A COMPARISON OF THE UDY DYE-BINDING AND KJELDAHL PROCEDURES FOR PROTEIN ANALYSIS OF GRAIN SORGHUM, *Sorghum bicolor* (L.) Moench

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CHAPTER I

INTRODUCTION

The problem of nutrition in the world is related primarily to a shortage of protein. Protein for human and livestock nutrition is available from two sources, animal products and plant products. Animal proteins are nutritionally complete proteins, but most plant proteins are deficient in one or more amino acids essential for tissue synthesis and body growth.

Approximately two thirds of the world's population rely upon the cereals (wheat, rice, maize, sorghum, and millet) as the main source of protein nutrition. Better nutrition for these people may well depend upon the improvement of the inherent nutritional quality of the cereals.

Grain quality has been largely ignored in sorghum improvement programs in the United States, except as it influences yield, agronomic characteristics in germination, and susceptibility to seed or soil borne organisms and birds. This lack of information on nutritive quality is due in part to the unavailability of rapid, inexpensive, and accurate measurement techniques. This is particularly true of protein where only the Kjeldahl nitrogen test is universally accepted. The sorghum breeder who seeks to improve protein quality and quantity must screen large numbers of hybrid selections and their segregating populations, which can be a formidable task using the Kjeldahl method.

Perhaps the most promising alternative to Kjeldahl is the Udy

dye-binding method. The objective of this study was to determine the suitability of the Udy dye-binding method for use in a grain sorghum protein improvement program.

CHAPTER II

LITERATURE REVIEW

Grain sorghum, *Sorghum bicolor* (L.) *Moench*, is the third largest U.S. cereal crop and is the most important food item in parts of Africa, Asia, and Latin America (28). Sorghum is a robust, hardy plant, able to grow and produce under a wide range of environmental conditions and as such has great potential for increased use as a food for human consumption as well as a feed for domestic livestock (27).

Karper and Quinby (16), in 1947, listed the following as objectives of most sorghum improvement work then under way: more suitable maturity, more palatable seed, seed that will stand exposure with least damage, dwarfness to make machine harvesting easier, insect resistance, disease resistance, improved forage quality, and endosperms with waxy type starch. Miller, *et al*. (21) noted that hybridization, while increasing yields, generally caused a decrease in protein content. Plant breeders have only recently begun to emphasize protein content and quality as a part of their breeding programs.

Pickett (26) has found grain protein content of the world collection of sorghum to range from 7.0 to 26.0%. The average protein percentage of sorghum grain is about 12% (16,39). Most significant increases in all cereal crop variety and hybrid yields in the past have been accompanied by significant decreases in protein percentage, with 1.5 to 2.0% loss being common (26). However, when hybrids were made

among diverse inbred lines with high protein, considerable heterosis for yield was seen and while the overall trend was for decrease in protein, the relationship was not absolute and many hybrids were observed with protein percent as high as the parents or even slightly higher. Collins and Pickett (8) have noted maternal effects in the protein percentage values from seed of F_1 reciprocals when crosses were made between divergent parents. The F_1 seed of the cross, made by using the high protein line as the female, was much higher in protein percentage than the reciprocal made from using the low protein line as female.

Environmental Effects on Grain Protein

Protein content is known to be influenced by several non-genetic factors such as soil type, fertilization, moisture, planting date, and temperature. Heller and Sieglinger (12) noted considerable variation in composition within grain sorghum varieties grown at Perkins and Woodward, Oklahoma. They attributed this variation largely to temperature and moisture differences. Miller, _et al_. (21) observed that location within the state (Kansas) considerably affected protein content of sorghum grain. They also observed environmental effects at each location from year to year. However, the hybrids with high protein content at one location tended to have relatively high protein content at all other locations.

Burleson, Cowley, and Otey (5) noted that protein content of sorghum grain was increased from 6.58% to 7.92% and 10.39% by applications of 60 and 120 pounds per acre of nitrogen, respectively. Nitrogen recovery, based on a ratio of plant protein nitrogen over fertilizer nitrogen, was 83.2% and 89.6% when 60 and 120 pounds of nitrogen,

respectively, were applied. Campbell and Pickett (6) also found that nitrogen fertilization significantly increased protein production, but variation among lines was much greater, suggesting the importance of genetic factors.

Planting date and temperature are other non-genetic factors affecting protein content. Worker and Ruckman (40) reported that the average protein content of sorghum grain produced from April plantings was 10.12% as compared with 14.02% from July plantings. This difference is probably due to temperature since they also noticed that cooler weather after antheses was advantageous to protein production. The use of such exotic approaches as the development of tetraploid lines (29) and treatment with simazine to enhance nitrate reductase activity (20) have also been suggested to increase protein content.

The fact that protein content is influenced by environmental conditions certainly does not diminish the potential for genetic improvement. The heritability of protein percentage is not fully understood but the potential for improvement is generally thought to be significant (7). Pickett (25) stated that gene action for percent protein was predominantly due to additive genes, however, there was also a significant amount of non-additive gene action which was apparently caused by epistasis and dominance. Chapman and McNeal (7) studied the gene effects for grain protein in five spring wheat crosses. They found additive gene action was highly significant for all crosses, dominance was significant in only two crosses, and significant epistasis was absent in all crosses. They stated that in all cases the additive effects appeared to have been contributed by the parent with the higher grain protein. These results suggest considerable potential for improving

grain protein percentage through selection.

Methods of Protein Estimation

To accomplish varietal improvement programs, relatively quick, inexpensive, and accurate methods for determining protein are needed to facilitate screening large numbers of breeding lines and hybrid selections (24). The measurement of percent protein has been made almost exclusively by two principal methods; the Kjeldahl method, which is the accepted standard, and the newer dye-binding method, which offers certain advantages in speed and economy.

Neill (23) described the Kjeldahl protein test as a nitrogen test. This test includes the soluble proteins, amino acids, the gluten, and all other organic material containing nitrogen, and is reported as total protein. Protein is the term applied to a combination of amino acids which are united by chemical bonds. The amount of nitrogen times a factor of 5.7 for wheat and flour or a factor of 6.25 for feed grains gives the amount of crude protein (34). These factors are based on the average percent of nitrogen in the various protein molecules. This method is slow and rather expensive but it is highly repeatable. Geddles and Milton (11) reported that if only a single Kjeldahl determination is made, the result may be expected to be within plus or minus 0.2% of the correct value.

Fraenkel-Conrat and Cooper (10) discovered that the acid dye, Orange G, combined stoichiometrically with basic protein groups at pH 2.2. They concluded that the number of basic groups binding Orange G represented the sum of the guanidine, imidizol, and E-amino groups of the proteins. These groups were furnished by the basic amino acids

lysine, arginine, and histidine (18,31). This method was applicable to both soluble and insoluble proteins.

Udy (35) developed a technique by which the binding quality of these basic groups on certain protein molecules could be used to quantitatively measure wheat flour protein fractions. Udy (36) found that wheat proteins reacted with the disulfonic acid dye, Orange G, at pH 2.2 to form an insoluble complex. The dissociated sulfonic acid groups of the dye reacted with the strongly basic R groups of lysine, arginine, and histidine in the protein molecules to form an insoluble protein-dye complex. The amount of dye bound per gram of sample may be used to provide an accurate estimate of protein content. In practice, the estimate is based on the concentration of unbound dye as measured colorimetrically using a light filter (470 mμ).

Composition of Grain Sorghum Protein

The cereal breeder who seeks to improve nutritional quality must be concerned with both the quantity and quality of the protein (15). A study conducted by Hubbard, Hall, and Earle (13) found the composition of sorghum kernels to be 82% endosperm, 10% germ, and 8.0% bran. Wall and Blessin (39) found comparable results and indicated that the endosperm contained 12.3% protein, the germ contained 18.9% protein, and the bran contained 6.7% protein. Shoup, et al. (32) indicated that protein in the bran and germ was relatively high in the amino acids lysine, arginine, and glycine, but lower in other essential amino acids than whole grain.

The soluble proteins of sorghum grain, which comprise about 85% of the total protein, have been divided into four fractions (30,38,39).

These fractions include the prolamine or kafirin fraction, which is soluble in ethanol, the glutelin fraction, which is soluble in dilute alkali, the globulin fraction, which is soluble in salt, and the albumin fraction, which is soluble in water. Virupaksha and Sastry (38) indicated that prolamine or kafirin, which comprises about 47% of the total protein, and glutelin, which comprises about 27% of the total protein, are the principal proteins of the sorghum grain. Albumin and globulin, the two remaining soluble fractions, together account for less than about 12% of the total protein.

Sorghum proteins, like those of other grains, are generally lower in the essential amino acids lysine, tryptophan, and threonine than dietary requirements established for nonruminant animals and humans (39). Wall and Blessin (39) stated that the prolamine or kafirin fraction is low in these limiting essential amino acids. Prolamine contains a high percentage of glutamic acid, proline, and isoleucine. Skoch, et al. (30) also indicated that the prolamine fraction was low in lysine and threonine as well as histidine, arginine, serine and glycine. They found that glutelin, the other major soluble protein of sorghum grain, was several times higher than prolamine in lysine, histidine, arginine, threonine, serine and glycine.

