most difficult amino acids to digest that are present in the protein matrix of the samples) should be used in a method comparison such as this, and results should be reported alongside those for the rest of the samples assayed. An instrument that is unable to achieve the method N recovery and digestion verification requirements of the standard should have its results excluded from the overall method comparison.

There is a need to review the scope of the analytical procedures and to rationalize and update the standard methods for determining protein in dairy products. The Dumas method needs to be given Codex Alimentarius recognition as an alternative to the Kjeldahl method for the determination of N in foodstuffs.

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APPENDIX B

Protein Nitrogen Combustion Method Collaborative Study I. Comparison with Smalley Total Kjeldahl Nitrogen and Combustion Results

David L. Berner* and Janet Brown¹

American Oil Chemists' Society, Champaign, Illinois 61826-3489

During 1993–1994, a collaborative study of the determination of the nitrogen content of oilseed meals by the nitrogen combustion method was conducted among 24 laboratories in seven countries for the analysis of cottonseed, soybean (two samples), peanut, canola and safflower (two samples). These meals were also analyzed by the $\text{CuSO}_4/\text{TiO}_2$ Kjeldahl method (*Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., 1989, Method Ba 4d-90) in the 1993–1994 Smalley Check Sample Program Oilseed Meal Series [Brown, J., *INFORM* 5:640 (1994)]. Some participants used commercial nitrogen combustion instruments. In the Smalley Program, $\text{CuSO}_4/\text{TiO}_2$ Kjeldahl analysis gave nitrogen values that ranged from 0.05 to 0.13% lower than values obtained by the combustion method in the collaborative study. Nitrogen values obtained by the combustion method on an optional basis in the Smalley Program were generally lower by 0.01 to 0.03% than nitrogen values obtained by the combustion method in the collaborative study reported here.

KEY WORDS: Copper sulfate, copper sulfate/titanium dioxide, Kjeldahl, mercuric oxide, nitrogen, nitrogen combustion, oilseed meals, protein nitrogen, seed meals, TKN.

In 1987, because of increasing concerns about the disposal of mercury waste from the mercuric oxide (HgO) Kjeldahl method for total Kjeldahl nitrogen (TKN), the American Oil Chemists' Society (AOCS) adopted a copper sulfate (CuSO_4)-catalyzed Kjeldahl method, AOCS Official Method Ba 4b-87 (1). The CuSO_4 Kjeldahl method was not satisfactory for two reasons: In comparison with the HgO Kjeldahl method, CuSO_4 gave a negative bias for protein and it required a longer digestion time.

In 1990, to identify a more satisfactory Kjeldahl method and any bias associated with both it and the CuSO_4 method, the AOCS Examination Board initiated a comparison study, coordinated by Examination Board Chairperson Richard Benson, of three Kjeldahl methods: HgO, CuSO_4 and $\text{CuSO}_4/\text{TiO}_2$ ("mixed catalyst"). In the study, six laboratories analyzed a total of 380 samples of soybean meal by the three Kjeldahl methods. The results of this study (2) indicated that, in comparison with the HgO method, CuSO_4 and the $\text{CuSO}_4/\text{TiO}_2$ mixed catalyst gave protein negative biases of -0.25 and -0.17% , respectively. The $\text{CuSO}_4/\text{TiO}_2$ mixed catalyst gave a digestion time close to that of HgO and less than CuSO_4 . Based on this study, the $\text{CuSO}_4/\text{TiO}_2$ method was adopted in 1990 as AOCS Official Method Ba 4d-90 (3), and it became the official referee method. In a later study by Falk (4), the $\text{CuSO}_4/\text{TiO}_2$ method was used to determine protein nitrogen in cottonseed and cottonseed meal. In that study, when collaborators used the catalyst and sample weights specified in the

method, a more satisfactory digest was obtained with 30 mL sulfuric acid. All AOCS methods for determining protein nitrogen with HgO and CuSO_4 were declared obsolete ("Surplus") in 1991.

The Dumas nitrogen combustion method offers savings through reduced time, chemicals and waste disposal, and it eliminates the use of hazardous chemicals. Coupling the Dumas method with appropriate computer software and standardization techniques gave a viable alternative to the traditional Kjeldahl method for determining protein nitrogen. In 1987, the Association of Official Analytical Chemists (AOAC) conducted a collaborative study (5) in which the Dumas nitrogen combustion method was compared with the AOAC CuSO_4 Kjeldahl method (6); the two methods compared favorably. In 1989, the AOAC conducted a collaborative study (7) in which the Dumas nitrogen combustion method was compared with the AOAC HgO Kjeldahl method (8); in this study, the combustion method gave results that were higher for protein nitrogen by $+0.04\%$. On the basis of the AOAC study (8), the AOCS adopted the combustion method as Recommended Practice Ba 4e-93 in 1993. The method was not adopted as an AOCS Official Method because of insufficient data for oilseeds and oilseed meals. Bicsak coordinated a collaborative study (9) in which the combustion method was compared with the HgO Kjeldahl method. In that study, the combustion method gave results that were higher for protein nitrogen by $+0.04\%$.

EXPERIMENTAL PROCEDURES

During 1993–1994, we coordinated an international collaborative study of the Dumas nitrogen combustion method that included 24 participants from seven countries. The purpose of the study was twofold: To determine the variability associated with the analysis of oilseed meals and to determine the bias of the combustion method vs. the CuSO_4 Kjeldahl method. In the study, the seven oilseed meals analyzed for nitrogen content consisted of cottonseed, soybean (two samples), peanut, canola and safflower (two samples). The meals were from the same lots of oilseed meals analyzed by the $\text{CuSO}_4/\text{TiO}_2$ Kjeldahl method, AOCS Official Method Ba 4d-90 (3), in the 1993–1994 Smalley Check Sample Program. One soybean meal and the cottonseed and peanut meals were submitted as blind duplicates. The meals were ground to a particle size of approximately 0.7 mm in a Herringbone grinder.

Participants were permitted to use commercial nitrogen combustion instruments but were requested to note the instrument used. AOCS Recommended Practice Ba 4e-93 (3) was suggested as a general procedure. For a nitrogen standard, participants were given 2-amino-2-(hydroxymethyl)-1,3-propanediol or [tris(hydroxymethyl)amino methane] ("TRIZMA"), 99.92%, containing 11.56% nitrogen, obtained from the National Institute of Standards Testing (NIST) (Gaithersburg, MD). Duplicate analyses were performed.

*To whom correspondence should be addressed at AOCS, P.O. Box 3489, Champaign, IL 61826-3489.

¹Current address: American Dairy Science Association, 309 W. Clark St., Champaign, IL 61820.

Collaborative study samples were analyzed at approximately the same time as the Smalley samples. In addition to performing the required nitrogen analysis by the $\text{CuSO}_4/\text{TiO}_2$ Kjeldahl method, Smalley participants analyzed the Smalley samples by the nitrogen combustion method on an optional basis.

Smalley results were statistically analyzed with the dBase computer program developed by Richard Benson at Cargill (Minneapolis, MN) (unpublished results). Outliers were removed at ± 3 sigma (approximately 99.7% confidence limits). The Smalley results were verified with a SuperCalc 4 program, developed by one of us (DLB), to give mean values and reproducibility values S_R and RSD_R [%CV (coefficient of variation)], after removal of outliers. No repeatability values could be calculated for Smalley results because duplicate analyses were not conducted in the Smalley Program. For the statistical analysis of the collaborative study results, International Standards Organization (ISO) procedure 5725-1986 (AOCS Procedures M 1-92 and M 4-86) (3) was followed, through a Lotus program supplied by David Firestone, to give repeatability (S_r , RSD_r and r) and reproducibility (S_R ,

RTSD_R and R) parameters. The accuracy of the three computer statistical programs was confirmed by analyzing data with known statistical constants; all three programs gave the same values.

RESULTS AND DISCUSSION

Statistical analysis and comparison of the collaborative study results with those obtained for the same samples in the Smalley Check Sample Program are summarized in Table 1. Individual analysis of blind duplicate results for cottonseed, soybean and peanut meals, collaborative study sample pairs 2-7, 1-6 and 5-10, respectively, showed no significant differences, so the results were pooled. The bias found for the nitrogen combustion method vs. the $\text{CuSO}_4/\text{TiO}_2$ Kjeldahl method is shown in Table 2 (10).

In comparison with the $\text{CuSO}_4/\text{TiO}_2$ Kjeldahl method [AOCS Official Method Ba 4d-90 (3)], the nitrogen combustion values from the collaborative study were higher by 0.09%, while the Smalley Program gave values for nitrogen that were higher by 0.07%. In the AOAC study

TABLE 1

Statistical Results for an International Study of the Protein Nitrogen Combustion Method^a

	Samples ^b						
	A	B	C	D	E	F	G
Number of labs after outliers	24	24	24	24	23	24	23
Determinations, n	92	91	91	47	45	47	45
Outliers	2	3	4	0	2	0	2
Smalley, combustion (nitrogen, %)	6.61	7.85	8.22	7.86	7.20	3.33	3.35
Smalley, Kjeldahl (nitrogen, %)	6.55	7.77	8.12	7.78	7.13	3.29	2.36
Collaborative study, combustion (nitrogen, %)	6.62	7.88	8.25	7.89	7.21	3.34	3.32
Collaborative study, combustion (nitrogen, %)	6.62	7.88	8.25	7.89	7.21	3.34	3.32
Repeatability ^c							
S_r	0.06	0.05	0.03	0.04	0.03	0.04	0.05
RSD_r	0.85	0.60	0.39	0.46	0.37	1.25	1.47
$r = (2.8 \times S_r)$	0.17	0.14	0.08	0.11	0.08	0.11	0.14
Reproducibility ^c							
S_R	0.07	0.06	0.07	0.08	0.04	0.11	0.06
RSD_R	1.04	0.81	0.80	0.97	0.60	3.23	1.70
$R = (2.8 \times S_R)$	0.20	0.17	0.20	0.22	0.11	0.31	0.17

^aTwenty-four laboratories participated, each analyzing 10 samples of oilseed meal and obtaining two values (except for samples A, B and C, which were submitted in duplicate and for which four values were obtained).

^bSample key: A = cottonseed meal, collaborative study samples 2 and 7; Smalley sample 9. B = soybean meal, collaborative study samples 1 and 6; Smalley sample 1. C = peanut meal, collaborative study samples 5 and 10; Smalley sample 7. D = soybean meal, collaborative study sample 8; Smalley sample 4. E = canola meal, collaborative study sample 3; Smalley sample 3. F = safflower meal, collaborative study sample 4; Smalley sample 5. G = safflower meal, collaborative study sample 9; Smalley sample 8.

^cStatistical parameters relate only to percent nitrogen values obtained in collaborative study.

TABLE 2

Comparison of Nitrogen Combustion and $\text{CuSO}_4/\text{TiO}_2$ Kjeldahl Results

Meal	Combustion (nitrogen %)	Kjeldahl (nitrogen %)	Combustion ^a (bias)
Cottonseed	6.62	6.55	+0.07
Soybean	7.88	7.77	+0.11
Peanut	8.25	8.12	+0.13
Soybean	7.89	7.78	+0.11
Canola	7.21	7.13	+0.08
Safflower	3.34	3.29	+0.05
Safflower	3.32	3.26	+0.06

^aAverage bias +0.09% nitrogen; +0.56% protein, based on factor of 6.25.

(7) in which the nitrogen combustion method was compared with the HgO Kjeldahl method, the nitrogen combustion method gave values for nitrogen that were higher by 0.04%.

The AOCS study (2), in which the $\text{CuSO}_4/\text{TiO}_2$ and HgO Kjeldahl methods were compared by six laboratories, analyzing a total of 380 samples of soybean meal, the $\text{CuSO}_4/\text{TiO}_2$ Kjeldahl method gave protein values that were 0.174% lower for protein (0.03% lower for nitrogen) than the HgO Kjeldahl method.

Thus, at least part (0.03% nitrogen) of the 0.07 to 0.09% bias for nitrogen observed, when comparing the $\text{CuSO}_4/\text{TiO}_2$ Kjeldahl method with the nitrogen combustion method, may be due to the use of the $\text{CuSO}_4/\text{TiO}_2$ mixed catalyst. The remaining bias (0.04 to 0.06% nitrogen) is close to the 0.04% bias for nitrogen observed in the AOAC (7) and the Federal Grain Inspection Service (FGIS) (9) studies, which compared the nitrogen combustion and the HgO Kjeldahl methods.

In the FGIS collaborative study conducted by Bicsak, recoveries of nicotinic acid, lysine-HCl and tryptophan were 100.53, 99.74 and 100.29% of theoretical, respectively (9). The FGIS study gave an average bias of +0.04% for nitrogen with the nitrogen combustion method vs. the AOAC HgO Kjeldahl method. A cause for the positive bias associated with the nitrogen combustion method is sometimes attributed to "nonprotein nitrogen," possibly from the presence of nitrites (nitrites would not be digested by the Kjeldahl method). A contribution by nitrites has not been documented. The most likely explanation is that the nitrogen combustion method is more efficient (9).

Based on this study and previous AOAC (7), AOCS (2) and FGIS (9) studies, we conclude that for the determination of protein nitrogen in oilseed meals, the nitrogen combustion method will show a +0.07 to +0.09% bias for nitrogen when compared with the $\text{CuSO}_4/\text{TiO}_2$ Kjeldahl method and a +0.04 to +0.06% bias for nitrogen when compared with the HgO Kjeldahl method. This bias is most likely associated with the greater efficiency of the nitrogen combustion method.

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APPENDIX C

FOOD COMPOSITION

Comparison of Kjeldahl Method for Determination of Crude Protein in Cereal Grains and Oilseeds with Generic Combustion Method: Collaborative Study

RONALD C. BICSAK

U.S. Department of Agriculture, Federal Grain Inspection Service, Quality Assurance and Research Division,
PO Box 20285, Kansas City, MO 64195

Collaborators: R. Boles; R. Cathey; V. Collins; K. Hannasious; J. Haselhorst; L. Henderson; L. Jann; L. Meschi; R. Molloy; M. Stillions; K. Swanson; D. Tate; J. Webb; G. Wilkins

Seven laboratories participated in a collaborative study to extend the applicability of the AOAC generic combustion method for determination of crude protein in animal feed (990.03) to include determination in cereal grains and oilseeds. In the study, method 990.03 was compared with the AOAC mercury catalyst Kjeldahl method for determination of protein in grains (979.09) and crude protein in animal feed (954.01). The study also evaluated the effect on the results of fineness of grind. For determination of crude protein in grains and oilseeds by the combustion method, standard deviations for repeatability and reproducibility ranged from 0.10 to 0.37 and from 0.25 to 0.54, respectively, and relative standard deviations for repeatability and reproducibility ranged from 0.77 to 2.57% and from 1.24 to 3.15%, respectively. The combustion method was adopted first action by AOAC International for determination of crude protein in cereal grains and oilseeds containing 0.2–20% nitrogen.

