

A Room-Temperature, Rapid Method for the Determination of Protein in Wheat and Other Grains by the Biuret Reaction¹

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ABSTRACT

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A rapid, room-temperature, biuret method for determining protein in grains was developed. Samples of wheat ground to pass a 0.105-mm screen were mixed for 5 min in a blender with 2 ml of isopropanol, 700 mg of basic cupric carbonate (bright green), and 100 ml of 0.500M NaOH in 30% isopropanol. After filtering with suction through a glass fiber

filter, absorbance was measured at 550 nm. A calibration curve for wheat of absorbance vs. protein concentration gave a slope of $0.350 \text{ cm}^{-1}\text{g}^{-1}\text{L}$, compared to 0.310 for the official AACC method using heat. Satisfactory results were also obtained with rice and soybeans.

The original purpose of a rapid method for determining protein in wheat and other grains was to provide a quick and accurate result for grain and feed dealers, using a technique that could be performed by a technician with nominal capital expenditure (Johnson and Craney 1971). In response to this need, six methods were published in the period from 1971 to 1975. The procedure advocated in two articles, which required heating, was given first approval as an official method of the American Association of Cereal Chemists in October 1974 (AACC 1983, Method 46-15). To test the method, 10 wheat samples were submitted to members of the AACC Subcommittee on Quality Tests for Wheat and Flour in 1974 (AACC 1974). In the minutes of the same subcommittee in 1975, the analyses were reported to have given a correlation coefficient of 0.98 and a standard error of 0.30% (AACC 1975).

The adjective "rapid" was first applied to protein determination in grains by Johnson and Craney in 1971. Their method was applied by Misra and co-workers to the analysis of corn (Misra et al 1973). The reaction period was 30 min, as was that of Theis-Maimone (1975). Noll et al (1974) used 10-30 min. Deacon (1972) and Craney (1972) shortened the period to 4 min by their procedures, while the method of Greenaway and Johnson (1974) and Greenaway and Davis (1975) took 5 min. Their procedure became the official method.

Studies of the biuret reaction and of the official method in the Food Analysis Laboratory of the State University of Campinas indicated that it might be possible to devise a fast, room-temperature method that would be more convenient, more sensitive, and at least as accurate.

Description of Rapid Methods

Most procedures follow a similar pattern; the specific differences are listed in Table I. The grain is ground in a mill to a specified particle size. Samples are weighed into a stoppered Erlenmeyer flask. Two milliliters of isopropanol (IP) is added to prevent lumping of the sample when the alkaline reagent is added. The latter is a solution of KOH or NaOH in a mixed solvent of IP and water. The copper reagent, basic cupric carbonate (BCC), or 25% w/v $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, is added, and the mixture is agitated by the method indicated in the table, with or without heating, for a specified period. The mixture is then filtered with suction through a glass-fiber filter, and the absorbance of the filtrate is measured against that of a suitable blank at 550 nm by means of a spectrophotometer or filter photometer. A series of samples covering a range of protein contents is analyzed by both the rapid method and the Kjeldahl method, and a calibrating regression equation is prepared for standardizing subsequent analyses. The procedure of Noll et al (1974) was unique in using a biuret reagent

solution containing 0.150M KOH and CuSO_4 , solubilized with NaK tartrate. They added no IP; instead they suspended the weighed, powdered samples in 1 ml of a solution of acetic acid, urea, and cetyltrimethylammonium bromide.

Noll et al (1974) were the only authors to investigate the effect of particle size. They ground samples in a Wiley mill to pass 40-, 60-, and 80-mesh screens (0.42, 0.250, and 0.177 mm, respectively). The highest absorbance was obtained with the 80-mesh sieve.

The concentration of 0.085M KOH in Table I requires explanation. Four of the articles listed, plus that of Greenaway and Davis (1975) and the official method, used 60% IP solution containing 5.61 g of KOH per liter. This is exactly one 10th of the molar weight of KOH. However, in the official method and the article by Greenaway and Davis (1975), the concentration of KOH is given as 0.085N! A check of catalogs regarding chemically pure KOH revealed that a purity of 85% is standard in the industry.

