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DETERMINATION OF THE NITROGEN CONTENT OF CEREAL GRAIN BY COLORIMETRIC METHODS[1]

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ABSTRACT

Two modifications of colorimetric methods previously published are presented for the determination of cereal nitrogen content. In method 1, the nitrogen content of white-grained varieties of wheat and barley is determined by a modification of the biuret method (Pinckney). In this method, extraction of the protein and color development occur simultaneously in an alkaline copper tartrate solution. The use of potassium sodium tartrate in place of glycerol in the biuret reagent results in a 13% increase in sensitivity in this method. Those varieties of barley with blue-or black-colored aleurone layers may be conveniently analyzed by method 2 which involves treatment of the extract prepared by the biuret extraction procedure with Folin-Ciocalteu phenol reagent (Lowry *et al.*). The results from both methods are as reproducible as those obtained with the Kjeldahl method of nitrogen determination. (Pinckney: *Cereal Chem.* **26:** 423; 1949. Lowry *et al.*: *J. Biol. Chem.* **193:** 265; 1951.)

The percentage of the total nitrogen content actually extracted into solution in the extraction procedure common to both methods increases as the total nitrogen content increases. Plotting the extracted nitrogen instead of the total nitrogen against the colorimeter reading increases the degree of correlation to only a small extent. The amount of brown-colored material extracted by alkaline solutions from barley and wheat is much greater in the case of barley. The variation in the amount of this extracted brown material is great enough in the case of barley to lessen the degree of correlation between Kjeldahl nitrogen content and the color produced in method 1. The accuracy of method 2 is not affected by this extraneous brown color.

The main factor causing imperfect correlation between Kjeldahl nitrogen content and the color produced in both methods is the variation in the absorbance coefficients of the extracted proteins.

Since, in this laboratory, a large number of determinations of total nitrogen content of both barley and wheat are required each year for plant-breeding purposes, it was decided to adopt colorimetric methods of analysis. Two methods are currently used and both methods are described in this paper.

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Method 1 is the modification by Pinckney (as reported by Halloran and Moss, 3) of his original method (7), but using a modified extracting solution developed in this laboratory. This method is used for the analysis of red- and white-grained varieties of wheat and white-grained varieties of barley.

Method 2 is an adaptation of the method of Lowry *et al.* (4) and is used for analysis of those varieties of barley which have black or blue hulls, pericarps, aleurone layers, or endosperm outer layers. This method may also be suitable for the analysis of dark-grained varieties of wheats.

A study was made of some factors which could affect the reliability and accuracy of both methods.

Suggested Methods, and Materials

Extraction Procedure Common to Both Methods

It has been found convenient to use the same reagents, apparatus, and procedures in both methods until the clarified extract has been obtained.

Reagent. The modified biuret reagent used in the common extraction procedure is prepared as follows: 15 ml. of a 10*N* potassium hydroxide solution and 2.5 g. of potassium sodium tartrate (A.R.) are dissolved in approximately 900 ml. of distilled water; 30 ml. of a 4% copper sulfate pentahydrate solution are then added slowly with continual mixing. Finally, the volume is made to 1 liter.

Procedure. Portions of the samples, 500 ± 5 mg., are weighed in a suitably shaped aluminum or plastic scoop on a rapid-weighing balance and then transferred to dry, wide-necked extraction bottles fitted with push-in plastic stoppers. The weighed portions are completely wetted with 2 ml. of carbon tetrachloride. Then 50 ml. of the modified biuret reagent are added and the stoppered bottle shaken on a mechanical shaker for 60 minutes. Portions of the extracts are centrifuged for 10 to 15 minutes at 3,100 R.C.F. (4,500 r.p.m. in an M.S.E. Super Minor centrifuge[2]; Measuring and Scientific Equipment Ltd., 14/28 Spenser St., London S.W.1).

Method 1[3]

Procedure. The clarified extract obtained in the common extraction procedure is read in a suitable colorimeter at 550 mμ (EEL colorimeter, filter O.G.R.I. or 625; Evans Electroselenium Ltd.,

[2]Mention of proprietary or trade names is not to be construed as an endorsement over other products of a similar nature, but is made for information purposes only.