Kjeldahl nitrogen determination has been the standard method for over 100 years for determination of crude protein in a wide variety of products. During this time, the analytical chemist has endured its long analysis times and use of hazardous chemicals. The Dumas method, a combustion procedure, is another 100-year-old method for determining

crude protein. The method does not use hazardous chemicals or require long analysis times, but it has not been as widely accepted as the Kjeldahl method. Modern advances in electronic instrumentation and computers have improved the capabilities of the Dumas method, making it faster, safer, and more reliable than the Kjeldahl method. In the improved Dumas method, nitrogen freed by pyrolysis at high temperature in pure oxygen is quantified by a thermal conductivity detector. Equivalent protein is calculated from the nitrogen value by a microprocessor. Analysis time varies from 4 to 11 min depending on the sample size and the instrument model.

A generic combustion method for determination of crude protein in animal feeds was collaboratively studied by Sweeney (1) and adopted by AOAC as method 990.03 (2). The present collaborative study was conducted to compare 990.03 with the AOAC mercury catalyst Kjeldahl methods (3) for determination of protein in cereal grains (979.09) and determination of crude protein in animal feed (954.01). The purpose of the study was to extend the applicability of method 990.03 to additional products.

The generic description in 990.03 allowed use of 3 different brands of equipment. All equipment had to meet the performance criteria in that method. Three manufacturers were represented in this study: LECO Corp., Perkin-Elmer Corp., and UIC, Inc.

The study also included samples to evaluate the effect of fineness of grind on results.

Collaborative Study

The experimental design addressed systematic error (inter-laboratory bias), precision (within-laboratory repeatability using blind duplicates), and accuracy (recovery of known standards). Three protein concentration levels (8–13%, 17–23%, and 35–40%) were selected to represent the total range of protein found in the products being considered. Two different products were then selected from each concentration level. From these products, the blind duplicates and the samples for

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The recommendation was approved by the General Referee and the Committee on Foods II and was adopted by the Official Methods Board of the Association. See "Changes in Official Methods of Analysis" (1993) *J. AOAC Int.* 76 Jan/Feb issue.

The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Dept of Agriculture over other firms or similar products not mentioned.

Table 1. Samples used in collaborative study of combustion method for determining crude protein in cereal grains and oilseeds

Concn level	Batch (type)	Sample	Estd protein, %	Screen size, mm	Description	
1	1	1	35	1.0	Soybean 1	
		6	35			
		16	35	2.0		
		25	35			
		12	40			
	22	40	1.0	Soybean 2		
	2	4	4	20	1.0	Canola 1
			11	20		
		27	27	23	1.0	Canola 2
			29	23		
2	1	28	18	1.0	Sunflower	
		30	18			
		5	13	1.0		
		17	13			
		13	13			2.0
	15	13				
	21	21	17	1.0	Wheat 2	
		24	17			
		7	12			1.0
	20	12				
3	1	2	8	1.0	Corn	
		10	8			
		3	8	2.0		
		18	8			
	2	9	9	1.0	Sorghum	
		23	9			
		19	71 ^a			—
26	71 ^a	—				
Reference standard	8	96 ^a	—	Lysine-HCl		
	14	96 ^a	—			

^a Calculated equivalent protein.

the alternative grind comparison were chosen. Two chemical reference materials were also selected for analysis. The total sample set was designed to provide 15 closely matched pairs (Table 1).

992.23 Crude Protein in Cereal Grains and Oilseeds Generic Combustion Method

First Action 1992

(Applicable to cereal grains and oilseeds containing 0.2–20% N, % crude protein, for wheat and its products = $N \times 5.70$,

% crude protein, for other cereal grains and oilseeds = $N \times 6.25$)

Method Performance (estimated % crude protein):

Soybean, 35 and 40%

$s_r = 0.29$; $s_R = 0.47$; $RSD_r = 0.77\%$; $RSD_R = 1.24\%$

Canola, 20 and 23%

$s_r = 0.19$; $s_R = 0.39$; $RSD_r = 0.87\%$; $RSD_R = 1.79\%$

Sunflower, 18%

$s_r = 0.37$; $s_R = 0.54$; $RSD_r = 2.00\%$; $RSD_R = 2.94\%$

Wheat, 13 and 17%

$s_r = 0.15$; $s_R = 0.27$; $RSD_r = 0.99\%$; $RSD_R = 1.74\%$

Table 2. Effect of fineness of grind (1 vs 2 mm) on collaborative results for determination of protein (%) in soybean, wheat, and corn by combustion method

Lab. ^a	N	Soybean		Wheat		Corn	
		Mean	SD	Mean	SD	Mean	SD
1	4	34.76	0.32	13.10	0.16	8.74	0.18
2	4	35.01	0.48	13.38	0.50	8.96	0.48
3	4	35.63	0.43	13.50	0.22	9.10	0.16
4	4	34.94	0.19	13.43	0.31	9.07	0.21
5	4	34.84	0.40	13.13	0.16	8.72	0.08
6	4	34.55	0.34	13.37	0.13	9.14	0.15
7.1	4	35.53	0.39	13.51	0.15	9.03	0.25
7.2	4	35.52	0.40	13.69	0.15	9.48	0.14
7.3	4	34.94	0.78	13.41	0.30	9.05	0.27
Screen size							
1 mm	18	35.27	0.43	13.51	0.22	8.95	0.25
2 mm	18	34.88	0.56	13.27	0.30	9.11	0.33

^a Laboratory 7 reported results for 3 different analyzer brands.

Barley, 12%

$s_r = 0.27$; $s_R = 0.40$; $RSD_r = 2.13\%$; $RSD_R = 3.15\%$

Corn, 8%

$s_r = 0.10$; $s_R = 0.26$; $RSD_r = 1.15\%$; $RSD_R = 2.88\%$

Sorghum, 9%

$s_r = 0.23$; $s_R = 0.25$; $RSD_r = 2.57\%$; $RSD_R = 2.84\%$

Lysine-HCl, 96%*

$s_r = 0.36$; $s_R = 0.72$; $RSD_r = 0.38\%$; $RSD_R = 0.75\%$

Nicotinic acid, 71%*

$s_r = 0.32$; $s_R = 0.83$; $RSD_r = 0.45\%$; $RSD_R = 1.18\%$

*Calculated equivalent protein.

A. Principle

Nitrogen freed by pyrolysis and subsequent combustions at high temperature in pure oxygen is quantified by thermal conductivity detection. Equivalent protein is calculated.

B. Apparatus

Any instrument or device designed to measure nitrogen by combustion provided that it meets system suitability requirements, E.

(a) *Furnace*.—Capable of maintaining minimum operating temperature of 950° for pyrolysis of sample in pure (99.9%) oxygen. Some systems may require higher temperatures.

(b) *Isolation system*.—Capable of isolating liberated nitrogen gas from other combustion products for subsequent measurement by thermal conductivity detector. Device for converting NO_x products to N₂ or measuring N as NO₂ may be required and included in instrument.

(c) *Detection system*.—Capable of interpreting detector response as % nitrogen w/w. Features such as calibration of standard material, blank determination, and barometric pressure compensation may be included. Any required calibration

must be based on theoretical % nitrogen in pure standard organic material such as EDTA.

(d) *Grinder*.—Capable of grinding samples to pass No. 20 sieve.

(e) *Analytical balance*.—Accurate to 0.01 mg.

(f) *Barometer*.—Hg type, readable to 0.1 mm.

C. Reagents

(a) *Accuracy standards*.—(1) *Nicotinic acid*.—99.9% minimum purity. (2) *Lysine-HCl*.—99.9% minimum purity (tryptophan, 99.9% minimum purity, may be substituted).

(b) *Calibration standards*.—EDTA, 99.9% minimum purity, or other suitable standard of equal purity.

D. Samples

Grind samples to suitable fineness (determined for each different material analyzed) to attain $\leq 2.0\%$ relative standard deviation (RSD) for 10 successive nitrogen determinations for that material type.

$$RSD, \% = (s/N) \times 100$$

Table 3. Analysis of variances for soybean, wheat, and corn (with protein as dependent variable) due to model, laboratory, and fineness of grind effects

Statistic	Soybean	Wheat	Corn
Av. protein, %	35.08	13.39	9.03
R-square	0.61	0.55	0.58
Root MSE	0.387	0.222	0.226
<i>P</i> -value			
Model	0.0012	0.0054	0.0024
Laboratory	0.0036	0.0267	0.0033
Grind	0.0057	0.0033	0.0423

Table 4. Collaborative results for determination of crude protein (%) in cereal grains and oilseeds by Kjeldahl method 955.04 and generic combustion method

Sample ^a	Laboratory											
	1		3		5		2	4	6	7	8	9
	Kjel.	Comb.	Kjel.	Comb.	Kjel.	Comb.						
1	34.7	34.7	36.0	35.7	34.9	34.9	34.8	34.9	34.8	35.7	35.8	35.1
2	8.8	8.7	9.0	9.1	8.7	8.8	8.6	9.1	9.2	9.0	9.3	8.8
3	8.6	8.6	9.0	8.9	8.7	8.8	9.7	8.9	9.1	8.8	9.5	9.0
4	20.6	20.4	21.4	21.2	20.7	20.7	21.1	20.8	20.7	21.0	20.9	20.8
5	13.3	13.2	13.6	13.5	13.2	13.2	13.6	13.6	13.3	13.4	13.9	13.2
6	35.0	35.0	35.8	35.8	35.1	35.1	35.3	35.2	34.7	35.6	35.9	35.9
7	12.4	12.5	13.0	13.0	12.6	12.6	12.9	13.0	12.8	13.0	13.6	12.5
8	95.5	95.4	96.6	96.1	96.1	96.2	96.3	95.5	94.3	96.0	95.4	93.6
9	8.5	8.6	9.0	9.0	8.7	8.4	9.1	8.6	8.9	8.9	9.4	9.1
10	8.7	8.7	9.1	9.1	8.7	8.6	8.7	8.9	9.0	9.0	9.5	9.0
11	20.6	20.6	21.3	21.4	20.4	20.4	20.7	20.5	21.0	20.9	20.9	21.3
12	40.0	40.9	41.8	41.7	41.3	40.9	41.7	41.1	40.4	41.6	41.8	41.0
13	12.8	12.9	13.6	13.4	13.1	13.0	13.6	13.5	13.3	13.6	13.6	13.7
14	95.7	95.5	96.4	96.0	95.9	95.8	95.7	95.3	94.9	95.9	95.5	94.8
15	13.0	13.0	13.3	13.3	13.1	13.0	12.6	13.0	13.3	13.4	13.6	13.1
16	34.0	34.3	34.8	35.0	34.4	34.3	35.5	34.7	34.1	35.0	35.0	34.5
17	13.5	13.3	13.9	13.8	13.3	13.3	13.7	13.6	13.6	13.7	13.7	13.6
18	9.0	9.0	9.3	9.3	9.1	8.7	8.9	9.4	9.3	9.4	9.6	9.4
19	62.5	71.0	65.7	70.8	71.3	71.3	69.8	69.5	70.4	71.6	71.6	71.2
20	12.6	12.6	13.0	13.1	12.6	12.6	12.5	12.3	12.9	13.5	13.2	12.2
21	17.1	17.0	17.5	17.4	17.0	16.9	17.3	17.3	17.3	17.9	17.4	17.7
22	40.4	40.5	41.1	41.1	40.4	40.6	41.0	40.8	40.2	41.8	41.0	41.1
23	8.8	8.7	9.0	9.0	8.8	8.6	8.9	9.2	9.0	9.1	8.8	8.9
24	16.8	17.1	17.6	17.6	17.0	17.0	17.2	17.2	17.3	18.1	17.6	17.2
25	34.9	34.9	36.0	36.0	35.0	35.1	34.4	34.9	34.7	35.9	35.5	34.2
26	69.0	70.9	66.0	71.1	70.9	71.4	69.0	69.3	70.7	71.4	70.7	71.4
27	23.0	23.0	23.9	23.7	23.1	22.9	23.3	22.3	23.0	24.0	23.8	23.0
28	18.5	18.8	19.7	19.6	18.0	18.3	18.6	20.1	19.4	18.5	18.2	17.9
29	22.6	23.0	23.6	23.6	23.1	22.9	23.2	22.8	23.4	24.0	23.8	23.4
30	18.1	18.2	18.8	18.6	18.1	17.7	18.1	17.4	18.8	18.5	18.0	17.9

^a See Table 1 for sample description.

where s = standard deviation, N = mean % nitrogen.

Some materials may require analysis of larger sample sizes to achieve this precision, depending on fineness of grind attained.

E. System Suitability

System equipped as in B(a)-(c) must meet or exceed minimum performance specifications as follows:

(1) Capable of measuring nitrogen in materials containing 0.2-20% nitrogen.

(2) Demonstrate system accuracy based on 10 successive determinations of nitrogen in nicotinic acid and 10 successive determinations of nitrogen in lysine-HCl or tryptophan. Means of determinations must be ± 0.15 of respective theoretical values, with standard deviations ≤ 0.15 . System accuracy must not be tested with same material used for calibration.

F. Calculations

For wheat and its products:

$$\text{Crude protein, \%} = N \times 5.70$$

For other cereal grains and oilseeds:

$$\text{Crude protein, \%} = N \times 6.25$$

Ref.: J. AOAC Int. (1993) 76, July/August issue.