The shortage of the essential amino acid lysine is a characteristic of plant proteins in general and of cereal proteins in particular. Current emphasis on breeding for greater lysine content has necessitated a fast, accurate, and economical procedure for determining the amount of this amino acid. Wall and Blessin (39) noted that in sorghum grains with higher levels of protein, usually there is a lower concentration of lysine in the protein. They further noted that in high protein

grain, prolamine or kafirin constituted a greater proportion of the total endosperm protein than in the low protein varieties. They concluded that the decrease in protein quality observed in most high protein sorghum grains is caused by almost all of the increase in protein being due to higher kafirin contents. The study by Virupaksha and Sastry (38) also confirmed that increased protein content in sorghum varieties may be attributed mainly to an increase in the prolamine fraction of the grain. They stated that any change in protein composition which would increase the prolamine fraction will result in a decrease in the lysine content, and an increase in the glutelin fraction will result in an increase in the lysine levels of the seed.

Working with corn, _Zea mays_, Laible, _et al_. (17) found a highly significant correlation between lysine content, as determined by the bioassay technique of Henderson and Snell, and the optical density as measured by the Udy colorimeter. The correlation between total nitrogen from Kjeldahl and lysine content was found to be a highly significant negative value. Mossberg (22) also confirmed that the correlation between dye-binding capacity and lysine content was much better than the correlation between Kjeldahl nitrogen and lysine. These results suggest that the dye-binding procedure is adequate for use in a breeding program to screen selections for higher lysine content, whereas the Kjeldahl method is not.

Correlation of Udy and Kjeldahl

The use of the Udy dye-binding method for protein determination is a relatively recent development and only limited research has been conducted on its effectiveness. The Udy method has been found to be well

correlated with the Kjeldahl method on samples of wheat and wheat flour (3,36). MacKenzie and Perrier (19) also noted a good correlation between Kjeldahl nitrogen and the dye-binding properties of the plant proteins for six feed and forage crops including sorghum grain. They observed that the relation for all crops was nonlinear when plotted semi-logarithmically, demonstrating that each crop had a different dye-binding characteristic. They concluded that these differences illustrate the variety in protein types among different plant materials. Ashworth, Seals, and Erb (2) found the correlation between Udy and Kjeldahl for whole milk ranged from .92 to .99. The samples with the lowest correlation of .92 also had the highest average percent protein.

The Udy dye-binding method has encountered some criticism because of the fact that constituents other than protein seem to be bound by the dye. Udy (36) stated that starch and bran appeared to bind significant amounts of the dye. Bunyan (4) noted that when Kjeldahl protein was plotted against bound dye concentration, the resulting regression lines did not always pass through the zero percent protein point, suggesting that protein is not the only constituent binding the dye. He further suggested that the possibility of encountering samples with atypical amino acid composition means that estimation of protein content from dye-binding could be completely misleading in certain cases. Deyoe and Shellenberger (9) found that amino acid composition of sorghum grain protein can be significantly altered by hybridization and location.

Many of the same criticisms can also be applied to the Kjeldahl method. Kjeldahl is also based upon a constant or average amino acid composition for all protein molecules within a particular product.

Because both methods are based upon a common fallacy, they can only be expected to give an estimation of true protein content.

CHAPTER III

GENERAL MATERIALS AND METHODS

The sorghum material used in this study consisted of both hybrid and purebred varieties grown at the Agronomy Research Station, Perkins, Oklahoma in 1968 and 1969. The material ranged from a low of 8.0% protein to a high of 17.4% protein.

Protein content was determined for all material used in the study by both the Udy dye-binding method and the macro Kjeldahl method. A representative sample, consisting of 5 to 10 grams, of each variety was hand cleaned to remove foreign material including badly shrunken and diseased kernels. Each sample was then ground to a particle size of .015 mm using a Weber cyclone hammermill equipped with a vacuum collecting device. The ground samples were thoroughly blended and 1,000 mg subsamples were weighed out for protein determination by both Kjeldahl and Udy. Duplicate determinations were made for each sample in each method.

The dye-binding method used in this study was the standard procedure described by Udy (37). The 1,000 mg samples of sorghum grain was transferred into a two-ounce reaction bottle and 40 ml of the standard reagent dye, obtained from the Udy Analyzer Company, were added. This mixture was shaken vigorously for two hours on an Eberbach shaker. The shaker will hold 44 samples at once and the samples were prepared and placed on the shaker at one minute intervals, which permitted

reaction of a large number of samples while maintaining the optimum reaction time. The colorimeter, equipped with a flow through cuvette, was turned on one to two hours prior to the analysis. After this warm up period, the colorimeter cuvette was filled with a reference dye that has a standard transmission of 42%. The colorimeter meter is set to this reading. At the end of the required shaking time the sample solution was filtered into the cuvette through a funnel equipped with a fiber-glass filter disc. The percent transmission was read when the colorimeter needle had stabilized after approximately 20 to 30 seconds. This colorimeter reading was converted to percent protein by the use of a standard wheat conversion chart developed by Udy. Duplicate determinations were made for each sample.

The macro Kjeldahl method used in this study was the Winkler modification as described by Jacobs (14). The 1,000 mg sample of sorghum grain was transferred into a 1,000 ml macro Kjeldahl flask and 10 grams of sodium sulfate, 2 to 3 granules of selenium, and 25 ml of concentrated sulfuric acid were added. The flask was placed on the digestion rack for 90 minutes during which time the organic material is reduced to carbon dioxide, water, and ammonia. The ammonia, containing the nitrogen from protein degradation, is trapped in the form of ammonium sulfate, a substance with a high boiling point. After cooling, 300 to 350 ml of water were added to each sample. After the addition of 75 ml of sodium hydroxide (50% solution) and zinc boiling chips, the flasks were placed on the distillation rack. The ammonia, liberated from ammonium sulfate in an alkaline medium, was distilled into receiving flasks containing 50 ml of a 5% solution of boric acid with methyl red or methylene blue added as an indicator. The first 150 to 200 ml of

distillate will contain all of the ammonia. The ammonia was titrated directly with 0.1253 N sulfuric acid until the solution began to regain its blue color or until no green color could be seen. One ml of the standard acid required for titration is equivalent to one percent protein.

CHAPTER IV

SHAKING TIME AND GRAIN CONDITION AS RELATED TO PROTEIN DETERMINATION

Careful consideration must be given to shaking time if the Udy dye-binding method is to give accurate results. Udy (37) has concluded that the optimum shaking time for sorghum grain is two hours. However, Apichatabootra (1) found that the reaction was not complete at the end of two hours. He found that an increase in shaking time from one to two to three hours continued to give higher determinations with the dye-binding method, while an increase in digestion time from 90 to 180 minutes had no effect on the Kjeldahl method. He also found that decreasing particle size from .024 to .015 mm gave noticeably higher determinations with the dye-binding method, but did not influence the Kjeldahl results. Both methods gave a linear relationship between sample size and total protein present.

The purpose of the present study was to determine the length of time required for completion of the dye-binding reaction and to determine the effect of various grain treatments on protein estimation by Udy and Kjeldahl.

Materials and Methods

The sorghum grain used in this study consisted of three red seeded varieties (B Wheatland, B Redlan, and BOKY 54) and one white seeded

variety (ROKY 62). Each variety was analyzed by the dye-binding method with four different shaking times (2, 3, 4, and 5 hours) and three different grain conditions (pearled, new ground grain, and old ground grain). Each variety was also analyzed by the Kjeldahl method with the three different grain conditions. The pearled samples were prepared by using a hand operated barley pearler to completely remove the seed coat. The remaining endosperm was ground for analysis. The old ground grain consisted of whole grain that had been ground and stored for several months under cold storage. The new ground grain consisted of whole grain that had been ground only a few days prior to analysis.