Calculation of Sensitivities and Accuracy

The success of a rapid method of protein determination can be assessed by: 1) the time required for the analysis, 2) the accuracy, and 3) the sensitivity obtained. The sensitivity of the method determines the magnitude of the instrument reading for a given concentration of protein.

The analytical division of IUPAC (1978) recommends that the sensitivity of an analytical determination be defined as the slope of the calibration curve relating the concentration (or quantity) of the substance being determined to the observed response. In Beer's law, the linear relationship between concentration and absorbance indicates that absorptivity (a , the slope) is the sensitivity of a spectrophotometric method (Strong 1952).

This conclusion requires converting the data or the regression equations given in the rapid-methods articles to the form $A = abc + k$, where A is absorbance, b is cell thickness, and k is the ordinate intercept. Concentration (c) was calculated by dividing the protein values by the total volume: reagent plus 2 ml of IP (generally) plus 1 ml of CuSO_4 (if used). Except for Misra et al (1973), all articles provided regression equations and data that could be used to calculate sensitivities ($\text{cm}^{-1}\text{g}^{-1}\text{L}^{-1}$) (Table II). These can be interpreted as absorbances of solutions with 1 g of protein per liter (or milligrams per milliliter) in a 1-cm cell. Accuracy is calculated from regression data by the value of the correlation coefficient, r and/or by the standard error of estimate.

Comparison of Sensitivities and Accuracies

Although the grains used by Deacon (1972) and by Greenaway and Johnson (1974) differed (soybeans and wheat, respectively), the procedures were similar; however, the sensitivity obtained by Deacon (0.356) was considerably higher than that of Greenaway and Johnson (0.310). Deacon heated to a higher temperature (70 vs. 60°C) but attained it in 2 min, versus 3 min for Greenaway and Johnson, and in each case gave two additional minutes of

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mixing without heating. Both used magnetic stirring. The principal difference was the base reagent, Greenaway and Johnson using 0.085M KOH in 60% IP and Deacon 0.500M NaOH in 30% IP. This suggests that the latter is a better reagent because the sensitivity is greater and the reaction therefore more complete. Consistent with this conclusion is the fact that clinical chemists have standardized a procedure for the determination of serum protein that uses an NaOH concentration of 0.600M (Doumas et al 1981).

The first rapid method (Johnson and Craney 1971) was done at room temperature and gave a fairly high sensitivity (0.323) after the sample was shaken for 15 min and let stand another 15 min. Craney (1972) continued using room temperature, substituting violent mixing in a blender for shaking and shortening the period drastically to 4 min, by which she obtained a lower, but reasonable, sensitivity of 0.289. Subsequent workers resorted to raising the temperature to increase sensitivity and keep reaction time short. However, it does appear that room temperature reaction with a reagent more like that of Deacon might give reasonable sensitivity in a short time.

The fact that the sensitivity obtained by Johnson and Craney (1971) for corn (0.453) is considerably higher than that found by Misra et al (1973) (0.407) with the same procedure makes the figures questionable. Their value for *r* is relatively low (0.95). The problem may have been increased absorbance due to the corn starch. Mitsuda and Mitsunaga (1974) found that solubilized starch reacts with the biuret reagent to give an absorbing product. Of the 15 sets of analyses, 13 had *r* values of 0.987–1.000, indicating that the methods are capable of good accuracy.

Research Plan

The objective of the present study was to devise a simple, rapid, room-temperature method with high sensitivity that would indicate a substantially complete reaction. Analysis of the differences in the methods and the resulting sensitivities, as discussed above, suggested that the following be investigated: 1) a higher concentration of base than 0.085M, 2) a lower concentration of IP than 60%, and 3) the high-velocity mixing technique of Craney (1972) by use of a blender.

