Comparison of Kjeldahl and Dumas Methods for Determining Protein Contents of Soybean Products

S. Jung[a,b,*], D.A. Rickert[a], N.A. Deak[a], E.D. Aldin[a], J. Recknor[c], L.A. Johnson[a,b], and P.A. Murphy[a,b]

[a]Department of Food Science and Human Nutrition, [b]Center for Crops Utilization and Research, and [c]Department of Statistics, Iowa State University, Ames, Iowa 50011-1210

ABSTRACT: The Kjeldahl and Dumas methods for quantifying nitrogen content were compared using nine soybean products having protein contents ranging from 0.5 to 90%. In addition to comparing day-to-day variability of the Dumas method, differences between and variabilities of two Kjeldahl systems and Kjeldahl operators were also evaluated. The Kjeldahl method gave slightly, but significantly, lower values than did the Dumas method. Both the Kjeldahl and Dumas methods had equivalent variabilities (same SD about the means). The ratios between the means for the Kjeldahl and Dumas (K/D) protein values ranged from 0.66 to 1.03. The conversion equation $y = -0.00536 + 0.97188x$ ($R^2 = 0.9997$) was developed and validated to convert from Dumas to Kjeldahl protein concentrations.

Paper no. J10592 in *JAOCS 80,* 1169–1173 (December 2003).

KEY WORDS: Dumas nitrogen, Kjeldahl nitrogen, protein, soybeans, soy protein.

Consumer interest in soy protein products has increased rapidly in Western cultures in recent years. This trend is due in part to the high-quality protein of soy foods and soy protein ingredients and in part to their associated health benefits. In 1999, the U.S. Food and Drug Administration announced that manufacturers of foods containing soy protein could make the health claim that 25 g of soy protein per day may improve cardiovascular health (1). Consequently, precisely determining protein contents of soy products is very important.

The international reference method used to determine the protein contents of food and feeds is the Kjeldahl method (2). Both macro- and micro-Kjeldahl methods have been developed for different sample sizes. The basis of the Kjeldahl method is digestion of the sample with sulfuric acid in the presence of catalysts. Organic nitrogen is reduced to ammonium sulfate, which is distilled in the presence of sodium hydroxide, liberating ammonia gas. The distillate is collected into boric acid solution, and the borate anions formed are titrated with standardized hydrochloric acid solution. The milliequivalents of acid required for titration are used to calculate the nitrogen content in the sample (3). This more than 100-yr-old method has been modified over the years to be more convenient and is used in analytical laboratories as a routine method. However, this method has the disadvantages of using corrosive and/or toxic chemicals with consequent waste production and risk to human health, long analysis time, and multiple steps providing many opportunities for error.

The Dumas method (4), or nitrogen combustion method, is an attractive alternative to the widely used Kjeldahl assay. Numerous recent technical breakthroughs have improved the method's accuracy, and the adoption of several automated features has made the method easy to use. The basis of the Dumas method is the conversion of all nitrogen forms in the sample to nitrogen oxides through combustion at 800–1000°C, reduction of these forms to nitrogen gas (N_2), and subsequent measurement by use of a thermal conductivity detector. The Dumas method requires less than 5 min per sample, can be semiautomated, avoids the use of corrosive and hazardous chemicals, and is a relatively safe procedure.

Several studies have compared the effectiveness of the Dumas method with that of the Kjeldahl method for various food products including dairy products (5), oilseeds and cereal grains (6,7), meat and meat products (8), and vegetable leaves (9). In most cases, the Dumas method consistently gave higher nitrogen values than did the Kjeldahl method. Inorganic forms of nitrogen, such as nitrates and nitrites, are not measured by the Kjeldahl method if they are not adequately reduced during digestion. In contrast, all nitrogen sources, both organic and inorganic, are measured by the Dumas method (10). Watson and Galliher (11) emphasized that replacing the Kjeldahl method with the Dumas method for plant materials containing high concentrations of nitrogen associated with nitrates, such as in lettuce, potatoes, or tomatoes, must be done with caution. However, determinations of nitrogen concentrations of animal feed (e.g., meat meal, soy protein concentrate, and feather meal), infant formula, cereals (wheat, corn, and long-grain rice), and dairy products have been shown to be similar when measured by the Kjeldahl and Dumas methods (5,10).