[3]Refer to Pinckney (3,7).

Harlow, Essex, England) between 105 and 150 minutes after the commencement of shaking.

Method 2[4]

Reagents. Reagent A, 2% sodium carbonate in 0.1*N* sodium hydroxide solution.

Reagent B, 0.5% copper sulfate pentahydrate in 1% potassium sodium tartrate solution. The addition of a few drops of dilute sodium hydroxide solution is necessary to obtain a clear solution.

Reagent C (alkaline copper solution). Mix 50 ml. of reagent A with 1 ml. of reagent B. Discard after 1 day.

Reagent D (diluted Folin-Ciocalteu reagent). Dilute the Folin-Ciocalteu reagent (1) to make it 1*N* in acid. Folin-Ciocalteu reagent (1) can be obtained commercially, or it may be prepared as follows: reflux gently for 10 hours in a 1.5-liter flask a mixture consisting of 100 g. sodium tungstate dihydrate, 25 g. sodium molybdate dihydrate, 700 ml. water, 50 ml. 85% phosphoric acid, and 100 ml. concentrated hydrochloric acid. Add 150 g. lithium sulfate, 50 ml. water, and a few drops of bromine water. Boil the mixture for 15 minutes without the condenser to remove excess bromine. Cool, dilute to 1 liter, and filter. The reagent should have no greenish tint. (Determine the acid concentration of the reagent by titration with 1*N* sodium hydroxide to a phenolphthalein end point.)

Procedure. Transfer, using a Lang-Levy automatic zero micropipet, 0.100 ml. of the centrifuged extracts obtained by the extraction procedure to test tubes, 6 in. by 5/8 in. Add 10 ml. of reagent C and mix. After at least 15 minutes, add 1 ml. of reagent D with immediate and vigorous mixing. Allow at least 30 minutes for full color development before reading in a suitable colorimeter at 760 mμ (EEL colorimeter, filter O.R.I. or 608).

Calculation of Results. A suitable number and range of samples, the nitrogen content of which has previously been determined by a Kjeldahl method on a moisture-free basis, are treated by the appropriate method above. The colorimeter readings are plotted against the corresponding percentage total nitrogen contents on a moisture-free basis and a line of best fit drawn by inspection or alternatively from a numerical analysis of the data. The relation between nitrogen content and absorbance is linear in both methods. The unknown samples are then read off the line, the results being reported as percentage total nitrogen on moisture-free basis. Also, as pointed out (7), the value for nitrogen actually extracted may be substituted

[4] Refer to Lowry *et al.* (3).

for percentage total nitrogen. The reproducibility of both procedures has been found to compare favorably with that of the Kjeldahl method of nitrogen determination, confirming claims made previously (3,7) for the biuret method.

Table I shows the number of samples considered, the range of percentage nitrogen contents (on moisture-free basis) involved, the correlation coefficient, the error[5] of the determination of the nitrogen content by the appropriate colorimetric method assuming that the nitrogen content as determined by the Kjeldahl method is correct, and the equation for the relation between % nitrogen content (on a moisture-free basis) and absorbance (or colorimeter reading) for some typical standard curves obtained during use of the methods.

TABLE I

ACCURACY OF ESTIMATION OF NITROGEN IN WHEAT AND BARLEY SAMPLES BY METHODS 1 AND 2

CEREAL AND METHOD[a]	RANGE OF % N (mfb)[b]	CORRELATION COEFF.	ERROR OF COLORIMETRIC DETERMINATION OF NITROGEN[c] (± % N(mfb))	REGRESSION EQUATION[d]
	%		%	
Wheat – 1	1.88–2.96	r = 0.987**	0.045	% N(mfb) = 0.883 C– 0.723
Barley – 1	1.82–3.22	r = 0.984**	0.062	% N(mfb) = 0.884 C– 1.239
Barley – 1	1.16–3.63	r = 0.997**	0.062	% N(mfb) = 5.141 A– 1.042
Barley – 2	1.31–3.43	r = 0.994**	0.066	% N(mfb) = 1.087 C– 1.013

[a] In each case, 32 samples were treated by the appropriate method.
[b] % N(mfb), percentage nitrogen content on moisture-free basis. The nitrogen content was determined in replicate by a macro-Kjeldahl procedure; the colorimetric values are the result of single determinations.
[c] The error was determined by the standard linear regression method.
[d] The regression equation showing the relation between %N(mfb) and the colorimeter reading. In these equations, C = EEL colorimeter reading and A = absorbance (Unicam S.P. 600).

