Reliability of Five Methods for Protein Determination in Barley and Malt[1]

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Protein was determined in 44 barley and 49 malt samples by the Kjeldahl method. The data were used to establish a regression line of Kjeldahl protein and protein contents as estimated by biuret, dye-binding, alkaline distillation, and infrared reflectance (on two commercial instruments) methods. Protein contents of additional samples (76 of barley, 72 of malt) were determined by the Kjeldahl method (actual Kjeldahl protein). Protein contents of those were estimated by each of the five methods and were converted to the Kjeldahl nitrogen by use of the regression lines (predicted Kjeldahl nitrogen). Reliability of the five methods was evaluated by comparison of predicted with actual Kjeldahl protein of those additional samples. Six-rowed barleys contained, on the average, more protein than two-rowed barleys; varietal ranges in protein contents were smaller than location ranges; differences between barley and malt protein were small. Protein contents affected particle size of ground barley more than that of ground malt; ground high-protein barleys contained more large particles than the low-protein barleys. Malting reduced the particle size difference between high- and low-protein samples and increased amounts of fine particles. Correlation coefficients for linear regression between protein determined by the Kjeldahl and any of the other methods for calibration were significant at the 99% level and were generally above 0.97. The Kjeldahl method was the most precise and the biuret method the least precise. The biuret and dye-binding methods gave best agreement (for averages of two determinations) with the Kjeldahl method, infrared methods were intermediate, and alkaline distillation was the poorest.

Key words: *Alkiline distillation, Biuret, Dye binding, Infrared reflectance, Kjeldahl, Protein methods.*

Protein content of malting barley is important because it affects malting, brewing, and their end products (2,3,4,9,12,18). Kjeldahl-N determination is the commonly accepted method of protein estimation in barley and malt. The main objections to that procedure are the corrosive reagents and the time, facilities, and personnel it requires. Consequently, rapid and simple tests have been tried as substitutes.

The three main procedures for protein determination on the basis of nitrogen content are the Kjeldahl, the Dumas, and the neutron activation methods. The principles, limitations, and reliability of those tests have been reviewed in detail elsewhere (15). The specific methods used in this study are summarized briefly here. The dye-binding capacity of proteins can be used to estimate the protein contents of agricultural products and food (20), including barley (11,16). The empirical alkaline-distillation method has been developed for determination of protein in wheat and barley (17). The biuret method, presumably, has been revised to eliminate interferences from barley aleurone components (7) and to adapt it to simple and routine testing (8,10,13). Finally, investigations conducted by K. H. Norris and coworkers at the Instrumentation Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture have led to the development of an instrument which uses infrared reflectance to measure the moisture, protein, and oil contents of oilseeds and cereal grains. Since 1971, two firms (Dickey-john, Inc. and Neotec Instruments, Inc.) have been marketing commercial infrared reflectance instruments (19,21).

We compared the reliability of protein determination in barley and malt by the Kjeldahl, biuret, dye-binding, alkaline distillation, and infrared reflectance procedures. Theoretically, the factors that affect reliability are: specificity (absence of interfering substances), accuracy (degree of conformity of a measurement to a standard or true value), precision (agreement among repeated measurements of the same parameter), and sensitivity (the smallest detectable composition difference between two samples). In practice, however, the main criterion for reliability of a protein assay is comparison with the Kjeldahl determination.

MATERIALS AND METHODS

Barley and Malt

The barleys (120 samples) and malts (121 samples) used in this study are listed in Table I, and were supplied by the Barley and Malt Laboratory, ARS, USDA, Madison, Wis. The barleys were plant breeders' samples; the malts were prepared on a laboratory scale as described by Dickson and Burkhart (5). As expected, average protein contents between the barleys and malts (Tables II and III) differed little. Protein for six-rowed barleys and malts was higher than for two-rowed varieties. For 1974, overall average was 14.7% for six-rowed and 12.6% for two-rowed barleys. The protein ranges for individual samples were 10.4–21.5% for barley and 10.3–20.3% for malt.