Results and Discussion

The dye-binding method as used for sorghum grain is not an equilibrium reaction. It is apparent from Figures 1, 2, and 3 that as shaking time was increased from the standard two hours, percent protein as determined by Udy showed a general increase. However, the rate of increase was not constant among varieties, suggesting that different varieties reacted at different rates. ROKY 62, the only white variety used in the study, demonstrated little change in protein percentage after two hours, indicating that the reaction was essentially complete. The red varieties increased in protein percentage until four hours shaking time. Beyond that point, each variety reacted differently but consistently in the whole grain samples. Whole grain samples of both new and old ground grain showed that Redlan leveled off after four hours, Wheatland continued to increase in protein percentage, and BOKY 54 decreased in protein percentage. Thus, Wheatland was the only variety that failed to reach equilibrium at the end of five hours

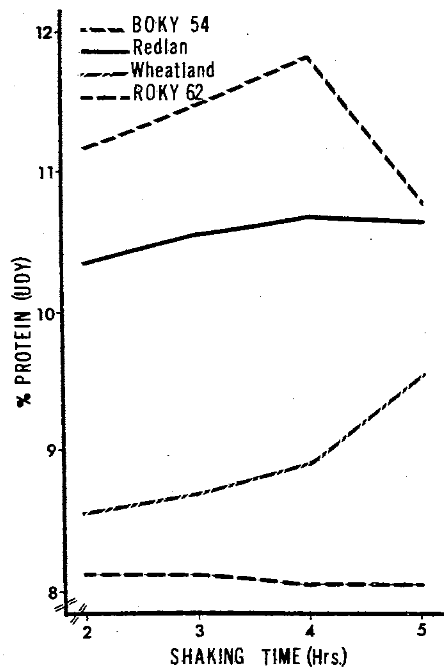

Figure 1. The Effect of Shaking Time on Protein Determinations by the Dye-Binding (Udy) Method Using Newly Ground Whole Grain of Four Grain Sorghum Varieties

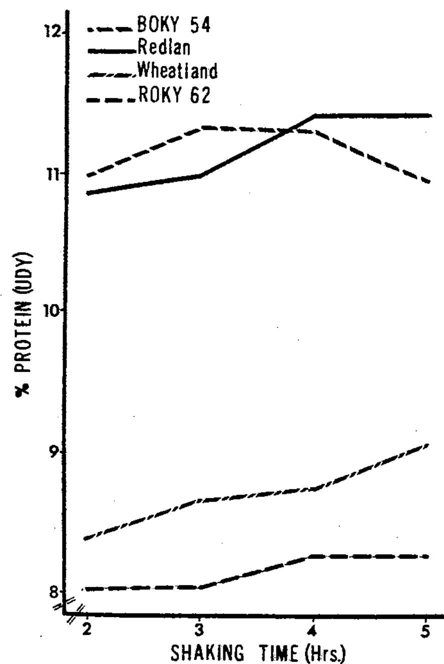

Figure 2. The Effect of Shaking Time on Protein Determinations by the Dye-Binding (Udy) Method Using Old Ground Whole Grain of Four Grain Sorghum Varieties

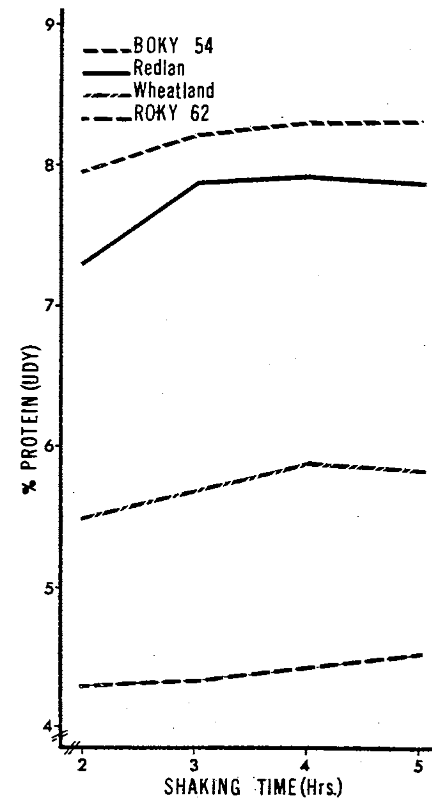

Figure 3. The Effect of Shaking Time on Protein Determinations by the Dye-Binding (Udy) Method Using Pearled Grain of Four Grain Sorghum Varieties

shaking time. The reason for the decrease in protein percentage demonstrated by BOKY 54 between four and five hours shaking time is unknown. One possible explanation is the presence of a slowly soluble grain pigment reducing light transmission and thus protein percentage as determined by Udy, or perhaps the prolonged shaking began to cause a breakdown in the protein-dye bonding. The possibility of pigment interaction is discussed in the next chapter. Note in Figure 3, that when all varieites were pearled, their reaction curves were very similar with much less varietal variation due to time even though their protein relationships were maintained.

The three different grain conditions used in this study were selected to determine if pearling the grain or storing the ground grain for prolonged time periods influenced protein analysis. The results of protein analysis by both Udy and Kjeldahl are presented in Table I. The old ground grain had been stored in paper packets which had absorbed some oil from the samples. When these samples were analyzed by Udy and Kjeldahl, the results agreed closely with new ground samples. There was no indication that storing the ground grain had any significant effect on protein determination.

The data were analyzed statistically and the analysis of variance is presented in Table II. There was a highly significant difference due to grain conditions by the Udy method and a significant difference by the Kjeldahl method. This difference was mainly due to the pearled grain. When pearled grain was analyzed by Udy, the percent protein declined by as much as 48% in the case of ROKY 62 and the least reduction was about 29% for BOKY 54. The difference was much less pronounced by Kjeldahl and no general trend was evident. Pearled ROKY 62 decreased

TABLE I

COMPARISON OF PERCENT PROTEIN BY UDY AND KJELDAHL METHODS WITH THREE GRAIN CONDITIONS AND THREE SHAKING TIMES FOR UDY

Grain Condition	New Ground Grain				Old Ground Grain				Pearled Grain			
Method	Udy			Kjel.	Udy			Kjel.	Udy			Kjel.
Shaking Time (hrs)	2	3	4		2	3	4		2	3	4	
Variety	%	%	%	%	%	%	%	%	%	%	%	%
ROKY 62	8.16	8.16	8.06	8.30	8.01	8.01	8.30	8.00	4.28	4.35	4.41	7.75
Wheatland	8.60	8.73	9.00	9.00	8.40	8.64	8.73	8.95	5.46	5.69	5.88	8.85
Redlan	10.35	10.58	10.66	11.00	10.82	10.98	11.43	10.95	7.55	7.85	7.90	11.25
BOKY 54	11.18	11.47	11.62	11.80	10.99	11.36	11.33	11.75	7.96	8.21	8.30	11.90

TABLE II

ANALYSIS OF VARIANCE OF PERCENT PROTEIN
BY UDY AND KJELDAHL

Source	d.f.	Mean Squares	
		Udy	Kjel.
Reps (R)	1	0.0145	0.0067
Varieties (V)	3	61.1378**	19.0050**
Conditions (C)	2	111.9129**	0.0279*
V X C	6	0.5853**	0.0662**
Error (a)	11	0.0167	0.0058
Time (T)	3	0.5181**	
V X T	9	0.1639**	
C X T	6	0.0215	
V X C X T	18	0.0617**	
Error (b)	36	0.0227	
C.V.		1.4896	0.7615

*Significant at 0.05 level of probability.

**Significant at 0.01 level of probability.

slightly in protein percentage over whole grain and the other three varieties increased slightly but these differences were statistically significant only because of a very small error term.

These results indicate that the normally good correlation between Udy and Kjeldahl, when whole grain is analyzed, deteriorates when the grain is pearled. The bran of sorghum grain is normally low in protein, averaging about 6.5% as compared to 12% for whole grain (39). This

would seem to indicate that removing the bran would increase protein percentage of the grain, however the pearling process used in this study also removed a portion of the germ, the amount depending upon the relative hardness or softness of the kernel. The germ is high in protein, averaging about 19% according to Wall and Blessin (39). Thus, the results obtained from Kjeldahl in this study would seem reasonable, since the removal of the bran and a portion of the germ should have little effect on the total percent protein. Shoup, et al. (32) found that the composite bran and germ had about the same protein percentage as whole grain. However, the average protein values mentioned for kernel components were determined by the Kjeldahl method and are subject to any discrepancies of this method.

The extreme sensitivity of the Udy method to removal of the seed coat indicates that the method cannot always be expected to agree with Kjeldahl. Any alteration in the normal proportion of seed coat to endosperm in the whole grain that might be caused by shrunken kernels could be expected to produce considerable difference between the two methods. Shoup, et al. (32) and others have indicated that the bran and germ do not have the same amino acid composition as the whole grain. In fact, the bran and germ are relatively higher than whole grain in lysine and arginine, two of the amino acids measured by the Udy method. It is not known if this alteration in amino acid composition is sufficient to explain the difference between the methods due to pearled grain. The Kjeldahl method should also be sensitive to amino acid composition although perhaps not to the same extent as Udy. The possibility is also present that a constituent other than protein is reacting with the Udy

dye. The effect of altering grain composition is discussed more thoroughly in Chapter VI.

CHAPTER V

GRAIN COLOR AS RELATED TO PROTEIN DETERMINATION

Protein determination by the dye-binding method is dependent upon the optical density of the dye remaining in the filtrate after reaction with the grain protein. Any source of additional color, such as water soluble pigments, should theoretically produce a protein estimation lower than actual protein content. The seed coat of sorghum grain contains several pigments and preliminary results by Apichatabootra (1) indicated the possiblity of interference of grain pigment with the test dye in the Udy analysis.

The purpose of this study was to determine the effect, if any, of grain pigments on Udy protein determination. The study was also designed to give a good comparison of the Udy and Kjeldahl methods over a wide range of protein percentages.