Comments on Procedures

To facilitate the practical use of these methods, the following comments and suggestions are made.

Grinding. The samples of whole wheat and barley are ground in a Christie and Norris Junior Laboratory Mill (Thos. Robinson and Son Ltd., Railway Works, Rochdale, Lancashire, England) with a 1-mm. seive and placed in screw-top round sample bottles.

Mixing. To allow adequate mixing, the sample should not occupy more than three-quarters of the volume of the bottle. The samples are mixed mechanically for at least 1 hour on a machine which consists of a series of parallel rollers (all driven in the same direction), mounted on a framework which tilts from side to side. Replicate

[5] The error has been determined by the standard linear regression method.

nitrogen determinations on samples taken from the bottle without further mixing, whether by the Kjeldahl method or colorimetric methods, have always shown the usual degree of reproducibility associated with these methods.

Shaking. The duration and method of shaking are not critical. The duration may vary from 30 to 120 minutes and the method may vary from gentle mixing to vigorous shaking without affecting the results.

Discussion of Reagents and Procedures

Advantages of the Methods. The considerable economies made in time, capital equipment, and maintenance costs with both methods outweigh, for many purposes, the loss in accuracy when the results obtained are compared with those obtained with the Kjeldahl method. Two operators can carry out over 200 determinations per day when using Pinckney's method (3,7) as set out above in method 1, and about 120 determinations per day when using the procedure of Lowry *et al.* (4) as described in method 2. All the equipment and apparatus used is relatively inexpensive and readily available.

Choice of Methods. Varieties of barley grain with blue- or black-pigmented hulls, pericarp, aleurone, or outer endosperm layers must be determined by method 2 since, in the extraction procedure common to both methods, the naturally occurring color is extracted and absorbs strongly at the wave length of maximum absorbance of the copper-protein chelate. This causes large errors in method 1. In method 2 the original extract is diluted about 100 times, and the color developed with the Folin reagent is read at 760 mμ.

Ordinary noncolored barleys may be used to derive the standard curve in method 2 without introducing any error in the determination of the nitrogen content of those varieties of barley with dark-colored grains.

Moisture Content. Since the material for analysis for plant-breeding purposes is fully matured in the field before harvest and the moisture content of these samples is not normally required, variations in moisture content have been ignored. The moisture content of the majority of samples will fall very close to the mean of the whole harvest, and the variation of the remainder from the mean does not cause a serious error.

Lipids and Turbidity. The extraction bottles must be dry to avoid formation of doughy lumps which are not subsequently wetted with carbon tetrachloride; the samples must be completely wetted with carbon tetrachloride to avoid turbidity from unextracted lipid material.

The Modified Reagent. The use of sodium hydroxide instead of potassium hydroxide in the reagent of method 1 is not recommended; the absorption characteristics of the reagents prepared with each alkali are identical, but, when barley or wheat samples are extracted with these reagents, the absorbances of the colors developed with the reagent prepared with sodium hydroxide are only 70% of those of the colors developed with the reagent prepared with potassium hydroxide. Although several experiments were carried out to investigate this reproducible difference, no evidence can be presented to account for this phenomenon.

Pinckney's latest modification of his method (3) has been further modified in this laboratory by the use of potassium sodium tartrate (9,2) instead of glycerol. The use of potassium sodium tartrate has several advantages over glycerol in that it is virtually free of impurities giving rise to precipitation of cuprous oxide, and gives an increase in sensitivity since the copper tartrate chelate has a lower absorbance coefficient than the copper glycerol chelate, and also the copper tartrate chelate solution exhibits maximum absorbance at 675 mμ compared with 630 mμ for the copper glycerol chelate solution. This is shown in Fig. 1 where both reagents contained the same concentrations of potassium hydroxide and of copper sulfate. The molar ratio of copper sulfate to sodium potassium tartrate was 1:1.8; that of copper sulfate to glycerol was 1:7.1.

