Use of Dye-Binding and Biuret Techniques for Estimating Protein in Brown and Milled Rice[1]

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ABSTRACT

Linear regression equations relating colorimetric protein dye-binding and biuret values (absorbance) to Kjeldahl protein content of milled and brown rice were determined. Two dye-binding techniques, based on the reaction of acid orange 12 dye with protein, and modified for single-sample analysis and for multiple-sample determinations, were investigated. The rice samples, representing 45 varieties, ranged from 4.6 to 12.9% protein (% Kjeldahl N X 5.95). Correlation coefficients for milled rice were −0.986 and −0.961 for the single-sample and multiple-sample dye-binding techniques, respectively. For brown rice, corresponding "r" values were −0.969 and −0.984. Correlation coefficients for the biuret test were 0.964 and 0.981 for milled and brown rice, respectively. All four methods of determining protein were employed to analyze both milled and brown samples of 32 varieties of rice from a different crop. Mean protein content of milled and brown rice estimated by the two dye-binding techniques were not statistically significantly different from those obtained by Kjeldahl determinations; whereas statistically higher mean protein values were found with the biuret test. The colorimetric techniques described are satisfactory, rapid, simple, and relatively inexpensive methods for routine use in determining protein in rice-breeding programs.

Considerable interest has been shown in developing varieties of rice (*Oryza sativa* L.) which have higher protein levels. To accomplish the objective through varietal improvement programs, relatively quick, inexpensive, and accurate methods for determining protein are needed to facilitate screening large numbers of rice breeding lines and hybrid selections.

Colorimetric dye-binding and biuret methods are used extensively to estimate protein content in various foods and food products. The biuret method was successfully applied to the determination of protein in cereals (1-3), soya products (4), and meats (5). It involves the peptization of proteins with potassium hydroxide and treatment with copper sulfate. Under controlled conditions the intensity of the color produced is proportional to the protein concentration. In work with dyes, Fraenkel-Conrat and Cooper (6) showed that the dissociated sulfonic acid groups of Orange-G dye react with basic groups on the protein molecule to form an insoluble protein-dye complex. Dye-binding techniques have been used to determine protein content of wheat and wheat products (7), milk (8), meats (5), meat, fish, bean, and nut meals (9), and soya products (4), and for routine barley protein analyses (10).

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However, the usefulness of dye-binding techniques for determining protein in milled and brown rice has not been fully explored.

This paper describes two modified analytical dye-binding methods for estimating protein content in milled and brown rice and compares the efficiency of the biuret and dye-binding techniques on the basis of values obtained from Kjeldahl protein determinations.

MATERIALS AND METHODS

Collection and Preparation of Samples

Forty-five rice varieties grown on experimental-yield trial plots in the Philippines during the wet season of 1966 were obtained from the International Rice Research Institute (IRRI). Samples of rough rice were dehulled in a Satake laboratory grain testing mill and the brown rice was milled and polished in a standard McGill No. 3 rice mill. A separate lot was dehulled for determination of protein in brown rice. Both milled and brown rice samples were ground in a Weber pulverizing mill to pass through a 0.010-in. screen. All analytical determinations were made in duplicate and the results expressed on 11% moisture basis. Averages of duplicate observations were used in the statistical calculations.

Modified Dye-Binding Methods

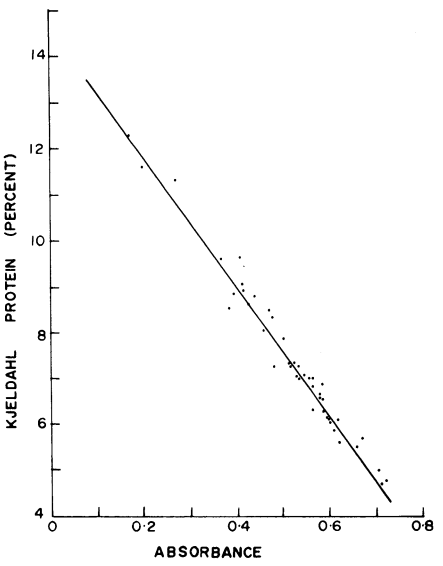
Single-Sample Technique. The apparatus[2] and dye-binding technique used for single-sample protein analysis in certain cereal grains have been described in detail (7,11). As modified for rice, the technique was as follows: Samples of ground rice (800 mg.) were weighed into the React-R-Mill, which is a special tube with metal ends containing a sliding metal plunger. Exactly 40.0 ml. of acid orange 12 dye (obtained from the Udy Analyzer Co. and prepared according to manufacturer's instructions) was added and the sample was vigorously shaken in the React-R-Shaker. Milled rice was shaken for 3 min. to complete reaction of the dye with protein; 5 min. of shaking time was required for brown rice. The reacted sample was filtered and the absorbance of the unbound (excess) dye was determined colorimetrically at 485 mμ. The quantity of unbound dye was calibrated in terms of protein content.