The reported differences in nitrogen contents in foods as measured by the Kjeldahl and Dumas methods indicate a need to compare the techniques in analysis of soy protein and soy food samples, for which limited comparative data are available. Without accurate conversion between the two Kjeldahl methods and the Dumas method, it is not possible to compare results obtained by the two methods correctly. Thus, the purpose of this study was to compare the protein contents determined by the Dumas and the Kjeldahl methods for soy pro-

*To whom correspondence should be addressed at Department of Food Science and Human Nutrition, Iowa State University, 2312 Food Science Bldg., Ames, IA 50011-1061. E-mail: jung@iastate.edu

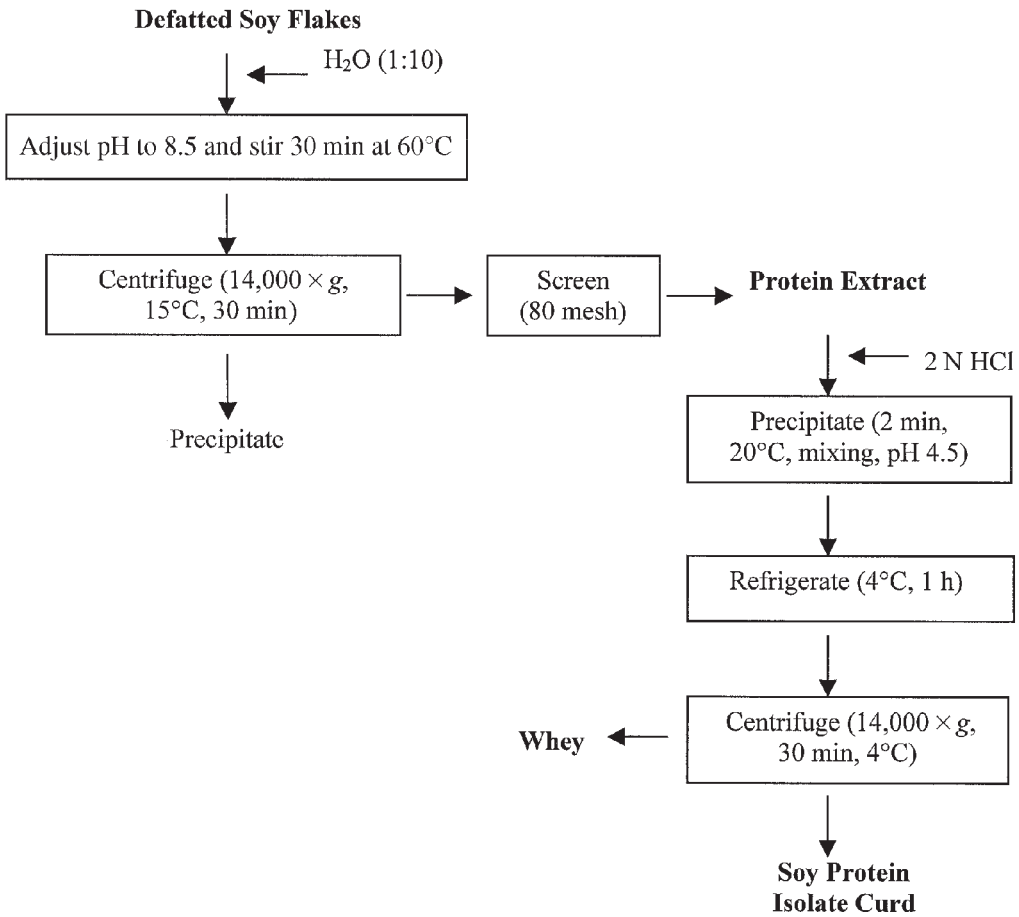
tein samples over a range of protein concentration from 0.5 to 90% and to develop and validate an accurate conversion procedure. A wide range of protein contents was selected so that the conversion method will apply to concentrations found in most soy products.

EXPERIMENTAL PROCEDURES

Soy products. Commercial soy products procured from Archer Daniels Midland Co. (Decatur, IL) were soy protein concentrates (Arcon®SM, Arcon®T, Arcon®F, Arcon®VF) and soy protein isolate (Profam®955). Another concentrate prepared in our crops-processing pilot-plant facility at Iowa State University (ISU) was also used for this study. The soybeans (variety 1274 RR) came from Golden Harvest (Fort Collins, CO). Defatted soy flakes were prepared from IA 2042 variety from the 2000 harvest at the oilseeds extraction facility of the Food Protein R&D Center of Texas A&M University (College Station, TX) by extracting flaked soybeans with hexanes. The soy flakes and soybeans were ground with a standard coffee grinder and passed through a 35-mesh screen. Tofu (Hinoichi, House Foods America, Garden Grove, CA) and soymilk (West Soymilk Drink, Melville, NY) were purchased from a local supermarket and stored at 4°C until used. Protein extract, whey, and soy protein isolate curd fractions were prepared on the day of analysis in our laboratory from 50 g of defatted soy flakes (Scheme 1).