[1]Mention of firm names or trade products does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

TABLE I
Description of Barleys and Malts Used in Study

Year		Varieties	Locations
1974	2-rowed	Firlbecks III, Shabet, Vanguard, Larker[a] Klages, ID 181053, WA 766467, WA 765267, Hannchen, MT 548448	Aberdeen, Idaho, Twin Falls, Idaho, Fairfield, Mont., Pullman, Wash., Laramie, Wyo.
1973	6-rowed	Barbless, Manchuria, Trail, Larker, Conquest, Bonanza, Minn. M-16 (Manken), ND 1156	Fargo, N. Dak., Langdon, N. Dak., Brookings, S. Dak.
1974	6-rowed	Barbless, Manchuria, Trail, Larker Conquest, Bonanza, Prilar, Manken, Minn. M-18, ND 231	East Lansing, Mich., Langdon, N. Dak., Crookston, Minn., Carrington, N. Dak., Brookings, S. Dak.

[a]A 6-rowed barley.

TABLE II
Range, Mean, and Coefficient of Variation (CV) of Varietal Kjeldahl-Protein Means for Barley and Malt

Type and Variety	Barley			Malt		
	Range	Mean	CV%	Range	Mean	CV%
1974 2-rowed						
Firlbecks III	10.8–14.9	12.9	14.3	10.7–15.1	12.9	15.0
Shabet	11.3–14.7	12.9	11.4	11.1–14.7	12.9	12.4
Vanguard	10.8–13.9[a]	12.4[a]	10.9[a]	10.6–14.0[a]	12.2[a]	12.0[a]
Larker[b]	11.9–15.6	14.2	10.3	11.8–16.5	14.0	14.0
Klages	11.2–14.3	12.6	10.4	10.9–14.4	12.6	11.1
ID 181053	10.7–13.8	12.1	12.6	10.5–14.1	12.1	14.4
WA 766467	10.6–13.7	12.0	11.6	10.4–13.4	11.9	11.4
WA 765267	10.4–13.4	11.9	10.9	10.3–13.2	11.7	11.1
Hannchen	11.6–14.9	13.7	11.0	11.1–14.6	13.4	12.9
MT 548448	11.1–14.6[a]	12.5[a]	12.3[a]	11.3–14.6[a]	12.4[a]	12.5[a]
1973 6-rowed						
Barbless	15.5–16.8	16.2	4.14	15.4–16.9	16.2	4.69
Manchuria	14.4–15.0	14.7	2.17	15.1–15.4	15.2	1.06
Trail	13.8–14.4	14.1	2.20	13.4–14.8	14.3	5.22
Larker	14.5–14.9	14.7	1.50	14.5–15.2	14.9	2.48
Conquest	14.0–14.4	14.2	1.32	14.2–14.3	14.3	0.49
Bonanza	14.1–14.9	14.5	2.98	14.3–15.6	15.0	4.48
M-16	14.3–14.5	14.4	0.659	14.3–14.6	14.5	1.26
ND-1156	14.4–15.1	14.8	2.43	15.0–15.4	15.2	1.33
1974 6-rowed						
Barbless	13.2–18.4	16.0	13.0	13.1–18.7[c]	15.8[c]	14.9[c]
Manchuria	12.6–17.1	15.3	12.4	12.7–17.2	15.3	11.9
Trail	11.5–19.1	14.9	20.2	11.4–18.1	14.8	18.8
Larker	11.4–18.2	14.1	17.0	11.2–17.0	14.7	15.2
Conquest	12.2–18.3	15.4	15.6	11.9–17.2	15.0	20.0
Bonanza	11.4–17.7	14.7	16.4	11.1–16.6	14.4	15.6
Prilar	14.1–17.3[d]	15.6[d]	9.50[d]	11.6–18.0	15.1	16.3
Manken	11.6–18.6	15.2	17.9	11.8–17.6	15.0	14.9
M-18	14.0–17.4	15.6	10.8	11.8–17.0	14.5	13.4
ND-231	12.6–21.5[d]	16.3[d]	20.1[d]	12.5–20.3	16.1	18.3

[a]Missing from Laramie, Wyo.
[b]A 6-rowed barley.
[c]Missing from Brookings, S. Dak.
[d]Missing from East Lansing, Mich.

