# Comparison of Combustion and Kjeldahl Methods for Determination of Nitrogen in Oilseeds[1]

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**The recent development of combustion-type nitrogen analyzers capable of handling relatively large samples with semi-automatic operation offers a potential replacement for the Kjeldahl method for direct determination of nitrogen. Nitrogen analyses for canola seed, flaxseed, sunflower seed, mustard seed and soybeans on a LECO (St. Joseph, MI) FP-428 Nitrogen Analyzer were evaluated against results from the Grain Research Laboratory's (GRL) Kjeldahl system. The nitrogen analyzer gave significantly higher values than the Kjeldahl method, resulting in a correction of low values in the GRL Kjeldahl, caused by the inability to use mercury as catalyst. The standard error for results from the analyzer was comparable to that for the Kjeldahl method. The nitrogen analyzer also was faster than the Kjeldahl method and had less environmental impact. The combustion method has replaced the Kjeldahl method for routine nitrogen determinations in oilseed surveys conducted by the GRL.**

KEY WORDS: Canola, combustion analysis, Dumas, flaxseed, Kjeldahl, mustard seed, protein, rapeseed, soybean, sunflower seed.

The current Grain Research Laboratory (GRL) Kjeldahl laboratory (Winnipeg, Manitoba, Canada) was built in 1972, primarily to support the Canadian Grain Commission's segregation of hard red spring wheat into protein classes (1). Designed for high throughput of cereal samples, the present laboratory consists of 48 Kjeldahl units that can complete an analysis of protein in wheat in about 45 min. Daily throughput, rated for a 3-shift, 24-hour operation, was 1600 samples. The laboratory also supplied Kjeldahl protein determinations for other crop monitoring and research activities within the GRL (2). This included monitoring the protein content of Canadian oilseeds, including canola, flax or linseed, sunflower seed, soybeans and mustard seed.

Problems with the analysis of protein in oilseeds were noted in the late 1970s when we began to subscribe to the Smalley Check Sample Survey (AOCS, Champaign, IL). Results for check samples were consistently low, often more than expected variance would allow. The low results may be related to the GRL's discontinued use of mercury catalyst in 1976, but it is not possible to definitively assign the problem to catalyst differences because data for GRL Kjeldahl analyses of Smalley check samples with mercury catalyst are not available. Attempts to remedy the problem, at least for canola, included an in-depth study of the amounts of reagents and time of digestion (3). While this resulted in a procedure that improved yield, the increased digestion time resulted in frothing, and the modifications were not incorporated into routine operation. More recently, experimentation with the digestion procedure using hydrogen peroxide met with only limited success. Participation in a round-robin study on the use of copper sulfate catalyst demonstrated again that GRL oilseed protein determinations were lower than protein analyses from other laboratories, even when using the same catalyst (4). In recent Smalley Check Sample Series (Table 1), results from the GRL Kjeldahl laboratory have averaged considerably lower than the mean for all participants.

As our present system seemed to have consistently low results, we decided to investigate alternative methods of analysis. The technique of combustion analysis developed by Dumas in 1831 (5) has been used for organic elemental analysis for many years. The small sample size and labor-intensive application of the early apparatus has precluded its use for routine nitrogen determination in agricultural commodities, but recently, the apparatus has been modified

