Technical News Feature

Nitrogen Analysis of Whole Seeds

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ABSTRACT

Whole seeds were digested in sulfuric acid with catalyst in conical beakers on a hot plate and the digest was analyzed for nitrogen. The nitrogen analysis of the digest can be performed by distillation-titration or colorimetrically, using manual or automatic procedures. Linear regression analyses of 8 samples of 16 soybeans each gave values that agreed within 2% of those obtained by the standard Kjeldahl procedure. Precision of the method is 1.8 relative standard deviation compared with 1.4 relative standard deviation for the standard method. The whole seed procedure gives results that are independent of seed size and has been applied to samples of both oilseeds and cereal grains.

INTRODUCTION

The need for large numbers of nitrogen analyses in plant breeding research is well recognized. The standard method for nitrogen analysis is the macro-Kjeldahl procedure, which is time-consuming and requires a considerable investment in specialized equipment. Numerous alternative or secondary methods have been employed including gas chromatography (1), infrared reflectance (2,3) and automated or semiautomated Kjeldahl (4-6) procedures. In some cases, these alternative procedures require expensive or single-purpose equipment or an extensive series of standards of the type of seeds being analyzed. In the standard Kjeldahl procedure for analysis of plant materials, most of the operator time is consumed in grinding, weighing and titration. Elimination or speeding up of these steps would greatly increase time efficiency.

We describe here a modified Kjeldahl procedure, which eliminates grinding, simplifies weighing and can employ normal titration or an automated colorimetric procedure. The digestion is performed on whole seeds, which eliminates the grinding step and makes subsequent weighing and sample handling much simpler. The ammonia in the digest can be determined by one of several manual or automatic colorimetric procedures or the traditional distillation-titration procedure may be employed. The choice of procedure will depend on equipment availability and on the number of samples to be analyzed.

EXPERIMENTAL PROCEDURE

Materials

Certified Beson soybeans #38-4484 were used in method development. Soybean samples used as protein standards were obtained from breeders throughout the United States. Cereal grain samples of seed-grade quality were obtained from a local seed store. All samples were sufficiently clean so that no cleaning was necessary.

All chemicals used were reagent grade or better. The catalyst consisted of 65 g powdered potassium sulfate and 5 g powdered red mercuric oxide mixed in a laboratory ball mill.

Digestion

Seeds were dried in a forced-air oven for 3 hr at 135 C. The cooled, dried seeds were accurately weighed into a 250-ml conical beaker (Corning #1080). Approximately 2.5 g catalyst and 50 ml concentrated sulfuric acid were added and the beaker was covered with a watch glass. Samples were then digested for 16 hr on a hot plate preheated to 400 C. Digestion of 4-g samples for less than 12 hr gave low and variable results and thus the 16-hr digestion time was chosen to insure complete digestion. After cooling, the samples were quantitatively transferred to 100-ml volumetric flasks and diluted with water to ca. 95 ml. Caution must be exercised during the transfer and dilution to avoid hot spots which can cause bumping. Swirling the flask during addition was found to be satisfactory. The flasks were cooled to room temperature and diluted to 100 ml. Aliquots of the sample were then used for ammonia determination.

Colorimetric Analysis

The nitrogen content of the diluted digests can be determined colorimetrically by either the alkaline phenol-hypochlorite (5) or the nitroprusside-salicylate (6) procedure. The alkaline phenol-hypochlorite procedure can be run either manually or automatically, but the dialysis step makes the nitroprusside-salicylate procedure more adaptable to an automated system. For our analysis we chose an automated alkaline phenol-hypochlorite procedure, as described by Uhl (4), except that the digestion was as previously described and a dilution manifold replaced the digestion manifold. For soybeans a dilution of 600-fold was necessary, but cereal samples required only a 200-fold dilution.

Distillation-titration Analysis

Diluted digests can also be analyzed for ammonia by running an aliquot through the normal steam distillation-titration procedure for Kjeldahl analysis. Samples for standard curves were analyzed by distillation-titration as well as by the colorimetric procedure. Our distillation apparatus was a micro-Kjeldahl type and 0.01N HCl was used for titration.