Results and Discussion

Seven laboratories participated in the study. One laboratory used 3 different brands of combustion nitrogen analyzers; 6 laboratories used the LECO® FP-428 model nitrogen analyzer (Leco Corp.). Although 8 laboratories were originally contacted to analyze the samples by the Kjeldahl method, only 3 were able to complete the Kjeldahl determinations for the study. Laboratories that used the mercury catalyst Kjeldahl

Table 5. Comparison of laboratory averages for determination of protein (%) by combustion method

Sample ^a	Av., Kjeldahl	Av., combustion	Kjel. - Comb.
1	35.19	35.16	0.03
2	8.83	8.95	-0.12
3	8.75	9.00	-0.25
4	20.89	20.84	0.05
5	13.36	13.44	-0.08
6	35.30	35.39	-0.09
7	12.67	12.88	-0.21
8	96.06	95.41	0.65
9	8.73	8.89	-0.16
10	8.82	8.95	-0.13
11	20.77	20.85	-0.08
12	41.03	41.24	-0.21
13	13.17	13.41	-0.24
14	96.01	95.47	0.54
15	13.12	13.13	-0.01
16	34.38	34.70	-0.32
17	13.54	13.58	-0.04
18	9.10	9.22	-0.12
19 ^b	66.49	70.78	-4.29
20	12.75	12.78	-0.03
21	17.18	17.36	-0.18
22	40.66	40.90	-0.24
23	8.87	8.90	-0.03
24	17.14	17.36	-0.22
25	35.31	35.06	0.25
26 ^b	68.62	70.65	-2.03
27	23.33	23.22	0.11
28	18.75	18.83	-0.08
29	23.09	23.35	-0.26
30	18.34	18.15	0.19
Average	28.01	28.26	-0.25
Average ^b	25.18	25.23	-0.05

^a See Table 1 for sample description.

^b Kjeldahl analysis of nicotinic acid (samples 19 and 26) showed significant difference from theoretical; results for those samples were eliminated from calculation of the second set of averages.

method and also had a nitrogen analyzer and were willing to participate in the collaborative study were extremely difficult to locate. The collaborative study by Sweeney (1) established a statistically sound correlation between the Kjeldahl methods and the combustion method. Because the purpose of the present study was to extend the applicability of the combustion method, the Kjeldahl data were not essential for validation.

The sample set (Table 1) consisted of 15 matched pairs of blind duplicates to establish the within-laboratory repeatability of the method. The samples were ground with a 1 mm screen, and 3 of the samples (soybeans, corn, and wheat) were also ground with a 2 mm screen to establish whether any significant difference existed due to fineness of grind or particle size.

The moisture content and oil content of cereal grains and oilseeds contribute to the difficulty in grinding these types of

method for feeds (990.03) would necessitate predrying of the cereal grain and oilseed samples, thereby adding excessive sample preparation time. Sample size also becomes a critical consideration as the nonhomogeneity of the sample increases because of the nature of the material and/or the fineness of grind. The size of the ground sample analyzed must represent the sample as a whole. For these reasons, 1.0 and 2.0 mm screens were chosen to prepare the samples on an "as-is" basis.

Using the calculated *P*-values for each type of grain tested, there appears to be a statistically significant difference due to grind effects (Tables 2 and 3). The types of samples used in this study are very homogeneous when ground with a 1 mm screen, with the exception of sunflower seeds, which required additional care in blending (after grinding). Different products grind differently with the same size screen. Therefore, guidelines must be set for grinding each type of product. The traditional 1 g sample was chosen for the Kjeldahl method to decrease the variance of analysis due to sample variation and grinder effects. All but one of the combustion models tested accept sample sizes of at least 200 mg for the types of samples studied. Therefore, the recommended requirements for determining an instrument's precision were based on the following criteria: type of sample, fineness of grind, and sample size for the individual laboratory and the particular brand of analyzer used.

Three different brands of combustion analyzers and 2 different models of 1 brand of instrument were used in this collaborative study. The manufacturers' recommended sample sizes and analysis times varied considerably from instrument to instrument. In general, the sample size is proportional to the analysis time. The manufacturers' recommended sample sizes for the products tested were 20 mg for the Perkin-Elmer® (Perkin-Elmer Corp.), 150 mg for the LECO, and 500 mg for the Heraeus® (UIC, Inc.). A comparison to determine whether any significant differences exist between models due to sample size was not included in this collaborative study.

Results are shown in Table 4. A general observation is that the combustion method gives slightly higher protein results than does the Kjeldahl method. An average difference of -0.05% protein was obtained by comparing the Kjeldahl values with the combustion values (Table 5) after discarding the nicotinic acid data (poor recovery of nicotinic acid by several laboratories skewed the data). The combustion method data from the performance criteria procedure using standard reference materials (Table 6) showed a standard deviation for 10 analyses by each of the 9 laboratories of 0.03 for nicotinic acid, 0.02 for lysine-HCl, and 0.02 for tryptophan (2 laboratories). Data in Table 7 demonstrate the accuracy and precision of the combustion method in determining the nitrogen content of a sample.

Recommendations

I recommend that the scope of the generic combustion method for crude protein in feeds (990.03) be extended to include cereal grains and oilseeds. I also recommend that the

Table 6. Performance of combustion method for determination of nitrogen in standard reference materials

Analysis No.	Nicotinic acid, av. % N	Lysine-HCl, av. % N	Tryptophan, av. % N
1	11.49	15.28	13.77
2	11.47	15.29	13.74
3	11.44	15.30	13.78
4	11.48	15.32	13.74
5	11.42	15.32	13.72
6	11.43	15.28	13.76
7	11.42	15.28	13.73
8	11.37	15.27	13.76
9	11.43	15.31	13.79
10	11.40	15.31	13.74
No. labs	9	9	2
Av., %	11.44	15.30	13.75
SD	0.03	0.02	0.02
Theoretical, %	11.38	15.34	13.71

Kjeldahl method for determining protein in cereal grains (979.09).

I further recommend that the following be substituted for C(3) when method 990.03 is applied to cereal grains and oilseeds:

A suitable fineness of grind must be determined (for each different material analyzed) to achieve a precision which gives a relative standard deviation (RSD) of $\leq 2.0\%$ for 10 successive

determinations of nitrogen. $RSD, \% = (SD/\text{mean } \% N) \times 100$. Some materials may require analysis of larger quantities of the material to achieve this precision, depending on the attainable fineness of grind.

Acknowledgments

I thank the following collaborators for their contribution:

Table 7. Statistical summary of collaborative results for study of combustion method for determining crude protein in cereal grains and oilseeds

Sample	Description	s_r	s_R	$RSD_r, \%$	$RSD_R, \%$
1 & 6	Soybean	0.26	0.44	0.75	1.24
16 & 25 ^a	Soybean	0.52	0.57	1.49	1.62
12 & 22	Soybean	0.32	0.49	0.78	1.20
Average		0.29	0.47	0.77	1.24
4 & 11	Canola	0.20	0.29	0.95	1.39
27 & 29	Canola	0.19	0.48	0.80	2.06
Average		0.19	0.39	0.87	1.79
28 & 30	Sunflower	0.37	0.54	2.00	2.94
5 & 7	Wheat	0.17	0.22	1.23	1.63
13 & 15 ^a	Wheat	0.21	0.27	1.55	2.02
21 & 24	Wheat	0.14	0.31	0.82	1.78
Average		0.15	0.27	0.99	1.74
7 & 20	Barley	0.27	0.40	2.13	3.15
2 & 10	Corn	0.10	0.26	1.15	2.88
3 & 18 ^a	Corn	0.32	0.33	3.50	3.66
9 & 23	Sorghum	0.23	0.25	2.57	2.84
8 & 14	Lysine-HCl	0.36	0.72	0.38	0.75
19 & 26	Nicotinic acid	0.32	0.83	0.45	1.18

^a 2 mm grind. Results not used to calculate average for this sample type. All other grain and oilseed samples ground to 1 mm.

R. Boles and R. Cathey, University of Missouri-Columbia, Columbia, MO

J. Haselhorst, V. Collins, and R. Molloy, U.S. Department of Agriculture, Federal Grain Inspection Service Technical Center, Kansas City, MO

L. Jann and D. Tate, Illinois Department of Agriculture, Springfield, IL

L. Meschi, UIC, Inc., Joliet, IL

K. Swanson, Perkin-Elmer Corp., Norwalk, CT

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APPENDIX D

Combustion Method for Determination of Crude Protein in Meat and Meat Products: Collaborative Study

MARCLA KING-BRINK and JOSEPH G. SEBRANEK

Iowa State University, Department of Animal Science, Ames, IA 50011

Collaborators: C. Anthony; P. Coleman; B. Cottingham; R. Culmo; R. Curtis; L. Dingman; R. Johnson; G. Lehman; J. Loughran; S. Martinez; J. Moody; C. Paisley; H. Radloff; A. St. John; E. Schrader; J. Sizemore; J. Wenger; G. White

Twelve laboratories participated in a collaborative study to compare a combustion method with the AOAC mercury catalyst Kjeldahl method (928.08) for the determination of crude protein in meat and meat products. Three different combustion instruments were used; consequently, the combustion method for this study is written in generic terms describing the principle, the apparatus specifications, and the performance requirements needed. Fifteen sample pairs were used for the study; each pair consisted of the same commercial meat product from each of 2 different manufacturers. Protein content of all samples ranged from about 10 to 20%. In addition, nicotinic acid and lysine monohydrochloride were used as standards to assess combustion equipment performance. All laboratories and all instruments performed the combustion method satisfactorily on the basis of results for the standards. For the meat samples, repeatability standard deviations (s_r) ranged from 0.11 to 0.40 for the Kjeldahl method and from 0.12 to 0.41 for the combustion method; the repeatability relative standard deviations (RSD_r) ranged from 0.82 to 2.41% and from 0.60 to 2.23% for the Kjeldahl and combustion methods, respectively. Reproducibility standard deviations (s_R) ranged from 0.20 to 0.49 for the Kjeldahl method and from 0.18 to 0.46 for the combustion method, whereas the reproducibility relative standard deviations (RSD_R) ranged from 1.59 to 2.84% for the Kjeldahl method and from 1.32 to 3.35% for the combustion method. Overall grand means were 15.59% protein for the Kjeldahl method and 15.75% protein for the combustion

method. The combustion method was adopted first action by AOAC International.

Combustion methods for protein analysis that release nitrogen at high temperatures and quantitate the nitrogen by thermal conductivity were shown to be a practical alternative to the classical Kjeldahl method (1, 2). Several different manufacturers currently provide instruments that measure nitrogen in meat and meat products. The combustion method has inherent advantages over the Kjeldahl method in terms of speed (about 3 min per sample) and freedom from concentrated acid and base and the mercury catalyst.

Although the combustion method was studied and adopted for protein analysis of materials such as animal feeds (2, 3), it was not approved for meat and meat products. Because an alternative to the Kjeldahl method is of great interest to the meat industry, a collaborative study of the combustion method for meat and meat products was initiated.

Collaborative Study

Twelve laboratories, using 3 different commercially available combustion instruments, participated in the study. Nine laboratories used the LECO FP-428, 2 used the Foss Heraeus Macro-N Analyzer, and 1 used the Perkin-Elmer PE2410. To avoid requiring a particular manufacturer's instrument for this method, the combustion method was generally described with performance guidelines to be met for analysis of standard nicotinic acid and lysine hydrochloride. The standards and guidelines for accuracy were used to ensure that each instrument was capable of sufficient accuracy. The amino acid standards (Sigma Chemical Co., St. Louis, MO) and EDTA for instrument calibration were provided to each collaborator. Collaborators were asked to report the results from 10 successive analyses of each of the 2 amino acid standards, using the combustion and the mercury catalyst Kjeldahl methods.

Meat samples provided to collaborators consisted of 15 closely matched pairs, 30 samples in total. Samples were selected by choosing 15 typical commercial meat products and then choosing 2 commercial manufacturers of each product.

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The recommendation was approved by the General Referee and the Committee on Foods I and was adopted by the Official Methods Board of the Association. See "Changes in Official Methods of Analysis" (1993) *J. AOAC Int.* 76 Jan/Feb issue.

Samples were prepared by grinding in a tabletop meat grinder (Biro Model 8-22, Biro Co., Marblehead, OH), following U.S. Department of Agriculture (USDA) guidelines (4) to ensure uniform fineness of samples. The preparation consisted of passing emulsified meat products through the grinder (1/8 in. plate) twice and passing nonemulsified (coarse) products through 3 times. After grinding, ca 250 g units were double-bagged in polyethylene and frozen at -30°C for 72 h before shipment to collaborators. Each sample was identified with a random 3-digit number obtained from a random number table. No indication was made to collaborators of which samples were paired. Collaborators were instructed to keep the samples refrigerated (5°C or less) and to knead or massage each sample bag for 20 s before opening. Collaborators were encouraged to run all analyses within 1 week of receiving the samples.

Each collaborator was also provided a copy of the combustion method requirements for accuracy, a general description of the combustion method, and a set of report sheets to use for data recording. Sample size used by the various collaborators ranged from ca 200 mg to ca 900 mg.

992.15 Crude Protein in Meat and Meat Products—Combustion Method

First Action 1992

(Applicable to meat and meat products with 10–20% crude protein)

Method Performance:

$s_r = 0.12\text{--}0.41$; $s_R = 0.18\text{--}0.46$; $\text{RSD}_r = 0.60\text{--}2.23\%$; $\text{RSD}_R = 1.32\text{--}3.35\%$

A. Principle

Combustion method determines nitrogen released at high temperature into pure oxygen and measured by thermal conductivity. Nitrogen is converted to protein equivalent by using the appropriate factor, 6.25 for meat and meat products.

B. Apparatus

Note: Manufacturer's recommendations must be followed for safe and accurate operation of instruments. For proper laboratory precautions in handling compressed gases required for instruments, see "Compressed Gas Cylinders" in "Appendix: Laboratory Safety", *Official Methods of Analysis* (1990) 15th Ed.

(a) *Combustion instrument.*—Suitable for detecting 1–5% nitrogen (ca 5–30% protein) in meat and meat products to within $\pm 0.15\%$ of theoretical nitrogen content of standard, with standard deviation of ≤ 0.15 for 10 successive determinations on same standard. Instrument capable of analyzing single sample of at least 200 mg (to reduce impact of nonhomogeneity of meat and meat products). Instrument with oven capable of operating at $\geq 850^{\circ}$ in pure oxygen (for complete release of nitrogen from samples), capable of isolating nitrogen from other combustion products (i.e., CO_2 , H_2O) for subsequent quantitation, and capable of thermal conductivity measurement of nitrogen (Leco FP428, Leco Corp., St. Joseph, MI 49085, USA;

Macro-N Analyzer, Ross Heraeus Analysensysteme GmbH, Hanau 1, Germany; and PE2410, Perkin-Elmer Corp., Norwalk, CT 06859, USA, are suitable). Calibrations, required by most instruments, must be conducted by using theoretical percent nitrogen in pure primary standard organic compounds, such as EDTA.

(b) *Food chopper.*—With 1/8 in. (or less) plate, capable of grinding meat samples.

C. Reagents

The following reagents are typical but may vary depending on instrument. Consult manufacturer's instructions for specific instruments.