BCC should be chosen as a cupric reagent over CuSO₄ and solubilized CuSO₄ for two reasons: 1) precipitation of Cu²⁺ as Cu(OH)₂ lowers the base concentration, and 2) solubilized CuSO₄ gives an excess of reagent that varies with the amount of protein in the sample, which could interfere with the measurement.

Specific steps would be as follows: 1) prepare a wheat sample and analyze it by the Kjeldahl method, 2) analyze the sample by the official method to provide results for comparison, 3) investigate the variables listed above, plus any others that might arise, and 4) test grains other than wheat.

MATERIALS AND METHODS

Samples

Wheat was variety IAC 24, obtained from the Winter Wheat and Cereals Section of the Instituto Agronômico de Campinas (IAC); the local name of the variety was Tukurui. Rice was a commercial product, purchased in a local market. Soybeans and corn (both dried) were commercial products, purchased in a local market.

TABLE I
Summary of Published Rapid Biuret Methods for Determination of Protein in Grains

Authors	Grain	Biuret Reagent				Reaction Conditions			
		Cupric Reagent	Base (moles)	Percent Isopropanol	Base (ml)	Mixing	Temperature (°C)	Minutes	
Johnson and Craney (1971)	Various ^a	BCC ^b (1 g)	0.085	60	50	Shaker	Room	30	
Craney (1972)	Wheat ^c	BCC ^b (1 g)	0.085	60	100	Blender	Room	4	
Deacon (1972)	Soybeans ^d	CuSO ₄	0.500	30	100	Magnetic	70 ^e	4 ^e	
Misra et al (1973)	Corn	Used procedure of Johnson and Craney (1971)							
Greenaway and Johnson (1974) ^f	Wheat ^c	Used reagents of Johnson and Craney (1971)				
Noll et al (1974)	Various ^h	CUSO ₄ ⁱ	0.150 ⁱ			Magnetic	60 ^g	5 ^g	
Theis-Maimone (1975)	Rice ^c	Used reagents of Deacon (1972)				Vortex	40	10–30 ^j	
						Shaker	40	30	

^a Ground in mill with 0.024-in. (0.61-mm) screen "so that approximately 75% of the sample passed through a 70-mesh (0.210-mm) sieve"; 1-g sample.

^b Basic cupric carbonate.

^c Ground in mill with 0.024-in. (0.61-mm) screen; 1-g sample.

^d Ground to a fine powder; 200-mg sample.

^e Reaching 70° in 2 min with stirring; stirred 2 min more unheated.

^f Became official AACC method 46-15.

^g Reaching 60° in 3 min with stirring; stirred 2 min more unheated.

^h Ground in mill with 80-mesh screen; samples with 2–15 mg of protein in 1.0 ml of AUC (0.01M acetic acid, 3M urea, and 0.01M cetyltrimethylammonium bromide).

ⁱ 10 ml of 0.0048M CuSO₄, solubilized in 0.150M KOH and 50% isopropanol with 0.0089M NaK tartrate.

^j Incubated 10–30 min at 40° after stirring with vortex mixer.

TABLE II
Sensitivities (cm⁻¹g⁻¹L) for All Samples Analyzed by Rapid Methods

Samples	Johnson and Craney (1971)	Misra et al (1973)	Craney (1972)	Deacon (1972)	Greenaway and Johnson (1974)	Noll et al (1974)	Theis-Maimone (1975)
Wheat flour	0.312					0.216	
Wheat	0.323		0.289		0.310	0.311	
Corn	0.453	0.407					
Sorghum	0.389						
Oats	0.320						
Barley	0.255						
Rice							0.299
Soybeans				0.356			
Gluten						0.316	

Reagents and Equipment

The BCC was either from Vetec (Rocha, Rio de Janeiro, Brazil) or Riedel-de Haën AG (Seelze-Hanover, Germany). The latter was green and C.P. grade. KOH was 0.085*M*, in 60% IP. It was prepared initially, as described in the official method and other publications, from 5.61 g of 85% KOH (Labsynth, Diadema, SP, Brazil) per liter of 60% IP (Merck, Hohenbrun, Germany), then standardized and adjusted to 0.085*M*. NaOH (Labsynth) was prepared in various concentrations (0.085, 0.200, 0.350, 0.500, and 0.750*M*) in 30% IP. It was also prepared at one concentration (0.500*M*) in 25, 35, 40, and 50% IP.