TABLE III
Range, Mean, and Coefficient of Variation (CV) of Location Kjeldahl-Protein Means for Barley and Malt

Type and Location	Barley			Malt		
	Range	Mean	CV%	Range	Mean	CV%
1974 2-rowed						
Aberdeen, Idaho	13.4–15.2	14.3	4.41	13.2–15.2	14.2	5.06
Twin Falls, Idaho	10.4–12.0	11.2	5.27	10.4–12.0	11.0	5.19
Fairfield, Mont.	10.7–14.9	12.4	11.10	10.5–14.1	12.3	11.90
Pullman, Wash.	10.9–12.7	11.8	5.42	10.7–12.4	11.7	5.06
Laramie, Wyo.	12.9–15.6[a]	14.1[a]	6.38[a]	12.8–16.5[a]	14.3[a]	7.69[a]
1973 6-rowed						
Fargo, N. Dak.	14.0–16.8	14.7	6.11	13.4–15.6	14.8	4.89
Langdon, N. Dak.	13.8–15.5	14.7	3.33	14.3–16.3	15.0	4.27
Brookings, S. Dak.	14.1–13.4	14.7	4.84	14.2–16.9	15.0	5.93
1974 6-rowed						
East Lansing, Mich.	11.4–13.2[b]	12.1[b]	5.56[b]	11.1–13.1	11.9	5.46
Langdon, N. Dak.	13.2–15.0	14.2	3.59	13.3–15.0	14.3	3.37
Crookston, Minn.	13.6–15.9	14.8	4.92	13.7–16.4	15.0	5.54
Carrington, N. Dak.	16.2–18.4	16.9	3.96	16.2–18.7	17.1	4.21
Brookings, S. Dak.	17.1–21.5	18.3	7.11	16.3–20.3[c]	17.3[c]	8.05[c]

[a]Varieties Vanguard and MT 548448 missing from this location.
[b]Varieties Prilar and M-18 missing from this location.
[c]Variety Barbless missing from this location.

TABLE IV
Sieve Analyses of Barley Samples

Sample Identification	Sieve Analysis (%) for Openings of μm						
	>295	>208	>147	>124	>104	>74	<74
Carrington, N. Dak. (10 samples, average protein 16.94%)							
Range, %	1.9–6.4	7.7–17.9	12.4–19.0	7.8–20.2	10.5–21.5	4.6–8.1	20.8–34.2
Mean, %	3.85	13.25	16.42	14.17	17.81	6.62	27.88
Coefficient of variation, %	36.6	22.0	12.8	31.3	24.4	17.8	15.8
Twin Falls, Idaho (10 samples, average protein 11.22%)							
Range, %	1.0–2.1	6.4–12.3	14.1–26.2	9.6–23.1	9.6–24.2	5.7–22.3	10.3–34.6
Mean, %	1.51	8.82	21.63	15.53	16.76	10.93	24.82
Coefficient of variation, %	22.9	23.3	17.5	30.2	23.7	44.7	28.6
ND 231 Variety (5 samples, average protein 16.32%)							
Range, %	2.8–5.8	10.6–14.5	17.0–20.0	13.6–16.9	12.1–24.1	3.9–6.2	13.6–28.9
Mean, %	4.19	13.12	19.84	15.98	18.48	5.72	22.67
Coefficient of variation, %	34.1	16.4	6.4	6.9	28.7	16.8	32.7
WA 765267 Variety (5 samples, average protein 11.92%)							
Range, %	1.2–2.1	7.0–10.4	16.6–25.2	13.7–23.1	9.0–17.2	6.8–9.2	16.8–32.4
Mean, %	1.66	9.09	22.49	19.42	13.96	7.92	25.46
Coefficient of variation, %	24.7	18.9	16.8	18.7	26.9	13.2	24.2

TABLE V
Sieve Analyses of Malt Samples

Sample Identification	Sieve Analysis (%) for Openings of μm						
	>295	>208	>147	>124	>104	>74	<74
Carrington, N. Dak. (10 samples, average protein 17.15%)							
Range, %	1.9–3.7	6.2–11.9	13.5–18.9	6.7–18.9	8.6–16.4	8.2–17.2	22.3–46.3
Mean, %	2.61	7.95	15.69	12.84	11.59	14.06	35.26
Coefficient of variation, %	23.0	23.9	10.9	34.1	26.9	19.5	22.4
Twin Falls, Idaho (10 samples, average protein 11.01%)							
Range, %	1.1–3.4	3.9–12.1	6.7–17.3	6.9–20.6	7.9–12.8	9.6–31.8	22.0–50.8
Mean, %	2.43	6.78	12.48	12.50	10.49	14.97	40.35
Coefficient of variation, %	29.22	36.1	25.5	41.8	16.9	44.4	24.2
ND 231 Variety (5 samples, average protein 16.09%)							
Range, %	1.9–3.9	5.5–9.2	13.6–15.6	6.7–21.0	8.9–16.5	11.2–21.6	15.5–44.0
Mean, %	3.23	7.79	14.97	12.60	12.68	14.54	34.19
Coefficient of variation, %	26.2	21.7	5.8	52.8	27.6	32.1	35.0
WA 765267 Variety (5 samples, average protein 11.72%)							
Range, %	2.1–3.4	5.3–8.2	13.7–19.3	7.7–24.0	6.8–11.1	10.8–16.1	22.4–41.6
Mean, %	2.61	7.24	16.69	14.67	9.26	13.71	35.82
Coefficient of variation, %	21.2	20.1	14.9	45.0	17.5	19.0	21.4

Grinding of Barleys and Malts

Particle size is, next to composition, the most important factor that affects infrared reflectance readings (14,15). Therefore, variations in particle size should be minimized.