TABLE 1

**Performance of GRL Kjeldahl Laboratory (Winnipeg, Manitoba, Canada) in Smalley Check Sample Program 1989–1991[a]**

| | | | Nitrogen (%) | | | | |
|---|---|---|---|---|---|---|---|
| Series | Year | Number of samples | Mean Smalley | Mean GRL | Mean Diff. | Diff./Std. Dev. | Number of low GRL samples |
| Canola | 1989 | 5 | 3.68 | 3.49 | 0.19 | 0.75 | 5 |
| | 1990 | 5 | 3.59 | 3.51 | 0.09 | 0.69 | 5 |
| | 1991 | 5 | 3.75 | 3.68 | 0.08 | 0.70 | 5 |
| Soybean | 1989 | 10 | 6.53 | 6.40 | 0.13 | 1.92 | 10 |
| | 1990 | 10 | 6.51 | 6.44 | 0.07 | 1.19 | 8 |
| | 1991 | 10 | 6.62 | 6.47 | 0.15 | 1.66 | 10 |
| Sunflower | 1989 | 8 | 3.18 | 2.96 | 0.22 | 5.40 | 8 |
| | 1990 | 8 | 3.27 | 3.26 | 0.01 | 0.50 | 6 |
| | 1991 | 8 | 3.12 | 2.97 | 0.15 | 2.39 | 7 |
| Oilseed meal | 1989 | 10 | 7.58 | 7.24 | 0.34 | 4.18 | 8 |
| | 1990 | 10 | 7.47 | 7.23 | 0.24 | 4.14 | 9 |
| | 1991 | 10 | 7.60 | 7.21 | 0.41 | 3.63 | 10 |

[a]Diff. = difference; Std. Dev. = standard deviation; GRL = Grain Research Laboratory.

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to allow larger sample sizes and also semi-automatic operation (6). After considerable study and experimentation, we chose the LECO FP-428 (LECO Corp., St. Joseph, MI) apparatus as the instrument most suitable to our purposes, although several other instruments on the market performed as well analytically.

This work describes our experiences with combustion determination of total nitrogen in comparison to the results obtained from the GRL's Kjeldahl laboratory. In addition to samples from the AOCS Smalley Program, nitrogen determinations on samples from several Canadian oilseed crops, including canola, flaxseed, soybeans, sunflower seed and mustard seed, were compared. In all cases, the nitrogen analyzer gave higher values for nitrogen than the GRL Kjeldahl.

## MATERIALS AND METHODS

*Combustion analysis.* The LECO apparatus has three phases in the analysis cycle: purge, burn and analyze. In the purge phase, the encapsulated sample is placed in the loading head, then sealed, and the apparatus is purged of any atmospheric gases that have entered during sample loading. The ballast volume and gas lines are also purged at this point. At the beginning of the burn phase, the sample is dropped into a hot furnace (850°C) and flushed with ultra-pure oxygen for rapid combustion. The products of combustion are passed through the thermoelectric cooler to remove most of the water, then collected in the ballast volume. The ballast volume has a free-floating piston, which moves up during collection of the gas products and is forced back down during gas removal. All the gas products in the ballast volume are allowed to become a homogenous mixture at a pressure of approximately 975 mm and a constant temperature. In the analyze phase, the piston is forced down, and a 10-cc aliquot of the sample mixture is collected. The sample aliquot is swept through hot copper and tungsten trioxide, to remove oxygen and sulfur and to change $NO_x$ to $N_2$, and then through carbosorb and magnesium perchlorate, to remove carbon dioxide and water. The remaining combustion product, $N_2$, is measured in a thermal conductivity cell. The instrument was calibrated daily with ethylenediaminetetraacetic acid (EDTA) as a nitrogen standard. The LECO conditions were based on those published by the Association of Official Analytical Chemists (AOAC) (7): Sample size (125 mg); furnace (850°C); flow profile (high); gases (oxygen 99.99%, helium 99.99%); calibration standard (EDTA); total analysis time, after calibration is completed (about 3 min/sample); cost estimate ($3 per sample). Cost estimates are based on a base of $58.00 per hour: $3 per sample, based on batch analyses (does not take into account setup and calibration costs).

*Kjeldahl determination.* The Kjeldahl conditions, based on Reference 1, were: sample size, 0.5 g; catalyst $CuSO_4/TiO_4$, 10.5 g; digestion time, 40 min; total analysis time, 4 h; cost estimate, $5 per sample. These conditions are the best compromise to the AOCS method (8) that can be achieved in the GRL protein laboratory.

*Samples.* Samples of oilseeds were obtained from the AOCS Smalley Check Sample Series, the Western Canadian Cooperative Test for Canola and harvest surveys for oilseeds carried out by the Canadian Grain Commission (2). Reference compounds were supplied by LECO.