Materials and Methods

The sorghum grain used in this study consisted of 10 red seeded varieties and 10 white seeded varieties, each set of varieties ranging in protein percentage from approximately 9.0 to 17.5%. These varieties were selected from a large group of F_2 segregating populations which had been analyzed for protein content by the dye-binding method during a previous study (1). Protein content of each variety was determined by the Udy and Kjeldahl methods previously described. Duplicate trials

at different times were conducted for each variety in each method.

Results and Discussion

Identical one gram samples of each variety were analyzed for percent protein in duplicate by Udy and Kjeldahl. The means of these two trials by Udy and Kjeldahl are shown in Figures 4 and 5. Figure 4 gives a comparison of the two methods on ten red seeded varieties and Figure 5 gives the same comparison of ten white seeded varieties. It can be seen that the two methods agree rather well at the low and medium protein levels regardless of grain color, but it is evident that as protein percentage of the grain goes above 15 to 16%, Udy gives consistently higher determinations than Kjeldahl. When each color is statistically analyzed in Table III, methods are shown to be nonsignificant for white grain, but the same trend is still evident at the higher protein levels and the nonsignificance is due to the good correlation at the low and middle protein ranges. No variety in this study with a Kjeldahl determination of 16% or greater, gave a lower Udy determination. Again the reason for this difference is uncertain, but a change in relative kernel composition and amino acid composition is suggested. In general, the higher protein varieties do not have large, plump, well developed kernels common in varieties with low to medium protein percentage.

The original purpose of this study was to determine the effect, if any, of colored pigments in the grain on Udy protein determination. However, the experiment could not be designed so as to statistically prove or disprove this theory. The analysis of variance of percent protein by Udy and Kjeldahl, as presented in Table IV, shows color to be statistically significant with both methods, but red and white varieties

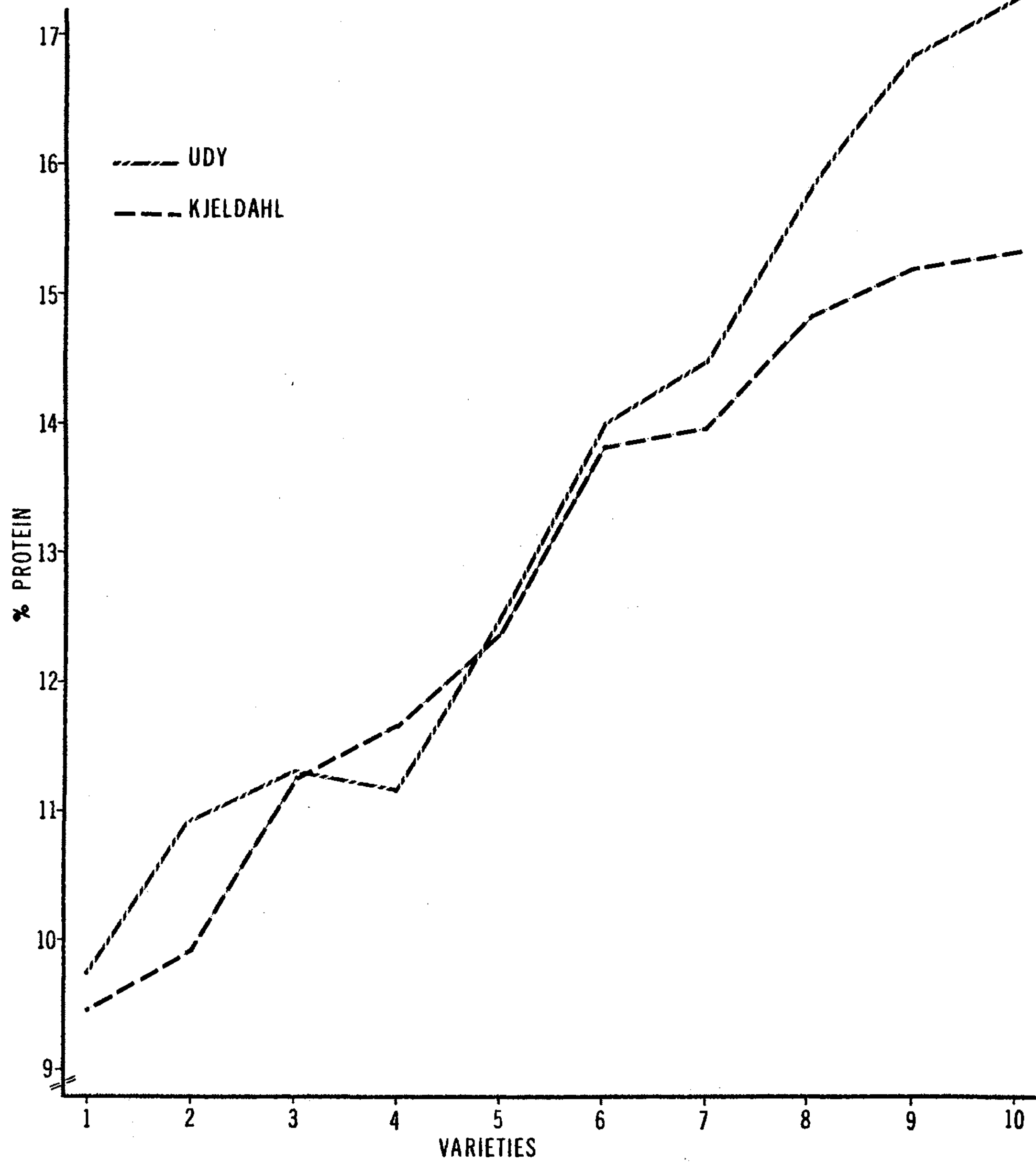

Figure 4. Comparison of Percent Protein by Udy and Kjeldahl on Ten Varieties of Red Grain

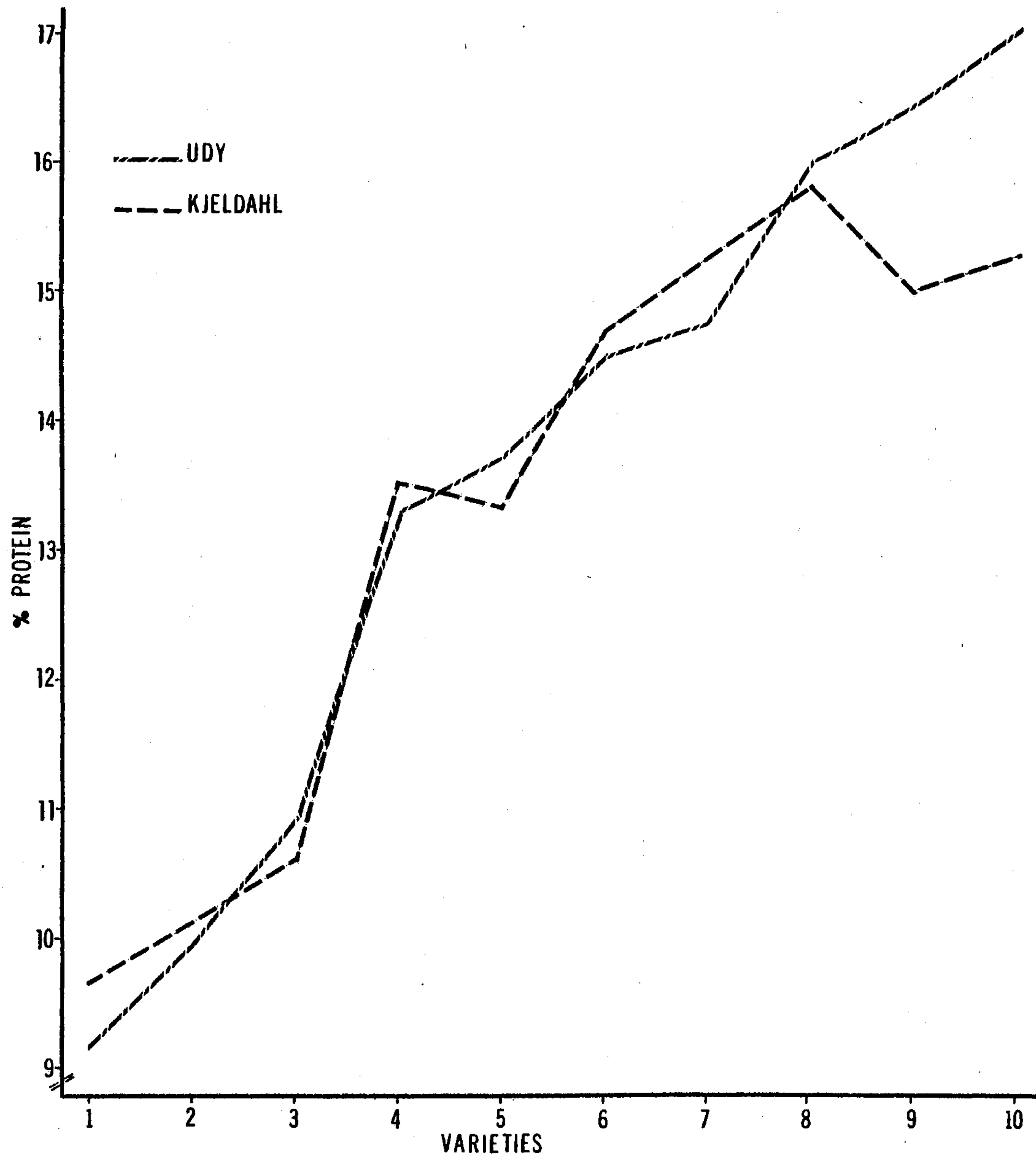

Figure 5. Comparison of Percent Protein by Udy and Kjeldahl on Ten Varieties of White Grain