(a) *Compressed oxygen gas.*—99.99%.

(b) *Compressed helium gas.*—99.99%.

(c) *Compressed inert gas.*—Nitrogen (or equivalent), oil and water free.

(d) *Nitrogen standard.*—Ethylenediaminetetraacetic acid (EDTA), 9.59% nitrogen, or other suitable organic material of high purity and known nitrogen content (e.g., nicotinic acid or lysine hydrochloride).

(e) *Quartz wool.*

(f) *Glass wool.*

(g) *Alumina oxide pellets.*

(h) *Anhydrous magnesium perchlorate (MgClO_4).*

(i) *Sodium hydroxide on silicate carrier.*

(j) *Cu sticks.*

(k) *Cu metal turnings.*

(l) *Al foil combustion cups.*

Reagents available from several commercial manufacturers of combustion analyzer instruments [See B(a)].

D. Preparation of Sample

Pass samples through grinder 2 \times in succession for emulsified meat products; mix thoroughly after each grinding. Pass 3 \times in succession for nonemulsified (coarse or whole muscle) products.

E. Determination

Set instrument operating parameters (oven temperature, oxygen flow, calibration values, etc.) according to manufacturer's instructions. Let furnace and instrument reach operating temperature and stabilize. Warm-up time may be ca 6 h from cold start. Establish system blanks as appropriate for analysis and calibrate to blanks if necessary. At least 5 blank analyses are recommended. Calibrate instrument by using 3–5 analyses of nitrogen standard as follows:

(1) Accurately weigh 110–150 mg EDTA to the nearest 0.1 mg, or equivalent amount of nitrogen if using other nitrogen standard, into tared combustion cup or foil and transfer cup or foil to open loading port on instrument. Enter or record protein conversion factor required, if appropriate for instrument used.

(2) Close port, move sample into furnace, and begin analysis.

(3) When analysis is complete (3–5 min), repeat sequence for next sample.

Table 1. Samples used for collaborative study of combustion method for determination of crude protein in meat and meat products

Sample pair	Sample No.	Estd protein, % ^a	Description	Supplier
1	377	17	Ground beef	Fareway
	741	17		HyVee
2	414	20	Lean ground beef	ISU Meat Lab
	573	20		Cub Foods
3	185	19	Ground pork	Cub Foods
	822	19		ISU Meat Lab
4	439	18	Ground turkey	Louis Rich
	946	18		Longacre
5	471	11	Frankfurters	Dubuque Beef
	759	11		Nissan Beef
6	298	12	"Light" frankfurters	Oscar Mayer
	582	12		Hermel
7	163	13	Turkey frankfurters	Jennie-O
	935	13		Schweigert
8	434	10	Bologna	HyVee
	723	10		Oscar Mayer
9	637	12	"Light" bologna	Oscar Mayer
	866	12		Kahns Bulk at Cub
10	198	14	Turkey bologna	HyVee
	586	14		Wilson
11	118	17	Canned ham	Dubuque
	777	17		Farmland
12	364	17	Ham, water added	Wilson
	629	17		Farmstead
13	255	20	Dried beef	Carl Buddig
	299	20		Wilson
14	513	13	Pork sausage	ISU Meat Lab
	856	13		Purnells Old Folks
15	647	14	Summer sausage	Huisken
	941	14		ISU Meat Lab

^a Protein estimated from previous work with commercial meat products by assuming typical values would be found.

(4) Adjust instrument as necessary on the basis of results from nitrogen standard.

(5) Analyze samples by repeating steps (1) to (3).

(6) Read nitrogen results directly from instrument.

F. Calculation

$$\text{Crude protein, \%} = \% \text{ nitrogen} \times 6.25$$

(Note: Results with this method average 1.01 × results with 928.08.)

Ref.: J. AOAC Int. (1993) 76, July/August issue.

Results and Discussion

Sample pairs selected for the study are presented in Table 1. We anticipated that by choosing different manufacturers of the same product (for example, frankfurters) the protein content of each pair would be very similar but not identical, an objective of the Youden pair approach (5). Collaborators analyzed each sample once by the AOAC mercury catalyst Kjeldahl method (928.08) (1) and once by the combustion method. The data reported by each of the 12 laboratories are presented in Table 2. In general, sample pairs were closely matched, with about 5%

Table 2. Data for collaborative study on crude protein (%) determination in meat and meat products by AOAC Kjeldahl (K) method and a combustion (C) method

Pair	Sample		Laboratory											
	No.	Method	1	2	3	4	5	6	7	8	9	10	11	12
1	377	K	16.58	16.19	16.06	17.08	16.17	16.64	16.54	16.80	16.50	16.53	16.33	16.32
		C	16.48	16.72	17.10	16.74	16.62	16.30	16.62	16.91	15.88	16.15	17.58	16.56
	741	K	17.25	16.81	16.63	16.00	16.87	17.05	17.23	16.88	17.13	16.83	16.60	16.64
		C	17.38	17.41	17.61	16.83	16.74	16.92	17.18	17.50	17.38	16.55	17.71	16.81
2	414	K	20.46	20.38	20.02	21.02	19.49	20.39	20.68	20.15	19.31	20.21	20.09	19.99
		C	20.25	20.59	20.31	20.40	20.77	19.82	20.58	20.30	18.56	20.65	20.28	20.03
	573	K	18.98	17.82	18.38	19.56	18.94	18.87	18.20	18.40	18.25	18.55	18.40	18.39
		C	18.71	18.96	18.80	18.41	18.88	18.78	18.29	18.84	18.44	18.99	19.52	18.81
3	165	K	19.73	19.12	19.58	20.33	18.95	19.63	20.57	19.53	19.38	19.71	19.36	19.45
		C	19.35	19.90	20.00	19.44	19.53	19.59	20.08	19.85	19.62	19.62	21.12	19.88
	822	K	19.77	19.56	19.23	20.30	19.10	19.73	19.74	19.51	18.50	19.72	19.36	19.45
		C	18.44	19.84	19.68	18.82	19.51	19.58	19.97	19.84	19.94	20.03	20.31	19.70
4	439	K	18.81	18.00	18.49	19.18	18.76	18.84	18.71	18.76	17.81	18.41	18.46	19.02
		C	18.54	19.06	18.87	18.63	18.61	18.73	18.68	19.02	17.63	17.98	19.34	18.72
	946	K	18.25	18.38	17.63	18.52	18.36	18.46	18.44	18.27	18.31	18.58	17.79	18.05
		C	18.36	18.21	17.30	18.16	18.47	18.14	18.68	18.35	18.25	18.37	19.25	18.68
5	471	K	11.65	11.44	11.92	11.82	11.35	11.49	11.71	11.32	10.75	11.55	11.46	11.65
		C	11.61	11.78	11.66	11.68	11.73	11.33	11.79	11.69	11.19	11.58	12.03	12.15
	759	K	11.65	11.50	11.08	11.63	11.56	11.32	12.04	11.76	10.94	11.33	10.98	11.03
		C	11.53	11.74	11.50	11.49	11.51	11.22	11.68	11.74	10.88	11.00	11.45	11.99
6	298	K	11.63	11.19	11.76	11.86	11.70	11.26	11.55	11.72	11.69	11.66	11.64	11.27
		C	11.57	11.70	11.89	11.54	12.16	11.78	11.90	11.74	11.81	11.74	11.88	11.92
	582	K	11.95	12.12	11.53	11.58	11.97	12.03	11.87	11.54	12.19	12.03	11.76	11.76
		C	11.78	12.08	11.87	11.78	11.78	12.10	11.94	11.71	12.19	12.25	12.18	11.76
7	163	K	13.44	13.19	13.13	13.40	13.47	13.33	13.30	13.51	12.88	13.40	13.42	13.33
		C	13.25	13.58	13.61	13.31	13.38	13.18	13.85	14.19	13.08	13.89	14.26	13.88
	935	K	13.60	13.38	13.44	13.37	13.82	13.24	13.46	13.68	12.75	13.38	13.48	13.31
		C	13.44	13.68	13.54	13.68	13.55	13.88	13.92	13.46	12.94	13.56	13.71	13.37
8	434	K	10.90	10.69	10.76	10.67	10.98	10.53	10.51	10.89	10.58	10.78	10.82	10.80
		C	10.69	10.99	10.03	10.63	10.88	10.83	10.98	11.55	10.69	10.85	11.86	11.18
	723	K	11.68	11.44	11.06	11.64	11.49	11.02	11.87	11.63	11.00	11.53	11.54	11.40
		C	11.45	11.87	11.50	11.34	11.46	11.43	11.58	11.55	11.25	11.68	12.14	12.04
9	637	K	11.68	11.50	11.42	11.42	11.41	11.11	11.82	11.88	11.69	11.54	11.28	11.25
		C	11.40	11.67	11.42	11.23	11.54	11.38	11.53	12.10	11.63	11.45	12.17	11.89
	866	K	13.94	13.58	13.53	13.55	13.68	13.92	13.63	13.41	13.69	13.45	13.56	13.57
		C	13.27	13.88	13.74	13.42	13.76	13.35	13.70	14.14	13.88	13.58	13.85	13.52
10	198	K	14.65	14.62	15.22	15.16	14.34	14.38	14.77	14.16	14.88	14.60	14.50	14.53
		C	14.55	14.78	14.66	14.79	14.47	14.42	14.81	15.02	14.56	14.52	14.95	14.59
	598	K	14.67	14.75	14.29	15.16	14.87	14.45	14.75	14.99	15.35	14.70	14.70	14.71
		C	14.66	14.98	14.78	14.92	14.73	14.48	15.01	15.50	15.00	14.83	14.98	14.50
11	118	K	17.04	16.81	16.98	17.45	16.67	16.51	17.27	17.13	18.08	16.98	16.84	16.83
		C	16.91	17.27	17.24	17.13	17.44	16.88	17.14	17.45	16.88	16.87	17.41	17.09
	777	K	16.50	16.69	16.23	16.82	16.50	16.54	16.22	17.04	16.00	16.46	16.44	16.30
		C	16.52	16.82	16.82	17.18	16.73	17.56	16.66	17.31	16.19	16.33	16.91	16.45

Table 2. (Continued)

Pair	Sample		Laboratory											
	No.	Method	1	2	3	4	5	6	7	8	9	10	11	12
12	364	K	17.36	17.12	16.92	17.81	17.54	17.08	16.94	17.80	17.06	17.44	17.26	17.01
		C	17.08	17.64	17.68	18.00	18.03	17.11	17.42	18.05	17.01	17.44	17.90	17.53
	629	K	17.05	16.94	16.48	17.72	16.82	18.48	17.18	17.23	16.50	17.60	16.91	16.99
		C	17.04	17.30	16.84	18.46	17.00	17.71	16.95	17.27	16.56	17.12	17.41	17.12
13	255	K	19.83	19.50	19.56	20.29	19.09	19.61	20.49	19.42	19.81	19.46	18.88	19.07
		C	19.81	19.59	19.78	19.51	19.70	19.35	19.62	19.79	19.56	19.14	19.95	19.47
	299	K	20.49	20.44	20.08	21.37	20.83	20.39	20.41	20.64	20.25	20.16	19.95	20.27
		C	20.63	20.92	20.74	21.88	20.48	20.94	20.63	21.10	20.06	20.33	20.99	20.57
14	513	K	15.43	15.06	15.25	16.12	15.34	15.14	15.21	15.53	15.81	15.41	15.17	15.63
		C	15.13	15.82	15.65	15.42	15.36	15.18	15.82	16.16	15.88	15.53	15.86	15.84
	858	K	13.10	13.00	12.31	13.29	12.73	12.49	12.59	12.45	12.31	13.09	12.57	12.98
		C	12.81	13.23	12.98	12.93	12.77	11.84	13.37	13.06	12.44	12.86	13.24	13.09
15	647	K	14.44	14.50	14.00	14.55	14.34	14.20	14.00	14.24	13.81	13.97	14.52	14.17
		C	14.39	14.45	13.84	14.18	14.19	13.99	14.40	14.64	13.44	14.33	15.08	14.79
	941	K	19.69	19.19	19.14	20.34	19.43	18.75	19.36	19.63	19.50	19.31	18.99	18.16
		C	19.24	18.87	19.51	19.26	19.21	18.93	19.72	19.47	19.56	19.49	19.50	19.41

or less difference between the 2 samples of the pair. Three of the pairs (sample pairs 9, 14, and 15), however, showed more than 10% difference between the 2 samples of the pair. These samples are products (light bologna, pork sausage, and summer sausage, respectively) that are not uniform across the industry, and consequently, the greater differences were not surprising.

The performance testing of the combustion method by the collaborators showed that all laboratories performed well in combustion analysis of standard nicotinic acid and lysine hy-

drochloride (Table 3). Only 1 (laboratory 10) of the 24 means shown in Table 3 was outside the recommended ± 0.15 , and only 2 (laboratories 2 and 6) of 24 exceeded the recommended standard deviation of 0.15 (by 0.01 and 0.02, respectively).

Examination of the data from both methods for gross outliers was done by preparing 2-sample X-Y plots of the 15 sample pairs (5).

No gross outliers were observed for the Kjeldahl values from any of the 12 laboratories. In addition, use of the Cochran test, the single Grubbs test, and the double Grubbs test showed that none of the Kjeldahl data exceeded critical values for these tests for outliers. For the combustion method, one sample pair (414, 573) from laboratory 9 was in the low quadrant of the X-Y plot and exceeded the critical value of the single Grubbs test. Consequently, for the combustion method, the data for this pair (414, 573) from laboratory 9 were excluded from the rest of the comparisons.

The calculated estimates of precision are shown in Table 4, with the sample pairs arranged in order of increasing protein content. Repeatability standard deviations (s_r) were very similar for the 2 methods, ranging from 0.11 to 0.40 for the Kjeldahl method and from 0.12 to 0.41 for the combustion method (Table 5). Reproducibility standard deviations (s_R), likewise, were similar, ranging from 0.20 to 0.49 and from 0.18 to 0.46 for the Kjeldahl and combustion methods, respectively. The repeatability relative standard deviations (RSD_r) ranged from 0.82 to 2.41% for the Kjeldahl method and from 0.60 to 2.23% for the combustion method. The ranges for the reproducibility relative standard deviations (RSD_R) were 1.59 to 2.84% for the Kjeldahl method and 1.32 to 3.35% for the combustion method.