All samples were ground on a Udy-modified Weber pulverizer to pass a screen with 0.010-in. slotted openings, which enable rapid processing with little loss. Particle size of the ground barley and malt samples was determined by sieving 20-g samples for 10 min on an 8-in. diameter Ro-Tap Testing Sieve Shaker, U.S. Tyler Co. Two Carmichael cleaners with nylon brushes were placed on each sieve to assist in separation. The samples were sieved through the following standard Tyler sieves:

Tyler sieve mesh	48	65	100	115	150	200
Sieve opening (μm)	295	208	147	124	104	74

Two series (each of two sets) of samples were selected for the sieve analyses of barley and malt. The two sets (barley and malt) of the first series consisted of 10 varieties each from Carrington, N. Dak., and Twin Falls, Idaho, which had the highest and lowest location protein levels, respectively. The two sets (barley and malt, each from five locations) of the second series consisted of the cultivars ND 231 and WA 765267, which had the highest and lowest varietal protein levels, respectively. In each series, average protein was higher for 6-rowed than for 2-rowed barleys (malts).

The results of the sieving analyses (Tables IV and V) were corrected for recoveries, which were at least 95%. In the barley series (Table IV), there were more coarse particles (295 plus 208 μm) in high-protein than in low-protein samples, indicating that the vitreous, high-protein barleys resist complete disintegration to a fine powder more than the soft, low-protein barleys. Differences in distribution were less pronounced and consistent among small (104 μm and below) than among large particles.

Modifications in kernel structure caused by malting consistently affected particle-size distribution of ground samples (Table V). Differences in concentrations of coarse particles were small between high- and low-protein malts because malts disintegrated extensively during grinding. High protein contents contribute to toughness of the barley endosperm; physical modification during malting converts the hard and vitreous barley into friable and mellow malt, which disintegrates easily during grinding (9). Fine particles (104 μm and below) increased from 46.9–52.5% in ground barleys to 58.8–65.8% in ground malts.

Analytical Methods

Moisture and Kjeldahl protein were determined according to ASBC Methods of Analysis (1). Protein is expressed as N × 6.25 on a dry matter basis. The other protein methods were biuret (6), dye binding (20), infrared reflectance (according to the manufacturer's instructions—Dickey-john for Model GAC-2 and Neotec Corp. Grain Quality Analyzer for Model 41), and alkaline distillation (17).

All determinations were made in duplicate. First, 44 samples of barley and 49 samples of malt were selected to cover the whole range of protein contents (according to the Kjeldahl method) and a linear calibration regression line was established for the relation between Kjeldahl protein and protein content as estimated by each of the other five methods. Those lines were subsequently used to predict the protein contents of the other samples (76 samples for barley and 72 samples for malt) that were not used to develop the regression equations. Actual Kjeldahl protein of those additional samples was also determined. Reliability of each rapid method was evaluated by comparison of predicted with actual Kjeldahl protein.

The approximate times required for protein determinations by the methods are:

Method	*Time to Run a Single Assay (min)*	*No. of Single Assays per Day*
Kjeldahl	150	100
Biuret	10	50
Dye-binding		
Single sample reactor	15	100
Shaker tray	60	200
Infrared	0.5–1	250–300
Alkaline distillation	90	125

The infrared methods require a fairly lengthy precalibration, but less additional time than the other methods for a duplicate assay. In principle, calibration lines from the infrared methods should remain valid for the life of the electronic components. In practice, however, differences in variety, in location, in crop year, and replacement of filters in the instrument require recalibration. In the biuret, dye-binding, and alkaline distillation methods, the need for recalibration depends mainly on interacting major compositional differences. In all methods, calibration equations or curves must be prepared for the whole range of analytical values; extrapolations outside that range are unreliable.