## RESULTS AND DISCUSSION

The method as set up gave good recovery of nitrogen (%) by combustion from reference compounds in ten determinations, including nicotinic acid which gives low recovery from many Kjeldahl analyses, glycine and ammonium *p*-toluenesulfonate, respectively: theoretical value (4.74, 5.67, 7.41); average (4.72, 5.67, 7.42); standard deviation (0.04, 0.06, 0.03); coefficient of variation (0.88, 1.04, 0.46). Nicotinic acid, however, tended to produce heavy soot under the operating conditions used.

During initial studies, a grant from the Canola Council of Canada supplied a summer student with the objective of analyzing samples from the cooperative test trials of breeders lines. This project was carried out to provide information that would allow plant breeding establishments to assess this new method as a potential method for protein analysis in canola breeding programs.

The relationship between single combustion and Kjeldahl tests on samples for the 1990 cooperative test samples are shown in Tables 2 and 3. The summer student set up the instrument and carried out the analysis with minimal supervision, not by design but due to the unexpected absence of regular supervisors. The standard error for the 1990 analyses was relatively large but was reduced significantly when a trained technician analyzed the 1991 cooperative tests. During this study, it was noted that the combustion method gave higher results for nitrogen than the Kjeldahl method. In both years, the linearity between the two types of determination was consistent and close to 1.0 over a fairly large range of samples, suggesting that there was no interaction between the protein content and the difference in methods.

For canola seed, the difference was consistent for several types of samples (Table 4), and discounting the 1990 Coop samples because of the inexperience of the technician, an average increase of 0.119% nitrogen was noted.

We have also studied other oilseeds (Table 5) and have found that, with the possible exception of sunflower seed, the combustion method gave higher values than the Kjeldahl method for these also. As we officially adopted this method for all oilseed surveys, starting in August

TABLE 2

**Summary of Results from Paired Combustion and Kjeldahl Determinations of Nitrogen (%) in Samples from the 1990 and 1991 Western Canadian Cooperative Tests for Canola**

| | 1990 | | | 1991 | | |
|---|---|---|---|---|---|---|
| Statistic | Combustion | Kjeldahl | Difference | Combustion | Kjeldahl | Difference |
| Mean | 4.29 | 4.11 | 0.18 | 4.18 | 4.09 | 0.09 |
| Minimum | 3.62 | 3.37 | 0.25 | 3.64 | 3.66 | −0.02 |
| Maximum | 4.90 | 4.66 | 0.24 | 4.60 | 4.49 | 0.11 |
| Standard deviation | 0.28 | 0.27 | 0.01 | 0.21 | 0.16 | 0.05 |

TABLE 3

**Statistical Analysis of Results from Paired Combustion and Kjeldahl Determinations of Nitrogen (%) in Samples from the 1990 and 1991 Western Canadian Cooperative Tests for Canola[a]**

| Paired *t*-test | | | Simple linear regression analysis | Combustion (Y) vs. Kjeldahl (X) | | Difference (Y) vs. Kjeldahl (X) | |
|---|---|---|---|---|---|---|---|
| Differences | 1990 | 1991 | Regression | 1990 | 1991 | 1990 | 1991 |
| Mean | 0.18 | 0.11 | Constant | 0.22 | 0.12 | −0.08 | 0.12 |
| Standard deviation | 0.07 | 0.04 | Standard error of Y estimated | 0.07 | 0.05 | 0.07 | 0.04 |
| Number | 153 | 248 | R-squared | 0.931 | 0.938 | 0.053 | 0.000 |
| T (D = 0) | 31.16 | 41.12 | Number of observations | 152 | 248 | 152 | 248 |
| 95% C.I. | | | Degrees of freedom | 150 | 246 | 150 | 246 |
| Upper | 0.19 | 0.12 | X Coefficient(s) | 0.991 | 0.997 | 0.060 | −0.003 |
| Lower | 0.17 | 0.11 | Standard error of coefficient | 0.022 | 0.016 | 0.021 | 0.016 |
| | | | T (X coefficient = 0) | 3.674 | 3.900 | 0.236 | −0.236 |

[a]Student's *t*-value difference; C.I. = confidence internal; D = difference.