TABLE III

ANALYSIS OF VARIANCE OF PERCENT PROTEIN OF WHITE AND RED GRAIN WITH METHODS POOLED

Source	d.f.	Mean Squares	
		White	Red.
Corrected Total	39	6.1479	5.9690
Reps (R)	1	0.0578	0.0137
Methods (M)	1	0.6605	2.7773*
Error (a)	2	0.1355	0.0447
Varieties (V)	9	25.9265**	24.8463**
M X V	9	0.5939**	0.6644**
Error (b)	18	0.0086	0.0183
C.V.		2.7337	1.6201

*Significant at 0.05 level of probability.

**Significant at 0.01 level of probability.

TABLE IV

ANALYSIS OF VARIANCE OF PERCENT PROTEIN BY UDY AND KJELDAHL WITH COLORS POOLED

Source	d.f.	Mean Squares	
		Udy	Kjel.
Reps (R)	1	0.2993	0.0360
Colors (C)	1	0.7840*	3.0250*
Varieties in Color	18	15.6616**	10.3540**
Error	19	0.0181	0.0087
C.V.		1.0001	0.7142

*Significant at 0.05 level of probability.

**Significant at 0.01 level of probability.

of exactly the same protein percentage, throughout the range used, were not available. A red and white variety was matched at each protein level as nearly as possible, but the statistical analysis picked up varietal difference as well as color difference, if any existed. Table III indicates that there is a better fit between the two methods on white varieties but again, variation at the high protein levels seems more important. If a color effect is present it is probably minor and relatively unimportant when the Udy method is used for routine screening of breeding material.

CHAPTER VI

SAMPLE WEIGHT AND GRAIN COMPOSITION AS RELATED TO PROTEIN DETERMINATION

A desirable protein test must be able to accurately measure protein percentage over the wide range normally encountered in sorghum grain. To do this, the test must be able to detect quantitative differences in protein content within standard sized samples. Therefore, a good protein test should give a linear relationship as sample size and thus total protein content is increased or decreased. If this linear relationship does not exist, the test will not be able to accurately detect variation in protein percentage within widely diversified lines or varieties.

Protein determination by Udy or Kjeldahl is dependent upon an average or standard amino acid composition of the grain. Shoup, *et al.* (32) indicated that endosperm, germ, and bran differ considerably in their amino acid composition. This suggests that any variation in the relative percent of endosperm, germ, and bran composing the whole grain, such as might occur because of shrunken or immature grain, could produce abnormal protein determinations. This study was conducted to (a) determine if a linear relationship does exist for protein content and sample weight and (b) to determine the effect of changing the relative composition of the sample to be analyzed.

Materials and Methods

A large sample of Wheatland grain sorghum consisting of several hundred grams was selected for use in this study. A portion of this sample was pearled, using a hand operated barley pearler, and the resulting bran was collected and ground in the cyclone mill. This bran consisted of the entire seed coat as well as a portion of the germ which was also removed during the pearling process. The remaining portion of the whole grain sample was also ground.

In order to simulate variation in grain composition, three different mixtures were prepared by blending bran and whole ground grain. Mixture one consisted of the whole ground grain with no bran added. Mixture two consisted of one part bran to four parts whole ground grain. Mixture three consisted of one part bran to one part whole ground grain. Five sample weights, each differing by .05 grams, were analyzed for each mixture in order to determine if a linear relationship existed. Protein determinations were made by both Udy and Kjeldahl. The addition of bran was expected to increase protein percentage, therefore the range in sample weight for each mixture was altered so that the range in total protein content would fall within that normally encountered in sorghum grain. Mixture one ranged in sample weight from 1.10 to 1.30 grams, mixture two from .90 to 1.10 grams, and mixture three from .70 to .90 grams.

Variation in composition can occur naturally because of shrunken or immature grain produced under environmental stress. In order to determine the effect of this condition, six samples of immature grain due to drought were analyzed for protein. Each sample was placed on a sizing screen and the largest and smallest seed in each sample were

collected and ground. Seed size varied among varieties but in each case there was considerable difference between largest and smallest seed. A standard one gram sample of these varieties was used for protein determination.

Results and Discussion

Results from Chapter IV suggested that a change in kernel composition would produce a significant difference in percent protein as determined by Udy and Kjeldahl. In this study, the results of both artificial and natural changes in kernel composition were investigated. The results of an artificial change in kernel composition are shown in Figure 6. Sample weight was plotted against total protein for the three different grain-bran mixtures described previously. It is evident that the protein to sample weight relationship for each method is linear regardless of the mixture. This means that both methods can accurately detect quantitative protein differences. However, it is obvious that the regression lines for Udy and Kjeldahl are not parallel, indicating that the linear relationship is not the same for both methods. A standard one gram sample of mixture one (whole ground Wheatland grain) gave a Udy determination of 9.17% protein and a Kjeldahl determination of 9.15% protein for almost perfect correlation. However, as sample weight was increased, total protein by Udy increased at a faster rate than total protein by Kjeldahl. The slope for the Udy regression line was 0.7940 as compared to a slope of 0.4275 for Kjeldahl for a difference of 0.3765.

A standard one gram sample of mixture two (1 part bran: 4 parts whole ground grain) gave a Udy determination of 12.96% protein and a

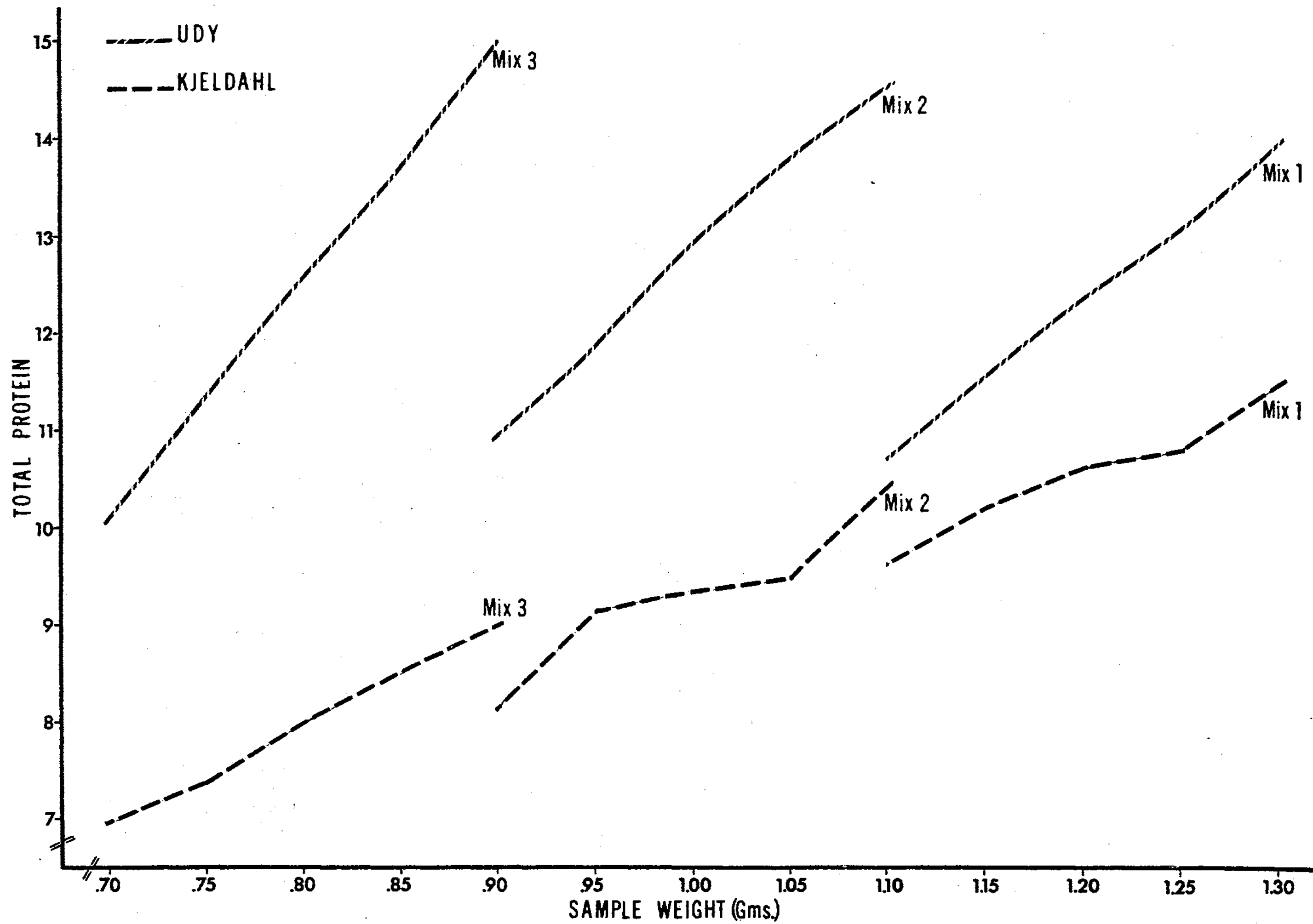

Figure 6. The Effect of Mixture and Sample Weight on Protein Determinations by Udy and Kjeldahl on Three Bran-Grain Mixtures With Five Sample Weights per Mixture