RESULTS AND DISCUSSION

The calibration curves were based on the following model:

$$P_i = \beta_{0k} + \sum_{j=1}^{C_k} \beta_{jk} M_{ijk} + e_{ik} \qquad \begin{matrix} i = 1,2, \ldots, s \\ k = 1,2, \ldots, 5 \end{matrix} \qquad [1]$$

where P_i is the protein value determined by the Kjeldahl method for sample i, M_{ijk} is j-th value determined by method k (one of the five methods) for sample i, C_k is the number of calibration constants determined by method k, s is the total number of samples (s = 44 for barley and s = 49 for malt), and e_{ik} is the error. For the regression equation developed for calibration, duplications were averaged. Except for the infrared methods ($C_k = 6$ for infrared I and $C_k = 4$ for infrared II), $C_k = 1$. Correlation coefficients for linear regression for samples used in calibration (44 samples of barley and 49 samples of malt) were highly significant (generally above 0.97).

Correlation coefficients with Kjeldahl protein for the calibration series were:

	Barley	*Malt*
Biuret	0.983	0.980
Dye-binding	0.974	0.984
Infrared I	0.931	0.965
Infrared II	0.986	0.972
Alkaline distillation	0.985	0.970

Estimates $b_{0k}, b_{ik}, \ldots, b_{pk}$ of $\beta_{0k}, \beta_{ik}, \ldots, \beta_{pk}$, in equation 1, were calculated. The corresponding protein content for each sample was calculated with:

$$P_{ik} = b_{0k} + \sum_{j=1}^{C_k} b_{jk} M_{ijk} \qquad \begin{matrix} i = 1,2, \ldots, t \\ k = 1,2, \ldots, 5 \end{matrix} \qquad [2]$$

where t = 76 for barley, and t = 72 for malt.

To determine the agreement of each method with the Kjeldahl method, we calculated an estimate of the average mean of squared error:

$$\lambda_k^2 = \frac{1}{t} \sum_{i=1}^{t} (P_{ik} - P_i)^2 \qquad k = 1,2, \ldots, 5 \qquad [3]$$

The values λ_k^2 are given in Tables VI and VII for barley and malt, respectively. If the calibrations were perfect, the value λ_k^2 would be zero, since the predicted result P_{ik} for method k would equal the true value P_i for each sample i. Therefore, the order of the magnitude of λ_k^2 can be used to compare the calibration with the predicted result of method k with respect to the Kjeldahl method. Biuret and dye-binding methods consistently gave the lowest average mean square error; infrared methods were intermediate, and the alkaline distillation method was the highest.

We computed λ_k^2 for all samples and separately for samples in three protein ranges (Tables VI and VII). The ranges below 12%, 12–15%, and above 15% represent desirable, medium-high, and

TABLE VI
Values of λ^2_k Given by Equation 3 for Various Methods of Protein Determination in Barley

Method	All Samples[a]	Below 12% Protein[b]	12-15% Protein[c]	Above 15% Protein[d]
Biuret	0.336	0.738	0.250	0.256
Dye binding	0.897	1.207	0.635	1.328
Infrared I	1.624	1.689	1.408	2.119
Infrared II	1.980	1.900	1.880	2.289
Alkaline distillation	2.383	1.955	2.222	3.097

[a] t = 76.
[b] t = 13.
[c] t = 45.
[d] t = 18.

TABLE VII
Values of λ^2_k Given by Equation 3 for Various Methods of Protein Determination in Malt

Method	All Samples[a]	Below 12% Protein[b]	12–15% Protein[c]	Above 15% Protein[d]
Biuret	0.236	0.495	0.126	0.176
Dye binding	0.548	0.899	0.314	0.633
Infrared I	0.838	1.348	0.452	1.052
Infrared II	1.045	1.764	0.568	1.215
Alkaline distillation	1.540	2.186	0.721	2.453

[a] t = 72.
[b] t = 19.
[c] t = 35.
[d] t = 18.

