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Comparison of the Kjeldahl, Dye-Binding, and Biuret Methods for Wheat Protein Content

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ABSTRACT

The Kjeldahl, Udy dye-binding, and biuret methods have been compared by testing 367 samples of all wheat classes. It was shown that although the Johnson versions of biuret and Kjeldahl methods do not differ significantly for wheat classes, the dye-binding method does differ from the Kjeldahl for all classes except Hard Red Spring. Most mean differences were small, being less than 0.5% between dye-binding and Kjeldahl values, except for wheats of less than 10% protein content, which differed by approximately 1%. When a quadratic equation was developed from the data and used to prepare a new conversion table, dye-binding values were brought into good agreement with Kjeldahl and biuret values.

In grain marketing at the present time there is urgent need for a method of determining wheat protein content which is rapid, easy to perform, and which produces results not significantly different from results by the Kjeldahl method for all classes of wheat.

The Kjeldahl method is based on the measurement of nitrogen, a basic element in the protein molecule. The procedure is accurate and precise for nitrogen, but requires about 1 hr. to complete. The Udy dye-binding method (1), on the other hand, requires only approximately 5 min. per analysis. In this method proteins react with monosulfonic azo dye, acid orange 12, to form an insoluble complex. The estimate of protein from a conversion table is based on colorimeter measurement of unbound dye through its relationship to total nitrogen as determined by a standard procedure such as the Kjeldahl.

In the biuret method, originally applied to wheat by Pinckney (2), a blue-violet color is developed with the peptide linkage in the protein molecule in the presence of an alkaline solution containing a copper compound. A version of the biuret method developed by Johnson and Craney (3) requires about 30 min. per determination. The time for this method has been reduced to approximately 5 min. by use of heat rather than shaking to speed the reaction.

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PROCEDURE AND RESULTS

One hundred eighty-four samples of cargo wheat of all classes with a wide range in protein content were analyzed. These were procured for us by the U.S. Department of Agriculture, Consumer and Marketing Service (C&MS), Grain Division.

Large differences in protein content were observed between the Kjeldahl and dye-binding (d.b.) methods and between the biuret and d.b. methods for wheat with protein contents below 10% (soft wheat). Data from the cargo wheat were, therefore, analyzed statistically in three groups: 1) those of 10% protein content and above, 2) those below 10%, and 3) combined.

Another 100 samples were analyzed for protein content by the three methods, 20 from each of the following classes: 1) Hard Red Winter, 2) Hard Red Spring, 3) Soft Red Winter, 4) White, and 5) Durum. The mean protein content values for all samples, both cargo and wheat classes, are shown in Table I. Since the same letter appears above the mean result for the Kjeldahl and biuret methods in each group, it indicates no significant differences between these methods. It should be pointed out, however, that among individual samples, differences may be greater than those between the means.

On the other hand, only Hard Red Spring wheat has an "a" above the mean for each method. The d.b. method differs significantly from both the Kjeldahl and biuret mean values for all remaining groups.

Correlation coefficients and standard errors of estimate for the same groups of wheat samples discussed above are shown in Table II. By the d.b. method it may be estimated from the standard error of estimate shown that approximately 99% of the values would check within ±0.82% of the Kjeldahl values for combined cargo wheats tested. By the biuret method, 99% would be expected to check with the Kjeldahl to within ±0.41%.

Twenty-seven samples which differed most widely in percent protein content (values not shown) were selected from the entire number of wheats tested. The

TABLE I. MEAN PROTEIN CONTENTS OF DIFFERENT CARGO WHEAT GROUPS AND WHEAT CLASSES BY THE KJELDAHL, BIURET, AND DYE-BINDING METHODS[a]

Wheats	No.	Mean Protein Content, %		
		Kjeldahl	Biuret	Dye-Binding
Cargo Sample				
10% protein and over	157	14.11a	14.16a	13.84b
Less than 10% protein	27	9.23a	9.23a	8.10b
Total cargo	184	13.37a	13.42a	12.97b
Wheat Classes				
Hard red winter	20	11.54a	11.60a	11.31b
Hard red spring	20	14.43a	14.48a	14.49a
Soft red winter	20	10.37a	10.30a	9.87b
Soft white	20	9.46a	9.54a	9.02b
Durum	20	13.24a	13.31a	12.88b
Total Classes	100	11.83a	11.81a	11.51b

[a]The same letter above the mean within each group in the table indicates no significant difference at the 0.05 probability level by the Duncan multiple range test.