Since, as pointed out by Pinckney (7), copper is removed from the

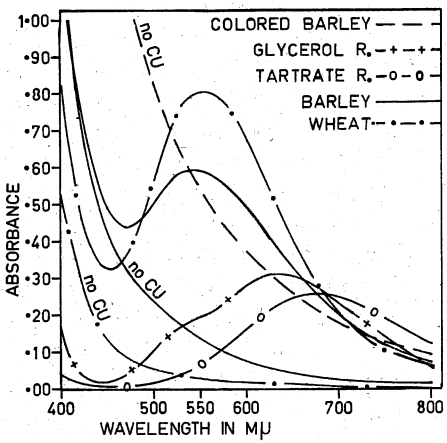

Fig. 1. Absorption spectra of the biuret reagents prepared with potassium sodium tartrate and glycerol, of the biuret colors prepared from a sample of wheat and a sample of barley, and of the extraneous color developed in the absence of copper from these same samples of wheat and barley and also from a colored barley.

copper tartrate complex to react with the protein, the amount of copper tartrate present will decrease proportionally as the protein content of the test solution increases. The absorbance at 550 mμ will be the sum of the absorbances of the copper protein chelate, the remaining copper tartrate chelate, and the extraneous color (see below). The smaller decrease in absorbance of the copper tartrate chelate as compared with the copper glycerol chelate on removal of a given amount of copper by protein results in a 13% increase in sensitivity, or a greater net increase in absorbance at 550 mμ for a given amount of protein. This may be illustrated by an experiment in which 32 samples of wheat were treated with both reagents and the appropriate correlations determined. With the reagent prepared with potassium sodium tartrate, an increase of 1% in nitrogen content (on moisture-free basis) gave an increase in absorbance of 0.222, whereas with the reagent prepared with glycerol the same increase in nitrogen content gave an increase in absorbance of 0.196. The molar ratio of potassium sodium tartrate to copper sulfate (1.8:1) used in this laboratory is adequate to ensure stability of the reagent for several days at least. Since 11 liters of reagent are used per day, greater stability than this is not required. However, higher ratios of tartrate to copper may be used if desired.

Method 2—Extraction of Protein

The method of Lowry *et al.* (4) is about 100 times more sensitive than the biuret method. To avoid undue weighing and sampling errors, it is advisable not to reduce the amount of grain sample taken for analysis below 500 mg. Since the biuret method as employed in this laboratory gives satisfactory extraction of the proteins, the following procedure has been adopted. The biuret color is first developed in the usual manner. After centrifuging, suitable portions (0.100 ml.) are treated by the procedure of Lowry *et al.* (4; see method 2). The biuret reagent is used for the extraction in preference to alkali alone, because a larger proportion of the cereal protein present is soluble in this reagent. Although, theoretically, there is sufficient copper sulfate present in the portion taken to permit full color development in the method of Lowry *et al.* (4; see method 2) the use of reagent C yields more color than reagent A, the increase in absorbance being greater than the increase due to the reagent alone.

Color Stability. In method 1, the samples are read between 105 and 150 minutes after the commencement of shaking, as it has been found with both barley and wheat that there is a rapid decrease in absorbance at 550 mμ between 60 and 90 minutes after commence-

ment of shaking; no further change occurs between 105 and 150 minutes, after which a gradual decrease in absorbance takes place. This differs from the findings of Pinckney (7) with wheat, of Robinson and Hogden (8) and Mehl (5) with serum and other proteins, and of Jennings (unpublished) with ovalbumin. In these latter cases, however, the prepared protein solution was subsequently treated with alkaline copper sulfate, whereas in the method described here the extraction of protein and color development occur simultaneously.

It is necessary when using method 2 to adhere fairly closely to a convenient time schedule to avoid batch-to-batch variations in the absorbance of the developed colors.