TABLE VIII
Average Mean Squares of Replicate Measurements and its Confidence Interval (CI)

Material	Kjeldahl	Biuret	Dye Binding	Infrared I	Infrared II	Alkaline Distillation
Barley						
1973, 6-rowed	0.0240	0.290	0.0310	0.0478	0.00623	0.0910
1974, 2-rowed	0.00142	0.310	0.0255	0.0434	0.00915	0.0256
1974, 6-rowed	0.00181	0.187	0.0238	0.0484	0.00565	0.0798
All samples	0.00700	0.256	0.0261	0.0465	0.00705	0.0630
Lower 95% CI	0.00545	0.199	0.0204	0.0362	0.00549	0.0491
Upper 95% CI	0.00954	0.349	0.0356	0.0633	0.00961	0.0859
Width of the CI	0.00409	0.150	0.0152	0.0271	0.00412	0.0368
Malt						
1973, 6-rowed	0.00208	0.444	0.0132	0.0402	0.183	0.0717
1974, 2-rowed	0.00153	0.302	0.0209	0.0870	0.0176	0.0503
1974, 6-rowed	0.00288	0.323	0.0411	0.0896	0.0219	0.0915
All samples	0.00220	0.344	0.0272	0.0832	0.0589	0.0719
Lower 95% CI	0.00171	0.268	0.0211	0.0648	0.0459	0.0560
Upper 95% CI	0.00300	0.469	0.0370	0.113	0.0803	0.0980
With of the CI	0.00129	0.201	0.0159	0.0482	0.0344	0.0420

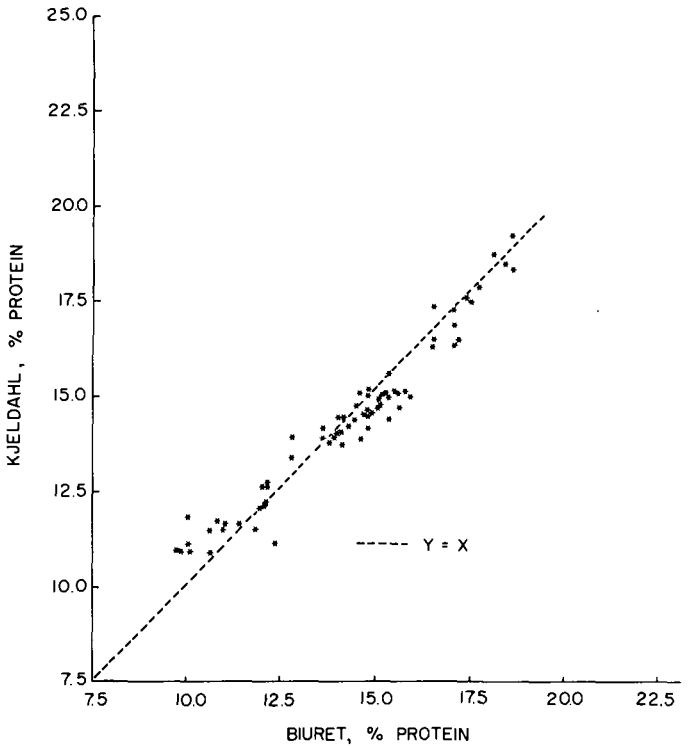

Fig. 1. Plot of protein determined by the biuret method on Kjeldahl protein in barley.

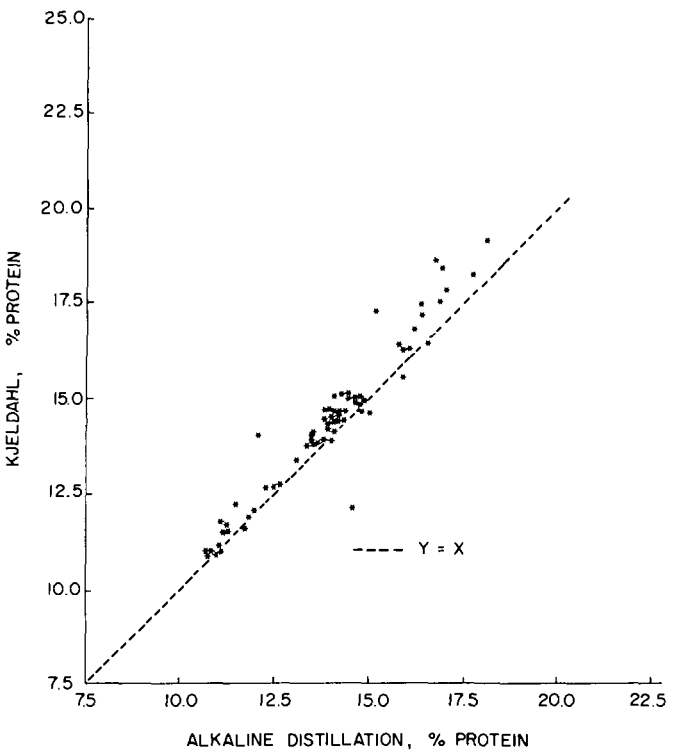

Fig. 2. Plot of protein determined by the alkaline distillation method on Kjeldahl protein in barley.

