Rapid Biuret Method for Protein Content in Grains

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ABSTRACT

A rapid, simple, and inexpensive method for determining protein content in small grains and corn is proposed. Powdered cupric carbonate is added to the finely ground grain which is then dispersed in a dilute alkali-alcohol solution. The mixture is shaken, filtered, and the absorbance value read at 550 nm. The color intensity of the filtrate is proportional to the protein concentration. Meter readings of 391 samples of various grains representing 3 crop years were correlated with Kjeldahl protein content. Correlation coefficients were highly significant for all grains tested. Total time per sample is about 5 min. when run sequentially. Reagent costs are about 5 cents per sample. With the exception of the special mill, only standard laboratory equipment is used in the test.

Since the publication of the classical method for determination of nitrogen by Kjeldahl in 1883 (1), this method, which utilizes sulfuric acid and a catalyst for digestion and subsequent distillation of the nitrogen as ammonia, has been considered one of the most dependable tests available for determining the protein content of grain. The Kjeldahl test was apparently first used by a flour mill in 1900, as reported by Whitcomb and Bell (2). However, it suffers from several disadvantages, being costly, complicated, and time-consuming.

Several other tests have been developed for measuring protein content in grain which involve reactions of protein with reagents to produce colored complexes. These include reactions with peptide linkage (biuret, 3–20); with free alpha-amino groups (Van Slyke method, 21); and with phenol (Folin-Ciocalteau, 22). Reactions of proteins with organic dyes have been used to estimate protein content of cereal grains (Udy dye-binding, 23–28).

Solubility or peptization of protein and resulting opalescence under controlled pH has been proposed as a quantitative method for measuring protein content in wheat (29,30). Pyrolysis of protein-containing material with volumetric measurement of the evolved N_2 gas has also been utilized as a measure of protein content (31).

None of the above-mentioned methods are modifications of the Kjeldahl method; instead, they employ different principles and are usually calibrated against this basic method. However, none have been used alternatively with the Kjeldahl test, possibly because they are not as precise and are not applicable to all grains.

In recent years, grain dealers and feedmen have encountered a wide variation in protein content in grain sorghums and other grains (32). There is a need for one method to measure quickly and accurately the protein content of all grains. The test should be simple enough to be operated by a technician with a minimum of training and require a nominal capital expenditure.

In the search for a protein test which would be applicable to all grains, attention was directed to the biuret reaction which has long been used as a quantitative test

for protein content in biological materials. The biuret reaction yields a violet coloration when a protein complex reacts with an alkaline copper solution.

In 1949, Pinckney (9) developed the biuret test as an inexpensive and rapid estimation of protein in wheat and flour. Although this method offered promise as a means of determining protein content in wheat at the local elevator level, it was not adopted by elevator operators. Pinckney's method was not satisfactory for grain sorghum or dark-colored barley varieties because the extraneous brown color that is extracted in the alkaline solution absorbs strongly at the wave length of maximum absorbance of the copper-protein chelate (14). We found that this interfering color could be eliminated when the combination of cupric carbonate and alcohol was used.

This paper describes a rapid method of determining protein content in grain using powdered cupric carbonate as a source of copper in the biuret reaction.

MATERIALS AND METHODS

Reagents

1. Alkaline-alcohol solution. In a 1,000-ml. volumetric flask place 5.61 g. potassium hydroxide pellets. Add 600 ml. isopropyl alcohol. Make up to volume with distilled H_2O.
2. Isopropyl alcohol.
3. Cupric carbonate (reagent grade).

Apparatus

1. Mill (Udy cyclone-hammer mill[1] with 0.024-in. screen or equivalent).
2. Balance (torsion or equivalent).
3. Shaker (wrist action or equivalent).
4. Vacuum filter assembly (No. 3 Gooch crucible with 2.1-cm. glass fiber filters).
5. Colorimeter (Leitz Photrometer[1] or equivalent).
6. 250-ml. Erlenmeyer flasks with No. 6 stoppers.
7. Automatic pipet, 50 ml.
8. 50-ml. test tubes.

Procedure

1. Grind a representative sample of about 50 g. in Udy mill.
2. Weigh 1.00±0.01 g. well-mixed, finely ground grain into 250-ml. Erlenmeyer flask.
3. Add 2 ml. of isopropyl alcohol and swirl.
4. Add 1.0±0.1 g. of powdered cupric carbonate.
5. Pipet 50 ml. of alkaline-alcohol solution into flask; stopper.
6. Shake vigorously on shaker for 15 min.
7. Let stand 15 min. to develop biuret color.
8. Prepare Gooch crucible for filtering by placing two glass fiber filters in crucible.

[1]Mention of specific instruments or tradenames is made for identification purposes only and does not imply any endorsement by the U.S. Government.

9. Shake by hand to mix flask contents. Turn on vacuum. Filter about 20 ml. and collect clear filtrate[2]. Turn off vacuum as soon as sample is collected as the filtrate may become concentrated through evaporation.