TABLE II. CORRELATION COEFFICIENTS AND STANDARD ERRORS OF ESTIMATE OF KJELDAHL, DYE-BINDING (d.b.), AND BIURET PROTEIN METHODS

Wheat	No.	Correlation Coefficient: Biuret vs. Kjeldahl	Correlation Coefficient: d.b. vs. Kjeldahl	Standard Error of Estimate: Biuret vs. Kjeldahl %	Standard Error of Estimate: d.b. vs. Kjeldahl %
Cargo Samples					
Hard wheat	157	0.96	0.94	±0.22	±0.26
Soft wheat	28	0.88	0.71	0.27	0.40
Hard and soft	185	0.99	0.99	0.16	0.32
Classes					
Hard red winter	20	0.99	0.97	0.16	0.25
Hard red spring	20	0.99	0.96	0.17	0.39
Soft red winter	20	0.90	0.78	0.12	0.17
Soft white	20	0.98	0.88	0.11	0.33
Durum	20	0.95	0.93	0.19	0.22
All classes	100	0.99	0.99	0.16	0.32

TABLE III. STATISTICS FOR COMPARING THE DEGREE OF CORRELATION BETWEEN THE BIURET AND DYE-BINDING (d.b.) WHEAT-PROTEIN METHODS, RESPECTIVELY, WITH THE KJELDAHL PROCEDURE

Variables	"r"	"syx" %	Regression Equation
Kjeldahl vs. biuret	0.995	0.22	$\hat{Y} = 0.0711 + 0.9927\ X$
Kjeldahl vs. d.b.	0.988	0.33	$\hat{Y} = 1.7938 + 0.8842\ X$
Kjeldahl vs. d.b. colorimeter reading	0.986	0.36	$\hat{Y} = 1.7322 + 0.2461\ X$
Kjeldahl vs. log of d.b. colorimeter reading	0.988	0.34	$\hat{Y} = -26.8964 + 24.1472$ Log X
Kjeldahl vs. d.b. colorimeter reading + (reading)^2	0.990	0.32	$\hat{Y} = -1.7839 + 0.4121X - 0.00188\ X^2$

greatest difference noted between the Kjeldahl and biuret methods was 0.5%; between the Kjeldahl and d.b., it was 1.6%. The group of samples were retested under different numbers to ensure objectivity. These results did not differ from the original.

A scatter diagram (not shown), to show the relationship between Kjeldahl protein and d.b. colorimeter readings, indicated that additional samples of wheat with higher protein content would strengthen the equations derived from the data. Therefore, another set of samples of the 1970 wheat crop was obtained from the Grain Division, C&MS. Eighty-three were selected at random–20 exceeding 15% protein content–and analyzed. The results were combined with the 284 samples previously tested and then analyzed statistically by computer (Table III).

The curvilinear equation (Table III) $Y = -1.78389 + 0.4121\ X - 0.00\ 138\ X^2$ was used to develop a new conversion chart (Table IV). This chart differs significantly from that released by Udy in 1968 for wheats in the lower protein range where there was greatest significant differences between methods. Figure 1 compares graphs prepared from the two conversion charts.

TABLE IV. PROPOSED CHART FOR CONVERTING METER READINGS OF DYE-BINDING METHOD TO PERCENT PROTEIN CONTENT IN WHEAT[a]

Meter Reading	Protein %	Meter Reading	Protein %	Meter Reading	Protein %
25	7.34	41	11.95	57	15.60
26	7.66	42	12.21	58	15.79
27	7.97	43	12.46	59	15.98
28	8.28	44	12.71	60	16.17
29	8.58	45	12.96	61	16.36
30	8.89	46	13.20	62	16.54
31	9.18	47	13.43	63	16.71
32	9.48	48	13.67	64	16.89
33	9.76	49	13.89	65	17.06
34	10.05	50	14.12	66	17.23
35	10.33	51	14.34	67	17.39
36	10.62	52	14.56	68	17.55
37	10.89	53	14.77	69	17.70
38	11.16	54	14.99	70	17.85
39	11.42	55	15.19	71	18.00
40	11.69	56	15.40	72	18.14

[a]This table is based on the regression equation:

$$\hat{Y} = -1.78389 + 0.41211\ X - 0.00188\ X^2$$

Note: If wheat moisture differs from 14%, use the following formula to convert to 14% moisture basis:

$$P\ (14\%\ m.b.) = \text{Percent protein from table } X \times \frac{(100 - 14)}{(100 - \%\ \text{wheat moisture})}$$

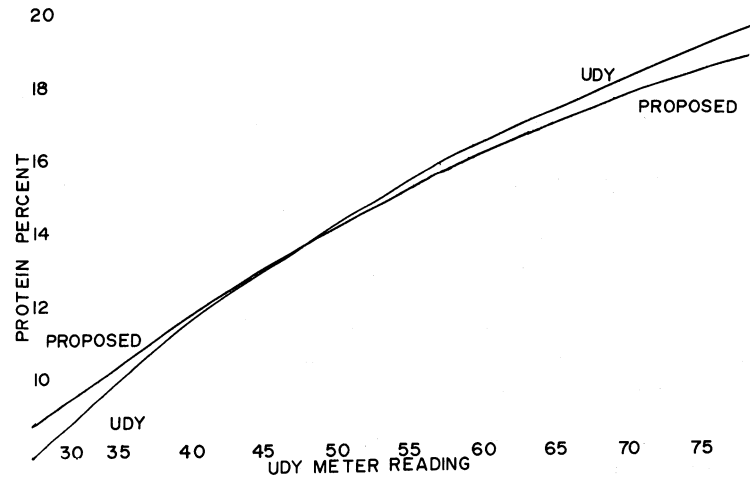

Fig. 1. Graph of proposed conversion chart vs. graph of Udy chart (wheat).