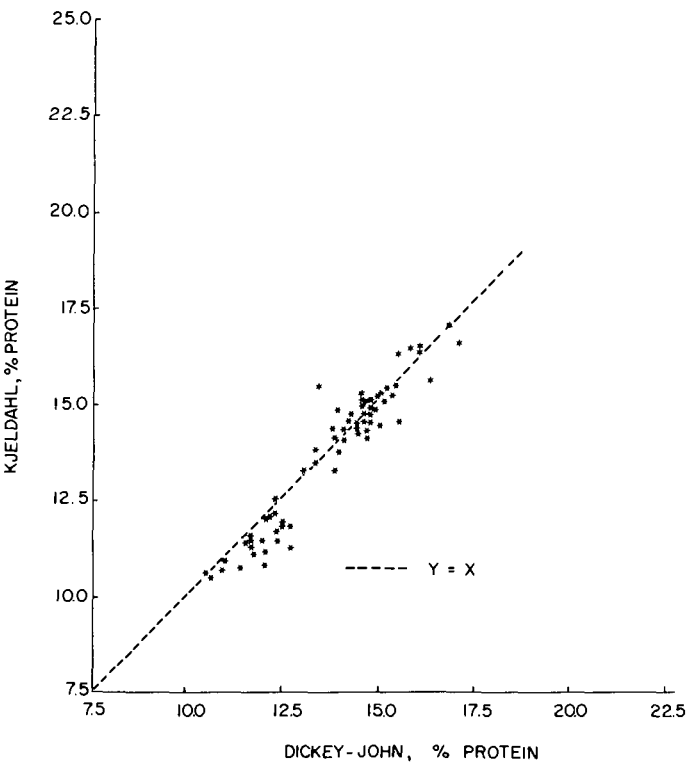

Fig. 3. Plot of protein determined by the infrared I method of Kjeldahl protein in malted barley.

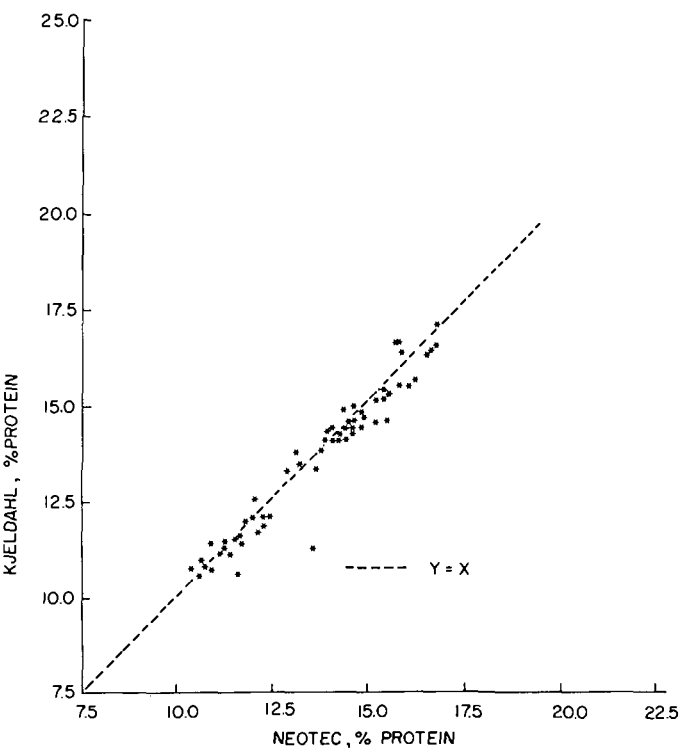

Fig. 4. Plot of protein determined by the infrared II method on Kjeldahl protein in malted barley.

excessively high protein levels in malting barleys, respectively. In general, the average mean square error was low for biuret and dye-binding methods,intermediate for infrared methods, and high for alkaline distillation methods. Except for the alkaline distillation method for barley, all other methods consistently gave lowest average mean error in the 12–15% range.

Kjeldahl protein *vs.* experimental data for individual estimates by four of the methods are plotted in Figs. 1–4. The four plots were selected to illustrate differences in distribution patterns of individual points.

