Rapid Determination of Protein in Soybean Meals

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ABSTRACT

Protein content of soybean meal is determined by a modified biuret method. Samples are stirred with 0.5N sodium hydroxide containing normal or isopropyl alcohol and copper hydroxide at 70 C. Protein is determined from the absorbance of the filtered solution at 550 nm. Satisfactory correlation with the standard Kjeldahl method was obtained.

The biuret procedure measures protein from the absorbance at 550 nm of copper complexes of the protein in an alkaline solution. The reaction is given by biuret (NH_2-CO-NH-CO-NH_2) and by proteins through reaction of their peptide linkages (-CO-NH-). No decomposition of protein is involved (1). Pomeranz (2) successfully used a biuret procedure with soybean meals, but a more rapid technique is needed. A common biuret reagent contains sodium potassium tartrate to prevent precipitation of copper hydroxide. We find, however, that tartrate lowers absorbance of the copper complex of soybean protein, while a significant portion of the total absorbance is due to the excess of dissolved copper hydroxide. At higher temperatures, e.g., 40 C, reddish precipitates have been reported (2). Johnson and Craney (3) used basic copper carbonate and dilute potassium hydroxide in 60% isopropyl alcohol to determine protein in cereal grains. The carbonate did not dissolve sufficiently to increase absorbance at 550 nm giving, in effect, a "colorless" biuret reagent. Unfortunately different batches of commercial copper carbonate required new calibrations (4). We modify this procedure by using copper hydroxide, and thus avoid the formation of the reddish color, even at 70-80 C. Isopropyl alcohol was reduced to 30%, since a portion of the blue reaction product was insoluble in 60% alcohol.

EXPERIMENTAL PROCEDURES

Copper hydroxide was formed by the action of alkali on copper sulfate solution. Stirring soybean meals first with dilute copper sulfate, followed by alkali, was not satisfactory. The material that dissolved in the copper sulfate solution was responsible for 30% of the total color, but only 6% of the protein dissolved before addition of alkali. The interference was due to carbohydrate and was minimized by using 0.5N alkali, rather than 1.0N, added just before the copper sulfate. Blending gave good results, but required too much time and gave low results when the copper sulfate was added before blending.

Soybean protein dissolved slowly in alcoholic alkali at ambient temperature, but dissolved in 2 min at 70 C. The hot 0.5N sodium hydroxide, containing 30% isopropyl or normal propyl alcohol, evidently converted insoluble protein into soluble form. Results have always been lower when alcohol was not present. Copper sulfate is added before heating. Lower absorbances resulted when the copper sulfate was added to the heated mixture, or to the heated mixture after cooling. Too much copper sulfate reduced the rate of filtration and removed some of the blue reaction product from the solution.

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TABLE I

Biuret Absorbance vs. Kjeldahl Protein for Commercial Soybean Meals

Kjeldahl protein, %	Biuret absorbance, 500 nm	Kjeldahl protein, %	Biuret absorbance, 550 nm
42.18	.295	46.76	.327
42.24	.289	46.77	.325
43.00	.297	47.33	.325
43.15	.304	47.50	.330
43.26	.302	48.09	.335
43.73	.305	48.19	.337
44.14	.310	48.20	.337
44.19	.310	48.32	.335
45.25	.309	48.67	.334
45.47	.310	48.76	.337
45.80	.316	52.10	.364
46.29	.320	54.85	.380

PROPOSED METHOD

Samples

Grind soybean meal samples to fine powder in a suitable mill such as a Mikro-Samplemill or equivalent. For calibration, protein values are determined by the Kjeldahl procedure (N x 6.25).

Reagents and Supplies

Alkaline alcohol solution: Dissolve 20 g sodium hydroxide in water, add 300 ml isopropyl, or normal propyl alcohol, and dilute to 1000 ml.

Copper sulfate solution (25% w/v): Dissolve 25 g of copper sulfate pentahydrate in warm water and dilute to 100 ml.

Glass fiber discs should be 21 mm Reeve Angel or equivalent.

