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

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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 $CuSO_4/TiO_2$ 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, $CuSO_4/TiO_2$ 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 $CuSO_4/TiO_2$ ("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 $CuSO_4/TiO_2$ mixed catalyst gave protein negative biases of −0.25 and −0.17%, respectively. The $CuSO_4/TiO_2$ mixed catalyst gave a digestion time close to that of HgO and less than $CuSO_4$. Based on this study, the $CuSO_4/TiO_2$ 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 $CuSO_4/TiO_2$ 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 Kjedahl 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 $CuSO_4/TiO_2$ 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)aminomethane] ("TRIZMA"), 99.92%, containing 11.56% nitrogen, obtained from the National Institute of Standards Testing (NIST) (Gaithersburg, MD). Duplicate analyses were performed.

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Collaborative study samples were analyzed at approximately the same time as the Smalley samples. In addition to performing the required nitrogen analysis by the $CuSO_4/TiO_2$ 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 $CuSO_4/TiO_2$ Kjeldahl method is shown in Table 2 (10).

In comparison with the $CuSO_4/TiO_2$ 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

[a]Twenty-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).

[b]Sample 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.

[c]Statistical parameters relate only to percent nitrogen values obtained in collaborative study.

TABLE 2

Comparison of Nitrogen Combustion and $CuSO_4/TiO_2$ 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

[a]Average 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 $CuSO_4/TiO_2$ and HgO Kjeldahl methods were compared by six laboratories, analyzing a total of 380 samples of soybean meal, the $CuSO_4/TiO_2$ 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 $CuSO_4/TiO_2$ Kjeldahl method with the nitrogen combustion method, may be due to the use of the $CuSO_4/TiO_2$ 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 $CuSO_4/TiO_2$ 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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