Instruments Used. The results reported in this paper have been obtained using either a Unicam SP600 Spectrophotometer (Unicam Instruments Ltd., Arbury Works, Cambridge, England) or an EEL Colorimeter. All readings in the Unicam SP600 Spectrophotometer have been made using cuvets of 2-cm. light path with distilled water in the reference cuvet, and in the EEL Colorimeter with the standard tubes ($3\frac{1}{8}$ in. by $\frac{5}{8}$ in. diameter) supplied with the instrument, again with distilled water in the reference tube.

Investigation and Discussion of Factors Affecting Accuracy

Extraneous Color. When plant materials are extracted with alkaline solutions a certain amount of extraneous brown color is usually associated with the alkaline extracts. This extraneous brown color is usually due to hemicelluloses, lignin, polyphenolic compounds, and natural pigments in the extract. The amount of this extraneous brown color in alkaline extracts of barley is much greater and more variable than in alkaline extracts of wheat. In neither barley nor wheat is the amount of this color related to total nitrogen or extractable nitrogen content. The extraneous color was developed by extraction of samples with 0.15*N* potassium hydroxide solution instead of the biuret reagent, using the recommended procedure. When read in cuvets of 2-cm. light path at 550 mμ, the range of the absorbances of the extraneous color of wheat was 0.029 to 0.041, with an average value of 0.035.

The variability in this color in barley is large enough to lessen the degree of correlation obtained in method 1. The degree of correlation is improved and the error of the determination of nitrogen content by method 1 is decreased if the absorbance of this extraneous color is subtracted from the absorbance of the biuret color and the difference (i.e., the color due to the copper protein chelate and the unreacted reagent) plotted against total nitrogen content, as shown

in Table II. Table II shows some typical absorbance values for the biuret color, the extraneous color, and the difference between these for white-grained samples of barley, and also values for two barleys with dark-colored grains. The values for the colored barleys are omitted from the calculations for the correlation coefficients, the errors of the determinations, and the equations showing the relationship between nitrogen content and absorbance. The effect of this extraneous

TABLE II

EFFECT OF EXTRANEOUS COLOR ON ACCURACY OF METHOD 1

	Barleys %N(mfb)[a]	Absorbance		
		Biuret Color	Extraneous Color	Biuret Minus Extraneous Color
	%			
White-grained				
	1.16	0.429	0.137	0.292
	1.45	.476	.109	.367
	1.75	.550	.132	.418
	1.82	.526	.117	.409
	2.16	.604	.125	.479
	2.21	.617	.110	.507
	2.34	.673	.148	.525
	2.51	.658	.125	.533
	2.60	.687	.127	.560
	2.74	.721	.124	.597
	3.02	.777	.117	.660
	3.22	.798	.116	.682
Colored[b]				
	2.43	.805	.382	.423
	2.97	1.240	0.531	0.709

	Corr. Coeff.	Error of Colorimetric Determination of Nitrogen[c] (± %N(mfb))	Regression Equation[d]
		%	
Biuret A vs. % N (mfb)	r = 0.993**	0.079	% N (mfb) = 5.350 A − 1.103
(Biuret A − extraneous A) vs. % N (mfb)	r = 0.996**	0.061	% N (mfb) = 5.300 A − 0.415

[a] %N(mfb), percentage nitrogen content on moisture-free basis.
[b] Excluded from correlation coefficient and other calculations.
[c] The error was determined by the standard linear regression method.
[d] The regression equation showing the relation between %N(mfb) and the absorbance (A) values in the calculated line of best fit.

color becomes negligible in the method of Lowry *et al.* (4), as set out in method 2. This is demonstrated by the fact that when 0.15*N* potassium hydroxide extracts of barley and wheat and a preparation of ovalbumin are serially diluted and these dilutions are treated by the method of Lowry *et al.* (4), as set out in method 2, the resultant

plots of absorbance against nitrogen content very nearly coincide.

Typical absorption spectra of the extraneous color (i.e., the color extracted by alkali in the absence of copper) from wheat, barley, and a barley with a black aleurone layer are shown in Fig. 1. Also included in Fig. 1 are the absorption spectra of the developed biuret color (using the modified reagent recommended above) from the same samples of wheat (2.96% nitrogen, moisture-free basis) and barley (1.86% nitrogen, moisture-free basis). The influence of the extraneous color on the shape and point of maximum absorption of the absorption spectra of the biuret colors derived from barley and wheat is shown in Fig. 1.