TABLE V

ANALYSIS OF VARIANCE OF PERCENT PROTEIN BY UDY AND KJELDAHL ON BRAN-GRAIN MIXTURES

Source	d.f.	Mean Squares	
		Udy	Kjel.
Corrected Total	29	2.0865	1.6811
Reps (R)	1	0.0080	0.2521
Mixtures (M)	2	0.6140**	16.9187**
Error (a)	2	0.0051	0.0941
Sample Wts. (S)	4	14.3005**	3.4578**
S X M	8	0.2549**	0.0529*
Error (b)	12	0.0018	0.0182
C.V.		0.5672	3.3038

*Significant at 0.05 level of probability.

**Significant at 0.01 level of probability.

Kjeldahl determination of 9.35% protein. The addition of 20% bran produced an increase of .20% Kjeldahl protein compared to a corresponding Udy increase of 3.79% protein. When sample weight of mixture two was increased from .90 to 1.10 grams, the slope of the Udy regression line was 1.6349 as compared to a slope of 0.4725 for Kjeldahl for a difference of 1.1624.

A standard one gram sample of mixture three (1 part bran: 1 part whole ground grain) gave a Udy determination of 17.04% protein as compared to a Kjeldahl determination of 9.90% protein. The addition of 50% bran produced an increase in Kjeldahl protein of .75% compared to a

corresponding Udy increase of 7.97% protein. An increase in sample weight of mixture three from .70 to .90 grams produced a Udy regression line with a slope of 2.9890 as compared to a slope of 0.5250 for Kjeldahl for a difference of 2.4640.

These results leave little doubt that a fixed kernel composition is essential for good correlation between Udy and Kjeldahl. There is also a strong indication that correlation between the methods is good only at or near a standard sample weight. It is impossible to say that one method gives wrong determinations and the other gives correct determinations, but several factors favor the Kjeldahl method. An extrapolation of the Kjeldahl regression line crosses the zero sample weight very close to the zero percent protein point, but this is not true of Udy. The large increase in protein percentage, which Udy indicates as a result of bran additions, is not substantiated by nutritive studies or other protein tests. It seems reasonable to conclude that Udy is overestimating protein percentage of samples with greater than normal bran percentage.

Several varieties of immature grain were analyzed by Udy and Kjeldahl to determine if naturally altered samples would produce the same results as the artificial blends. Fifty to one hundred grams of each variety were sized and a sample of the smallest and largest grain in each variety was analyzed. The small seed were very badly shrunken and malformed and even the large seed were shrunken to an extent and were not normal well developed seed. The results of this comparison are presented in Table VI. The smallest grain in each variety gave higher determinations by Udy than by Kjeldahl in all cases. When the largest grain in each variety was compared, the determinations by Udy

were closer to Kjeldahl and one variety (OK 6105) gave a higher Kjeldahl value. There was a mean difference between Udy and Kjeldahl of almost 2.0% protein when the smallest grain in each variety was analyzed but a mean difference of less than 1.0% protein when the largest grain in each variety was analyzed. These data suggest a significant difference between methods due to grain size within the same sample. It also adds further evidence as to the importance of kernel composition to good correlation between Udy and Kjeldahl.

TABLE VI

COMPARISON OF PERCENT PROTEIN BY UDY AND KJELDAHL ON SAMPLES OF IMMATURE GRAIN

Variety	Smallest Grain in Sample		Largest Grain in Sample	
	Udy	Kjel.	Udy	Kjel.
ROKY 47	16.03	14.00	14.89	14.05
OK 6821	15.06	12.58	13.48	12.00
AKS 614	15.06	12.50	13.60	12.45
Martin	15.40	14.33	14.08	13.45
OK 6105	15.62	14.45	14.08	14.10
WD 6709	14.89	12.28	13.60	12.00
AWD 4 X RWD 10	14.08	13.00		
OK 64189	14.26	13.60		
AWD 18 X RWD 13	14.38	12.50		

CHAPTER VII

CONSTRUCTION OF A GRAIN SORGHUM CONVERSION CHART

The protein molecules of each crop species have a characteristic amino acid composition which cause each crop to have different dye-binding characteristics when the Udy method is used for protein determination. The dye-binding characteristics of a given crop are related to Kjeldahl protein by means of a conversion chart prepared by plotting the colorimeter readings (converted to dye concentration) against percent protein as determined by Kjeldahl. The chart is then used to convert colorimeter readings (dye concentration) to percent protein. If proper care is taken in preparation of the chart, very close correlation with Kjeldahl is achieved on most samples. However, no allowance is made for variation in protein composition within a given crop species, therefore samples which vary from a normal protein composition will produce abnormal determinations. Previous results suggest that sorghum grain may have greater variation in protein composition than wheat, rice, and some other cereals where the dye-binding method has been demonstrated to be well correlated with Kjeldahl over a wide protein range.

The purpose of this study was to construct a conversion chart for sorghum grain which will give better correlation with Kjeldahl over the range of protein encountered in grain sorghum.

Materials and Methods

The sorghum grain used in this study consisted of 19 representative samples which covered the protein range normally encountered in sorghum grain. Udy colorimeter readings were made for all samples and these readings were converted to dye concentrations (unbound dye remaining after reaction with protein). Dye concentration at a given colorimeter reading has been determined by Udy and is a constant value when a fixed volume (40 ml) of dye is added to each sample. Kjeldahl protein determinations were made for each sample as previously described. A linear regression line was fitted using Kjeldahl protein values as the independent variables and predicting the corresponding Udy concentration values for each sample. The means of two trials for each sample were plotted with percent protein as determined by Kjeldahl on the abscissas and the predicted Udy concentrations on the ordinate.

Results and Discussion

The relationship between Udy protein and Kjeldahl protein for several products, notably wheat and milk, has been shown to be linear and well correlated over the entire protein range normally encountered. However, this study has not shown a similar relationship for sorghum grain. Below 15 to 16% protein, the correlation is good and the relationship is linear, but above 15% protein the points are scattered and the correlation is poor.

The standard conversion chart developed by Udy overestimated protein percentage as determined by Kjeldahl in all samples over 16% and many samples between 14 and 16% protein. Regression analysis of the 19 samples used in this study indicated that a linear regression line was

adequate to relate Kjeldahl protein to Udy dye concentration (colorimeter readings). Figure 7 shows the regression line produced by plotting standard Kjeldahl protein against predicted Udy dye concentrations. Using this graph, a conversion chart was prepared relating colorimeter readings to protein percentage for sorghum grain. The conversion chart developed for sorghum grain is presented in Table VII. Because of the observed variations at the higher protein levels in sorghum grain, the correlation between Udy and Kjeldahl protein determinations cannot be expected to be as close as indicated for some other products. However, the conversion chart presented here should give adequate correlation for use in preliminary screening of breeding material.