Comparison of the sample means (Table 4) shows the means from the combustion method to be slightly higher for all

Table 3. Collaborators' performance for combustion analysis of standards

Laboratory	Nicotinic acid		Lysine-HCl	
	Mean, % N ^a	SD ^b	Mean, % N ^a	SD ^b
1	11.32	0.13	15.34	0.04
2	11.31	0.09	15.22	0.17 ^c
3	11.39	0.05	15.40	0.02
4	11.42	0.02	15.33	0.01
5	11.43	0.05	15.29	0.03
6	11.38	0.16 ^c	15.32	0.03
7	11.49	0.03	15.38	0.02
8	11.46	0.06	15.32	0.02
9	11.39	0.02	15.34	0.02
10	11.26	0.03	15.06 ^c	0.12
11	11.49	0.01	15.37	0.05
12	11.41	0.10	15.26	0.05
Theoretical	11.38		15.34	

^a Mean of 10 determinations.

^b SD = standard deviation.

^c Value outside the performance requirements of ± 0.15 respective theoretical %N values or ± 0.15 standard deviation.

Table 4. Comparison of precision parameters for Kjeldahl and combustion methods for closely matched pairs in collaborative study on crude protein in meat and meat products

Sample pair	Kjeldahl					Combustion				
	Mean % protein	s_r	s_R	RSD _r , %	RSD _R , %	Mean % protein	s_r	s_R	RSD _r , %	RSD _R , %
8	11.09	0.19	0.22	1.75	2.02	11.26	0.25	0.30	2.19	3.36
5	11.46	0.28	0.32	2.41	2.84	11.58	0.14	0.29	1.20	2.50
6	11.72	0.27	0.27	2.28	2.28	11.88	0.18	0.18	1.53	1.53
9	12.54	0.18	0.20	1.31	1.59	12.64	0.16	0.27	1.23	2.14
7	13.36	0.11	0.22	0.82	1.68	13.59	0.29	0.33	2.14	2.46
14	14.08	0.27	0.32	1.90	2.29	14.26	0.25	0.37	1.75	2.59
10	14.72	0.30	0.30	2.04	2.07	14.22	0.12	0.24	0.79	1.60
15	16.80	0.29	0.33	1.75	1.99	16.83	0.36	0.36	2.08	2.12
1	16.76	0.22	0.37	1.29	2.22	16.90	0.28	0.41	1.65	2.43
11	16.77	0.40	0.40	2.37	2.37	16.99	0.28	0.36	1.64	2.14
12	17.13	0.24	0.37	1.40	2.16	17.40	0.34	0.43	1.97	2.49
4	18.42	0.33	0.34	1.77	1.86	18.50	0.41	0.46	2.23	2.47
3	19.60	0.22	0.39	1.11	1.99	19.76	0.12	0.26	0.60	1.32
2	19.41	0.36	0.49	1.89	2.53	19.64 ^a	0.25	0.28	1.27	1.42
13	20.02	0.33	0.42	1.63	2.11	20.18	0.33	0.36	1.63	1.77

^a Results by combustion method for sample pair 2 from laboratory 9 were excluded on basis of single Grubbs test.

pairs. When means are compared for each sample within pairs (Table 6), only one sample (941) resulted in a greater value by the Kjeldahl method. The average difference was 0.16% protein (0.025% N) between the 2 methods for all samples. Paired *t*-tests showed no significant difference between the methods. The grand means of all samples were 15.59% for the Kjeldahl method and 15.75% for the combustion method.

Comparing the 2 methods for analysis of the standards, nicotinic acid and lysine hydrochloride (Table 7), shows the greater difficulty experienced by several laboratories with the Kjeldahl method for the standards. Six of the 12 laboratories reported low values for nicotinic acid, and 5 had difficulties with the determinations on lysine hydrochloride. This difficulty is not unusual and has been observed previously (2). The combustion method, when applied to the standards, was more consistent than the Kjeldahl method when all 12 laboratories are considered.

Conclusions

The combustion method for crude protein performed very satisfactorily when applied to a variety of meat and meat prod-

Table 5. Range of precision estimates for Kjeldahl and combustion methods in collaborative study on crude protein in meat and meat products

Measure	Kjeldahl	Combustion
s_r	0.11-0.40	0.12-0.41
s_R	0.29-0.49	0.18-0.46
RSD _r	0.82-2.41	0.60-2.23
RSD _R	1.59-2.84	1.32-3.35

ucts by 12 different laboratories. Repeatability and reproducibility standard deviations were equivalent for the 2 methods. Further, data for only one sample pair for the combustion method in one laboratory were excluded as outliers. This means that only 2 of the 360 data points collected for the combustion method were outliers, an outcome indicating that both the method and the laboratories involved performed very well.

In addition, 3 different combustion instruments were used in this study with no obvious effects on the results; therefore, the combustion method does not require a particular instrument. Minimum sample size and performance requirements for accuracy should be included when the method is applied to meat and meat products.

Recommendation

We recommend that the combustion method be adopted first action for determination of crude protein in meat and meat products. To achieve homogeneity before sampling, a minimum sample size of 200 mg and careful sample preparation following USDA laboratory guidelines are recommended. Performance guidelines for combustion instruments should be ± 0.15 of theoretical N content of the mean of 10 determinations of nicotinic acid and of lysine hydrochloride, with a standard deviation of ≤ 0.15 . Safety precautions should be followed according to the respective manufacturer of the instrument used.

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Table 6. Comparison of sample means by combustion and Kjeldahl methods (all laboratories) for collaborative study on crude protein in meat and meat products

Sample pair	Sample No.	Mean protein, %		
		Kjeldahl	Combustion	Difference (comb. - Kjeldahl)
1	377	16.54	16.64	0.10
	741	17.07	17.17	0.10
2 ^a	414	20.18	20.37	0.19
	573	18.63	18.91	0.28
3	165	19.61	19.77	0.16
	822	19.58	19.75	0.17
4	439	18.58	18.67	0.08
	846	18.25	18.35	0.10
5	471	11.51	11.69	0.18
	759	11.40	11.48	0.08
6	298	11.58	11.60	0.22
	582	11.86	11.86	0.10
7	163	13.32	13.66	0.34
	805	13.41	13.58	0.15
8	434	10.74	10.93	0.19
	723	11.44	11.59	0.15
9	637	11.50	11.62	0.12
	866	13.57	13.66	0.09
10	198	14.85	14.88	0.03
	598	14.79	14.88	0.07
11	118	17.08	17.18	0.12
	777	16.49	16.79	0.30
12	384	17.28	17.57	0.29
	829	16.98	17.23	0.25
13	255	19.59	19.59	0.00
	299	20.44	20.77	0.33
14	513	15.43	15.84	0.21
	856	12.74	12.89	0.15
15	647	14.23	14.31	0.08
	941	19.38	19.35	-0.03
Overall		15.59	15.75	0.16

^a Results of combustion method from laboratory 9 excluded.

Table 7. Collaborators' results of 10 determinations by combustion and Kjeldahl methods for standards (% protein)

Laboratory	Nicotinic acid		Lysine-HCl	
	Kjeldahl	Combustion	Kjeldahl	Combustion
1	71.45	70.75	95.91	95.89
2	70.21	70.72	94.02	95.10
3	68.99	71.18	94.63	96.27
4	59.54 ^a	71.35	94.06	95.80
5	39.28 ^a	71.43	93.33 ^a	95.57
6	64.83 ^a	70.98	93.52 ^a	95.74
7	71.90	71.81	96.08	96.01
8	70.19	71.65	94.65	95.72
9	70.73	71.16	95.80	95.84
10	27.94 ^a	70.37	93.17 ^a	94.23
11	14.49 ^a	71.79	77.33 ^a	96.07
12	35.04 ^a	71.28	99.60 ^a	95.39
Mean	70.75	71.21	95.15	95.64
Theoretical		71.12		95.88

^a Excluded from mean.

P. Coleman and S. Martinez, Swift-Eckrich, Downers Grove, IL

B. Cottingham and L. Dingman, Hudson Farms, Inc., Rogers, AR

R. Culmo, Perkin-Elmer, Norwalk, CT

R. Curtis and J. Moody, South Carolina Department of Agriculture, Columbia, SC

G. Lehman, Meat Science Laboratory, Ames, IA

J. Loughran, A & L Great Lakes Laboratory, Ft. Wayne, IN

C. Paisley, IAMS Co., Aurora, NE

H. Radloff and G. White, Oscar Mayer Foods, Madison, WI

E. Schrader, Quaker Oats, Lawrence, KS

J. Sizemore and A. St. John, IAMS Co., Lewisburg, OH

J. Wenger, Lancaster Laboratories, Lancaster, PA

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APPENDIX E

Determination of Nitrogen in Fertilizer by Combustion: Collaborative Study

DONALD F. TATE¹

Illinois Department of Agriculture, Division of Plant Industries and Consumer Services, General Chemistry Laboratory, Springfield, IL 62794-9281

Collaborators: H. Agahigian; R. Biscak; B. Crepin; R. Culmo; G. Curran; M. Flock; P. Geib; B. Jinks; D. Jones; P. Kane; B. Kaufman; W. Longmire; S. Mullins; R. Neumann; N. Newlon; J. Nichols; J. Nuzzo; B. Obert; R. Oeckinghaus; V. Pabst; M. Pyles; P. Ransdell; B. Sanders; B. Saylor; R. Sensacir; M. Soltys; S. Spaer; D. Stilwell; A. St. John; D. Storer; K. Swanson; M. Wooden

Fourteen laboratories participated in a collaborative study to compare abilities of AOAC modified copper catalyst Kjeldahl method, 978.02, and the generic combustion method, 990.03, to analyze the nitrogen content of fertilizer materials. Combustion analyses are more time efficient, more accurate, and less hazardous than Kjeldahl analyses. There were 3 different types of instrumentation involved in the collaborative study: (1) Leco FP-428 Nitrogen Determinator; (2) Perkin-Elmer 2410 Series II Nitrogen Analyzer; (3) Carlo-Erba 1500 Series II Nitrogen Analyzer. Thirty samples of fertilizer containing 1–67% N included 2 ACS grade standard materials: ammonium nitrate, theory 35.00% N; and dicyandiamide, theory 66.64% N. A diammonium phosphate and urea mixture (3 + 1; 1.0 mm grind) and 2 ACS grade standard materials of ammonium nitrate and ammonium sulfate were supplied for repetitive combustion analyses. Overall method performance of the combustion method was at least as good as the modified Kjeldahl method. Repeatability standard deviation (S_r) values for the combustion method ranged from 0.09 to 0.34 vs the modified Kjeldahl method range of 0.06–0.49; reproducibility standard deviation (S_R) values for the combustion method ranged from 0.13 to 1.07 vs the range of 0.09–3.57 for the modified Kjeldahl method. The grand mean was 20.78% for the combustion method, and 20.79% for the modified Kjeldahl

method using various fertilizers. The average ranges of S_r and S_R for the methods were, respectively, 0.17 and 0.29 for the combustion method, and 0.19 and 0.54 for the modified Kjeldahl method. The method was adopted first action by AOAC INTERNATIONAL.

The introduction of the AOAC combustion method 990.03 (1) for the analyses of protein content in feeds has prompted study to find methods to analyze the nitrogen content of other agricultural materials. For over 100 years, the Kjeldahl methods have been the only official quantitative means for determining the nitrogen content in fertilizers. Now, due to sophisticated combustion technology, combustion instruments can perform analyses without the danger to the worker and environment.

Kjeldahl analyses expose the analyst to electrical and fire hazards. They are expensive, time-consuming, and difficult to perform. Use of combustion instrumentation eliminates all these problems. When using a combustion instrument, it is not necessary to create any standard or reagent solutions, and there is no concern over disposal of toxic chemical by-products from wet chemical techniques. There are only 4 sources of error in the combustion technique: (1) certification of the standard material used for calibration of the instrument; (2) analytical balance used to weigh the sample; (3) operator proficiency in weighing samples and interpreting analytical results; and (4) instrument stability. Finally, it takes 3 min to analyze a fertilizer by combustion method and the instruments can operate autonomically.

Collaborative Study

Collaborators analyzed 27 samples and 2 standard materials (1 duplicate) by the combustion method and by the modified Kjeldahl method. The solid sample set included 2 different sample matrixes of different grinds (1.0 mm and 0.5 mm) to

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The recommendation was approved by the Committee on Foods, Fertilizers and Related Topics, and was adopted by the Official Methods Board of the Association. See "AOAC International Official Methods Board News" (1993) *J. AOAC Int.* 76, 33A, and "Methods Adopted First Action" (1993) *The Referee*, 17, March issue.

¹ Current address: Illinois Dept of Agriculture, Bureau of Environmental Programs, PO Box 19281/State Fairgrounds, Springfield, IL 62794-9281.

establish intralaboratory precision. The sample set also included a variety of different liquid samples, including 1 duplicate, to establish the instrument ability to analyze liquid fertilizers. The test samples were randomly labeled.

Three other materials were supplied to the collaborators for repetitive analyses by the combustion method. There were 2 standard materials, ACS grades of ammonium nitrate and ammonium sulfate, and a custom mix of diammonium phosphate and urea (3 + 1). The standard materials provided a performance check on setting up the instrument correctly, and the 3 + 1 mix (1.0 mm grind) verified the grind necessary to achieve precise results.

Fourteen collaborators submitted data from analyses by the combustion method. Seven Leco FP-428 Nitrogen Determinators, 4 Perkin-Elmer 2410 Series II Nitrogen Analyzers, and 3 Carlo-Erba 1500 Series II Nitrogen Analyzers were used. The combustion method was designed for use with different instruments to enable instrument manufacturers to meet the instrument performance requirements of the method. Once the instrument has been calibrated to correctly analyze ammonium nitrate, it would also accurately analyze the other fertilizer materials.

Since some laboratories did not perform the modified Kjeldahl method routinely, only 12 collaborators reported results for this method.

993.13 Nitrogen (Total) in Fertilizers—Combustion Method

First Action 1993

(Applicable to determination of 1–67% total nitrogen content in liquid and solid fertilizer materials)

Method Performance:

Liquid and solid fertilizers, 1.04–33.99% N
 $s_x = 0.11–0.26$; $s_R = 0.14–0.39$; $RSD_r = 0.77–10.99\%$; $RSD_R = 1.15–13.80\%$

A. Principle

Nitrogen is released from fertilizer sample through combustion at high temperature under high purity oxygen. Nitrogen is quantitatively measured by thermal conductivity detection and reported as w/w percent nitrogen in sample.

