Nitrogen Determination for Rapid Quality Control of Oilseed Meals[1]

R. M. McCREADY, G. FULLER and MONA GAUGER,
Western Regional Research Laboratory,[2] Albany, California 94710

Abstract

The classic Kjeldahl procedure for determing protein (nitrogen) in oilseed meals is satisfactory but too time consuming for quality control in plants handling large amounts of meal. This paper describes attempts to decrease the time required for nitrogen analysis of safflower seed and meal using equipment available in the quality control feeds laboratory. A dye-binding method on undigested proteins, digestion followed by colorimetric ammonium determination, digestion followed by distillation of ammonia, and several different digestion catalysts were tested. Digestion with the AOAC catalysts for 15 min and distillation of ammonia for 10–15 min for 90 ml of distillate followed by titration gave results on safflower meals in agreement with the AOAC method and is recommended for accelerated protein tests.

Introduction

Traditional methods of analysis often are time consuming and do not meet the needs of chemical control in continuous production processes. The need to analyze product streams and to apply timely corrective adjustments in continuous process lines is extremely important in plants handling large amounts of bulky materials such as feeds. For example, commercial vegetable oil plants producing by-product safflower meal for animal feed price their meal on the basis of its protein content. A guide method for the determination of protein on-line would permit high and low protein meals to be blended on the process line to a standard product.

Neutron activation analysis offers a means of completing a nitrogen analysis every 5 min with 15 min elapsed time for the first in the series (1,2). This procedure utilizes very sophisticated equipment not yet available to all segments of the feeds industry.

Gehrke et al. (3) automated protein analyses using the Technicon AutoAnalyzer where 20 determinations can be done each hour with 40 min elapsed time for the first in the series. This procedure was not considered here because of the difficulty in obtaining a small uniform sample from fairly coarse ground meal. In addition, the equipment is specialized and too sophisticated for most feeds control laboratories.

Rapid protein tests on wheat, corn and milk can be made using Orange-G dyestuff in Udy's colorimetric dye-binding method (4). The equipment for the tests is commercially available (Udy Analyzer Co., Boulder, Colo.). This procedure should be applicable to analysis of oilseed meals because it has been applied to high protein feedstuffs (5) and to dried herbages (6). Work is in progress here to apply the method to oilseed meals.

The method for protein in feeds generally used is to determine total nitrogen by the Kjeldahl procedure (7) and multiply the nitrogen value by a factor (usually 6.25 for oilseed meals) to calculate the protein content. Bradstreet reviewed the Kjeldahl method for organic nitrogen compounds (8). The method is convenient and does not require expensive equipment but may take as long as 3 hr to run.

Rapid methods for protein, applicable to oilseed meals were studied here in an attempt to decrease the time of nitrogen analyses utilizing equipment available in feeds control laboratories.

Udy's colorimetric dye-binding method using Orange-G showed promise but was not satisfactory under the conditions applied here. An accelerated Kjeldahl digestion and distillation with both colorimetric and titrimetric determination of ammonia were tested and the results of these experiments are reported.

Results and Discussion

Orange-G, a disulfonic acid dye, binds the free amino, imidazole and guanidyl groups of proteins to form insoluble complexes. The reaction has been applied to the quantitative determination of proteins in cereals, milk and other proteinaceous materials by measuring the concentration of the excess dye remaining in solution after contact with a solution of known amount.

Application of the procedure to protein in safflower meal was examined to determine optimum conditions. The reaction of dye-buffer solution with safflower protein was incomplete in 5 min and complete in 10 min. Fifty milliliters of 0.1% dye buffer solution

TABLE I

Effect of Various Digestion Catalysts and Time of Digestion on Nitrogen Recovery From Safflower Meal

Digestion procedure	Reference	Digestion time min	Nitrogen %	Relative recovery %
A. 0.7 g mercuric oxide, 15.0 g potassium sulfate, and 25 ml conc. sulfuric acid	7 (AOAC)	120	6.80	100
AA. As above	7	15	6.81	100.1
B. 0.25 g selenium, 10.0 g potassium sulfate, 20 ml conc. sulfuric acid	9	15	6.80	100
C. 0.07 g selenium, 0.07 g copper sulfate, 7.0 g potassium sulfate, and 20 ml conc. sulfuric acid	10	15	6.76	99.4
D. 20 ml of sulfuric acid-selenium oxide-perchloric acid (300 ml conc. sulfuric acid plus 33 ml water containing 3 g selenium oxide, cool, add 7 ml 72% perchloric acid)	11	15	5.92	87.0
E. 20 ml conc. sulfuric acid, heat to fuming, add 30% hydrogen peroxide dropwise to clear	12	15	6.86	100.8
F. 15 ml 30% hydrogen peroxide, 1 drop mercury, 12 ml of sulfuric acid-phosphorus pentoxide solution (200 g conc. sulfuric acid plus 100 g phosphorus pentoxide)	13	5	6.84	100.5
FF. As above	13	3	6.83	100.4

[1] Presented in part at the AOCS Meeting, Minneapolis, October 9, 1969.
[2] W. Utiliz. Res. Dev. Div., ARS, USDA.

TABLE II
Nitrogen Analyses of Safflower Seeds and Meal by the Official and Accelerated Methods

Sample	Nitrogen, %		
	Official	Accelerated	S.D.[a]
Meal			
1	6.80	6.80	0.04
2	3.67	3.57	.....
3	7.15	7.18	.....
4	2.67	2.70	.....
5	11.69	11.64	.....
6	3.80	3.80	.....
Seeds			
1	2.62	2.55	0.04
2	3.14	3.16	0.07
3	3.18	3.19	0.11

[a] Standard deviation, 10 replications.

provides ample excess for 0.5 g of crude safflower protein concentrate, the highest protein content which we examined (72%). One hundred milliliters of dye-buffer is more effective than 50 ml because blending is improved. Dye buffer solution used here was at pH 2.2, but no variation in binding occurred between pH 1.8 and 2.5. Nonionic surfactants did not affect the dye-binding reaction but aided wetting of samples by dye-buffer during blending. This was particularly important for samples of high oil content such as whole seeds.