10. Read absorbance of the filtrate at 550 nm. and refer to standard curve for protein content. Use 10 X 10-mm. cell for all grains except wheat and flour, which require a 5 X 10-mm. cell, in which case the duplicate values are added together.

DISCUSSION AND CONCLUSIONS

Macro-Kjeldahl protein values were used as standards and were determined by the Kjeldahl-Gunning-Arnold method of the AOAC (33). Percent nitrogen was converted to protein content by the factor 6.25 or, for wheat and flour, 5.7.

A total of 391 analytical comparisons was made to establish the regression lines between protein content determined by the Kjeldahl and by the rapid method. The grains tested represented a range in total protein from 7.0 to above 18.0%. Moisture content of the samples tested ranged from 7.2 to 10.9%. Protein comparisons were made on an "as-is" moisture basis. Included were grain sorghum, corn, oats, barley, HRS, durum, HRW, SRW, white wheat, and wheat flour (hard and soft) representing 3 crop years. All whole grain samples were ground in the Udy mill so that approximately 75% of the sample passed through a 70-mesh sieve.

Regression lines showing the relation between biuret absorbance values and Kjeldahl protein values for each grain are presented in Fig. 1. Plots of the points for each commodity are: grain sorghum (Fig. 2), corn (Fig. 3), oats (Fig. 4), barley (Fig. 5), wheat (Fig. 6), and wheat flour (Fig. 7). The values for grain sorghum, corn, oats, and barley represent averages of duplicate determinations. The duplicate absorbance values for wheat and wheat flour were added together because a half-size cell (5 X 10 mm.) was used, as the color density becomes too great to read accurately in the 10 X 10-mm. cell.

A statistical summary is presented in Table I which shows the number of tests for each grain, the coefficient of correlation, and the standard error of estimate.

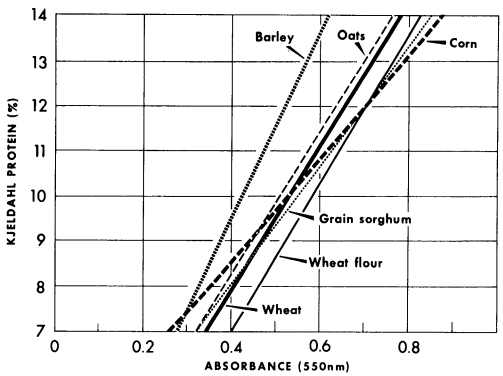


Fig. 1. Relation between biuret absorbance values and Kjeldahl protein for specified grains.

[2]If the filtrate is not clear, refilter.

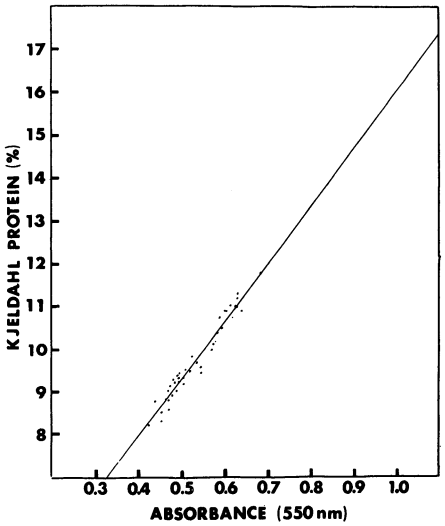


Fig. 2 (left). Relation between biuret absorbance values and Kjeldahl protein for grain sorghum. Y = 13.36X + 2.64 (r = 0.98**).

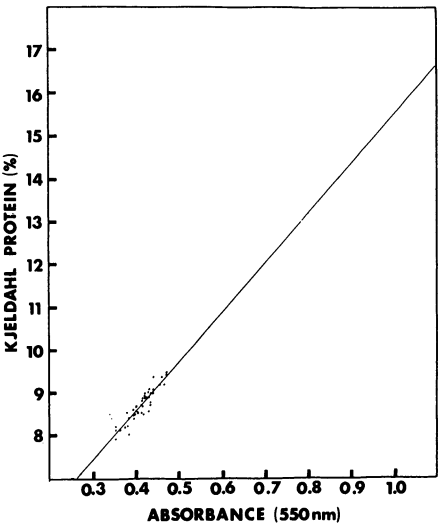


Fig. 3 (right). Relation between biuret absorbance values and Kjeldahl protein for corn. Y = 11.486X + 4.0 (r = 0.95**).

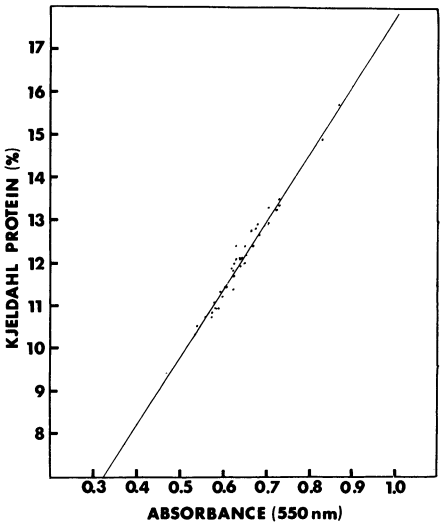


Fig. 4 (left). Relation between biuret absorbance values and Kjeldahl protein for oats. Y = 15.93X + 1.84 (r = 0.99**).