A filter photometer (Zeiss ELKO, Oberkochen, Germany) using filter 555, with maximum transmittance at 550 nm and half bandwidth of 25 nm, was used.

Grain Preparation

Grinding was done with a hammer mill (Tigre, São Paulo, SP, Brazil, type CV 2), initially using a 100-mesh (0.147-mm) screen and later a 150-mesh (0.105-mm) screen. After grinding, the samples were stored in closed glass containers at 4°C. The preground soybean sample was defatted by Soxhlet extraction with petroleum ether, then reground to pass a 150-mesh screen.

Total Nitrogen Determination by Kjeldahl

A rapid Kjeldahl digestion with hydrogen peroxide, developed in the Food Analysis Laboratory of the State University of Campinas, was applied to the wheat samples. It was related to the work of Hach et al (1987) but used simpler apparatus. Wheat samples weighing 100–200 ± 0.1 mg were weighed into 100-ml Kjeldahl flasks. With the operator wearing safety glasses, 5 ml of concentrated H₂SO₄ was added, and heat was applied in a Kjeldahl digestion apparatus with vapor removal until the wheat was well charred and the H₂SO₄ began to boil. The mixture was then cooled to room temperature to avoid the possibility of violent reaction, and 3 ml of 30% hydrogen peroxide (Merck) was added. The flask was replaced in the digestion apparatus and the contents boiled gently. If the solution did not become clear and colorless in 30 min, it was again cooled to room temperature, 3 ml more of H₂O₂ was added, and the solution was gently boiled. This was generally sufficient for clarification. Excess H₂O₂ was destroyed by the boiling. (The total time for digestion was 30 min to 1 hr.) Still wearing safety glasses, the operator proceeded with the remainder of the official AOAC (1984) micro-Kjeldahl method for total nitrogen. The other grains were analyzed completely by the AOAC micro-Kjeldahl method.

Moisture Determination

Ground samples of 2–3 g ± 1 mg were weighed in aluminum dishes and dried to constant weight at 130°C.

Rapid Protein Procedure

The following were added to a 250-ml flask: a weighed quantity of sample (1.000 g unless stated otherwise), 2.00 ml of IP, a weighed amount of BCC (initially 500 mg), 100.0 ml of alcoholic base solution, and, when stirring magnetically, a magnetic stirring bar.

For magnetic mixing, the solution was stirred for the specified time on the mixer unit (Corning PC-351), with or without heating. When mixing by shaking, the flask containing sample and reagent was clamped by the neck in the wrist-action shaker and the stopper was fixed in place with a rubber strap. The flask was adjusted to an angle that gave good mixing and the shaker was turned on for the specified period. For ultrasonic mixing, the base of the flask containing the sample and reagent was immersed in water contained in the ultrasonic generator, which was switched on for the desired time. When the blender was used for mixing, the measured quantities of sample and reagents were added to the blender jar and mixed for the specified period at the chosen speed.

Reaction mixtures were filtered with suction through a 7-cm glass fiber filter (Whatman 934-AH) in a Büchner funnel. If not completely clear, the filtrate was passed through the same filter a second time, which was usually sufficient. Although the literature

says that the same filter can be reused for economy, the filter was always changed in this research.

All absorption measurements were made on the Zeiss ELKO filter photometer with 1-cm cells. The reference for measurements in this instrument was the alcoholic base solution. Any absorption by dissolved free cupric ion in the sample would be constant at a given base concentration and unimportant in relative measurements. For the determination of regression equations, absorption by dissolved free cupric ion would be included in the constant term.