Protein analysis. Protein content was calculated from the nitrogen content of the material, using a nitrogen conversion factor of 6.25. All samples were prepared and analyzed on the same day, except for the day 2 samples in the Dumas day-to-day variability study. The day 2 samples were weighed out on day 1, then refrigerated overnight at 4°C.

Kjeldahl methods. A modified version of the micro-Kjeldahl method was used (12). Samples of 0.25 to 1 g, depending on protein concentration, were accurately weighed out and then digested with 6 mL of concentrated sulfuric acid in the presence of a catalyst by using a Labconco System (Labconco, Kansas City, MO). The catalyst was a mixture of cupric selenite (0.2 g) and potassium sulfate (0.3 g). The macro-Kjeldahl procedure used was the Corn Refiners Association method A-18 (13) and employed the Tecator Kjeltec System (Tecator, Hoganas, Sweden). Samples of 1 to 5 g were weighed and then digested with 17 mL of concentrated sulfuric acid plus one catalyst tablet (Pro-Pac Tablets N. TT-57; Alfie Packers Inc., Omaha, NE) containing 5.57 g of K_2SO_4, 0.033 g of $CuSO_4$, and 0.2 g TiO_2. Both methods used 40% NaOH to produce an alkaline distillation environment and 4% boric acid solution to collect the distilled ammonia. The titrations were performed with standardized 0.1 N hydrochloric



SCHEME 1

acid (SA5410; Fisher Scientific, Pittsburgh, PA). Tashiro's indicator was used to identify the end point of the titration (0.375 g of methyl red and 0.250 g of methylene blue in 300 mL of 95% ethanol).

Dumas method. Samples were analyzed according to AOAC method 993.13 (14) by using a RapidN III from Elementar Americas, Inc. (Mt. Laurel, NJ). Dry samples were wrapped and tightly pelleted in tin foil, whereas liquid samples were packaged in tin capsules. Aspartic acid (A9, 310-0; Sigma-Aldrich, St. Louis, MO) was used as the nitrogen calibration standard. The system was calibrated daily before analysis by running the following sequence: two blanks, two run-in samples, and three aspartic acid standards. The blank was an empty tin foil, and the run-in and aspartic acid samples contained approximately 200 mg of aspartic acid. The run-in samples were used to determine whether the nitrogen values obtained were acceptable based on the known nitrogen content of the aspartic acid, and the results obtained from the three aspartic acid samples were used to calculate the daily conversion factor, based on the unit's international standard curve. Oxygen dosing for optimal combustion was selected based on sample type. Dosing for blanks was 50 mL of O_2/min, whereas dosing for all other samples was 150 mL of O_2/min. After 15 sample analyses, a run-in was analyzed to verify satisfactory system performance.

Statistical analysis. There were four replications of each sample. The data were evaluated by using a statistical package by SAS Institute, Inc. (15). This package was used to perform *t*-tests and ANOVA, and to fit the data to a linear model. Statistical significance was determined by P values <0.05.

RESULTS AND DISCUSSION

This study compared the protein contents of soybeans and soy products having a wide range of concentrations, 0.5 to 90%, which was much broader than those used in other comparisons found in the literature (6,8). The Kjeldahl nitrogen contents were determined by both macro- and micro-Kjeldahl methods to evaluate differences between these two systems. Additionally, the Dumas method was performed over two consecutive days to determine the day-to-day variability of this method.

Comparison between the micro- and macro-Kjeldahl methods. Micro- and macro-Kjeldahl methods, each performed by a different analyst, produced significantly different results when all data were analyzed together (Table 1). For specific sample comparisons between some products (soy protein isolate, soy protein concentrate, soy flakes, soy protein isolate curd, and soybeans), there were significant differences between the micro- and macro-Kjeldahl methods. In addition, there was a system–product interaction for nitrogen concentration. To determine whether the difference between the two Kjeldahl methods was due to the system used or to analyst technique during analysis or titration, one analyst measured the nitrogen content of aspartic acid standard using both systems. The mean nitrogen values for the aspartic acid standard were 10.51 ± 0.03 and 10.45 ± 0.05% for the macro- and micro-Kjeldahl method, respectively. No significant difference between the means was observed, indicating that the difference between the two sets of Kjeldahl values was probably related to an analyst effect.