Multiple-Sample Technique. For batch or multiple-sample determinations, 800 mg. of ground rice was weighed into 50-ml. polyethylene bottles and 40.0 ml. of acid orange 12 dye was added. Sixty samples were shaken simultaneously on an Eberbach shaker at a rate of 60 (1.5-in.) strokes per min. One hour of shaking time was required to complete the reaction between dye and protein of milled rice. For brown rice, a 3-hr. shaking time was employed. After the shaking, the reacted samples were filtered and absorbance of the unbound dye was measured at 485 mμ.

Biuret Method

The biuret method employed the procedure reported by Pinckney (2) and used for rice by Webb (3). For milled and brown rice 1.0 g. ground sample was weighed into a test tube (25 × 150) mm. and mixed with 2 ml. of carbon tetrachloride.

[2]Equipment designed for this determination is manufactured by the Udy Analyzer Co., Boulder, Colorado.



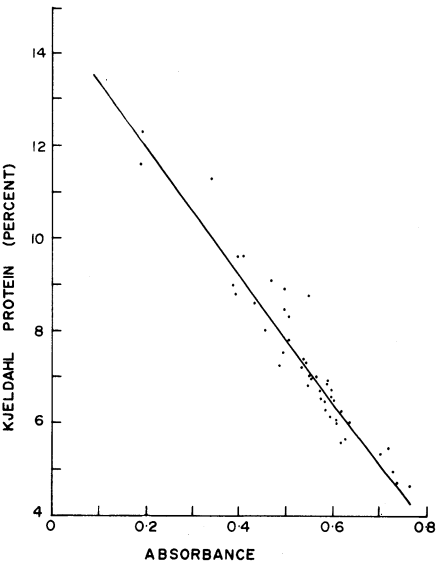


Fig. 1 (left). Relation between absorbance of unbound acid orange 12 dye and Kjeldahl protein for milled rice using the single-sample dye-binding technique. Y = −14.09X + 14.68 (r = −0.986).**

Fig. 2 (right). Relation between absorbance of unbound acid orange 12 dye and Kjeldahl protein for milled rice using the multiple-sample dye-binding technique. Y = −13.60X + 14.67 (r = −0.961).**

Exactly 40 ml. of biuret reagent B (2) was added and the samples were shaken for 90 min. Forty-eight samples were shaken at one time on a mechanical shaker operating at 50 (5-in.) strokes per min. After the shaking, a 15-ml. aliquot was centrifuged until clear and absorbance was determined at 550 mμ with the reagent as the blank.

Crude Protein

Total nitrogen content was determined by the official AACC micro-Kjeldahl method (11). Crude protein content was % N × 5.95.

RESULTS AND DISCUSSION

Relation between Dye-Binding Absorbance Values and Kjeldahl Protein Content

Figure 1 presents graphically the absorbance of the unbound dye as a function of protein content for milled rice by the single-sample technique. The same relationship for the multiple-sample dye-binding procedure is shown in Fig. 2. In this study, absorbance values of the unbound (excess) dye were related to protein content and were used in the preparation of regression lines. Table I summarizes the regression equations and correlations obtained for both milled and brown rice by the single-sample and multiple-sample dye-binding techniques. Correlation coefficients were highly significant for both techniques with milled and brown rice.

TABLE I. SUMMARY OF REGRESSION EQUATIONS AND CORRELATION COEFFICIENTS OF DYE-BINDING AND BIURET ABSORBANCE VALUES WITH KJELDAHL PROTEIN FOR MILLED AND BROWN RICE

Method	Regression Equation[a]	n	r	Sy. x
Milled rice				
Dye-binding—single sample	Y = −14.09X + 14.68	45	−0.986**	±0.28
Dye-binding—multiple sample	Y = −13.60X + 14.67	45	−0.961**	±0.48
Biuret	Y = 15.48X − 0.083	42	0.964**	±0.46
Brown rice				
Dye-binding—single sample	Y = −13.87X + 14.12	45	−0.969**	±0.40
Dye-binding—multiple sample	Y = −14.12X + 14.78	45	−0.984**	±0.29
Biuret	Y = 16.04X − 0.233	42	0.981**	±0.30

[a]Y = Predicted protein content (%); X = absorbance.