Amount of Nitrogen Actually Extracted. The amount of nitrogen actually extracted (as determined by a micro-Kjeldahl procedure) by the recommended extraction procedure from 500-mg. portions (of approximately 10% moisture content) of barley samples of varied total nitrogen contents was subtracted from that calculated to be present (allowance being made for the moisture content).

The unextracted nitrogen was calculated as percentage of the original sample on a moisture-free basis. This figure was relatively constant and was not related to total nitrogen content. The unextracted nitrogen content covered the range 0.10 to 0.31% with an average value of 0.18%. (Any amide nitrogen liberated and lost from the extracts would be included in the figure for unextracted nitrogen.)

This experiment was repeated, with omission of the copper sulfate and potassium sodium tartrate from the reagent. Similar results were obtained, except that the unextracted nitrogen content was higher, the average being 0.43% (moisture-free basis).

As a result, in both these experiments a lower percentage of the total nitrogen present in the 500-mg. portion was extracted from barley samples of low total nitrogen content than from samples of high total nitrogen content. Table III shows the results obtained with a number of barley samples in these two experiments. The finding that greater amounts of barley proteins are brought into solution by alkaline copper sulfate solutions than by alkaline solutions alone is in accord with the work on corn proteins by Mertz and Bressani (6). However, the addition of sodium sulfite (6) had no effect on the amount of nitrogen extracted or the amount of biuret color produced under the conditions applied in the experiments reported in this paper.

The results (Table III) were plotted as three curves: the amounts of nitrogen actually extracted, the amounts of nitrogen actually present, and the percentage nitrogen content on a moisture-free basis in

TABLE III

UNEXTRACTABLE NITROGEN CONTENT AND PERCENTAGE OF TOTAL NITROGEN CONTENT EXTRACTED FROM BARLEY[a]

BARLEY NITROGEN (mfb)[b]	MOISTURE	NITROGEN		TOTAL NITROGEN EXTRACTED	NITROGEN NOT EXTRACTED	UNEXTRACTED NITROGEN (mfb)	ABSORBANCE OF BIURET EXTRACT
		In 500 mg.	Extracted				
%	%	*mg*	*mg*	%	*mg*	%	
1.16	10.9	5.17	4.28	82.7	0.89	0.20	0.441
1.53	11.0	6.81	6.10	89.6	0.71	0.16	.500
1.82	11.0	8.10	7.08	87.4	1.02	0.23	.520
In absence of Cu tartrate			6.35	78.2	1.75	0.40	.107
1.86	11.2	8.26	7.35	89.0	0.91	0.20	.562
2.10	10.7	9.38	8.93	95.2	0.45	0.10	.628
2.16	11.0	9.62	8.87	92.3	0.75	0.17	.599
In absence of Cu tartrate			7.93	82.5	1.69	0.38	.117
2.47	11.6	10.92	10.35	94.9	0.57	0.13	.653
2.51	11.2	11.14	10.58	95.0	0.56	0.13	.652
2.51	10.6	11.22	9.82	87.6	1.40	0.31	.654
In absence of Cu tartrate			9.03	80.5	2.19	0.49	.111
2.53	10.3	11.35	10.63	93.7	0.72	0.16	.680
2.74	10.5	12.26	11.13	90.8	1.13	0.25	.710
In absence of Cu tartrate			10.20	83.2	2.06	0.46	.129
3.02	10.6	13.50	12.42	92.6	1.08	0.22	.759
3.22	10.5	14.41	13.59	94.3	0.82	0.18	.782
In absence of Cu tartrate			12.47	86.5	1.94	0.44	.121
3.63	10.8	16.18	15.63	96.6	0.55	0.12	0.868

[a] 500-mg. portions of barley were extracted by method 1.
[b] %N(mfb), percentage nitrogen content on moisture-free basis.