To test the comparison of the two charts, another set of samples (53) was analyzed. Results from each chart, and respective Kjeldahl values, were tabulated from the data analyzed. Results from the Udy chart differ significantly from Kjeldahl values, mainly because of the wide differences observed in the low-protein wheats (Table V).

Even though our Udy colorimeter was standardized against dye solutions of known concentration, there was concern as to whether the new conversion table would work as well when used by other analysts. Consequently, 12 samples of pulverized wheat meal were issued to members of the AACC Udy Protein Subcommittee. Typical results (Table VI) show that when the proposed conversion chart is used, results agree significantly better with the Kjeldahl value for wheats of low protein content. In wheats with protein content above 11% there was no significant improvement in the results.

DISCUSSION AND RECOMMENDATION

Our experiment consisted of 367 wheat samples, involving more than 2,200 tests for protein content. Analysis of variance for each group, except Hard Red Spring in the first set of 284 samples, showed that the differences between methods were highly significant. Even with a highly significant difference, however, the actual difference might be acceptable if it falls within a recognized tolerance. In Table I, for instance, all except the soft wheats appear to be acceptable. Agreement is good between the Kjeldahl and biuret methods over all wheat classes and protein levels.

The major differences between the dye-binding and the Kjeldahl and biuret methods occurred in wheats of low protein content. Although Fig. 1 indicates that protein contents read from the Udy Conversion Chart may be significantly high at protein levels above 18%, wheat samples at this level were not available for confirmation. Samples over 18% in protein content are rare.

TABLE V. COMPARISON OF KJELDAHL VALUES, DYE-BINDING RESULTS FROM UDY CONVERSION CHART, AND RESULTS FROM THE PROPOSED CHART

	Wheat Protein Content, %		
	Dye-binding		
Sample	Udy chart	Proposed chart	Kjeldahl
1	12.0	12.2	12.3
	14.6	14.6	15.1
	10.3	10.8	10.7
	16.8	16.7	17.1
	7.7	8.7	9.0
	18.2	17.9	18.4
	8.1	9.0	9.0
	13.1	13.2	13.2
	14.9	14.9	15.2
53	7.9	8.8	9.1
Mean Value:	12.74%	12.98[a]	13.04[a]
LSD (0.05) = 0.10%			

[a]Underscored means not significantly different at the 0.05 probability level.

TABLE VI. COMPARISON OF PROPOSED CONVERSION CHART WITH UDY'S BY AACC PROTEIN SUBCOMMITTEE

Collaborative Sample	Kjeldahl Average (3) %	Protein Content, % Dye-Binding					
		Collaboration 1		Collaboration 2		Collaboration 11	
		Proposed chart	Udy chart	Proposed chart	Udy chart	Proposed chart	Udy chart
1	9.3	9.3	8.6	9.2	8.4	8.7	7.9
2	9.0	9.1	8.2	8.9	8.1	8.7	7.9
3	9.3	9.0	8.1	9.3	8.6	9.0	8.1
4	9.2	9.0	8.1	9.2	8.3	8.8	7.9
5	10.3	10.3	9.7	10.4	9.9	10.1	9.5
6	11.0	11.4	11.2	11.3	11.0	11.2	10.8
7	10.9	11.4	11.1	11.3	11.0	11.1	10.7
8	13.1	13.0	12.8	13.1	13.1	12.9	12.8
9	15.0	15.0	15.1	14.9	15.1	14.6	14.6
10	13.2	13.4	13.5	13.6	13.6	13.2	13.7
11	16.0	16.2	16.3	15.8	16.2	15.8	15.9
12	15.3	15.2	15.2	15.0	15.2	15.3	15.3

Results in Table II show the extent and the protein levels at which the d.b. method failed to agree with the Kjeldahl and biuret methods. Tables V and VI show that this failure is corrected by the development and use of a new conversion table based on a curvilinear regression equation derived from the data from present tests.

It is recommended that the Udy Protein Subcommittee adopt the proposed conversion chart as the AACC official Udy method. A protein test which inaccurately reports protein contents that are below the true content may cause serious control problems, especially for the baker. For instance, Japanese prefer Pacific Northwest wheat because of its low protein content. Japanese bakers, therefore, would have serious control problems with flours having protein contents higher than those estimated.

It is also recommended that the comparative testing of protein methods be done at 5-year intervals to observe differences which might develop due to the introduction of new varieties into commercial channels. The dye-binding method is dependent upon certain amino acids and the biuret reaction is a function of polypeptides; therefore, any changes over the years which induce change in wheat, bran, or endosperm composition could also affect the response of the method.

Acknowledgments

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