RESULTS AND DISCUSSION

Sample Size

To determine the optimal sample size, 15 1-seed, 15 4-seed and 15 16-seed samples of soybeans were analyzed. These samples were analyzed by both distillation-titration and colorimetric procedures with very good agreement. In Table I the distillation-titration results are compared with the average of standard Kjeldahl analyses of 5 differently ground 100-g soybean samples. Each ground sample was analyzed 5 times and the mean values agree very well. Statistically, we found that the average of 15 1-seed analyses was as good as one 16-seed analysis. The 16-seed samples averaged 3 g in weight and had a relative standard deviation (RSD) of 1.8 as compared to 1.4 for the large-ground samples. Statistical analysis also indicated that samples larger than 30 seeds would show little improvement

TABLE I

Sample Size for Whole Seeds

No. of seeds	Weight range (g)	Average weight/bean	Mean % nitrogen	Standard deviation	Relative standard deviation
1	0.109-0.259	0.202	6.01	0.491	8.2
4	0.709-0.958	0.191	5.97	0.214	3.6
16	2.838-3.345	0.192	5.97	0.106	1.8
524-540[a]	100	0.188	5.97	0.086	1.4

[a]Five different 100-g samples were prepared and these results are an average of the 5 samples each analyzed 5 times.

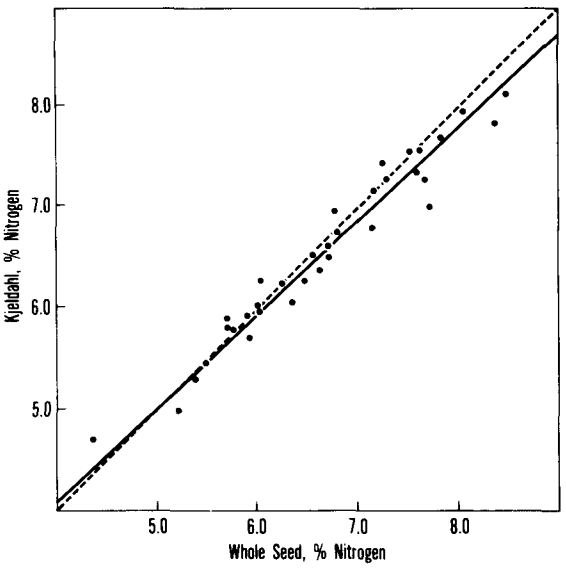

FIG. 1. Kjeldahl nitrogen vs whole seed nitrogen analyses of 34 soybean samples.

in precision. We concluded that a 4-g sample would be acceptable in subsequent analyses to get results comparable to standard Kjeldahl analyses.

Soybean Analysis

Thirty-four soybean samples with protein values ranging from 27 to 52% were analyzed for nitrogen by standard Kjeldahl and the whole seed method. The results of these analyses are plotted in Figure 1. Linear regression analyses of the data produced the (solid) line $y = 0.88X + 0.64$. Although this line compares with the theoretical (dotted) line, which shows that a deviation of as much as 2% can be expected between Kjeldahl and whole seed analysis, results from the whole seed procedure tended to give nitrogen percentages slightly higher than the Kjeldahl procedure. These analyses were run on 4-g samples and give further evidence that a 4-g whole seed sample can be analyzed for nitrogen by digestion without grinding.

Analysis of *Phaseolus vulgaris*

To determine the applicability of the method to other oilseed crops, 8 samples of *Phaseolus vulgaris* were analyzed. These results are shown in Table II. These samples represented 8 different varieties with considerable difference in seed size, as can be seen by the number of seeds necessary to make 4 g. Even with the smaller number of seeds, agreement between Kjeldahl and whole seed analyses is good.

Analysis of Cereal Grain Seeds

To determine the general applicability of the whole seed method, several different cereal grain samples were analyzed. The results of these analyses are shown in Table III. The nitrogen values for 4 varieties of foxtail millet are also reported in Table III. Again, good agreement was found between the whole seed and Kjeldahl procedures.

TABLE II

Analysis of *Phaseolus vulgaris*

Sample	Weight	No. of seeds	Whole seed % nitrogen	Kjeldahl[a] % nitrogen
1	4.003	127	3.95	4.13
2	4.076	88	4.22	4.48
3	4.017	20	3.34	3.58
4	4.272	12	3.34	3.40
5	4.091	11	3.79	3.82
6	4.100	24	4.22	4.16
7	4.146	12	3.14	3.34
8	4.222	14	2.93	3.02

[a]Average of duplicate analyses.

TABLE III

Analysis of Cereal Samples

Sample[a]	Whole seed nitrogen (%)	Kjeldahl[b] nitrogen (%)
Proso millet	2.06	2.04
Japanese millet	1.84	1.80
Wheat (hrw)	1.82	1.84
Corn	1.33	1.35
Rye	1.97	2.04
Foxtail millet #1	1.89	1.85
Foxtail millet #2	2.02	2.03
Foxtail millet #3	1.32	1.34
Foxtail millet #4	2.22	2.21

[a]4-g samples.
[b]Average of duplicate analyses.

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