Study of Analytical Variables

In all of the series of tests, the criterion for improvement was an increase in the absorbance of the extract. Tests were done first on wheat.

Base and solvent composition. Samples were analyzed 1) by the official method of Greenaway and Johnson (Table I), 2) using KOH in 30% IP at 60°, and 3) using this reagent at room temperature. Since some experimenters in Table I used NaOH with satisfactory results, NaOH was compared with KOH. Samples were analyzed using 0.085*M* KOH or 0.085*M* NaOH in 30% IP at room temperature. All subsequent experiments used NaOH as base.

Experiments on IP concentration were continued at room temperature. Since two researchers used 0.500*M* base and since we concluded that 0.085*M* is too low a concentration of base for adequate reaction, a concentration of 0.500*M* base was arbitrarily chosen. Solutions in 25, 30, 35, 40, and 50% IP were prepared and tested. Next, the arbitrary choice of 0.500*M* NaOH needed to be justified. A series of NaOH concentrations was prepared: 0.085, 0.200, 0.350, 0.500, and 0.750*M* in 30% IP.

Mixing. In the official method, mixing is by magnetic stirring. All room temperature experiments described so far used this method of mixing for 5 min. The next test was to see whether this period was sufficient or excessive with the standardized reagent, 0.500*M* NaOH in 30% IP. Stirring periods of 1–15 min were tested. Mixing with a wrist-action shaker (TECNAL, Piracicaba, SP, Brazil) was also tested for periods of 1–10 min.

The mixing apparatus used by Craney (1972), a blender (model LIR-C, Cia. ARNO, São Paulo, SP, Brazil), was tested next. Since mixing periods of 5 min were satisfactory by other techniques and since it is not desirable to operate a blender continuously for more than 5 min, this period was used and the speed was varied. Use of ultrasonic mixing (Bransonic-2, Branson Ultrasonic Corp., Danbury, CT, 470-ml chamber) for 5 min was also tested.

BCC. The fact that different batches of BCC can give different results, requiring recalibration of the analytical curve, was stated by Craney (1972), Greenaway and Johnson (1974), and Greenaway and Davis (1975). Work in our laboratory showed that commercial BCC is generally a hydrated form of the mineral malachite, which is green. The BCC used so far in the experiments was produced by the Vetec Company and was gray-green. A BCC produced by Riedel-de Haën was found to be bright green, more like the color of natural malachite. Analyses were made using the BCC of Riedel-de Haën in subsequent analyses.

Up to this point, 500 mg of BCC had been used in all experiments, but it was decided that this variable should also be tested. Quantities of 500, 600, 650, 700, 750, and 800 mg were added to the reaction mixture.

Sensitivity Curve and Sample Particle Size

To establish the sensitivity curve for protein in wheat, samples weighing 0.200, 0.400, 0.600, 0.800, 1.000, and 1.200 g were analyzed. In addition to these samples, which were ground to pass a 100-mesh (0.149-mm) screen, the wheat was reground to pass a 150-mesh (0.105-mm) screen to test the effect of sample particle size. Samples of the same weights as above were used. The weight of BCC added in each case was 700 mg.

Other Grains

The optimized procedure was applied to rice and soybean sam-

ples, ground to 150 mesh (0.105 mm). To establish the sensitivity curve for rice, the same weights were used as in the case of wheat. Because of the higher protein content of soybeans, smaller samples were used: 50, 100, 200, and 300 mg.

Effect of Heating

The objective of this research was to determine whether changes in mixing, concentration of base, concentration of isopropanol, and other variables would make heating unnecessary. However, with a room-temperature method successfully developed, we decided that the effect of also applying heat would be interesting to investigate.

Temperature and time needed to be chosen. Noll et al (1974) used 40°C for 10–30 min, and Theis-Maimone (1975) used 40°C for 30 min. Except for Craney (1972), who used room temperature, the other authors used 1–3 min at higher temperatures (60 and 70°C). Theis-Maimone (1975) showed that at 50°C and above, results were erratic, so conditions of 40°C for 30 min were chosen.