Equipment

Magnetic stirrer-hot plate must be able, after preheating,

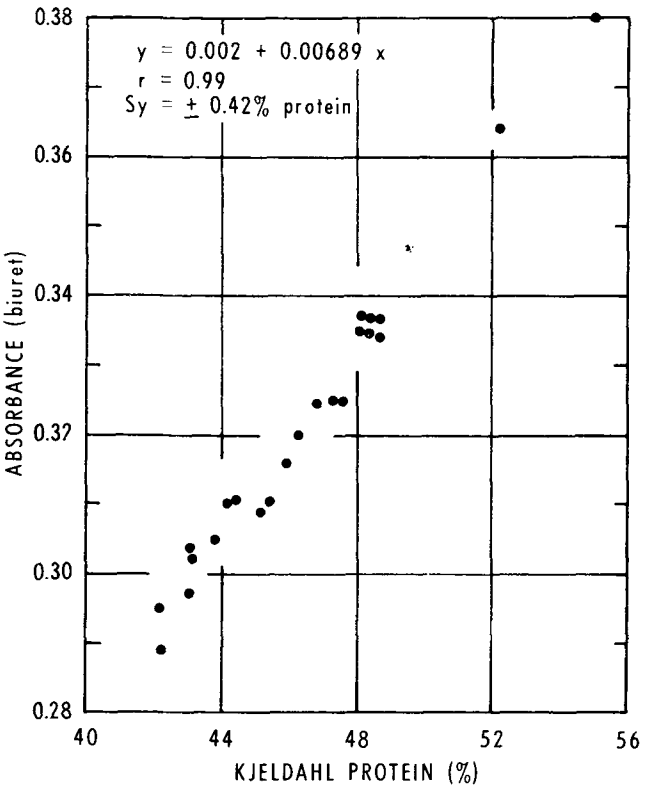

FIG. 1. Relation between biuret absorbance and Kjeldahl protein on commercial soybean meals.

to heat reaction mixture, while stirring rapidly, to 70 C in not over 2 min. A magnetic stirrer is also required.

Erlenmeyer flasks should hold 250 ml. With smaller flasks, such as 125 ml, time to reach 70 C would be longer.

Gooch crucibles should be Coors 2A or equivalent.

Spectrophotometer (or suitable colorimeter) is necessary.

Procedure

Weigh 200 mg finely ground soybean meal into a flask containing a suitable magnetic stirrer bar. Moisten meal with 2 ml isopropyl, or normal propyl alcohol, to prevent caking. Add 100 ml alkaline alcohol solution and 1 ml copper sulfate solution. Stir rapidly on a previously heated magnetic stirrer-hot plate to 70 C. Immediately transfer to an unheated magnetic stirrer and continue stirring to a total of 4 min. Filter by suction through a glass fiber disc in Gooch crucible. Measure absorbance of the filtrate at 550 nm. To assure clarity of the extract, discard first portion of filtrate and read absorbance on second portion. Per cent protein can be read from a standard curve, or from a table calculated from the regression equation.

RESULTS AND DISCUSSION

Twenty-four commercial soybean meals ranging from 42 to 55% protein, from various sources, were analyzed by the proposed procedure. Biuret absorbances vs. protein content as determined by Kjeldahl (N x 6.25) are given in Table I and the calibration curve shown in Figure 1. Normal propyl alcohol was used in the alkaline reagent. Results were the same as those previously obtained with a number of the same meals using isopropyl alcohol.

Close correlation between the biuret and Kjeldahl methods was found. The coefficient was 0.99 and the standard error of estimate ±0.42% protein. One additional meal was anomalous. It contained 48.6% protein, while the absorbance indicated only 46.6%. This meal was disregarded in the statistical analysis. Over 98% of its protein dissolved in the alkaline reagent at 70 C, but in the presence of copper hydroxide only 89% dissolved as compared with ca. 95% with other meals. This is probably the result of the effects of processing. Pomeranz (2) found that untoasted soybean meals gave higher absorbances than toasted meals. While untoasted meals are not used for food purposes, analysis for protein before toasting might be preferred.

Three isolated soybean proteins, containing 83, 90 and 92% protein, were also analyzed. They did not agree well with the meals, probably due to alteration of protein due to processing.

Normal propyl alcohol is more expensive than isopropyl alcohol, but can be used at slightly higher temperature. Ground soybeans required a higher temperature than the meals in the modified biuret method in order to rapidly hydrolyze the oil to give a clear filtrate. A temperature of 80 C was found to be satisfactory and can be obtained without loss of solvent using normal propyl alcohol. Results using ground soybeans and untoasted soybean meals will be reported in a later paper.

Statistics for 24 runs: Regression equation: $Y = 0.002 + 0.00689$ (% protein). Standard error of estimate = ±0.42% protein. Coefficient of correlation = 0.99.

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