

Methods of Protein Analysis and Variation in Protein Results

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Premiums on high-protein hard red spring wheat has created much interest in the protein test. The Kjeldahl method, a chemical procedure for nitrogen, is still the basic method used for protein analysis. The Kjeldahl method, the Udy dye binding method and the new infrared reflectance method for determining protein are described in this paper. In the analysis of wheat protein by the Kjeldahl method, the maximum amount of variance in results expected between different laboratories and within the same laboratory is larger than the increments of protein on which price premiums have been paid. The causes of protein variance and ways of reducing the variance are discussed.

In the past year, the price of hard red spring wheat has been substantially influenced by the protein content. Price premiums have been paid on increased increments of 0.1 percentage points of protein. The price expected for wheat on the basis of protein as determined by a North Dakota laboratory sometimes is changed because the protein found later at Minneapolis or Duluth differs more than the protein increment required for premium. With barley, an unexpected discount may be applied against the seller because the protein is found to be over the discount protein level at the marketing point. This paper discusses the protein test, how much variance should be expected between laboratories, and the causes of protein variance.

Protein Methods

1. **Kjeldahl Nitrogen Method.** Amino acids are the building blocks of protein, and they contain carbon, hydrogen, oxygen, sulfur and nitrogen (Table 1). In the basic method for protein, nitrogen is determined by a method of chemical analysis known as the Kjeldahl procedure. The nitro-

Table 1. Basis of Kjeldahl Protein Procedure.

1. Protein made of amino acid building blocks.
 2. Amino acids made of carbon, hydrogen, oxygen, sulfur, nitrogen.
 3. Protein calculated from measurement of nitrogen.
 4. Per cent nitrogen $\times 5.7 =$ per cent crude protein of wheat.
Per cent nitrogen $\times 6.25 =$ per cent protein of other grains.
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gen found, times a factor of 5.7 for wheat and 6.25 for other grains, gives the content of crude protein. Other protein methods like the Udy or the new infrared reflectance method are calibrated against the basic Kjeldahl method. The steps for the Kjeldahl protein determination are given in Table 2. This procedure requires the use of boiling concentrated sulfuric acid and strong caustic.

2. **Udy Dye Binding Method.** In this procedure, ground grain is shaken with an orange dye solution. This acid dye forms an insoluble dye-protein complex with the basic amino acid building blocks of the protein. The dye-protein complex reduces

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Table 2. Major Steps in Kjeldahl Protein Analysis.

1. Grind and weigh 1 gram ($\sim 1/28$ oz.) grain.
2. Digest in boiling concentrated sulfuric acid to convert protein nitrogen to ammonia (NH_3).
3. Add 50 per cent caustic to make ammonia volatile.
4. Boil off ammonia and collect by distillation.
5. Measure amount of ammonia collected by titration.

the amount of dye left in solution, the complex is removed by filtering, and the concentration of dye in solution is determined with a color measuring instrument. The amount of dye left in solution is inversely related to the protein content of the sample.

3. Infrared Reflectance Method. In 1971, instruments using infrared reflectance (Neotec and Infraalyzer) were introduced into the grain trade to measure protein, moisture and oil. In this method, infrared light of different wave lengths (obtained by filters) is directed onto a sample of ground grain in a cell (Figure 1). The light reflected from the grain depends upon the chemical composition of the grain, and the reflected light at different wave lengths is measured by a photo-cell detector. The response of the detector is fed into a small computer which calculates complex equations to obtain the protein, moisture or oil content of the sample. The instruments should gain rapid use in the grain trade, provided the accuracy is near that of the Kjeldahl method now used in protein laboratories.

Variance Expected In Protein Result

Discussion of protein variance will be confined mostly to the basic Kjeldahl procedure, because almost all protein laboratories are still using this method. Protein for samples in the grain trade are obtained from a single analysis on each sample. With a single analysis there is probability that occasionally there will be a wide variation from the actual value. More than one analysis on each sample would reduce this number of variations.

Data shown in Table 3 indicate how much variance in protein results should be expected between laboratories. Wheat flour samples were analyzed on a monthly basis by some 50 different laboratories in the United States. Wheat grain samples were also analyzed on a monthly basis by 13 different laboratories in the spring wheat area of the United States. The standard deviation, as a measure of deviation from the average value, was