After reacting the sample and reagent with 700 mg of BCC for 5 min, a portion was removed for filtering and measuring. The remainder was then stirred magnetically in a bath maintained at 40°C for 30 min. After another portion was removed for filtering, some samples were again heated and stirred at 40°C for additional 15- or 30-min periods.

RESULTS AND DISCUSSION

Analysis of Grain Samples

Results are given in Table III.

Study of Analytical Variables

Base and solvent composition. Table IV-A shows that decreasing IP concentration from 60 to 30% gave an expected increase in absorbance when the sample was reacted at 60°C. Although applying the new, 30% IP reagent to a room temperature procedure gave an expected, lower absorbance, its magnitude was still reasonable. Substitution of NaOH for KOH was not only satisfactory, but resulted in a 5% increase in absorbance.

Table IV-B shows two important results. One is that raising the NaOH concentration to 0.500M gave an absorbance at room temperature (0.382) that approached that of the official method at 60°C (0.389). The other is that 30% was confirmed as the best concentration of IP. There seems to be some unique value to 30% isopropanol in the base solution for stabilizing the formation of cupric complexes. Márques-Salomón (1976) showed that cupric complexes of sugars have more tendency to form in 0.100 and 0.500M NaOH when the solvent is 30% IP than when 28 or 32%. Table IV-C confirms the choice of 0.500M NaOH.

Mixing. Data in Table IV-D show that 5 min of magnetic stirring, using 30% IP reagent at room temperature, is optimum. In the first experiment with an alternative method of mixing (a wrist-action shaker), 5 min also appeared to be optimum (Table IV-E). Absorbance when using a blender for mixing was higher when it was operated at its maximum speed (Table IV-F).

The absorbance obtained by ultrasonic mixing was slightly less than with a shaker (Table IV-G). Magnetic stirring gave a significantly higher absorbance, but blending gave the highest absorbance and was adopted thereafter as the preferred method of mixing.

TABLE III
Preparation and Analyses of Grain Samples

Grain	Wheat	Rice	Soybeans
Screen, mesh	100	150	150
Moisture, %	12.00	ND ^a	ND
Kjedahl N, %	2.52 ± 0.02	1.134 ± 0.011	8.14 ± 0.13
Number of determinations	6	2	2
Protein, %	14.36 ± 0.12	6.75 ± 0.06	50.9 ± 0.8
Factor for N	5.70	5.95	6.25

^a Not determined

BCC. Use of the bright green product of Riedel-de Haën gave an increase of about 10% in absorbance (Table IV-H). Increasing the amount to 700 mg increased absorbance another 12% (Table IV-I).

TABLE IV
Effects of Experimental Variables on Absorbance and Standard Deviation^a