B. Apparatus

Combustion instrument.—Capable of measuring nitrogen by combustion utilizing high purity oxygen. Nitrogenous compounds liberated must be fully converted to N_2 gas. Other combustion by-products must be isolated either through gas chromatography or chemical scrubbing. Containing combustion chamber capable of maintaining minimum temperature of 950° for liberation of nitrogen from sample when high purity (99.99%) oxygen is introduced into chamber, either as aliquot or as carrier gas. Containing thermal conductivity detector capable of detecting N_2 gas liberated from combusted sample. Containing microprocessor capable of calibrating instrumentation with standard reference material; subtracting nitrogen im-

purities in instrument system and sample encapsulation process; tracking analyses time; retention of sample, standard, and blank data; converting detector response into w/w % N in sample; and controlling some, if not all, instrument operating parameters.

C. Standard Reference Materials

A certified reference material of % nitrogen content giving detector response in same analytical range as fertilizer sample to be analyzed is necessary for accurate analytical results. Uric acid should be obtained from National Institute of Standards and Technology (NIST) for comparison and measurement of reference materials obtained from other sources. Standard operating procedure should include weekly crosschecks of these standard materials. Use NIST Standard Reference Material (SRM) to calibrate instrument and to analyze for nitrogen content of other reference materials using instrument parameters as in E. Other primary standard materials for instrument calibration may be used if nitrogen content is verified by NIST SRM. Except for uric acid (which liberates HCN when heated), dry reference materials 2 h at 105° before use. Store reference materials in desiccator. Suggested reference materials:

(1) *Uric acid (33.23% N)*.—Clinical grade, 99.7% purity (SRM No. 913, NIST, Gaithersburg, MD).

(2) *Ammonium sulfate (21.20% N)*.—99.999% purity (Cat. No. 20450-1, Aldrich Chemical Co., Milwaukee, WI, is suitable).

(3) *Ammonium nitrate (35.00% N)*.—99.999% purity (Cat. No. 25606-4, Aldrich Chemical Co., is suitable).

(4) *Ammonium chloride (26.18% N)*.—99.99% purity (Cat. No. 32637-2, Aldrich Chemical Co., is suitable).

D. Performance Requirements

System must be capable of meeting or exceeding the following minimum performance specifications:

(1) Analytical system must be able to measure nitrogen in fertilizer materials containing 1–67% N.

(2) Accuracy of system must be demonstrated by performing 10 successive determinations of ammonium sulfate standard and 10 successive determinations of ammonium nitrate standard. Mean of 10 determinations for reference materials must be within ± 0.20 units of respective theoretical values. Standard deviations must be $\leq 0.20\%$ N for ammonium nitrate and $\leq 0.10\%$ N for ammonium sulfate standards.

(3) Grind fertilizer samples to suitable fineness to give relative standard deviation (RSD) $\leq 1.0\%$ for 10 successive nitrogen determinations. Moisture content of solid fertilizer sample must be same before and after grinding to ensure correct analytical result.

E. Instrument Setup

Follow manufacturer's recommendations for safe operation of instrument; refer to MSDS for safe handling of chemicals. Ventilate exhaust gases to appropriate fume removal system. For solid (but not liquid) reference materials or fertilizer samples, add at least 4 \times sample weight of powdered (use mortar

and pestle) sucrose to sample container if instrument utilizes only oxygen as carrier gas in combustion chamber.

Adjust combustion furnace temperature to 950° or higher according to manufacturer's recommendations. Calibrate instrument according to manufacturer's instructions using NIST Uric Acid. Adjust oxygen profile and other instrument parameters to achieve maximum combustion of ammonium nitrate standard before analyzing fertilizer samples. Check for full nitrogen recovery by alternately analyzing ammonium nitrate standard and instrument system blank. Readjust instrument parameters until instrument system blank value consistently returns to initial blank value and ammonium nitrate standard nitrogen value is within specifications (see D). Use manufacturer's recommended settings for other system constants.

F. Determination

Place instrument manufacturer's recommended weight of fertilizer sample into appropriate container. Diatomaceous earth may be used to adsorb liquid fertilizer samples. Add powdered sucrose when necessary (see E). Calibrate instrument with reference material, such as uric acid, before analyzing fertilizer samples. Check for instrument analytical drift and chemical reagent failure by analyzing instrument system blank periodically during sample analyses. Increase in blank value indicates chemical reagent failure or incomplete combustion. Recalibrate instrument with reference material whenever instrument parameters change significantly as noted by change in analytical time sequence for completed analyses. Always check for correct calibration and recalibrate, if necessary, whenever instrument's sealed combustion system is exposed to atmosphere.

Ref.: *J. AOAC Int.* 77, 829(1994).

Results and Discussion

Table 1 gives a description of test samples, the type of grind done on the materials, and how the material was characterized for statistical calculations. The sample set included 4 solid blind duplicates, 1 liquid blind duplicate, 5 solid Youden pairs, 2 liquid Youden pairs, 3 individual solids, and 3 individual liquids. Eleven of the fertilizers were ground with a 1.0 mm screen, and 5 of the fertilizers were double-ground with a 0.5 mm screen. A Brinkmann Ultra Centrifugal Mill (Model ZM 1) with a 12-tooth rotor was used for all grinds.

Dicyandiamide (DCD) was chosen due to its use in Europe (Culmo, R., Perkin-Elmer Corp., private communication, May 1991). All duplicate samples were taken out of the same container and sent in pairs to the collaborators.

Ammonium nitrate was sent in duplicate for comparison to the same material used in the combustion repetitive analyses. However, the ammonium nitrate analyzed blindly was not dried, while the same ammonium nitrate used for combustion repetitive analyses was to be dried by the collaborator at 105°C for 2 h before analyses.

Tables 2 and 3 contain the raw data for all 30 samples. Table 2 contains the combustion method analytical results; Table 3 contains the modified Kjeldahl method (1) analytical re-

Table 1. Samples used in the collaborative study of nitrogen in fertilizers

Sample guarantee	Type of repetition*	Grind, mm	Description
67-0-0	single	none	dicyandiamide standard (ACS grade)
48-0-0	single	1.0	urea fertilizer
35-0-0	duplicate	none	ammonium nitrate standard (ACS grade)
34-0-0	Youden	1.0, 0.5	ammonium nitrate fertilizer
32-0-0	single	none	urea & ammonium nitrate liquid fertilizer
28-0-0	duplicate	none	diluted urea liquid fertilizer
21-0-0	Youden	1.0, 0.5	ammonium sulfate fertilizer
18-46-0	duplicate	1.0	triple superphosphate fertilizer
16-6-0	Youden	1.0, 0.5	custom fertilizer
14-37-11	duplicate	1.0	custom-mix of 18-46-0 & 0-0-60 fertilizers
12-12-12	Youden	1.0, 0.5	nitric phosphate fertilizer
11-52-0	duplicate	0.5	monoammonium phosphate fertilizer
10-34-0	Youden	none	ammonium polyphosphate liquid fertilizer
10-30-0	Youden	none	custom liquid fertilizer slurry
8-24-0	Youden	none	custom liquid fertilizer slurry
7-22-5	single	none	custom liquid fertilizer
6-24-24	single	1.0	custom fertilizer
3-10-30	single	none	liquid fertilizer slurry
1-3-57	Youden	1.0, 0.5	custom-mix of 18-46-0 and 0-0-60 (1 + 17)

* Single = single blind sample; duplicate = blind duplicate samples; Youden = Youden closely matched pairs of samples.

sults. The combustion method data from Collaborators 2 and 8 and the modified Kjeldahl method data from Collaborators 2, 9, and 11 were excluded from the statistical calculations. The data failed either the Cochran or the Grubbs tests (2); the Cochran laboratory sum ranking test (3); or the ranking test for outliers (4). Seven Youden pairs and 5 blind duplicate sample plots were used to prepare the Cochran and Grubbs test. All 12 sample pairs were plotted and recalculated until they passed test criteria.

Table 4 contains the statistical calculations for the samples, excluding data that failed the Grubbs or Cochran tests. There was a noticeable difference in the analytical means when a sample was double-ground. A check of moisture content on these samples revealed an increase for most of the 0.5 mm double-ground samples. Apparently these fertilizer materials absorb moisture out of the air when they are double-ground.

Table 5 contains statistical information about analytical results which fell within the 95% confidence interval, excluding outliers. This created different average results and reproducible standard deviations than seen in Table 4. Some of the reproducible standard deviations are reduced by as much as one third of the original calculations.

Table 2. Collaborative study results for nitrogen in fertilizers by the combustion method

Sample	Grind, mm	Collaborator													
		1	2 ^a	4	6	7	8 ^b	9	10	11	12	13	14	15	16
Solid fertilizers															
Std:															
NH ₄ NO ₃	na ^b	34.85	24.76	34.80	34.42	34.73	38.42	34.94	34.55	32.12	33.57	34.40	34.05	35.01	34.59
Std:															
NH ₄ NO ₃	na	34.87	20.87	35.01	34.54	34.71	35.30	34.68	34.89	31.40	34.19	34.91	35.31	34.85	34.73
Std:															
dicyan- diamide	na	66.38	66.41	66.50	68.91	65.89	71.06	68.15	68.49	67.61	66.22	66.40	66.70	66.81	66.00
34-0-0	1.0	34.23	17.74	34.20	33.89	33.01	34.05	33.58	34.31	30.67	33.55	34.45	33.56	34.40	33.59
34-0-0	0.5	34.31	25.47	34.35	33.93	33.72	34.86	34.42	34.31	30.72	33.79	34.99	33.57	34.05	34.24
18-46-0A	1.0	17.63	17.79	17.54	17.90	17.83	18.31	17.81	17.52	17.58	17.74	17.45	17.88	17.65	17.88
18-46-0B	1.0	17.41	14.69	17.52	17.71	17.81	18.07	17.69	17.61	17.47	17.67	17.58	17.88	17.81	18.12
11-52-0A	0.5	10.93	11.16	10.98	11.11	11.16	11.27	10.98	10.82	10.55	11.14	10.90	10.97	11.19	11.30
11-52-0B	0.5	10.99	12.19	10.99	11.41	11.24	11.78	11.12	10.75	10.69	11.28	10.96	11.25	11.13	11.46
14-37-11	1.0	14.01	16.81	13.96	13.70	13.95	14.14	13.78	14.00	13.91	13.90	14.12	14.13	14.08	14.39
14-37-11	1.0	13.98	12.02	14.10	13.97	13.77	13.91	14.04	13.99	14.07	13.78	14.01	13.80	14.53	13.95
1-3-57	1.0	0.89	0.80	0.99	1.30	1.04	1.14	1.07	0.97	0.93	1.08	0.80	0.89	0.96	1.00
1-3-57	0.5	1.11	0.83	1.06	1.22	1.13	1.37	1.18	0.91	1.13	0.98	0.99	1.02	1.15	1.43
21-0-0	1.0	21.10	22.23	21.10	21.05	20.87	21.22	21.03	20.96	21.06	21.04	21.10	21.27	21.08	21.06
21-0-0	0.5	20.96	21.38	21.08	20.62	20.65	20.97	20.94	21.08	21.02	20.74	20.92	20.94	20.91	20.83
18-8-0	1.0	18.12	24.84	18.01	18.06	17.92	18.17	18.11	18.10	18.09	17.90	18.10	17.91	18.08	17.98
18-8-0	0.5	17.84	17.56	17.71	17.69	17.76	18.03	17.29	17.81	17.83	17.72	17.75	17.71	17.78	17.84
12-12-12	1.0	11.95	12.06	12.01	11.76	11.92	12.43	11.90	11.79	11.76	11.87	11.89	12.01	11.76	12.31
12-12-12	0.5	12.10	11.73	11.95	11.98	11.87	12.45	11.93	12.01	11.81	11.88	12.01	11.93	12.02	11.87
46-0-0	1.0	45.53	48.99	45.60	46.12	45.74	48.90	46.09	45.43	45.13	45.68	45.50	46.21	46.04	46.07
6-24-24	1.0	6.49	6.39	6.50	6.67	6.54	6.63	6.52	6.31	6.38	6.55	6.55	6.35	6.49	6.87
Liquid fertilizers															
10-34-0A	na	12.05	11.27	11.85	11.78	11.98	12.08	12.10	12.01	12.26	11.95	11.98	12.14	11.91	12.53
10-34-0B	na	9.76	10.14	9.72	9.75	9.93	10.18	9.88	9.80	9.83	10.05	9.69	9.92	9.59	7.57
28-0-0	na	29.10	20.43	30.00	29.16	28.84	30.30	28.90	28.88	29.09	28.63	28.76	29.44	29.00	28.24
28-0-0	na	28.80	22.03	28.79	29.18	28.86	29.26	23.06	29.10	28.78	29.19	28.97	29.45	28.82	28.60
UAN32-0-0	na	31.95	21.71	31.88	32.29	31.84	33.58	31.83	31.95	32.03	31.85	31.70	32.53	31.81	31.92
10-30-0	na	9.40	8.83	9.25	9.01	9.09	9.14	9.04	9.45	9.17	9.01	9.70	9.16	8.98	9.29
7-22-5	na	7.85	7.47	7.80	7.19	7.89	7.52	7.56	7.75	7.74	6.99	7.62	7.38	7.50	7.55
8-24-0	na	7.12	7.21	7.20	7.29	7.29	7.88	7.14	7.16	7.25	7.22	4.80	7.78	6.47	7.25
3-10-30	na	4.12	3.40	4.12	3.15	3.52	5.27	3.77	4.14	5.27	3.77	7.08	4.07	3.32	3.55
Average		19.531	17.305	19.555	19.462	19.420	20.180	19.564	19.495	19.112	19.362	19.509	19.567	19.499	19.494
Deviation		14.490	13.226	14.529	14.580	14.373	15.272	14.711	14.507	14.150	14.393	14.466	14.552	14.612	14.522

^a Collaborator's data excluded.

^b Not applicable.

Twenty percent of the combustion data for Collaborator 11 were outliers. Primarily this collaborator could not correctly analyze ammonium nitrate samples. This was to be expected since this collaborator did not get full recovery of the ammonium nitrate used in the repetitive combustion analyses. Apparently, this collaborator did not follow the instrument setup procedure of the combustion method. However, the other analytical results, except for 2 other outliers, were well within statistical limits. This author has noted the possibility of ana-

lyzing fertilizer materials other than ammonium nitrate without any complications; however, the instrument should be set up to correctly analyze this material to encompass all types of fertilizer.