Mixtures of low-fiber high-protein (LF-HP) and high-fiber low-protein (HF-LP) safflower meals were made and analyzed for nitrogen by the AOAC Kjeldahl method (7). Half gram samples of these meals, analyzed by the dye-binding method as described, did not show a linear relationship between dye-binding values and nitrogen contents determined by the Kjeldahl procedure.

When sample weights were adjusted to give different amounts of nitrogen per test with LF-HP and with HF-LP meals, the HF-LP gave a nonlinear erratic curve and the LF-HP gave a curve with a break and different slopes of the two linear portions.

Our results still show promise for developing a quick dye-binding method, but work is in progress to establish conditions whereby response to the dye between safflower meals of differing fiber contents is consistently and directly proportioned to nitrogen.

Experiments were conducted to modify the classic Kjeldahl digestion method on safflower meals by decreasing digestion and distillation times while maintaining accuracy. The trials were made using several digestion catalysts followed by distillation of the ammonia for about 30 min in the usual manner.

Table I summarizes the results of these tests. The catalyst in A is that recommended in the AOAC (7) and in AA the same but the digestion time was shortened to 15 min. In these experiments, all of the digestion catalysts gave good results except D, with which the digestion time of 15 min was insufficient. Any of the other digestion catalysts could be used in an accelerated analysis for protein, however the AOAC catalyst was selected because it is available in all feeds laboratories. This catalyst was used for further tests on decreasing the time of distillation of ammonia.

A number of experiments were conducted to reduce this distillation time. In the AOAC procedure 150 ml of water is added to the cooled concentrated sulfuric acid digest and 125 ml distillate collected over a period of about 30 min. In 10 min when less than 90 ml of distillate is collected, the yields of ammonia are near 100% but somewhat erratic. In 15 min 90 ml of distillate can easily be collected with results consistent and near 100% recovery of ammonia, as compared with the 30 min distillation followed by titration in the usual manner.

The colorimetric phenol hypochlorite method for ammonium of Berthelot (14) was tested for the determination of ammonia in the distillates. Agreement with the titration method was good, but due to the extreme sensitivity, multiple quantitative time-consuming dilutions were necessary. The method had no time-saving advantages here as compared with the conventional titration procedure.

The accelerated AOAC method using a digestion time of 15 min, a distillation time of 10–15 min for 90 ml of distillate followed by titration was selected as the preferred procedure. Analyses of selected commercial safflower meals and seeds are shown in Table II. These results show that the accelerated procedure as proposed is satisfactory for analyzing safflower meal and seeds and all analyses are in excellent agreement with one another.

The operations and times for various operations of the accelerated method proposed are summarized in Table III.

The minimum elapsed time for the first sample is about 25 min, but many samples can be run each hour provided that sufficient digestion and distillation equipment is available.

TABLE III
Operations Using the Proposed Accelerated AOAC Method

Operations in sequence		Operator's time, min	Elapsed time, min
Weigh	1.0 g oilseed meal	1	1
Digest	with 25 ml sulfuric acid, 0.7 g mercuric oxide, and 15 g potassium sulfate	2	15
Cool	.....	0	5
Add	125 ml ice water, 50 ml sodium hydroxide, 5 ml sodium thiosulfate	1	1
Distill	90 ml	1	10–15
Titrate	.....	2	2
Minimum time		7	~34

REFERENCES

1. Wood, D. E., P. L. Jessen and R. E. Jones, Cereal Sci. Today *12*, 115 (1967). Abstr., 52nd Annual Meeting of Cereal Chemists, Los Angeles, California, April 1967.
2. Doty, W. H., D. E. Wood and E. L. Schneider, J. Ass. Offic. Anal. Chem. *52*, 953 (1969).
3. Gehrke, C. W., F. E. Kaiser and J. P. Ussary, Ibid. *51*, 200 (1968).
4. Udy, D. C., Cereal Chem. *33*, 190 (1956).
5. Bunyan, J., J. Sci. Food Agr. *10*, 425 (1959).
6. Outen, G. E., J. M. A. Tilley and R. F. Wilson, Ibid. *17*, 285 (1966).
7. Horwitz, W., Editor, "Official Methods of Analysis," 10th Edition, Association of Official Agricultural Chemists, Washington, D.C., 1965, 2.043(a) and 2.044.
8. Bradstreet, R. B., Anal. Chem. *26*, 185 (1954).
9. Varley, J. A., Analyst *91*, 119 (1966).
10. Kent-Jones, D. W., and A. J. Amos, "Modern Cereal Chemistry," 5th Edition, Northern Publishing Co., Liverpool, England, 1957, p. 584.
11. Todd, J. R., and J. H. Byars, "Automation in Analytical Chemistry," 1966, Vol. 1, Medial, Inc., White Plains, New York, 1967, p. 140.
12. Jacobs, M. B., "Chemical Analysis of Foods and Food Products," D. Van Nostrand Co., New York, 1938, p. 24.
13. Kuehl, H., and P. G. Gottschalk, Cereal Chem. *6*, 512 (1929).
14. Berthelot, M. P., Repertoire de Chimie Appliqué *284* (1859).

[Received January 19, 1970]