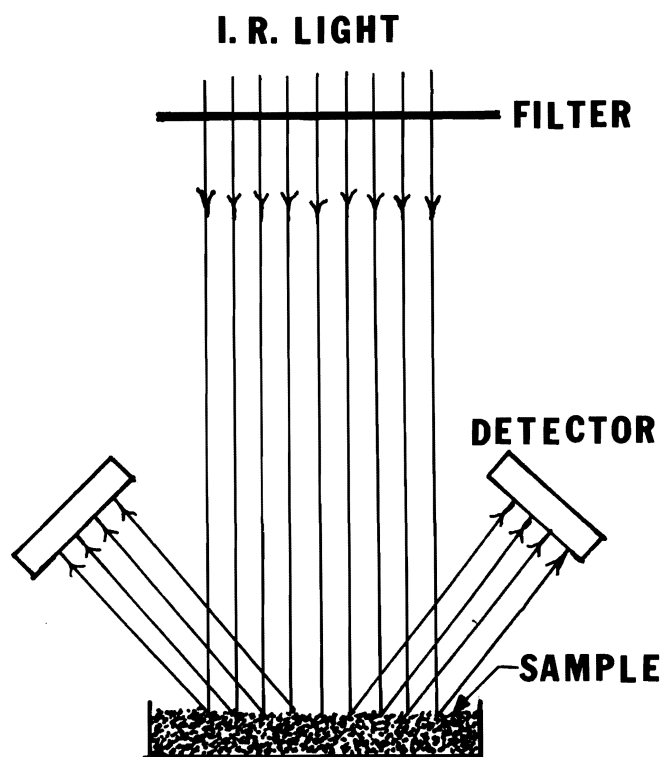


Figure 1. Illustration of infrared reflectance method for protein analysis.

lower for flour than wheat grain. The step of grinding the wheat sample by each laboratory should have caused at least a part of the higher variation for wheat. Another factor is the uneven distribution of protein found in ground wheat, which will be discussed later.

Table 3. Variance in Protein Results Between Laboratories on Samples Analyzed Monthly.

Sample	Number samples	Range in Protein %	Average standard deviation ³ %
Wheat flour (1975) ¹	12	11-12	0.14
Wheat grain (1975) ²	10	12-18	0.16

¹Monthly check sample from the American Association of Cereal Chemists analyzed by some 50 laboratories.

²Monthly check sample of wheat grain from Ingman Laboratories, Inc., analyzed by 13 laboratories.

³Standard Deviation - a measure of the deviation from the average value expressed as percentage protein.

Normal variation expected in protein results where there is a standard deviation of 0.16 per cent is given in Table 4. There would be a 67 per cent probability that a laboratory doing a single protein analysis would obtain a value deviating from the actual value by one $\pm$ standard deviation (SD) unit. For example, for a wheat of 14.0 per

Table 4. Expected Variance in a 14 Per Cent Protein Wheat Having a 0.16 Standard Deviation.

Probability %	Expected protein range %
67	13.84-14.16 (± 0.16)
95	13.68-14.32 (± 0.32)
99.74	13.52-14.48 (± 0.48)

cent protein, the value could be between 13.84 and 14.16 per cent protein (14.0 ± 0.16) 67 per cent of the time. There would also be, for a single analysis, a 95 per cent probability that the value would be within ± 2 S.D.; wheat of 14.0 per cent protein could show a range from 13.68 to 14.32 per cent protein (14.0 ± 0.32). What about the 5 per cent of the time when the variation is greater than ± 2 S.D.? There is a 0.26 per cent probability that the variation would be greater than ± 3 S.D. or for wheat, a variation greater than 0.48 percentage protein from the actual value. This wide variation can be expected in about one out of every 400 analyses. These variances in protein between protein laboratories is the reason why there can be differences in the total wheat price when premiums are paid on 0.1 per cent increments of protein.

The protein variance that can be expected in the same laboratory is indicated by the data presented in Table 5. The variance is lower than between different laboratories (Table 3), but it would be enough to cause some problems in price when premiums are paid on increments of 0.1 percentage protein.

Table 5. Variance in Protein Results in a Protein Laboratory at North Dakota State University.

Sample	Number analysis ¹	Protein content		Avg. %	Standard deviation %
		low %	high %		
Wheat grain ²	15	14.1	14.5	14.4	0.11
Wheat flour	15	14.1	14.3	14.2	0.06

¹A single analysis ran for 15 different days.

²A new lot of the grain sample was ground each day for the analysis.

The standard deviations on wheat grain by three methods of protein analysis as reported by Williams (1,2) are 0.15, 0.30 and 0.17 percentage protein by the Kjeldahl, Udy, and infrared reflectance methods, respectively. These data were obtained between different Canadian laboratories or between different infrared instruments. As shown by Williams (1,2) the new infrared reflectance method appears to be almost as good as the Kjeldahl procedure. Protein variance and suitability

for protein analysis by an infrared instrument is now being studied at North Dakota State University.