An interesting observation can be made about a collaborator whose modified Kjeldahl data did not fall within the 95% confidence interval. Collaborator 6 had 26.7% of the analytical results as outliers. This was a rather high percentage; however, this collaborator had passed the AOAC guidelines established for collaborator elimination. This collaborator had problem

Table 3. Collaborative study results for nitrogen in fertilizers by the modified Kjeldahl method

Sample	Grind, mm	Collaborator											
		1	2 ^a	4	6	7	8	9 ^a	10	11 ^a	12	13	14
Solid fertilizers													
Std: NH ₄ NO ₃	na ^b	34.79	35.25	34.86	34.49	34.17	35.05	32.38	33.75	15.92	33.36	34.57	34.23
Std: NH ₄ NO ₃	na	35.02	34.64	35.03	33.61	34.50	35.19	30.24	34.80	21.40	34.68	35.10	34.76
Std: dicyan-diamide	na	66.75	66.80	66.32	76.56	65.22	66.52	63.01	66.12	63.88	64.46	67.10	66.66
34-0-0	1.0	34.30	33.94	34.25	36.17	33.62	34.34	27.06	34.35	21.92	33.86	34.40	31.99
34-0-0	0.5	34.22	33.24	34.51	28.10	33.78	34.44	30.79	34.39	22.51	34.14	34.21	31.69
18-46-0A	1.0	17.64	17.77	17.85	17.40	17.71	17.83	17.03	17.65	17.03	17.68	17.60	17.89
18-46-0B	1.0	17.48	18.23	17.55	18.00	17.44	17.81	17.47	17.80	16.76	17.78	17.75	17.96
11-52-0A	0.5	10.93	11.45	11.10	11.17	11.29	11.17	10.96	10.91	10.59	11.04	11.10	11.31
11-52-0B	0.5	10.89	11.07	11.01	11.78	11.32	11.29	11.09	10.81	10.39	11.34	11.01	11.39
14-37-11	1.0	14.00	14.24	13.99	13.91	14.04	13.92	13.67	13.95	13.17	13.80	14.18	14.09
14-37-11	1.0	14.01	14.36	14.11	13.60	13.95	13.91	13.48	14.01	13.04	14.52	14.17	13.99
1-3-57	1.0	0.91	1.04	1.01	1.03	0.90	0.71	1.17	0.95	0.08	0.93	1.00	1.02
1-3-57	0.5	1.01	1.15	1.07	0.96	1.05	0.90	1.20	0.90	4.85	0.98	1.11	1.06
21-0-0	1.0	21.06	20.98	21.00	20.60	20.89	21.08	20.60	21.01	32.91	20.79	21.05	21.03
21-0-0	0.5	21.01	20.86	20.95	22.68	20.51	20.79	19.46	21.10	19.72	20.70	20.59	20.74
18-8-0	1.0	18.30	18.39	18.10	17.42	17.76	17.75	17.87	18.05	16.68	17.75	18.30	17.97
18-8-0	0.5	17.86	17.06	17.73	17.46	17.57	17.56	17.16	17.77	16.80	17.25	18.00	17.78
12-12-12	1.0	12.03	11.61	12.02	11.67	11.75	11.61	11.04	11.91	11.65	11.66	12.00	11.94
12-12-12	0.5	12.15	11.86	11.96	11.20	11.67	11.63	11.54	12.03	11.09	11.67	12.94	11.82
46-0-0	1.0	45.67	45.14	45.66	47.26	44.69	45.68	43.28	44.95	44.39	45.18	45.70	46.09
6-24-24	1.0	6.48	6.63	6.51	7.35	6.58	6.65	6.27	6.32	6.07	6.42	6.64	6.47
Liquid fertilizers													
10-34-0A	na	12.06	12.37	12.06	12.00	12.12	12.35	11.97	12.11	11.25	12.14	12.05	12.29
10-34-0B	na	9.77	10.28	9.76	9.93	10.04	10.16	9.80	9.82	9.44	9.92	10.01	10.14
28-0-0	na	29.21	29.71	29.75	27.89	28.57	29.34	28.41	28.82	21.73	28.76	28.99	29.40
28-0-0	na	28.79	29.50	28.90	25.52	28.96	29.36	28.85	29.50	21.38	28.81	28.95	29.42
UAN32-0-0	na	31.88	31.92	32.01	30.81	31.37	31.63	31.50	31.40	21.80	29.71	32.05	32.20
10-30-0	na	9.49	9.71	9.40	8.80	9.09	9.29	8.62	9.40	7.45	9.15	8.76	9.34
7-22-5	na	7.86	7.80	7.75	7.49	7.71	7.78	7.71	7.65	6.92	7.53	7.79	7.82
8-24-0	na	7.15	7.50	7.26	6.79	7.26	7.13	7.09	7.20	6.58	7.28	4.70	7.41
3-10-30	na	4.15	3.55	4.13	2.06	4.06	4.02	3.77	4.12	3.64	3.55	7.10	3.96
Average		19.56	19.60	19.59	19.46	19.32	19.56	18.48	19.45	16.70	19.23	19.63	19.46
Deviation		14.29	14.22	14.26	15.38	13.96	14.31	13.20	14.16	12.45	13.90	14.29	14.12

^a Collaborator's data excluded.

^b Not applicable.

with analyses of the standard materials and the ammonium nitrate fertilizer.

Table 6 presents the statistical differences between the methods after elimination of outliers. The combustion method has an S_R equivalent to or lower than the modified Kjeldahl method for all but 8 solid and 3 liquid materials. The combustion method gives an analytical result closer to the theoretical amount of nitrogen in DCD (theory 66.64% N). The average analytical results for the combustion methods were higher than

the modified Kjeldahl method in 52% analyses of solid materials and 67% liquids. There was a significant difference in the average analytical result for the UAN 32-0-0 liquid (Student's *t*-test verification), (5), which can be contributed to incomplete digestion by the modified Kjeldahl method. The combustion RSD_R averages were 0.30% lower for solid materials and 0.51% lower for liquids.

Table 7 contains the statistical parameters of both methods for blind duplicates and Youden pairs. The combustion analy-

Table 4. Performance of the modified Kjeldahl method and the combustion method

Sample	Grind, mm	Modified Kjeldahl ^a method			Combustion ^b method		
		\bar{X}	s_R	RSD _R , %	\bar{X}	s_R	RSD _R , %
Solid fertilizers							
Std. NH ₄ NO ₃	na ^c	34.363	0.547	1.59	34.336	0.806	2.35
Std. NH ₄ NO ₃	na	34.743	0.479	1.38	34.499	1.014	2.94
Std. dicyandiamide	na	67.301	3.568	5.30	66.739	0.613	0.92
34-0-0	1.0	34.142	1.077	3.15	33.620	1.029	3.06
34-0-0	0.5	33.276	2.127	6.39	33.817	1.017	3.01
18-46-0A	1.0	17.694	0.151	0.85	17.701	0.159	0.90
18-46-0B	1.0	17.730	0.200	1.13	17.707	0.205	1.16
11-52-0A	0.5	11.113	0.140	1.26	11.003	0.199	1.81
11-52-0B	0.5	11.204	0.303	2.70	11.106	0.240	2.16
14-37-11	1.0	13.987	0.110	0.79	13.984	0.178	1.27
14-37-11	1.0	14.030	0.243	1.74	13.998	0.203	1.45
1-3-57	1.0	0.840	0.099	10.54	0.977	0.151	15.50
1-3-57	0.5	1.004	0.075	7.47	1.109	0.136	12.27
21-0-0	1.0	20.946	0.159	0.76	21.060	0.094	0.45
21-0-0	0.5	21.008	0.656	3.12	20.889	0.151	0.72
18-8-0	1.0	17.933	0.290	1.62	18.032	0.084	0.47
18-8-0	0.5	17.664	0.227	1.29	17.727	0.148	0.63
12-12-12	1.0	11.843	0.170	1.44	11.911	0.155	1.30
12-12-12	0.5	11.897	0.480	4.03	11.945	0.083	0.70
46-0-0	1.0	45.853	0.743	1.63	45.760	0.341	0.74
6-24-24	1.0	6.602	0.299	4.53	6.518	0.148	2.28
Liquid fertilizers							
10-34-0A	na	12.131	0.116	0.96	12.053	0.193	1.60
10-34-0B	na	9.950	0.149	1.50	9.632	0.662	6.88
28-0-0	na	28.970	0.546	1.88	29.003	0.433	1.49
28-0-0	na	28.630	1.219	4.25	28.975	0.234	0.81
UAN32-0-0	na	31.451	0.763	2.49	31.873	0.226	0.71
10-30-0	na	9.191	0.264	2.88	9.213	0.218	2.36
7-22-5	na	7.709	0.128	1.67	7.568	0.273	3.61
8-24-0	na	6.909	0.846	12.24	6.981	0.803	11.50
3-10-30	na	4.128	1.298	31.46	4.157	1.069	25.73
Average		19.473	0.583	4.068	19.467	0.376	3.699
Deviation		14.520	0.729	5.890	14.483	0.327	5.581

^a Data from Collaborators 2, 9, and 11 excluded.

^b Data from Collaborators 2 and 8 excluded.

^c Not applicable.

ses gave a higher analytical result in 58% of 18 samples. Loss of nitrogen content is less likely in the combustion method than the modified Kjeldahl method due to fewer steps in analyses of a sample.

The repeatability standard deviation (S_r) for the combustion method was smaller than or equal to the modified Kjeldahl method for 50% samples due to less variability in consecutive instrumental analyses. The samples with a higher S_r for combustion were predominately mixtures of solid materials and mixtures of liquids. These sample matrixes may not have been homogeneous.

The reproducibility standard deviation (S_R) for the combustion method was smaller than or equal to the modified Kjeldahl method for 67% of 18 samples. This is primarily due to laboratory variation in Kjeldahl burners and different expertise in Kjeldahl operations. The average pooled S_R and s_r for the combustion method are 0.29 and 0.17, respectively. These average standard deviations reflect a pooled RSD_R of 1.40% and RSD_r of 0.82% for the range of 1–67% nitrogen content in fertilizers.

Table 8 presents the instruments used in this study, and statistical calculations of the 10 consecutive repetitive combustion analyses. Only 38% of the collaborators achieved an aver-

Table 5. Statistical evaluation of individual modified Kjeldahl analyses and combustion analyses at the 95% confidence interval

Sample	Grind, mm	Modified Kjeldahl ^a method				Combustion ^b method			
		n ^c	\bar{X}	S _R	RSD _R , %	n ^c	\bar{X}	S _R	RSD _R , %
Solid fertilizers									
Std. NH ₄ NO ₃	na ^d	9	34.363	0.547	1.59	11	34.537	0.424	1.23
Std. NH ₄ NO ₃	na	8	34.885	0.237	0.68	11	34.761	0.290	0.83
Std. dicyandiamide	na	8	66.144	0.879	1.33	11	66.611	0.443	0.67
34-0-0	1.0	9	34.142	1.077	3.15	11	33.888	0.463	1.37
34-0-0	0.5	8	33.923	0.930	2.74	11	34.098	0.302	0.88
18-46-0A	1.0	9	17.694	0.151	0.85	12	17.701	0.159	0.90
18-46-0B	1.0	9	17.730	0.200	1.13	12	17.707	0.205	1.16
11-52-0A	0.5	9	11.113	0.140	1.26	11	11.044	0.145	1.32
11-52-0B	0.5	9	11.204	0.303	2.70	12	11.106	0.240	2.16
14-37-11	1.0	9	13.987	0.110	0.79	11	13.994	0.178	1.27
14-37-11	1.0	9	13.969	0.171	1.22	12	13.949	0.119	0.86
1-3-57	1.0	8	0.969	0.052	5.39	11	0.947	0.117	12.40
1-3-57	0.5	9	1.004	0.075	7.47	11	1.060	0.096	8.85
21-0-0	1.0	8	20.889	0.099	0.47	11	21.041	0.070	0.33
21-0-0	0.5	8	20.799	0.206	0.99	12	20.889	0.151	0.72
18-8-0	1.0	9	17.933	0.280	1.62	12	18.032	0.084	0.47
18-8-0	0.5	9	17.664	0.227	1.29	11	17.767	0.057	0.32
12-12-12	1.0	9	11.843	0.170	1.44	11	11.875	0.096	0.81
12-12-12	0.5	8	11.768	0.389	3.31	12	11.945	0.083	0.70
46-0-0	1.0	8	45.453	0.466	1.02	12	45.760	0.341	0.74
6-24-24	1.0	8	6.509	0.112	1.72	11	6.486	0.103	1.59
Liquid fertilizers									
10-34-0A	na	9	12.191	0.116	0.96	11	12.010	0.127	1.06
10-34-0B	na	9	9.950	0.149	1.50	11	9.819	0.138	1.41
28-0-0	na	9	28.970	0.546	1.88	11	28.913	0.312	1.08
28-0-0	na	8	29.086	0.291	1.00	11	28.975	0.234	0.81
UAN32-0-0	na	8	31.669	0.461	1.46	11	31.923	0.150	0.47
10-30-0	na	9	9.191	0.284	2.88	11	9.168	0.162	1.77
7-22-5	na	9	7.709	0.128	1.67	11	7.621	0.208	2.74
8-24-0	na	8	7.185	0.182	2.53	11	7.197	0.300	4.17
3-10-30	na	8	3.756	0.712	18.96	11	3.891	0.571	14.67
Average			19.458	0.323	2.500		19.492	0.212	2.258
Deviation			14.429	0.270	3.433		14.505	0.132	3.469

^a Data from Collaborators 2, 9, and 11 excluded.

^b Data from Collaborators 2 and 8 excluded.

^c Number of Collaborators.

^d Not applicable.

age within the stated specifications for the ammonium nitrate, but 69% of the collaborators were able to meet the criterion of the repeatability standard deviation. Excluding Collaborator 11, the collaborators which did not fall within the stated criterion of the method were recovering approximately 99% of the ammonium nitrate. Collaborator 11 obtained 90% recovery caused by not adding enough sucrose to samples. Instruments which use oxygen exclusively as the carrier gas during the combustion process must have at least a 4 + 1 ratio (w/w) of

sucrose to the sample. A recovery of 99% for the ammonium nitrate may be sufficient for a screening process, but a higher recovery is desirable for quantifying the nitrogen content.