TABLE 1
Protein Contents (% as is) Obtained by Micro- and Macro-Kjeldahl Determinations[a]

Soy product	Micro-Kjeldahl	Macro-Kjeldahl
Soy protein isolate	88.51 ± 0.25[a]	87.68 ± 0.43[b]
Soy protein concentrate	67.86 ± 0.27[a]	66.84 ± 0.27[b]
Defatted soy flakes	53.70 ± 0.22[a]	53.17 ± 0.30[b]
Soy protein isolate curd	34.55 ± 0.22[a]	33.51 ± 0.15[b]
Soybeans	33.24 ± 0.22[a]	32.52 ± 0.37[b]
Tofu	7.93 ± 0.02[a]	7.91 ± 0.04[a]
Protein extract	4.16 ± 0.02[a]	4.07 ± 0.04[a]
Soymilk	1.51 ± 0.03[a]	1.48 ± 0.02[a]
Whey	0.51 ± 0.01[a]	0.49 ± 0.00[a]

[a]Means are followed by SD. Values in the same row followed by different letters are significantly different ($P < 0.05$).

Determination of day-to-day variability with the Dumas method. When each product was considered individually, day-to-day variations were not significant (Table 2). However, when the entire data range was considered, 0.6 to 90% protein, there was a significant difference between days (Pr > *F*: 0.03). When whey results, which had a 50% variation between day 1 and day 2, were not included in the statistical analysis, day-to-day variation was no longer significant. The whey samples had the lowest protein contents. The manufacturer recommends calibrating the Rapid NIII with a solution of THAM [2-amino-2 (hydroxymethyl-1,3-propanediol)] at a nitrogen concentration close to the nitrogen content in the samples when quantifications of low nitrogen concentrations are to be performed. We did not attempt this standardization in our study since our objective was to analyze the entire range of samples using the same procedure.

Comparisons between the Kjeldahl and Dumas methods. Table 3 compares the mean protein contents as determined by the Kjeldahl and Dumas methods, including the two Kjeldahl systems and the two different days for the Dumas method. The SD about the means for the Dumas method were similar

TABLE 2
Protein Contents (% as is) Obtained by the Dumas Method on Different Days[a]

Soy product	Day 1	Day 2
Soy protein isolate	90.43 ± 0.26	90.23 ± 0.21
Soy protein concentrate	69.72 ± 0.15	69.81 ± 0.23
Defatted soy flakes	55.43 ± 0.15	55.03 ± 0.18
Soy protein isolate curd	33.94 ± 1.33	34.97 ± 0.76
Soybeans	33.96 ± 0.73	33.63 ± 0.26
Tofu	8.24 ± 0.22	8.47 ± 0.19
Protein extract	3.94 ± 0.14	4.05 ± 0.12
Soymilk	1.47 ± 0.10	1.63 ± 0.14
Whey	0.56 ± 0.04	0.97 ± 0.38

[a]Means are followed by SD. None of the values in the same row between day 1 and day 2 are significantly different ($P < 0.05$).

TABLE 3
Protein Contents (% as is) Obtained by the Dumas and Kjeldahl Methods[a]

Soy product	Mean Kjeldahl	Mean Dumas	Ratio K/N[b]
Soy protein isolate	88.09[a]	90.33[b]	0.975
Soy protein concentrate	67.35[a]	69.76[b]	0.965
Defatted soy flakes	53.43[a]	55.23[b]	0.967
Soy protein isolate curd	34.03[a]	34.45[b]	0.987
Soybeans	32.88[a]	33.79[b]	0.973
Tofu	7.92[a]	8.35[a]	0.948
Protein extract	4.11[a]	3.99[a]	1.030
Soymilk	1.49[a]	1.55[a]	0.961
Whey	0.50[a]	0.76[a]	0.657

[a]Means are followed by SD. Mean protein contents for the Kjeldahl and Dumas methods in the same row followed by different letters are significantly different ($P < 0.05$).
[b]The K/N value is the ratio between the mean Kjeldahl value and the mean Dumas value.