Causes of Protein Variance

1. **Sampling.** A number of factors that can cause protein variance are listed in Table 6. Under sampling there are several areas where errors can occur. For example, the 4-5 lb. grading sample obtained by probe from a box car by a sampler may not be of the same protein content as the whole large bulk of wheat. Uneven areas of high and low protein make it difficult to obtain a truly representative protein sample.

Table 6. Factors That Can Affect Protein Results.

1. Sampling.
 - a. Of original grain (4-5 lb).
 - b. Subdividing grain sample (2 lb).
 - c. Sampling grading or submitted sample for grinding (1 oz).
 - d. Sampling ground sample for protein test (1 gram or $\sim 1/28$ oz).
2. Cleanliness of grain.
3. Moisture content of grain.
 - a. Original moisture.
 - b. Moisture loss on grinding.
4. Protein differences in particles of ground grain.
5. Laboratory techniques.

Bin samples brought into the protein laboratory by individuals should also be obtained by proper sampling procedures to get a truly representative sample. The assumption that the protein is exactly the same throughout the bin would be expected to be true only if there is a single variety involved, and the soil, fertilizer availability and moisture availability to the wheat plant were uniform during growth throughout the field or fields in which the wheat was grown. This assumption of even protein throughout the bin may be a major cause of error in submitted samples.

The 4-5 lb. sample obtained by a sampler is divided into two parts on a Boerner divider. One part of the sample is sent to the grading and protein laboratory in North Dakota and the other to the place of marketing. Improper dividing can cause differences in protein. After removing dockage, about a 1 oz. sample is obtained for grinding in the protein laboratory, and 1 gram ($1/28$ oz.) of the ground material is weighed for the protein test. Sampling errors can occur on the 1 oz. sample used for grinding or on the 1 gram sample for the test if there is uneven protein in the larger bulk of wheat from which the sample was taken.

2. **Cleanliness of Grain.** The second factor that might cause protein variance is the cleanliness of the grain (Table 6). The protein content of some dockage material is given in Table 7. Small amounts of high-protein soybeans and wild oats in wheat or barley samples could increase protein results, while low-protein straw and chaff could

Table 7. Protein Content of Dockage and Extraneous Material Found in Grain.

Material	Protein content %
Buckwheat	13
Pigeon grass	14
Soybeans	40
Straw and chaff	3
Wild oats	19

decrease them. The protein of buckwheat and pigeon grass is near that of wheat, so small amounts probably would not have a significant effect on protein. Routinely, grain is cleaned before a sample is taken for grading or a protein analysis.

3. **Moisture Content of Grain.** The third factor that can affect protein results is the moisture content of the grain. The extent of protein change in grain due to differences in moisture is illustrated in Table 8. First, a dry sample of 14.0 per cent protein contains 86 per cent nonprotein dry matter. This same sample with 10.0 per cent moisture now contains only 12.6 per cent protein because the sample has been diluted with water, and for the same reason the sample of 14.0 per cent moisture will contain only 12.1 per cent protein. When large enough moisture changes occur in grain there is also a change in the protein content. For this reason, grain samples to be tested should always be stored in air-tight containers.

Table 8. Protein Content at Different Moisture Levels.

Moisture content %	Per cent of dry sample			Protein content ¹ %
	water %	protein %	nonprotein %	
0	0	14.0	86.0	14.0
10	11	14.0	86.0	12.6
14	16	14.0	86.0	12.1

$$^1\text{Protein content} = \frac{\text{Protein as \% of dry sample}}{\text{Total wt. as \% of dry sample}} \times 100$$

Moisture can also be lost during the grinding of the wheat with resulting changes in protein. Table 9 illustrates the moisture changes with three mills commonly used for grinding grain for protein analysis. The Labconco and Hobart burr mills

Table 9. Moisture Loss on Grinding Wheat Grain for Protein Analysis.

Mill used	12.2% Moisture grain ¹		16.2% Moisture grain ¹	
	Moisture after grinding ² %	Change of protein content %	Moisture after grinding ² %	Change of protein content %
Labconco Burr	12.4	0.0	16.1	0.0
Hobart Burr	12.3	0.0	16.2	0.0
Udy Cyclone ³	10.9	+0.2	13.4	+0.5

¹Determined with the Matomco moisture meter.

²Determined by the 130° C. air-oven method.

³A 0.5 mm. sieve was used in the mill.

are used in most protein laboratories and the Udy cyclone mill for grinding samples for analysis by the Udy method. The two burr mills caused very little moisture loss on grinding wheat, even when the moisture content of the wheat was 16.2 per cent. The Udy cyclone mill caused considerable moisture loss and a change in protein. For example, a sample having an initial 12.2 per cent moisture in the wheat showed a 0.2 percentage increase in the protein content. With the 16.2 per cent moisture wheat, an increase of 0.5 percentage of protein occurred due to moisture loss. Protein changes due to moisture loss should be considered when using the Udy mill or similar type mill.