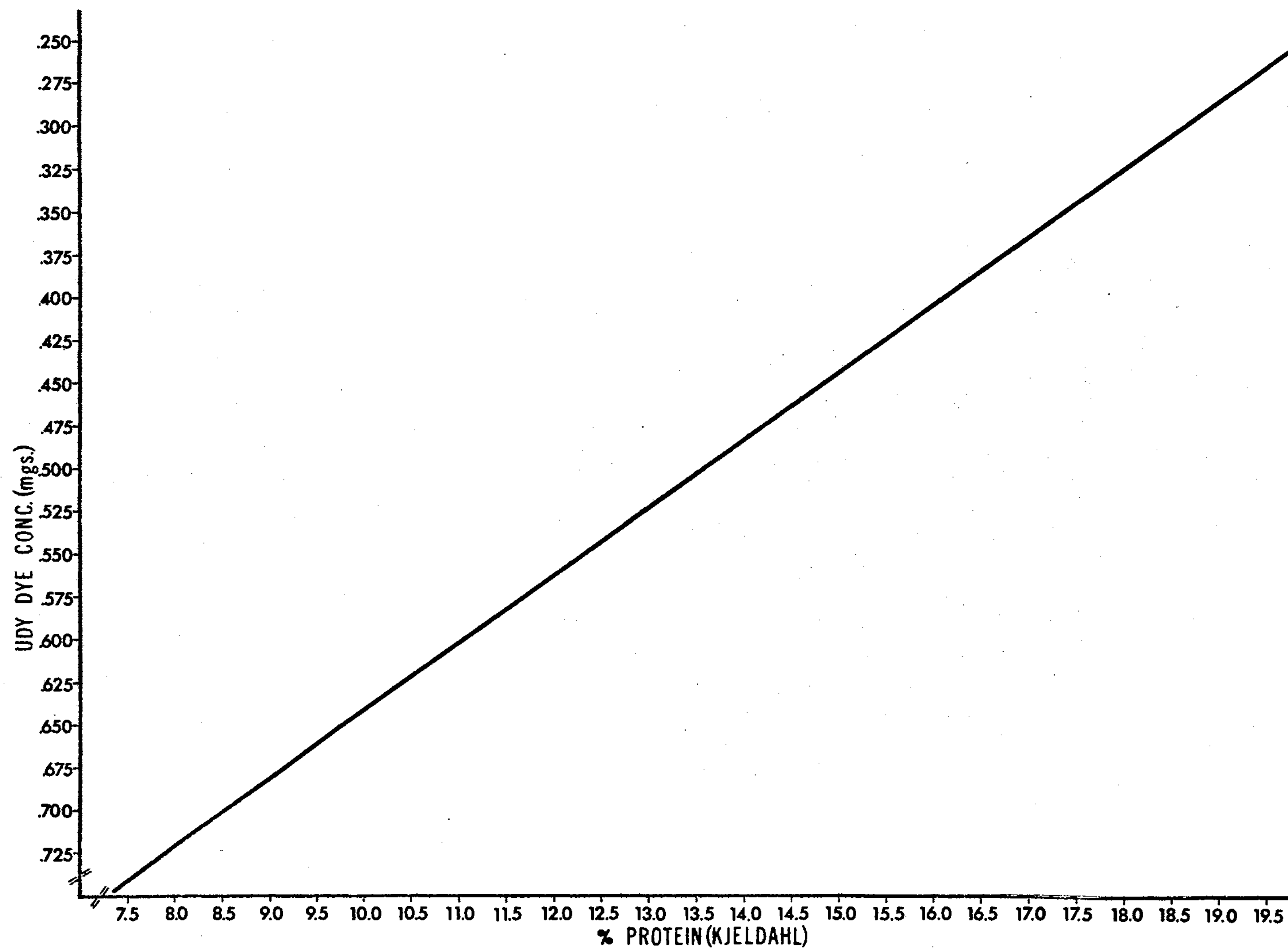

Figure 7. Linear Regression of Percent Kjeldahl Protein on Unbound Udy Dye Concentration

TABLE VII

STANDARD CONVERSION CHART FOR GRAIN SORGHUM

Protein	UIR	Conc.	Protein	UIR	Conc.
7.03	28.00	0.762	10.37	39.25	0.627
7.10	28.25	0.759	10.43	39.50	0.625
7.21	28.50	0.755	10.50	39.75	0.622
7.27	28.75	0.752	10.56	40.00	0.620
7.38	29.00	0.748	10.62	40.25	0.617
7.47	29.25	0.745	10.69	40.50	0.615
7.58	29.50	0.741	10.75	40.75	0.612
7.63	29.75	0.738	10.82	41.00	0.610
7.72	30.00	0.735	10.87	41.25	0.607
7.81	30.25	0.731	10.94	41.50	0.605
7.85	30.50	0.728	11.00	41.75	0.603
7.95	30.75	0.725	11.05	42.00	0.600
8.02	31.00	0.722	11.11	42.25	0.598
8.13	31.25	0.718	11.17	42.50	0.596
8.20	31.50	0.715	11.24	42.75	0.593
8.26	31.75	0.712	11.29	43.00	0.591
8.34	32.00	0.709	11.33	43.25	0.589
8.43	32.25	0.706	11.41	43.50	0.586
8.50	32.50	0.703	11.46	43.75	0.584
8.57	32.75	0.700	11.50	44.00	0.582
8.63	33.00	0.697	11.57	44.25	0.579
8.70	33.25	0.694	11.62	44.50	0.577
8.79	33.50	0.691	11.68	44.75	0.575
8.87	33.75	0.688	11.74	45.00	0.573
8.95	34.00	0.685	11.81	45.25	0.570
9.00	34.25	0.682	11.86	45.50	0.568
9.07	34.50	0.679	11.92	45.75	0.566
9.17	34.75	0.676	11.95	46.00	0.564
9.25	35.00	0.673	12.00	46.25	0.562
9.30	35.25	0.670	12.05	46.50	0.560
9.37	35.50	0.667	12.11	46.75	0.557
9.44	35.75	0.665	12.18	47.00	0.555
9.50	36.00	0.662	12.24	47.25	0.553
9.57	36.25	0.659	12.29	47.50	0.551
9.67	36.50	0.656	12.32	47.75	0.549
9.70	36.75	0.654	12.37	48.00	0.547
9.79	37.00	0.651	12.43	48.25	0.545
9.87	37.25	0.648	12.49	48.50	0.543
9.94	37.50	0.646	12.54	48.75	0.541
9.99	37.75	0.643	12.57	49.00	0.539
10.06	38.00	0.640	12.62	49.25	0.537
10.11	38.25	0.638	12.68	49.50	0.535
10.19	38.50	0.635	12.74	49.75	0.533
10.25	38.75	0.632	12.79	50.00	0.531
10.30	39.00	0.630	12.82	50.25	0.529

TABLE VII (Continued)

Protein	UIR	Conc.	Protein	UIR	Conc.
12.87	50.50	0.527	15.65	66.50	0.417
12.94	50.75	0.525	15.74	67.00	0.413
13.00	51.00	0.523	15.81	67.50	0.410
13.05	51.25	0.521	15.89	68.00	0.407
13.08	51.50	0.519	15.95	68.50	0.404
13.12	51.75	0.517	16.04	69.00	0.401
13.19	52.00	0.515	16.11	69.50	0.398
13.24	52.25	0.513	16.19	70.00	0.395
13.29	52.50	0.511	16.25	70.50	0.392
13.33	52.75	0.509	16.32	71.00	0.389
13.37	53.00	0.507	16.41	71.50	0.386
13.42	53.25	0.505	16.45	72.00	0.384
13.45	53.50	0.504	16.54	72.50	0.381
13.49	53.75	0.502	16.61	73.00	0.378
13.55	54.00	0.500	16.70	73.50	0.375
13.60	54.25	0.498	16.75	74.00	0.373
13.66	54.50	0.496	16.82	74.50	0.370
13.70	54.75	0.494	16.89	75.00	0.367
13.75	55.00	0.492	16.95	75.50	0.364
13.80	55.25	0.490	17.02	76.00	0.362
13.82	55.50	0.489	17.08	76.50	0.359
13.87	55.75	0.487	17.17	77.00	0.356
13.93	56.00	0.485	17.25	77.50	0.353
13.99	56.25	0.483	17.30	78.00	0.351
14.01	56.50	0.482	17.37	78.50	0.348
14.06	56.75	0.480	17.44	79.00	0.346
14.10	57.00	0.478	17.50	79.50	0.343
14.12	57.25	0.477	17.55	80.00	0.341
14.19	57.50	0.473	17.63	80.50	0.338
14.25	57.75	0.473	17.69	81.00	0.336
14.30	58.00	0.471	17.75	81.50	0.333
14.36	58.50	0.468	17.80	82.00	0.331
14.45	59.00	0.464	17.87	82.50	0.328
14.55	59.50	0.461	17.95	83.00	0.325
14.61	60.00	0.458	18.00	83.50	0.323
14.70	60.50	0.454	18.06	84.00	0.320
14.81	61.00	0.451	18.13	84.50	0.317
14.85	61.50	0.448	18.19	85.00	0.315
14.93	62.00	0.445	18.25	85.50	0.312
15.05	62.50	0.441	18.31	86.00	0.310
15.12	63.00	0.438	18.38	86.50	0.307
15.19	63.50	0.435	18.45	87.00	0.304
15.25	64.00	0.432	18.50	87.50	0.302
15.32	64.50	0.429	18.57	88.00	0.299
15.42	65.00	0.426	18.67	88.50	0.296
15.49	65.50	0.423	18.70	89.00	0.294
15.56	66.00	0.420	18.79	89.50	0.291

TABLE VII (Continued)

Protein	UIR	Conc.
18.82	90.00	0.289
18.90	90.50	0.287
19.00	91.00	0.283
19.06	91.50	0.280
19.12	92.00	0.278
19.20	92.50	0.275
19.25	93.00	0.272
19.31	93.50	0.270
19.34	94.00	0.267
19.43	94.50	0.265
19.50	95.00	0.262

CHAPTER VIII

SUMMARY AND CONCLUSIONS

The objective of this study was to determine the suitability of the Udy dye-binding method for use in a grain sorghum protein improvement program. The Kjeldahl and Udy methods were compared on corresponding grain samples under a variety of conditions. The effects of reaction time, grain condition and age, grain color, and kernel composition were determined.