TABLE 4
Protein Contents (% as is) Obtained by the Dumas and Micro-Kjeldahl Methods for Five Soy Protein Concentrates[a]

Soy concentrate	Dumas	Micro-Kjeldahl	Corrected Dumas values[b]
ArconS	75.71 ± 0.25[a]	74.13 ± 0.42[b]	73.58
ArconVF	67.29 ± 0.19[a]	65.49 ± 0.46[b]	65.39
ArconT	70.08 ± 0.11[a]	68.14 ± 0.54[b]	68.10
ArconF	67.16 ± 0.04[a]	65.72 ± 0.48[b]	65.27
ISU[c]	63.12 ± 0.25[a]	61.71 ± 0.18[b]	61.34

[a]Means are followed by SD. Mean protein contents in the same row followed by different letters are significantly different ($P < 0.05$).
[b]The corrected Dumas values were obtained by converting the Dumas experimental values using Equation 1, $y = -0.00536 + 0.97188x$ (where x is the Dumas value and y is the Kjeldahl value).
[c]Concentrate produced in the crops-processing pilot-plant facility at Iowa State University (Ames, IA).

to those of the Kjeldahl method ($P = 0.48$ and 0.41 for the Kjeldahl and Dumas methods, respectively), which was consistent with results obtained with cereal grains, oilseeds, and meat and meat products (6,8). Even though values for the two methods were highly correlated ($R^2 = 0.9997$, Fig. 1), the Dumas values were significantly higher than the Kjeldahl values ($P < 0.05$). The ratios between the means of the Kjeldahl and Dumas methods (K/D) were approximately 0.95 (± 0.02) for nearly all samples, except for the protein extract (1.030) and the whey (0.657) samples.

Higher protein contents obtained with the Dumas method were reported for meat and meat products with a nonsignificant average difference of 0.16 between the two methods (15.59 and 15.75% for the Kjeldahl and Dumas methods, respectively) (8). Schmitter and Rihs (16) reported higher nitrogen percentages (6.44 vs. 6.54% and 6.79 to 6.99%, respectively) when comparing the Kjeldahl and Dumas methods for two different soybean meals. These values gave corresponding K/D ratios of 0.984 and 0.971, similar to what we observed in our study. Bicsak (7) observed an average difference of –0.05% protein between Kjeldahl and Dumas values for cereal grains and oilseeds. Greater differences between Kjeldahl and Dumas methods were reported for fish and fruit, leading to K/D ratios of 0.8 and 0.73, respectively (10). Discrepancies between the two methods may be related to the quantification of nonamino nitrogen by the Dumas method from nitrates, nitrites, nucleotides, or nucleic acids in products such as plant materials, preserved meat, or cheese. However, soybeans and soy products contain negligible amounts of these components, and these small quantities cannot explain the difference we observed between the Dumas and Kjeldahl results. Indeed, as observed by Wiles *et al.* (5), there cannot be a difference of more than 0.005% nitrogen between the Dumas and Kjeldahl methods if the NO_3^- and NO_2^- contents do not exceed 150 µg/g. Similarly, nitrates in manure and sewage sludge samples cannot completely explain the higher nitrogen concentrations as determined by the Dumas method compared to the Kjeldahl method (11). In addition, for vegetable leaves (such as cucumber, sweet corn, and tomato), 25% of the nitrogen of the samples was not quantified by the Kjeldahl method, but the nitrogen from NO_3^- could not account for the observed difference between the methods (9). Other factors must interfere and lead to this difference between Kjeldahl and Dumas values.

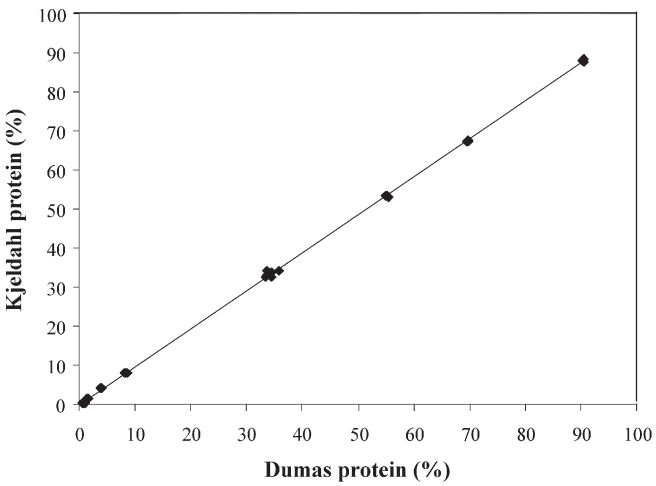


FIG. 1. Relationship between protein values (%) obtained by the Kjeldahl and Dumas methods ($y = -0.00536 + 0.97188x$, $R^2 = 0.9997$).

