Protein Estimation in Sesame Seed and Rapeseed Flours and Meals by a Modified Udy Dye Binding Method[1]

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ABSTRACT

The Udy standard dye binding method (shaker mixing) employing Acid Orange 12 was improved through a series of single-factor experiments designed to approximate the optimum combination of sample size, mixing period, particle size, drying conditions, shaker speed, and number of glass beads added for uniform mixing. The modified procedure yielded a higher recovery of protein and had high correlations with the Kjeldahl method, i.e., 0.991, 0.995, and 0.977 for sesame flour, rapeseed meal, and rapeseed flour, respectively. Mean values obtained by the Kjeldahl and modified dye binding methods from 12-17 analyses each on single lots of sesame flour, rapeseed meal, and rapeseed flour were 58.9% vs. 59.4% 36.1% vs. 36.0%, and 60.0% vs. 59.4%, respectively. A composite analysis of cereals, legumes, and oilseeds by the modified method had a high correlation (0.995) with Kjeldahl results.

INTRODUCTION

The increasing concern for nutrition among consumers and food producers has led to a need for a simple, rapid, and practical method to estimate proteins. The dye binding method offers these attributes wherein it is simple, rapid, precise, and does not require great skill (1)

The diversity of applications (1-5) of the dye binding method has increased greatly since Doyle C. Udy (6) first developed the method to estimate proteins in wheat. The literature reveals that the dye binding technique has demonstrated a close correlation to Kjeldahl and biuret methods (5,7,8) in several laboratories.

The present investigation was undertaken (a) to optimize and increase the recovery of proteins in oilseed flours and meals by modifying the Udy dye binding technique and (b) to compare the precision and accuracy of the modified method with the Kjeldahl method. The modified technique was applied to various foodstuffs to determine the feasibility of using a single equation for protein screening purposes.

MATERIALS AND METHODS

Apparatus

Apparatus used included a colorimeter with a short-path (ca. 0.35 mm) and 480 nm filter; batch shaker, automatic 40 ml pipette; glass-fiber filter discs; filter assembly; 60 ml polyethylene sample bottles; and glass beads (5 mm diameter).

Reagents

Udy's Reagent dye solution (1.300 g Acid Orange 12 per liter) and Udy's Reference dye solution (0.600 g Acid Orange 12 per liter) were used as reagents.

Methods

Udy standard dye binding method (shaker mixing) (1; D.C. Udy, Personal communication, 1972): Regression equation for sesame seed: Percent protein = (1.300-C)/0.0157, where C = concentration of unreacted dye (mg/ml) and the sample weight = 160 mg.

Proposed modified shaker dye binding method: Grind sample in Wiley mill to a desired particle size (40 mesh is sufficient). Weigh out a sample containing 65-85 mg protein into a sample bottle. Add six glass beads for uniform mixing. Add 40 ml of the reagent dye solution. Agitate up to a point where percent protein plateaus and use this time of mixing for subsequent experiments. Let the precipitate settle, filter within 3 hr into a cuvette, and read optical density of unreacted dye. Filtrates may be held overnight before reading, if necessary. Record the percent transmission and determine the concentration of unreacted dye from Udy's table (9). Calculate the percent protein using an equation derived from the relation of the modified dye binding and Kjeldahl methods: i.e., for rapeseed flour percent protein = (2.3091-1.9096C) x NF/Sg; for rapeseed meal, percent protein = (2.3814-1.9039C) x NF/Sg; and for sesame flour, percent protein = (2.2259-1.7434C) x NF/Sg, where C = concentration of unreacted dye (mg/ml), Sg = weight of sample in grams, and NF = nitrogen factor = 6.25. The general equation derived from composite analysis of cereals, legumes, and oilseeds: percent protein = (0.4052 + 0.0392 X') x NF, where X' = mg dye bound per gram sample, i.e., (1.300 - C) x 40/Sg, and NF = 6.25.

Kjeldahl Method: The semi-micro method employed is a modification of an AOAC approved procedure (10).