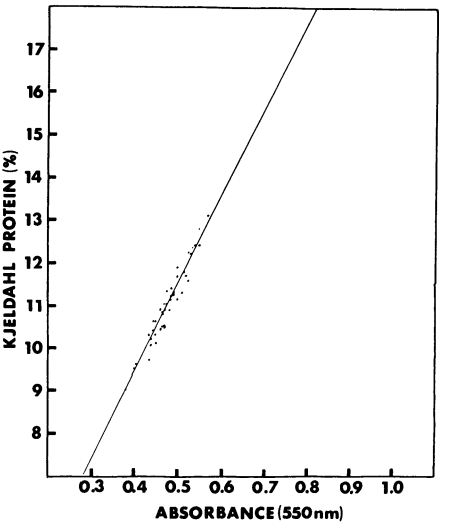
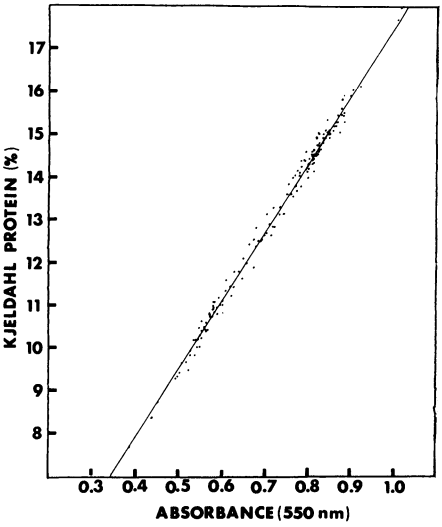


Fig. 5 (right). Relation between biuret absorbance values and Kjeldahl protein for barley. Y = 20.39X + 1.33 (r = 0.97**).



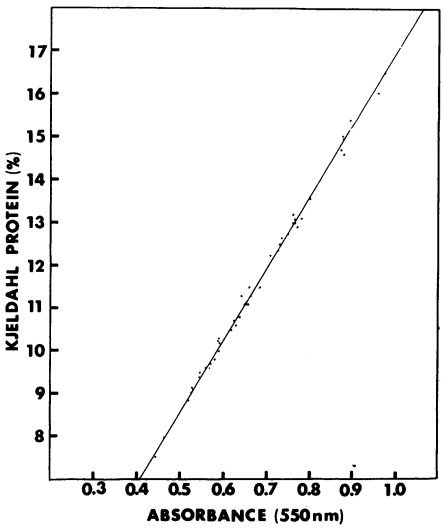


Fig. 6 (left). Relation between biuret absorbance values and Kjeldahl protein for all classes of wheat. Y = 16.07X + 1.47 (r = 0.99).**

Fig. 7 (right). Relation between biuret absorbance values and Kjeldahl protein for hard and soft wheat flour. Y = 16.68X − 0.26 (r = 0.99).**

Like the Kjeldahl, the rapid method can be used for determining the protein content of grains other than wheat. No hazardous chemicals are used in the rapid method. Time per sample is about 5 min. when samples are run sequentially, using six units at a time. Reagent costs for the rapid method are approximately 5 cents per sample for expendable materials as compared with about 10 to 15 cents per sample for the Kjeldahl method. Furthermore, no outlay for expensive equipment is necessary provided laboratory items such as a suitable mill, balance, shaker, glassware, chemicals, and colorimeter are available.

Advantages of the rapid method over the biuret method include: 1) use of stable powdered cupric carbonate rather than the unstable biuret reagent, 2) use of alkaline-alcohol solution to eliminate extraneous color formation, 3) elimination of centrifugation, and 4) applicability to all grains.

TABLE I. CORRELATION OF COEFFICIENT AND STANDARD ERROR OF ESTIMATE FOR THE RELATION BETWEEN BIURET ABSORBANCE VALUES AND KJELDAHL PROTEIN FOR SPECIFIED GRAINS

Sample	No. of Samples	Correlation of Coefficient	Standard Error of Estimate (% protein)
Grain sorghum	47	0.98	±0.19
Corn	48	0.95	±0.14
Oats	40	0.99	±0.17
Barley	44	0.97	±0.23
Wheat (all classes)	165	0.99	±0.18
Wheat flour (hard and soft)	47	0.99	±0.14

The average standard error of estimate for predicting protein content from meter readings for all grains tested was 0.18% protein. The standard deviation between duplicates for the rapid method is about 0.1%. This is comparable with the error reported in the literature of 0.11% wheat protein for the accepted Kjeldahl method (34).

As uniformity in results between colorimeters has not been established, each laboratory should construct a separate standard curve and conversion chart for each instrument. In establishing a standard curve for the analysis, select 35 or more samples having a good distribution over the normal protein range. After meter readings for the rapid method and Kjeldahl protein contents have been obtained, a regression equation for the standard curve can be calculated.

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