Variables	Absorbance ± SD	
A. IP^b concentration and temperature (based on official method^c)		
Temperature (max., °C)	IP (%)	Replicates
60	60	7
60	30	5
RT ^d	30	3
B. Concentration of IP in 0.500M NaOH (RT; magnetic mixing, 5 min)		
IP, %		
25		0.351 ± 0.006
30		0.382 ± 0.003
35		0.342 ± 0.007
40		0.280 ± 0.025
50		0.049 ± 0.009
C. Concentration of NaOH in 30% IP (RT; magnetic mixing, 5 min)		
NaOH, M		
0.085		0.305 ± 0.008
0.200		0.335 ± 0.013
0.350		0.320 ± 0.013
0.500		0.382 ± 0.003
0.750		0.338 ± 0.009
D. Period of magnetic mixing (0.500M NaOH in 30% IP; RT)		
Minutes		
1.0		0.318 ± 0.015
2.5		0.301 ± 0.009
5.0		0.382 ± 0.003
7.5		0.378 ± 0.017
10.0		0.367 ± 0.008
E. Time of mixing with wrist-action shaker (0.500M NaOH in 30% IP; RT)		
Minutes		
1.0		0.337 ± 0.011
2.5		0.337 ± 0.026
5.0		0.360 ± 0.019
7.5		0.352 ± 0.006
10.0		0.361 ± 0.004
F. Speed of blender for 5 min (0.500M NaOH in 30% IP; RT)		
Speed		
Minimum		0.424 ± 0.019
Maximum		0.452 ± 0.022
G. Mixing techniques, 5 min (0.500M NaOH in 30% IP; RT)		
Shaker		0.360 ± 0.019
Ultrasonic		0.357 ± 0.016
Magnetic		0.382 ± 0.003
Blender		0.452 ± 0.022
H. Sources of BCC, 500 mg (0.500M NaOH in 30% IP; mixing in blender, 5 min at maximum speed; RT)		
Manufacturer	Color	
Vetec	Gray-green	0.452 ± 0.022
Riedel-de Haën	Bright green	0.495 ± 0.018
I. Quantity of BCC (Riedel-de Haën; 0.500M NaOH in 30% IP; mixing in blender, 5 min at maximum speed; RT)		
BCC, mg		
500		0.495 ± 0.018
600		0.518 ± 0.014
650		0.553 ± 0.013
700		0.601 ± 0.007
750		0.555 ± 0.003
800		0.549 ± 0.028 ^f

^a Three replicates for each test, unless otherwise indicated.

^b Isopropanol.

^c Method of Greenaway and Johnson (1974), which became AACC Method 46-15.

^d Room temperature.

^e Basic cupric carbonate.

^f Five replicates.

Test of Procedure

Calibration curve for wheat and effect of sample particle size. The first set of samples, with larger particle size, gave points that were not linear (Fig. 1). Absorbances for the second set ground to pass a 150-mesh (0.105-mm) screen lay on a good straight line. After conversion to concentration (milligrams of protein per milliliter), least squares give the slope, intercept, coefficient of correlation (r), and standard error of estimate shown in Table V. The sensitivity for protein in wheat by the new

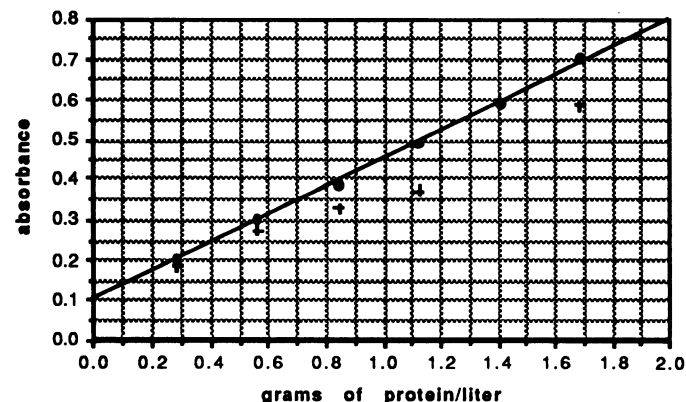


Fig. 1. Calibration curve for wheat. + = 100-mesh (0.147-mm) screens, • = 150-mesh (0.105-mm) screens.

TABLE V
Data and Regression Coefficients for Determining Protein in Wheat, Rice, and Soybeans

Mass ^a g	Wheat		Rice ^c		Soybeans	
	Protein (mg/ml)	A ^b	Protein (mg/ml)	A	Protein (mg/ml)	A
0.2	0.282	0.200	0.132	0.074		
0.4	0.564	0.303	0.265	0.132		
0.6	0.845	0.387	0.397	0.172		
0.8	1.127	0.487	0.529	0.222		
1.0	1.408	0.587	0.662	0.251		
1.2	1.690	0.700	0.794	0.313		
0.05					0.288	0.286
0.1					0.576	0.326
0.2					1.153	0.538
0.3					1.729	0.730
Sensitivity ^d	0.350		0.346		0.320	
k ^e	0.0986		0.0338		0.1703	
r	0.9992		0.996		0.994	
SEE ^f	0.007		0.008		0.022	

^a Weighed to ± 0.1 mg.