Procedures

Sesame flour: A single lot of sesame flour acquired from John Kraft Corporation, Paris, TX, was utilized in all experiments. Udy's standard method was used to determine the effect of drying conditions on protein content. In an attempt to improve accuracy and precision, Udy's standard method was modified through a series of one-factor-at-a-time experiments intended to determine approximately the optimum combination of the following variables: mixing time, shaker speed, particle size, sample size, and number of glass beads to be added for uniform mixing. As long as the response surface is "mound" shaped, as is commonly the case, a series of single-factor experiments should lead to approximately the same "optimum" as would be obtained through a large multifactor experiment. Protein values for vacuum dried sesame flour determined by the modified method were then compared with those obtained by Udy's standard method and with Kjeldahl crude protein values.

Rapeseed flour and meal: Single lots of these foodstuffs were obtained from the Canadian Department of Agriculture. The modified shaker technique was applied and the optimum periods of mixing required to equilibrate the dye and sample mixtures were determined. Results obtained on vacuum dried samples were compared to and correlated with Kjeldahl crude protein values.

Composite analysis of cereals, legumes, and oilseeds: The modified shaker method was applied to various commercial food products and correlated with Kjeldahl crude nitrogen values. The mg dye bound per gram of sample was plotted against the percent nitrogen. The protein content was reported on the "as is" basis.

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TABLE I

Statistical Data on Protein Content of Sesame Flour

	Kjeldahl method	Modified Udy dye binding	Udy dye binding
Tests:			
Number of samples[a]	12	14	10
Range	57.2-62.1%	58.4-60.0%	49.5-53.7%
Mean	58.9%	59.4%	51.4%
Standard deviation	1.093%	0.524%	1.280%
95% confidence interval	58.9 ± 0.7%	59.4 ± 0.3%	51.4 ± 0.9%
Coefficient of variation	1.86%	0.88%	2.50%

[a]Each sample represents the average of duplicate analyses.

TABLE II

Statistical Data on Protein Content of Rapeseed Flour

	Kjeldahl method	Modified Udy dye binding
Tests:		
Number of samples[a]	14	17
Range	54.0-63.4%	55.8-61.9%
Mean	60.0%	59.4%
Standard deviation	2.91%	1.743%
95% confidence interval	60.0 ± 1.7%	59.4 ± 0.9%
Coefficient of variation	4.85%	2.93%

[a]Each sample represents the average of duplicate analyses.

RESULTS AND DISCUSSION

Sesame flour: Analyses by Udy's standard procedure were conducted in duplicate on samples of sesame flour oven-dried either under a vacuum (55 C) or at atmospheric pressure (100 C). The average values obtained in three trials ranged from 49.8 to 51.1% with a mean of 50.5% for the 55 C samples, and from 49.6 to 53.5% with a mean of 51.8% for the 100 C samples. There was no significant difference in the mean protein contents obtained under the two drying conditions. Also, there was more variability in the samples dried under atmospheric conditions.

The addition of five glass beads to the reaction mixture greatly increased the protein recovery from 51.4% to 54.9%. Further investigation demonstrated that six or seven glass beads yielded optimum recovery of protein, i.e., 57.3% and 57.2%, respectively, after mixing for 120 min.

The protein values obtained by Udy's standard procedure using glass beads increased with the time of mixing, leveling off after 120 min. The mean values of the averages of duplicate determinations obtained in three trials after 30, 45, 60, 75, 90, 120, and 150 min of mixing were 52.4, 54.6, 56.3, 56.8, 58.0, 58.6, and 58.1%, respectively. The range for the 120 min determinations alone was 57.9-59.4%, and the mean of 58.6% was not significantly different from the Kjeldahl crude protein value (58.9 ± 0.7% from Table I).

The speed of the shaker had a direct effect on the recovery of proteins and was inversely related to the time of mixing. Using the Udy shaker, which had the highest speed, recovery of protein leveled off after 120 min while 180 min was required with a gyrotory shaker operating at slower speed. The amount of protein recovered was the same in each case (ca. 58%).

Flours possessing finer particles were found to require less time for mixing. Microscopic studies showed that the particle size of sesame flour ranged from 0.5 to 20 μm as compared to 0.5-10 μm for rapeseed flour, with larger particles being predominant in the sesame flour while most of the rapeseed flour particles were close to 5 μm. Dye binding equilibrium was attained after 30 min with rapeseed flour as compared to 120 min for sesame flour. The longer mixing time required for sesame flour may also be due to the unavailability of the protein molecules for the dye reaction. Other commodities that were analyzed showed that the 40 mesh screened samples yielded dye binding results that were in agreement with Kjeldahl values.