TABLE 4

**Summary of Results from Paired Combustion and Kjeldahl Analyses for Nitrogen in Canola Samples from Various Surveys**

| Source of samples | Year | Number of samples | Mean combustion nitrogen (%) | Mean Kjeldahl nitrogen (%) | Difference |
|---|---|---|---|---|---|
| Harvest survey | 1991 | 122 | 3.605 | 3.472 | 0.133 |
| Cooperative test | 1991 | 248 | 4.187 | 4.078 | 0.110 |
| Cooperative test | 1990 | 152 | 4.294 | 4.112 | 0.182 |
| Export shipments | 1991/1992 | 123 | 3.834 | 3.718 | 0.114 |

TABLE 5

**Summary of Results from Paired Combustion and Kjeldahl Analyses for Nitrogen in Samples from 1991 Harvest Surveys of Canadian Oilseeds**

| | Difference (%N) | | | | Number of |
|---|---|---|---|---|---|
| Seed type | Mean | Standard deviation | Minimum | Maximum | samples |
| Canola | 0.133 | 0.051 | −0.067 | 0.249 | 122 |
| Flaxseed | 0.155 | 0.061 | 0.000 | 0.322 | 114 |
| Sunflower | 0.056 | 0.066 | −0.122 | 0.232 | 44 |
| Soybean | 0.241 | 0.049 | 0.134 | 0.325 | 32 |
| Brown mustard | 0.128 | 0.041 | 0.028 | 0.190 | 44 |
| Oriental mustard | 0.148 | 0.037 | 0.090 | 0.264 | 31 |
| Yellow mustard | 0.151 | 0.074 | 0.001 | 0.452 | 50 |

1992, it was necessary to correct back data, either by re-analysis of archived composite samples or by application of a correction factor based on the above experiments.

For our first six months of routine operation, the coefficient of variation for nitrogen determination by the combustion method (± .03%) was only slightly higher than for the Kjeldahl method (± .024%) over the previous three years. Slightly higher errors in the combustion analysis may be due to the relatively small sample size (125 mg) used in our analysis. Because of the small sample size, it is especially important to obtain a fine grind of the sample. Oilseeds such as cottonseed and sunflower seed, which have fibrous hulls, may cause repeatability problems due to the difficulties in obtaining finely ground samples.

Poor performance in the Smalley Check Sample Series was the main reason for our adoption of the Dumas combustion methods for nitrogen. Re-analysis of the 1989–1991 samples (Table 6) showed results that were close but slightly higher than the Smalley mean for canola, flaxseed and oilseed meal but slightly lower than the Smalley mean for sunflower seed. The results were somewhat higher than the mean for soybeans. In a collaborative study, which compared nitrogen determined by combustion to nitrogen determined by Kjeldahl with mercury catalyst, Bicsak (9) reported results that ranged from 0.02%N lower to 0.04%N higher for combustion of canola; 0.05%N lower to 0.04%N higher for soybean; and 0.03%N lower to 0.01%N higher for sunflower seed. These results suggest that the combustion nitrogen determination method gives a more accurate determination of total nitrogen in oilseeds than the GRL Kjeldahl method. Higher results than the results found for the Smalley mean are not unexpected because many Smalley participants have not been able to use mercury catalyst.

TABLE 6

**Summary of Results for Samples from Smalley Check Sample Series Analyzed by Combustion Method[a]**

| Smalley Series | Year | Smalley mean nitrogen (%) | GRL Combustion–Smalley | | Combustion as percentage of Smalley |
|---|---|---|---|---|---|
| | | | Mean | Standard deviation | |
| Flaxseed | 1991/1992 | 3.91 | 0.07 | 0.03 | 101.8 |
| Oilseed meal | 1989/1992 | 7.55 | 0.12 | 0.10 | 101.6 |
| Rapeseed | 1989/1992 | 3.67 | 0.04 | 0.05 | 101.1 |
| Soybean | 1989/1992 | 6.55 | 0.19 | 0.13 | 102.9 |
| Sunflower | 1990/1992 | 3.19 | −0.07 | 0.01 | 97.8 |

[a]See Table 1 for abbreviation.

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