In the grain market, wheat protein content is expressed on an "as is" moisture basis. As already shown, the protein content on this basis changes with moisture content. The protein can also be expressed on a specific moisture basis so that there is no protein variance with moisture. Barley protein in the grain market is expressed on a dry weight basis (0 per cent moisture in sample). Wheat and flour moisture is expressed in scientific laboratories in the United States on a 14 per cent moisture basis.

4. **Protein Differences in Particles of Ground Grain.** The fourth factor that can affect protein results is protein differences in particles of ground grain (Table 6). The endosperm of grain is not uniform in protein. In hard wheat, Kent (3) has reported a protein content of 8 per cent in the center portion of the endosperm to 43 per cent near the bran layers. He also found that the flour contained material of high and low protein content. One would also expect ground grain to have a mixture of particles that would differ in protein content.

The data of Table 10 shows 3 to 4 percentage points difference in protein content between particles of hard red spring wheat when the Labconco and Hobart burr mills are used. The Udy cyclone mill produced particles that were found to differ only 1 percentage point in protein content. Thus

Table 10. Protein Content of Particles in Ground Hard Red Spring Wheat (Waldron variety).

Mill Used	Particle size, micrometers ¹					
	>1,000	920	670	398	358	223 <149
Labconco Burr Mill						
% Protein	16.6	15.9	14.1	13.7	14.0	14.6
% of grain	3.6	9.4	44.4	7.4	11.3	10.1
Hobart Burr Mill						
% Protein	16.2	15.7	14.5	13.7	13.8	14.2
% of grain	7.5	11.0	36.3	7.3	13.5	11.4
	Particle size, micrometers ¹					
	>420	358	237	<149		
Udy Cyclone Mill						
% Protein	14.5	14.8	14.6	15.5		
% of grain	13.1	60.8	13.7	12.4		

¹Particles of different sizes were separated on sieves.

a sampling error can occur when weighing 1 gram of ground sample for the protein test if the right amounts of different size particles are not selected. The ground sample should be thoroughly mixed before weighing to avoid this type of error.

5. Laboratory Techniques. Poor laboratory techniques can cause protein variance in the Kjeldahl method. These variances can be reduced to a minimum by properly trained and experienced technicians and by a continuous check on the accuracy of the determination. An analysis should be made on check samples each day. Chemical standardization of each new solution or reagent, and frequent checks on the accuracy of instruments and glassware should also be made.

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is true of most scientific research reporting, provided that it is accurate and more or less complete. This is not something that has been "granted" to experiment stations, but has been earned over the years through the witness of experience and satisfied customers—the farmers and ranchers who ultimately use the information.

Information from the Experiment Station is believable and believed. It has withstood the extreme tests of time, experimentation and practical application. It has successfully resisted the efforts of unscientific and non-scientific disbelievers to dent its armor of credibility.

But, we must remember that Experiment Stations are composed of people, too. And they suffer through all of the interpersonal communications problems that the rest of us do, too. The principal difference in their messages as reliable sources of information lies in their use of the methods of science in their search for truth. Once their methods uncover new evidence and new "facts" that indicate what they believe is so, then the

Conclusions

Protein analysis of grain is a complex procedure with many steps where errors can occur. A sampling error can be introduced prior to analysis when sampling the original grain, when an aliquot of the original sample is obtained for grinding, and when weighing the ground grain for the protein test. A change in moisture content of the grain before grinding or during grinding can also introduce an error prior to analysis. High or low protein dockage material in grain can cause a protein variance. Therefore, this material should be removed prior to grinding. Many factors can cause errors during the chemical analysis by the Kjeldahl method. To keep these to a minimum, technicians should be properly trained, and there should be frequent checks made on instruments and glassware for accuracy. Checks should also be made on all new solutions and chemical reagents used. Check samples should be analyzed daily to test the overall accuracy of the determination. Even with the best technician and equipment, a few results can still vary from the true or correct value.

Literature Cited

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communication problem becomes one of penetrating the perceptive shields of their intended audiences with believable and understandable interpretations of that evidence.

In this task, they have the support of a group of skilled interpersonal communication craftsmen whose business it is to help create messages that can attract attention and have meaning for those who can make use of that information. This is a fascinating business. The challenges we constantly meet to use all of the skills and techniques at our command to compose effective messages keeps our work filled with interest and variability.

It is the relative success of our working together to overcome these challenges that has brought about the large measure of credibility and believability that comes along with information based on Experiment Station research. Our future success lies in our ability to keep up with the new human communication technologies and use them to their fullest as our society continuously expands its interests in and uses of interpersonal communication.