In practice, the weighing of a sample within a given range is easier and faster than the weighing of an exact quantity. Sesame and rapeseed studies by the modified shaker technique demonstrated that samples containing 55-90 mg protein yielded transmission values in the 25-65% range, the protein estimates showing no significant difference.

Based upon the above observations, a modification of Udy's standard method was developed and compared statistically to the Kjeldahl and Udy standard methods. Several analyses were conducted in duplicate on sesame flour by the modified shaker dye binding method, the Udy standard method, and the Kjeldahl method. Results of the modified procedure in mg dye bound (X) were plotted against mg nitrogen (Y) as determined by the Kjeldahl method. Linear regression analysis ($Y = 0.3048 + 0.4358X$) yielded a high correlation of 0.991.

The predicted protein values of sesame flour samples obtained from a homogeneous lot estimated by using the modified dye binding equation had a range of 58.4-60.0% and a mean of 59.4% (Table I). Statistical analysis showed that the mean protein content from the modified shaker dye binding method was not significantly different from that of the Kjeldahl method as demonstrated by the 95% confidence intervals. The modified shaker dye binding method also showed greater accuracy relative to Kjeldahl than Udy's standard procedure as shown by the mean protein values. The relatively low standard deviation indicates that the method has greater precision than either the Udy method or the Kjeldahl method. Since all samples reported in Table I are from a single homogeneous lot of sesame flour, differences in standard deviations can be taken to indicate differences in precision of the method.

Rapeseed flour and meal: Rapeseed flour subjected to different mixing periods did not show any significant relationship between mixing time and protein value using data obtained by the modified shaker technique. The average

TABLE III

Statistical Data on Protein Content of Rapeseed Meal

	Kjeldahl method	Modified Udy dye binding
Tests:		
Number of samples[a]	12	12
Range	34.8-37.0%	35.1-36.4%
Mean	36.1%	36.0%
Standard deviation	0.595%	0.338%
95% confidence interval	36.1 ± 0.4%	35.9 ± 0.2%
Coefficient of variation	1.65%	0.94%

[a]Each sample represents the average of duplicate analyses.

results from two trials were as follows: 58.6, 58.5, 59.1, 58.5, and 58.1% protein when mixed for 30, 60, 90, 120, and 150 min, respectively. It was concluded from these observations that 30 min mixing was sufficient to achieve maximum recovery of protein.

Linear regression analysis on the averages of duplicate determinations on rapeseed flour ($Y = -1.7337 + 0.4774X$) and meal ($Y = -0.8896 + 0.4760X$) samples showed a high correlation (0.977 and 0.995, respectively) between the modified dye binding and Kjeldahl methods.

The predicted protein values based on averages of duplicate determinations on 17 rapeseed flour samples and 12 rapeseed meal samples obtained from single lots and estimated by the modified dye binding method showed no significant differences from Kjeldahl results (Tables II and III). The modified dye binding method had better precision than the Kjeldahl method, as shown by the lower standard deviation, supporting the conclusion made from the sesame flour data.

Quadratic regression analysis of the above data for

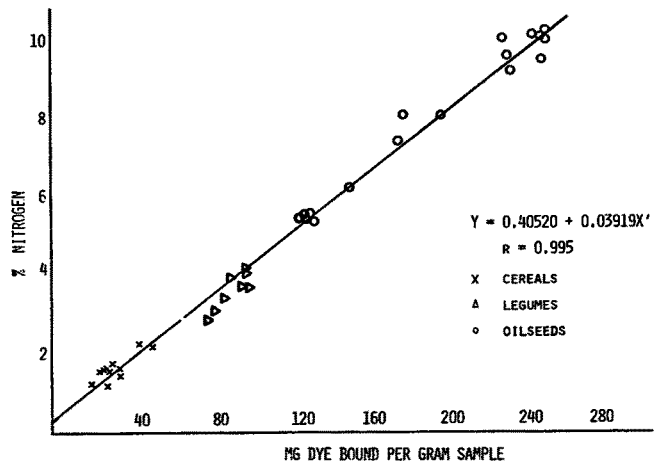

FIG. 1. Composite analysis of cereals, legumes, and oilseeds by the Kjeldahl method vs. modified shaker dye binding method.