Predictive equations. Based on our data, the relationship between the Dumas and Kjeldahl values in the case of soy products can be described by Equation 1 for the range of 0.5 to 90% protein:

$$y = -0.00536 + 0.97188x \qquad (R^2 = 0.9997) \qquad [1]$$

where x represents the Dumas value and y represents the Kjeldahl value.

If the whey protein values are not used in the regression analysis (1.5 to 90% protein), the correlation equation becomes:

$$y = -0.0175 + 0.97103x \qquad (R^2 = 0.9999) \qquad [2]$$

Validation. To validate Equation 1, five soy protein con-

centrates were analyzed by the Dumas and micro-Kjeldahl methods. The mean protein contents obtained for each substrate by using the two methods are grouped in Table 4. Differences between Dumas means and micro-Kjeldahl means were between 1.4 and 1.9%. When Dumas values were converted to Kjeldahl values using Equation 1, the converted theoretical Kjeldahl values differed from the mean experimental Kjeldahl values by less than 0.6%. This result confirms that our equation accurately converts soy protein concentrate nitrogen values determined by the Dumas method to Kjeldahl values.

ACKNOWLEDGMENTS

This journal paper of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, Project No. 6599, was supported by Hatch Act and State of Iowa funds.

REFERENCES

1. U.S. Food and Drug Administration, Food Labeling Health Claims: Soy Protein and Coronary Heart Disease, Food and Drug Administration. Final Rule, *Fed. Regist. 64*:57700–57733 (1999).
2. Kjeldahl, J., Neue Methods zur Bestimmung des Stickstoffs in Organischen Korpern, *Z. Anal. Chem. 22*:366–382 (1883).
3. Chang, S.K.C., Protein Analysis, in *Food Analysis*, edited by S. Nielsen, Aspen Publishers, Gaithersburg, MD, 1998, pp. 237–250.
4. Dumas, J.B.A., Procedes de l'Analyse Organique, *Ann. Chim. Phys. 247*:198–213 (1831).
5. Wiles, P.G., I.K. Gray, and R.C. Kissling, Routine Analysis of Proteins by Kjeldahl and Dumas Methods: Review and Interlaboratory Study Using Dairy Products, *J. AOAC Int. 81*:620–632 (1998).
6. Daun, J.K., and D.R. DeClercq, Comparison of Combustion and Kjeldahl Methods for Determination of Nitrogen in Oilseeds, *J. Am. Oil Chem. Soc. 71*:1069–1072 (1994).
7. Bicsak, R.C., Comparison of Kjeldahl Method for Determination of Crude Protein in Cereal Grains and Oilseeds with Generic Combustion Method: Collaborative Study, *J. AOAC Int. 76*:780–786 (1993).
8. King-Brink, M., and J.G. Sebranek, Combustion Method for Determination of Crude Protein in Meat and Meat Products: Collaborative Study, *Ibid. 76*:787–793 (1993).
9. Simonne, E.H., C.E. Harris, and H.A. Mills, Does the Nitrate Fraction Account for Differences Between Dumas-N and Kjeldahl-N Values in Vegetable Leaves? *J. Plant Nutr. 21*: 2527–2534 (1998).
10. Simonne, A.H., E.H. Simonne, R.R. Eitenmiller, and C.P. Cresman, Could the Dumas Method Replace the Kjeldahl Digestion for Nitrogen and Crude Protein Determinations in Foods? *J. Sci. Food Agric. 73*:39–45 (1997).
11. Watson, M.E., and T.L. Galliher, Comparison of Dumas and Kjeldahl Methods with Automatic Analyzers on Agricultural Samples Under Routine Rapid Analysis Conditions, *Commun. Soil Sci. Plant Anal. 32*:2007–2019 (2001).
12. AOAC, *Official Methods of Analysis of Association of Official Analytical Chemists*, 15th edn., Arlington, VA, 1990, Method 960.52.
13. Corn Refiners Association, *Standard Analytical Methods of the Member Companies of Corn Refiners Association, Inc.*, CRA, Washington, DC, 1980, Method A-18.
14. AOAC, *Official Methods of Analysis of Association of Official Analytical Chemists*, 16th edn., Arlington, VA, 1995, Method 993.13.
15. SAS, *SAS User's Guide: Statistics, Version 5,* Statistical Analysis System Institute, Inc., Cary, NC, 1985.
16. Schmitter, B.M., and T. Rihs, Evaluation of a Macrocombustion Method for Total Nitrogen Determination in Feedstuffs, *J. Agric. Food Chem. 37*:992–994 (1989).

[Received March 17, 2003; accepted September 25, 2003]