The following are summaries of the statistics for the data shown graphically in Figs. 1–4:

Plot vs. Kjeldahl Protein	*Correlation Coefficient (r)*	*Linear Regression Line Equation*	*Standard Error Estimate (s_e)*
Biuret (barley)	0.972	$y = 1.942 + 0.857x$	0.474
Alkaline distillation (barley)	0.962	$y = -0.670 + 1.070x$	0.552
Infrared I (malt)	0.956	$y = -1.03 + 1.069x$	0.529
Infrared II (malt)	0.971	$y = 0.149 + 0.978x$	0.434

Thus, correlation coefficients and standard errors of estimate for Kjeldahl *vs.* biuret protein and Kjeldahl *vs.* alkaline distillation protein in barley were essentially equal. Yet, the distribution patterns of individual points in Figs. 1 and 2 are different. In the alkaline distillation method, the deviation from a correlation of 1.00 is due to widely scattered points in the above 15% range (Fig. 2); in the biuret method, scatter spreads throughout the range (Fig. 1). The scatter in Fig. 2 is probably due to some "aberrant" compositional differences that affect the results of the highly empirical distillation method; the spread in Fig. 1 probably could be reduced by improvement in the analytical procedure that would make more reproducible and stoichiometric the interaction between the copper reagent and the barley proteins. Similar conclusions could be drawn from the comparison between the two infrared methods (for malted barley, Figs. 3 and 4). A more reproducible cell packing procedure would likely narrow the spread along the linear regression line in Fig. 3.

All experimental data were used to determine the relation between precision and protein contents. For each method, the mean and standard deviation for each replicate measurement were computed for each sample. A plot (not shown here) of the standard deviation of each replicate measurement $s_i = |X_{i1} - X_{i2}| / \sqrt{2}$ against the mean $\overline{X_i} = (X_{i1} + X_{i2}) / 2$ showed no consistent relation except (crop year 1974) for the dye-binding method in testing two-rowed barleys and six-rowed barleys. For those samples, precision of those two methods decreased linearly with the increase in protein content.

TABLE IX
Minimum Detectable Differences of Protein Contents of Not More than ± 2.5% at the 95% Level (Type I Error) with 90% Assurance (Type II Error) of Detecting the Difference

Material	Kjeldahl %	Biuret %	Dye Binding %	Infrared I %	Infrared II %	Alkaline Distillation %
Barley	0.28	1.66	0.53	0.50	0.08	0.68
Malt	0.49	1.92	0.54	0.95	0.80	0.88

The Kjeldahl method consistently yielded the lowest and the biuret method the highest pooled within-sample variances of replicate measurements (Table VIII). The width of the 95% confidence interval of the average mean square of replicate measurements is also given in Table VIII. Of the six methods tested, the Kjeldahl method was the most precise and the biuret the least precise.

Since each sample was tested in duplicate, we could estimate the minimum detectable protein content as an approximation of sensitivity (Table IX). In general, the sensitivity of the methods follows the pattern of precision. The Kjeldahl method was more sensitive than any of the other methods, except for the infrared II method in barley protein determination. The biuret method was the least sensitive. For each method, sensitivity was consistently higher in barley than in malt.

In the preceding analysis of precision, all data were combined to base the results on the largest available number of experimental results. The resulting equation relating the within-laboratory standard deviation (precision) to mean concentration constituted a single, pooled estimate of the precision for one laboratory. The precision of various laboratories could be estimated by a collaborative study.

SUMMARY AND CONCLUSIONS

1. When ground on a Udy-modified Weber pulverizer, high-protein barley samples contained more large particles than low-protein barleys. Malt modification reduced the difference between high- and low-protein samples and increased the percentage of fine particles (above that in barley) in both low- and high-protein barley malts.

2. Correlation coefficients for linear regressions between protein contents from Kjeldahl and dye-binding, biuret, alkaline distillation, or infrared reflectance (2 instruments) methods for calibrating 44 barley and 49 malt samples were significant at the 99% level and were generally above 0.97.

3. First, 44 samples of barley and 49 samples of malt were used for calibration; from the calibration curves protein contents were calculated for an additional 76 samples of barley and 72 samples of malt. Evaluation of the reliability of the methods, based on the latter samples, indicated that biuret and dye-binding methods agreed well with the reference Kjeldahl method, but showed a low degree of precision (agreement between replicate assays).

4. In barley, precision and sensitivity were highest in the Kjeldahl and infrared II methods, lowest in the biuret, and intermediate in the other methods. In malt, precision and sensitivity were highest in the Kjeldahl, lowest in the biuret, and intermediate in the other methods.

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