TABLE IV

Protein Content of Cereals, Legumes, and Oilseeds as Determined by the Kjeldahl and Modified Shaker Dye Binding Methods

Sample[a]	Modified Dye Binding (mg dye bound/ g sample [X])	Modified Dye Binding % Protein	Kjeldahl (% Protein)
Trial I			
All purpose flour	26.0	8.9	11.5
Promosoy-100	246.2	62.8	65.1
Soybean nuts (roasted)	133.1	35.1	35.7
Oatmeal	43.6	13.2	15.3
Whey powder	31.4	10.2	11.8
Gerber rice cereal	28.0	9.4	7.5
Gerber barley cereal	33.7	10.8	11.4
Gerber mixed cereal	33.2	10.7	10.2
Gerber Hi-protein	133.1	35.1	35.9
Gerber oatmeal	50.7	15.0	14.6
Rapeseed flour	232.3	59.4	61.4
Rapeseed meal	130.8	34.6	35.8
Cottonseed flour	251.6	64.2	60.2
Chick peas	79.9	22.1	18.9
Kidney beans	88.4	24.2	22.8
Mung beans	99.6	26.9	26.3
Low fat soy powder	176.6	45.8	47.2
Natural soy powder	152.4	39.9	40.4
Trial II			
All purpose flour	26.1	8.9	11.1
Oatmeal	44.0	13.3	15.8
Gerber rice cereal	27.8	9.4	7.7
Gerber barley cereal	32.5	10.6	10.7
Gerber mixed cereal	32.4	10.5	10.7
Gerber Hi-protein	131.7	34.8	35.6
Rapeseed flour	234.6	60.0	58.7
Rapeseed meal	130.1	34.4	35.0
Chick peas	81.1	22.4	19.5
Kidney beans	90.5	24.7	25.4
Mung beans	99.6	26.9	24.5

[a]Each sample represents the average of duplicate analysis.

sesame flour and rapeseed flour and meal did not show a significant improvement at the 5% level over linear regression; therefore, the linear regression equations were used for the estimation of protein content. The modified dye binding method was compared and correlated with the Kjeldahl technique because Udy has not published a method or an equation for rapeseed by the shaker technique. However, Udy recommended a React-R-Tube technique which required a large sample size (340 mg) for rapeseed. This sample size exceeded the sensitivity of the dye binding system wherein the protein content of the rapeseed flour and meal samples exceeded the available binding sites of the dye. The sample size ranges that we recommend are 80-160 mg for rapeseed flour and 150-290 mg for rapeseed meal.

Composite analysis of cereals, legumes, and oilseeds: Several investigators have reported that dye binding and Kjeldahl methods have a correlation of at least 0.90. Although other reports and our studies have shown that each commodity has a different dye binding capactiy, we investigated a composite analysis of various commodities such as cereals, legumes, and oilseeds. The primary purpose of this study was to examine the feasibility of using a single procedure and equation to estimate protein content of various food commodities for quality assurance analysis.

The plot of percent nitrogen (Y') vs. mg dye bound per gram sample (X') showed a linear relationship with a high correlation of 0.995 (Fig. 1). Using the linear regression equation $Y' = 0.4052 + 0.0392X')$ a general equation was derived to estimate proteins in cereals, legumes, and oilseeds. The predicted modified dye binding values of the varied commodities using the derived equation, percent protein = $(0.4052 + 0.0392X') \times$ nitrogen factor, showed that the deviations from the Kjeldahl method were up to 2% in cereals, 3% in legumes, and 4% in oilseeds (Table IV).

The proposed general equation should be useful in routine analysis of many foodstuffs provided that initial dye binding results are compared with Kjeldahl data. Agreement of the modified dye binding values with the Kjeldahl values is found when data for the product fits the regression line of percent nitrogen vs. mg dye bound shown in Figure 1, and then the use of the general equation is valid. However, if a product does not fit the regression line due to differences in dye binding capacity, then it is necessary to develop specific equations for the given commodity based on dye binding vs. Kjeldahl data. This new equation can then be used for routine analysis of that particular foodstuff.

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