Statistically, the slopes and the position of the regression lines for both dye-binding methods were similar. The standard deviation from the regression line in terms of percent protein for milled rice was 0.28 and 0.48 for the single- and multiple-sample techniques, respectively. For brown rice, the standard deviation was 0.40 and 0.29, respectively.

Relation between Biuret Absorbance Values and Kjeldahl Protein

Figure 3 shows the relation between biuret absorbance values and protein content determined by the Kjeldahl method for milled rice. Regression equations and correlation coefficients for milled and brown rice obtained with the biuret test are summarized in Table I. Correlation coefficients for both milled and brown rice

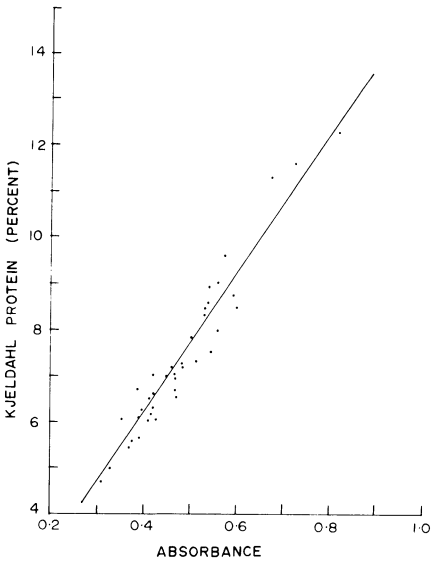


Fig. 3. Relation between biuret absorbance values and Kjeldahl protein for milled rice. Y = 15.48X −0.083 (r = 0.964).**

TABLE II. ANALYSIS OF VARIANCE FOR FOUR METHODS OF DETERMINING PROTEIN CONTENT IN MILLED AND BROWN RICE

Source	Milled Rice		Brown Rice	
	DF	MS	DF	MS
Method	3	7.69**	3	8.10**
Variety	31	14.96**	31	14.61**
Error	93	0.32	93	0.24

were highly significant. Standard deviation in terms of percent protein was 0.46 for milled rice and 0.30 for brown rice. Three varieties with red pericarp were excluded from the series of samples tested, because of possible interference due to colored pigmentation extracted by the alkaline biuret solution (1).

Comparison of Various Protein Tests

Milled and brown rice samples of 32 rice varieties grown at IRRI during the dry season of 1967 were analyzed separately for protein content by the two modified dye-binding techniques, the biuret test and the micro-Kjeldahl procedure. The regression equations presented in Table I were used to estimate protein content for the colorimetric procedures. An analysis of variance was used to test for differences between predicted protein means. Duncan's multiple range test was used to compare the means at the 1% level. For milled and brown rice, highly significant F values were obtained (Table II), and no differences were obtained between the means of the protein determined by the micro-Kjeldahl and the two dye-binding procedures (Table III). The mean protein contents of milled and brown rice (Table III) obtained by the biuret procedure were statistically (1% level) higher than the other methods.

Results of this study indicate that the dye-binding and biuret techniques described are satisfactory methods for use in estimating protein content of brown and milled rice, although the biuret method as used in this study may be less desirable than the dye-binding techniques. Dye-binding techniques offer a distinct

TABLE III. COMPARISON OF RANGE AND MEANS OF PROTEIN CONTENT OF MILLED AND BROWN RICE OBTAINED BY FOUR METHODS

Method	Milled Rice		Brown Rice	
	Mean (a) %	Range %	Mean (a) %	Range %
Micro-Kjeldahl	7.50a	5.74-11.69	8.25a	6.15-11.74
Dye-binding, single sample	7.75a	5.60-12.15	8.20a	6.10-12.15
Dye-binding, multiple sample	7.42a	5.55-11.65	8.04a	6.00-11.95
Biuret	8.18b	5.95-12.50	8.85b	6.30-12.45
	n = 32		n = 32	

[a]Means with the same letter are not different by Duncan's multiple range test (1%).

advantage because the procedure is simple and quick, and involves a minimum number of steps. The single-sample technique can be used to obtain results within 5 to 10 min., which includes grinding the sample. For routine protein determinations on large numbers of samples, the multiple-sample dye-binding or the biuret method may be preferred. These methods could probably be extended for samples with protein content greater than 12% by modification of sample size or reagent concentration and determination of appropriate regression equations.

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