The Udy reaction was found to require an average of four hours shaking time for completion. Some varieties reached equilibrium in two hours while others were still binding dye at the end of five hours reaction time. Digestion time in the case of Kjeldahl was not critical and was essentially complete for sorghum grain at the end of one hour.

The dye binding method was found to be extremely sensitive to pearling. The removal of the seed coat caused a reduction in percent protein by as much as 48% with the Udy analysis. The Kjeldahl method was much less sensitive to pearling with some samples showing a slight increase in percent protein and others a slight decline. There was no indication that age of the ground grain at time of analysis had any effect on protein determination by either method.

Grain color could not be shown to cause a statistical difference in protein determination using the dye-binding method. There still exists the possibility of a slight color interaction, but it would

probably be unimportant when using the method for screening analysis. The Udy and Kjeldahl methods were well correlated in the low and medium protein ranges, but the correlation progressively decreased as protein percentage of the sample increased above about 15%. The dye-binding method gave consistently higher determinations in the high protein range.

The effect of kernel composition on protein analysis was investigated and results indicated that a fixed kernel composition was essential for good correlation of the dye-binding method with the Kjeldahl method. When bran content of the sample was increased, the Udy method gave consistently higher determinations than the Kjeldahl method. The relationship between sample weight and total protein was linear for both methods indicating good detection of quantitative differences in protein content.

A grain sorghum conversion chart was constructed in order to better correlate Udy colorimeter readings with Kjeldahl protein, especially in the high range. The dye-binding method is faster, easier, and less expensive than the Kjeldahl method, but it is also much more sensitive to variation in molecular structure of the protein, and thus perhaps less accurate. However, the method does have the ability to detect a qualitative difference in protein because lysine, an essential amino acid, is one of the three amino acids used to estimate protein percentage. It would seem that the dye-binding method has definite advantages as a preliminary screening method in a protein improvement breeding program.

LITERATURE CITED

1. Apichatabootra, Apichai. 1970. Estimation of protein content in F_2 segregating populations of sorghum by the Udy dye-binding method. (unpub. M.S. theses, Oklahoma State University).

2. Ashworth, U.S., Rupert Seals, and R.E. Erb. 1960. An improved procedure for the determination of milk proteins by dye-binding. J. Dairy Sci. 43:613-623.

3. Banasik, Orville J., and K.A. Gilles. 1962. A new protein method. Cereal Sci. Today. 7:28-30.

4. Bunyan, J. 1959. Orange-G binding as a measurement of protein content. J. Sci. Food Agr. 10:425-430.

5. Burleson, C.A., W.R. Cowley, and G. Otey. 1956. Effect of nitrogen fertilization on yield and protein content of grain sorghum in the lower Rio Grande Valley of Texas. Agron. J. 48:524-525.

6. Campbell, A.R., and R.C. Pickett. 1968. Effect of nitrogen fertilization on protein quality and quantity and certain other characteristics of 19 strains of Sorghum bicolor (L.) Moench. Crop. Sci. 8:545-547.

7. Chapman, S.R., and F.H. McNeal. 1970. Gene effects for grain protein in five spring wheat crosses. Crop Sci. 10:45-46.

8. Collins, F.C., and R.C. Pickett. 1968. Protein quality and yield studies. Sorghum Newsletter. 11:61-62.

9. Deyoe, C.W., and J.A. Shellenberger. 1965. Amino acids and proteins in sorghum grain. J. Agr. Food Chem. 13:446-450.

10. Fraenkel-Conrat, Heinz, and Mitzi Cooper. 1944. The use of dyes for the determination of acid and basic groups in proteins. J. Biol. Chem. 154:239-246.

11. Geddes, W.F., and Nancy Milton. 1939. The analytical error of the Kjeldahl nitrogen test. Cereal Chem. 16:392-404.

12. Heller, V.G., and John B. Sieglinger. 1944. Chemical composition of Oklahoma grain sorghum. Okla. Agr. Expt. Sta. Bul. B-274.

13. Hubbard, J.E., H.H. Hall, and F.R. Earle. 1950. Composition of the component parts of the sorghum kernel. Cereal Chem. 27:415-420.

14. Jacobs, Morris B. The chemical analysis of foods and food products. D. Van Nostrand Company, Inc. Princeton, N.J.

15. Johnson, V.A., J.W. Schmidt, and P.J. Mattern. 1968. Cereal breeding for better protein impact. Econ. Bot. 22:16-25.

16. Karper, R.E., and J.R. Quinby. 1947. Sorghum-Its production, utilization and breeding. Econ. Bot. 1:355-371.

17. Laible, C.A., W.C. Wilcox, Erric Ross, and Myron Kiesewether. 1969. Estimating lysine content in Zea mays utilizing a Udy protein analyzer. Agron. Abst. pp. 60.

18. Lehninger, Albert L. 1970. Biochemistry. Worth Publishers, Inc., New York, New York. pp. 69-71.

19. MacKenzie, Arnold J., and Eugene R. Perrin. 1969. Colorimetric determination of protein in feed and forage crops. Agron. J. 61:332.

20. Maranville, J.W. 1969. Effects of simazine on certain aspects of nitrogen metabolism in sorghum. Agron. Abst. pp. 27.

21. Miller, G.D., C.W. Deyoe, T.L. Walter, and F.W. Smith. 1964. Variations in protein levels in Kansas sorghum grain. Agron. J. 56:302-304.

22. Mossburg, R. 1968. Evaluation of protein quality and quantity by dye-binding capacity: A tool in plant breeding. New Approaches to Breeding for Improved Plant Protein. pp. 151-160.

23. Neill, Claude D. 1962. The Kjeldahl protein test. Cereal Sci. Today. 7:6-12.

24. Parial, Lucila C., and L.W. Rooney. 1970. Use of dye-binding and biuret techniques for estimating protein in brown and milled rice. Cereal Chem. 47:38-43.

25. Pickett, R.C. 1968. Inheritance and improvement of protein quality and content in Sorghum vulgare. Pers. Report No. 4. Contract No. 1175, U.S. Agency for International Development, Dept. of State with Purdue Res. Foundation, Lafayette, Ind.

26. Pickett, R.C. 1968. Sorghum protein quantity and quality. Proceedings of the Twenty-Third Annual Corn and Sorghum Research Conference. pp. 138-140.

27. Rachie, K.O. 1969. Sorghum grain: Its worldwide significance and potential. Cereal Sci. Today. 14:271-276.

28. Rooney, L.W., and L.E. Clark. 1968. The chemistry and processing of sorghum grain. Cereal Sci. Today. 13:259-265.

29. Rooney, L.W., and R.D. Sullins. 1970. Chemical, physical, and morphological properties of diploid and tetraploid *Sorghum bicolor* (L.) Moench kernels. Crop Sci. 10:97-99.

30. Skoch, L.V., C.W. Deyoe, F.K. Shoup, J. Bathurst, and D. Liang. 1970. Protein fractionation of Sorghum grain. Cereal Chem. 47:472-481.

31. Schmidt, Carl L.A. 1944. The chemistry of the amino acids and proteins. Charles C. Thomas, Springfield, Ill. pp. 729.

32. Shoup, F.K., C.W. Deyoe, J. Campbell, and D.B. Parrish. 1969. Amino acid composition and nutritional value of milled sorghum grain products. Cereal Chem. 46:164-171.

33. Snedecor, George W., and William G. Cochran. 1969. Statistical methods. The Iowa State Univ. Press, Ames, Iowa.

34. Tkachuk, R. 1969. Nitrogen-to-protein conversion factors for cereals and oil seed meals. Cereal Chem. 46:419-423.

35. Udy, C. Doyle. 1954. Dye-binding capacities of wheat flour protein fractions. Cereal Chem. 31:389-395.

36. Udy, C. Doyle. 1956. Estimation of protein in wheat and flour by ion-binding. Cereal Chem. 33:190-197.

37. Udy, C. Doyle. Protein analyzer model S operating instructions. Udy Analyzer Co., Boulder, Colo.

38. Virupaksha, T.K., and L.V.S. Sastry. 1968. Studies on the protein content and amino acid composition of some varieites of grain sorghum. J. Agr. Food Chem. 16:199-203.

39. Wall, J.S., and C.W. Blessin. 1969. Composition and structure of sorghum grains. Cereal Sci. Today. 14:264-270.

40. Worker, George F. Jr., and Joseph Ruckman. 1968. Variations in protein levels in grain sorghum grown in the southwestern desert. Agron. J. 60:485-488.

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Thesis: A COMPARISON OF THE UDY DYE-BINDING AND KJELDAHL PROCEDURES FOR PROTEIN ANALYSIS OF GRAIN SORGHUM, *Sorghum bicolor* (L.) Moench

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