500-mg. portions of the same samples plotted against the absorbance of the colors produced in the biuret procedure. When the resultant plots were examined, the relative position of each sample to the other samples and to the relevant correlation curve was almost the same in each case. This shows that analytical results expressed on any of these three bases are directly comparable. The relevant correlation coefficient, the error of the determination of the nitrogen content by the colorimetric method, and the equation for the relation between nitrogen content and absorbance are given for each plot in Table IV.

Moisture Variation. As Table IV shows, the variation in moisture content (which is normally ignored in both methods) has no significant effect on the relation between absorbance and nitrogen content in the biuret method.

Absorbance Coefficient of Extracted Proteins. Variations in the amount of extraneous color produced, in the amounts of free amino acids and peptides present (which exhibit different spectral absorption characteristics and absorbance coefficients to proteins in the biuret reaction), or in the amounts present of such compounds as nucleic acids, are unlikely to account for the entire deviation from the theoretical correlation curve. It has been shown that different

TABLE IV[a]

RELATION BETWEEN ABSORBANCE (METHOD 1) AND EXTRACTED NITROGEN AND TOTAL NITROGEN CONTENTS OF BARLEY

ABSORBANCE OF BIURET COLOR CORRELATED WITH:	CORR. COEFF.	ERROR OF COLORIMETRIC DETERMINATION OF NITROGEN[b] (± MG. N OR %N(MFB))		REGRESSION EQUATION[c]
		mg	%	
N extracted, mg.	r = 0.994**	0.353		mg. N = (25.80A − 6.832)
N present, mg.	r = 0.993**[e]	0.371		mg. N = (25.61A − 5.882)
% N (mfb)[d]	r = 0.992**[e]		0.085	% N(mfb) = (5.716A − 1.302)

[a] All values used in calculating these results were derived from Table III.
[b] The error was determined by the standard linear regression method.
[c] The regression equation showing the relation between nitrogen content and the absorbance (A) in the calculated line of best fit.
[d] %N(mfb) : percentage nitrogen content on moisture-free basis.
[e] The difference between these two correlation coefficients is not statistically significant.

types of proteins exhibit different absorbance coefficients in both the biuret reaction (2) and the method of Lowry *et al.* (4), and it is suggested that differences in protein type and structure in the various varieties of wheat and barley account largely for nonperfect correlation in both methods. The examination of correlation curves, each derived from samples of several barley varieties, showed that each variety, regardless of nitrogen content, tended to maintain the same relative position to the correlation curve. For example, the proteins from Prior, Bonus, and Triple-Awned Lemma barleys showed absorbance coefficients which were respectively lower than, higher than, and very close to the mean absorbance coefficient of all barley varieties.

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Literature Cited

1. FOLIN, O., and CIOCALTEU, V. On tyrosine and tryptophane determinations in proteins. J. Biol. Chem. **73:** 627–650 (1927).
2. GORNALL, A. G., BARDAWILL, C. J., and DAVID, M. M. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. **177:** 751–766 (1949).
3. HALLORAN, G. M., and MOSS, H. J. The application of the biuret test to cereal breeding. Proc. Aust. Cereal Breeders and Genet. Conf. **1:** 14Aa–14A1 (1956).
4. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., and RANDALL, ROSE J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:** 265–275 (1951).
5. MEHL, J. W. The biuret reaction of proteins in the presence of ethylene glycol. J. Biol. Chem. **157:** 173–180 (1945).
6. MERTZ, E. T., and BRESSANI, R. Studies on corn proteins. I. A new method of extraction. Cereal Chem. **34:** 63–69 (1957).
7. PINCKNEY, A. J. Wheat protein and the biuret reaction. Cereal Chem. **26:** 423–439 (1949).
8. ROBINSON, H. W., and HOGDEN, CORINNE G. The biuret reaction in the determination of serum proteins. I. A study of the conditions necessary for the

production of a stable color which bears a quantitative relationship to the protein concentration. J. Biol. Chem. **135:** 707–731 (1945).
9. WEICHSELBAUM, T. E. An accurate and rapid method for the determination of proteins in small amounts of blood serum and albumin. Am. J. Clin. Pathol., Tech. Bull. **7:** 40–49 (1946).