^b Absorbance.

^c Masses equal for wheat and rice.

^d For protein determination, in $\text{cm}^{-1}\text{g}^{-1}\text{L}$.

^e Ordinate intercept.

^f Standard error of estimate, in milligrams of protein per milliliter.

procedure is thus established as $0.350 \text{ cm}^{-1}\text{g}^{-1}\text{L}$, compared with 0.310 for the official method of Greenaway and Johnson, which used heat (Table II).

Since the testing of variables for the analytical procedure was done with wheat ground to pass a 100-mesh screen, the occasional, relatively large standard deviations can be understood. The need for grinding the sample finer had not yet been discovered.

Other grains. The absorbances for increasing weights of rice and soybeans yielded the sensitivities, coefficients of correlation, and standard errors of estimate shown in Table V. The procedure was not successful with corn; the solutions were turbid and could not be clarified.

Effect of temperature. Absorbances for extracts of the three grains at the end of the new procedure and at the end of subsequent periods of heating at 40°C are given in Table VI. After studying the results, averages were calculated for absorbance ratios for the 5-min procedure and after 30 min of heating. The average values for wheat and rice, 98 and 96%, respectively, can be considered quite satisfactory, with soybeans at 87% somewhat less so.

Application of the method—Determining a regression equation. The reader will have noted that the sensitivity of the method for a particular grain was determined by the use of a series of weights of the same sample. The traditional procedure determines a "regression equation" or "regression curve" by analyzing a constant weight of a large number of samples of varying protein contents, determined by Kjeldahl. This remains to be done by anyone applying the method and is essential because of possible differences in equipment and reagents.

CONCLUSIONS

A new, room temperature, rapid method for the determination of protein in grains is proposed. To a blender, add 1.000 g of wheat, 1.500 g of rice, or 200.0 mg of defatted soybeans, ground to pass a 150-mesh (0.105-mm) sieve (adjust the sample weight to keep absorbance between 0.3 and 0.7); 2.0 ml of IP; 700 mg of BCC (Riedel-de Haën or equivalent); and 100.0 ml of 0.500M NaOH in 30% IP. Blend for 5 min at maximum speed. Filter with suction through a 7-cm glass fiber filter (Whatman 934-AH or equivalent). Measure the absorbance of the filtrate in a 1.000-cm cell at 550 nm against NaOH reagent or reagent blank (NaOH plus 2.0 ml of IP plus BCC, blended for 5 min and filtered). Calibrate using standards analyzed for total nitrogen by Kjeldahl.

The method is simpler than published methods requiring programmed heating. The sensitivity is greater than that of other rapid methods for wheat and rice. Subsequent heating of the reaction mixture at 40°C for 30 min showed that the absorbances for the room-temperature method were 98% (wheat), 96% (rice), and 87% (soybeans) of the absorbances after heating.

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TABLE VI
Absorbance (A) After 5-min, Room Temperature Reaction and After Subsequent Heating at 40°C for 30, 45, and 60 min

Conditions	Grain								
	Wheat			Rice			Soybeans		
	R1 ^a	R2	R3 ^b	R1 ^b	R2 ^b	R3 ^b	R1	R2	R3
Room temperature, 5 min	0.635	0.650	0.695	0.515	0.520	0.536	0.605	0.620	0.645
Subsequent heating at 40°C									
30 min	0.650	0.660	0.720	0.535	0.525	0.580	0.705	0.710	0.730
45 min	0.650	0.660
60 min	0.685	0.520	0.525	0.540
Ratio ($A_5:A_{30}$)	0.977	0.985	0.965	0.963	0.990	0.924	0.858	0.873	0.883
Average ratio		0.98			0.96			0.87	

^a R = replicate.

^b New grain sample.

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