There were no problems encountered with the instruments when analyzing the ammonium sulfate. Sixty-nine percent of the collaborators obtained a mean result within the specified criterion. All of the collaborators had a repeatability standard deviation (S_R) below the 0.20 specified in the method. In fact, the standard deviations were below or equal to half the criterion

Table 6. Statistical differences between the individual modified Kjeldahl analyses and the combustion analyses^a

Sample	Grind, mm	\bar{X}			s_R			RSD _R , %		
		Combustion	Kjeldahl	Difference	Combustion	Kjeldahl	Difference	Combustion	Kjeldahl	Difference
Solid fertilizers										
Std:										
NH ₄ NO ₃	na ^b	34.336	34.363	-0.027	0.806	0.547	0.259	2.35	1.59	0.76
Std:										
NH ₄ NO ₃	na	34.499	34.743	-0.244	1.014	0.479	0.535	2.84	1.38	1.56
Std:										
dicyandi- amide	na	66.739	67.301	-0.562	0.619	3.568	-2.855	0.92	5.30	-4.38
34-0-0	1.0	33.620	34.142	-0.522	1.029	1.077	-0.048	3.06	3.15	-0.09
34-0-0	0.5	33.817	33.276	0.541	1.017	2.127	-1.110	3.01	6.39	-3.38
18-46-0 a	1.0	17.701	17.694	0.007	0.159	0.151	0.008	0.90	0.85	0.05
18-46-0 b	1.0	17.707	17.730	-0.023	0.205	0.200	0.005	1.16	1.13	0.03
11-52-0 a	0.5	11.003	11.113	-0.110	0.198	0.140	0.059	1.81	1.26	0.55
11-52-0 b	0.5	11.106	11.204	-0.098	0.240	0.303	-0.063	2.16	2.70	-0.54
14-37-11	1.0	13.984	13.987	0.003	0.178	0.110	0.068	1.27	0.79	0.48
14-37-11	1.0	13.998	14.030	-0.032	0.203	0.243	-0.040	1.45	1.74	-0.29
1-3-57	1.0	0.977	0.940	0.037	0.151	0.099	0.052	15.50	10.54	4.96
1-3-57	0.5	1.109	1.004	0.105	0.136	0.075	0.061	12.27	7.47	4.80
21-0-0	1.0	21.060	20.946	0.114	0.084	0.159	-0.085	0.45	0.76	-0.31
21-0-0	0.5	20.889	21.008	-0.119	0.151	0.658	-0.505	0.72	3.12	-2.40
18-8-0	1.0	18.032	17.933	0.099	0.084	0.290	-0.206	0.47	1.62	-1.15
18-8-0	0.5	17.727	17.864	0.063	0.148	0.227	-0.079	0.53	1.29	-0.46
12-12-12	1.0	11.911	11.843	0.068	0.155	0.170	-0.015	1.30	1.44	-0.14
12-12-12	0.5	11.945	11.897	0.048	0.084	0.480	-0.396	0.70	4.03	-3.33
46-0-0	1.0	45.780	45.653	0.107	0.341	0.743	-0.402	0.74	1.63	-0.89
6-24-24	1.0	6.518	6.602	-0.084	0.148	0.299	-0.151	2.28	4.53	-2.25
Liquid fertilizers										
10-34-0 A	na	12.053	12.131	-0.078	0.199	0.116	0.077	1.60	0.96	0.64
10-34-0 B	na	9.632	9.950	-0.318	0.682	0.149	0.513	6.58	1.50	5.38
28-0-0	na	29.003	28.970	0.033	0.433	0.548	-0.113	1.49	1.88	-0.39
28-0-0	na	28.975	28.890	0.285	0.234	1.219	-0.985	0.51	4.25	-3.44
UAN 32-0-0	na	31.973	31.451	0.522	0.228	0.783	-0.557	0.71	2.49	-1.78
10-30-0	na	9.213	9.191	0.022	0.218	0.264	-0.046	2.36	2.88	-0.52
7-22-5	na	7.568	7.709	-0.141	0.279	0.128	0.145	3.61	1.67	1.94
8-24-0	na	6.981	6.909	0.072	0.803	0.846	-0.043	11.50	12.24	-0.74
3-10-30	na	4.157	4.128	0.029	1.069	1.298	-0.229	25.73	31.46	-5.73

^a Data from Collaborators 2, 9, and 11 by modified Kjeldahl method excluded; data from Collaborators 2 and 8 by combustion method excluded.

^b Not applicable.

specified for 77% of the collaborators. Collaborator 11 obtained a very high mean result on the ammonium sulfate even though the mean result on the ammonium nitrate was very low.

The 1.0 mm single-ground custom mix (3 + 1) posed no major problem in obtaining reproducible analytical results. Seventy-one percent of the collaborators met the specified criterion in the combustion method to obtain an RSD_R ≤ 1.0%.

Sample size and grind criteria used for analyses of fertilizer materials are important aspects to review when using combustion instrumentation, since most analysts believe that it is impossible to obtain good analytical results using small sample

sizes. Sample sizes were within the following ranges for each instrument: Carlo-Erba, 2-31 mg; Perkin-Elmer, 40-185 mg; and Loco, 25-362 mg. The majority of the sample sizes, including the Carlo-Erba, ranged from 50-110 mg. Sample size was not a critical component in analyzing these materials. Three instrument manufacturers had statistically valid analytical data. However, sample grind plays an important part in obtaining valid reproducible analyses. Statistical results of the combustion method obtained from 1.0 mm single-ground duplicate samples (18-46-0) and 0.5 mm double-ground duplicate samples (11-52-0) were the reverse of expectations. The repeat

Table 7. Comparison of the modified Kjeldahl and the combustion methods performances

Sample	Combustion method ^a					Modified Kjeldahl method ^b				
	\bar{X}	s_r	s_R	RSD _r %	RSD _R %	\bar{X}	s_r	s_R	RSD _r %	RSD _R %
Solid fertilizers										
DCD	66.74	—	0.61	—	0.92	67.30	—	3.57	—	5.3
46-0-0	45.76	—	0.34	—	0.74	45.65	—	0.74	—	1.63
NH ₄ NO ₃ ^c	34.74	0.34	0.34	0.97	0.97	34.55	0.49	0.54	1.43	1.56
34-0-0 ^c	33.99	0.26	0.39	0.77	1.15	34.12	0.23	0.26	0.66	1.05
21-0-0 ^c	20.97	0.10	0.13	0.50	0.60	20.89	0.14	0.16	0.65	0.78
18-46-0 ^c	17.70	0.09	0.18	0.54	1.03	17.71	0.18	0.18	1.04	1.04
18-8-0 ^c	17.68	0.13	0.13	0.70	0.70	17.60	0.11	0.26	0.64	1.46
14-37-11 ^c	14.00	0.18	0.19	1.27	1.34	13.99	0.09	0.14	0.65	0.98
12-12-12 ^c	11.93	0.13	0.13	1.13	1.13	11.87	0.27	0.36	2.24	3.03
11-52-0 ^c	11.05	0.11	0.23	0.97	2.04	11.16	0.17	0.24	1.51	2.13
6-24-24	6.52	—	0.15	—	2.28	6.60	—	0.90	—	4.53
1-3-57 ^c	1.04	0.11	0.14	10.99	13.80	0.97	0.06	0.09	6.22	9.04
Liquid fertilizers										
32-0-0	31.97	—	0.23	—	0.71	31.45	—	0.78	—	2.49
28-0-0 ^c	28.99	0.31	0.34	1.06	1.18	29.10	0.31	0.33	1.06	1.15
10-34-0 ^c	10.91	0.11	0.13	0.99	1.21	11.04	0.07	0.13	0.65	1.21
10-30-0 and 8-24-0 ^c	8.18	0.22	0.24	2.71	2.95	8.22	0.13	0.2	1.64	2.48
7-22-5	7.57	—	0.27	—	3.61	7.71	—	0.13	—	1.67
3-10-30	4.16	—	1.07	—	25.73	4.13	—	1.30	—	31.46
Average	20.78	0.17	0.29	1.68	3.45	20.79	0.19	0.54	1.52	4.06
Deviation	16.87	0.09	0.23	2.92	8.33	16.92	0.12	0.81	1.56	7.14

^a Data from Collaborators 2 and 8 excluded.

^b Data from Collaborators 2, 9, and 11 excluded.

^c Statistical calculation for Youden closely matched pairs and blind duplicates done by AOAC computer software: AOACBURFL.wk1, AOACYMP.wk1 (Phillips, J., Calculation of AOAC Performance Parameters, Rev. 11/2/90.)

ity and reproducibility standard deviations for the combustion method were 0.54 and 1.03, respectively, for a 1.0 mm single-ground sample, and 0.97 and 2.04 for a 0.5 mm double-ground sample, respectively. RSD_r and RSD_R for the modified Kjeldahl method were both 1.04% for a 1.0 mm single-ground sample, and 1.51% and 2.13% for a 0.5 mm double-ground sample, respectively. A 0.5 mm double-grind sample caused more variability in the analytical result, probably due to an increase in moisture content. The grind of a solid fertilizer is an important aspect to consider before attempting any analyses. However, it is not as critical as having the correct instrument parameters and having an experienced instrument operator.

The instrument operator must be proficient and experienced in interpretation of the analytical result. These instruments produce analytical results regardless of correct calibration or instrument condition. Computers have enhanced combustion technology and enabled faster and easier means of quantifying the combustion by-products, but the analyst is still the key component for determining correct analytical results.

A collaborator had problems comparing the uric acid (SRM 913) calibration standard against ammonium dihydro-

gen phosphate (SRM 194) and Leco EDTA (lot 891-129). The expected values were 12.15% and 9.59% N, respectively, but the collaborator obtained average analyses of 12.021% and 9.481% N when the instrument was calibrated with the uric acid (SRM 913). Because of problems of this kind, uric acid (SRM 913) was chosen as the standard for calibration. Other standard materials are affected by environmental conditions such as humidity. Uric acid has low affinity for water and does not have to be dried (6) before use as a calibration standard. The analyst should continually cross-check standard materials to verify their composition and purity since all analytical results obtained by combustion instruments rely on the quality of the standard material used for calibration.

Conclusion

The collaborators had problems analyzing the ammonium nitrate and the ammonium nitrate fertilizer by the modified Kjeldahl method (1) and the combustion method. There were a few noticeable differences in statistical calculations from the other fertilizer materials analyses. The combustion method

Table 8. Statistical information on repetitive combustion analyses of 3 different samples

Collab.	Instrument	ACS grade ammonium nitrate			ACS grade ammonium sulfate			Custom mix (3 + 1)		
		\bar{X}	s_r	RSD _r , %	\bar{X}	s_r	RSD _r , %	\bar{X}	s_r	RSD _r , %
1	Perkin-Elmer	34.958	0.114	0.327	21.121	0.063	0.301	25.127	0.102	0.408
2	Leco	34.947	0.697	1.995	21.383	0.149	0.698	25.146	0.395	1.572
4	Perkin-Elmer	35.018	0.163	0.467	21.105	0.069	0.329	25.095	0.069	0.273
6	Carlo-Erba	34.279	0.142	0.414	21.278	0.059	0.279	24.169	0.239	0.969
7	Leco	—	—	—	—	—	—	25.361	0.159	0.625
8	Carlo-Erba	34.804	0.099	0.284	21.338	0.026	0.124	25.532 ^a	0.378	1.471
9	Leco	34.788	0.195	0.562	21.114	0.074	0.350	25.246	0.104	0.411
10	Perkin-Elmer	34.998	0.042	0.121	21.193	0.066	0.310	25.188	0.083	0.330
11	Leco	31.529 ^a	0.626	1.968	21.625 ^a	0.115	0.532	25.284	0.154	0.607
12	Leco	34.577	0.373	1.077	21.195	0.074	0.350	25.068	0.181	0.721
13	Perkin-Elmer	34.980	0.037	0.104	21.182	0.020	0.096	25.196	0.022	0.088
14	Carlo-Erba	34.708	0.079	0.227	21.250	0.032	0.149	24.850	0.282	1.136
15	Leco	34.785	0.141	0.404	21.115	0.094	0.444	24.086	0.424	1.760
16	Leco	34.586	0.271	0.783	20.981 ^a	0.132	0.627	25.179	0.209	0.830
Average		34.782	0.196	0.564	21.207	0.066	0.312	25.130	0.167	0.664
Deviation		0.221	0.184	0.528	0.085	0.036	0.167	0.160	0.104	0.415
RSD _r , %		0.64			0.45			0.64		

^a Data excluded from statistical calculations; result fell outside 95% confidence interval.

gave a truer analysis of the dicyandiamide than the modified Kjeldahl method, as can be seen by the reproducible standard deviations of 0.613 and 3.568, respectively. The average analytical result for the combustion method on the UAN 32-0-0 liquid fertilizer sample was significantly higher than the results for the modified Kjeldahl method, 31.97% and 31.45% N, respectively. The combustion method reproducible standard deviation for the UAN 32-0-0 was about one third of the modified Kjeldahl method, 0.23 vs 0.78, respectively.

A 1.0 mm single grind gave a more accurate analysis than a 0.5 mm double grind of solid fertilizer when using a Brinkmann Ultra Centrifugal Mill (Model ZM1) with a 12-tooth rotor, because certain solid fertilizers pick up moisture when they are double-ground through a 0.5 mm screen. This extra moisture causes an increase in analytical variability and a change in the true analytical result.

There are some special considerations when using these instruments to analyze fertilizer materials. Some collaborators had problems with their combustion systems clogging up and noticed corrosion of metallic components. Fertilizers have high concentrations of halogens which can form corrosive acids that erode some metals after the gases cool. Some type of filter which can trap these halogens before they enter the rest of the combustion system is necessary if fertilizer materials are routinely analyzed. The in-line use of iron chips trapped by glass wool is one remedy at the present time.

Comments

The following changes should be made to the combustion method. The mean of the standard ammonium nitrate material used to demonstrate accuracy of the system must be within \pm

0.20 of the respective theoretical value, unless this material is quantified. If the material is quantified, then the mean must be within ± 0.10 of the respective value; the standard deviation for the standard ammonium sulfate should be ≤ 0.10 when used to demonstrate the accuracy of the system. The analyst should follow all safety precautions stipulated by the MSDS sheets for any chemicals used in analyses.

Recommendations

On the basis of data obtained in this study, it is recommended that the combustion method for determination of nitrogen in fertilizer be adopted first action.

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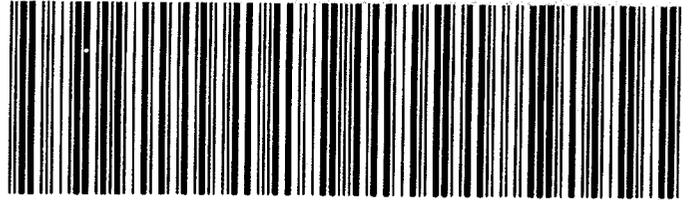
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