

RELATIONSHIP OF PROTEIN CONTENT
OF MATURE WHEAT AND VEGETATIVE GROWTH

by

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INTRODUCTION

With population explosion, one of the major concerns in the world today is the adequacy of the food supply. It has also been reported that one-third of the population are either undernourished, malnourished, or both. Especially protein-calorie malnutrition is the most serious problem in the lesser developed countries(17,25).

Wheat, the most important of the cereal grains, today is the world's largest food crop contributing one-fourth to one-third of the total energy to the human race(48). Considering the nourishment offered by an abundant wheat supply, it is the most economical of foods. A diet consisting of large amounts of wheaten foods and only small amounts of protein from animal sources provides adequate protein nutrition for the human(54). It has been shown that if the entire calorie requirements were supplied by a cereal, wheat alone would provide more than the minimum amount of protein needed. With the limitation of the animal protein production and the urgent need of protein food, it would be impossible to feed the world without the economical grain - wheat.

Like other living organisms, wheat, a living thing is readily affected by numerous factors during its growth and development. The main factors which influence wheat quality are climate, soil and variety. These factors are interdependent. They may influence each other. However, sometimes a single factor may be so strong that it minimizes the effects of the others. For instance, the lack of moisture at critical periods of growth may have a stronger influence on quality than varietal characteristics or the amount of available nutrients in the soil(48).

Climate may be defined as the atmospheric conditions which prevail within a place in regard to temperature, rainfall and prevailing winds. The

temperatures prevailing at the time of ripening together with the available moisture are apparently two of the most important factors which affect quality. Soil is the storehouse of the mineral elements necessary for plant growth. Soil nitrogen is the key element influencing the protein content of wheat. Water in the soil is the chief transporting medium for nutrients from the soil into the plant. Generally, available soil moisture during the weeks preceding maturity determines the quality characteristics of the wheat. Variety is a type of wheat within a larger class. The adaptability of wheat classes to given areas has been well established. Reviewing the literature, the importance of climate is generally emphasized over the variety.

It is well established that the protein content of wheat is essential for quality(7,30). It is also known that environmental factors play a big role influencing wheat quality. It has been recognized that approximately 80% of the variation in quality of hard red winter wheat produced for bread making is accounted for by protein content alone while 20% of the variation in baking response is due to variety and other quality factors(26). Since protein content is influenced mainly by the environment factors and the other 20% variation is influenced mainly by the inherited factors, it is therefore important to study the environment effect on the protein content. During the last half century considerable study has been made upon the effect of environment on the chemical composition of wheat(8,9,29). Especially, the central role of nitrogen on wheat quality as related to the environment has been studied for decades.

The changes in the composition of the wheat kernel during maturation have been studied recently(23,37,39). But the past work has not indicated the relationship of protein content of mature wheat and vegetative growth.

Many flour milling organizations have made elaborate and thorough surveys of the quality of the wheat in various states for years. This has permitted the classifying of wheat for quality characteristics according to locality. These surveys have been performed after the wheats have been harvested. Sometimes the wheat has been marketed and mixed in central terminals before full advantage can be taken of the survey information. If the survey information could be made available one month or so prior to the harvest, the data would be more meaningful and useful. If this information could be made available, the variation in the bread making quality of the wheat could be established and millions of dollars can be saved by the processor.

In this study, deposition of nitrogen in the wheat plant at the early head stage was compared with that found in mature grain. Four varieties at 11 locations were selected in addition to nitrogen fertilization plots at one location. It was the purpose of this study to determine whether whole plant analysis of nitrogen could be used to predict the protein content of the mature grain. If such evidence could be developed, it would be of great value to wheat growing regions and to processors as related to marketing of the harvested grain.

REVIEW OF LITERATURE

Climate

Thatcher(49) pointed out that three factors of climate were of importance as related to wheat quality. These were (1) rainfall, (2) length of growing season, and (3) temperature during the growing season. He presented evidence indicating high temperatures during ripening produce high protein content wheat, and concluded also that the length of the period of kernel formation rather than that of the whole growing period determined the composition of the grain. He found that the average weight of the kernel varied directly with the length of the development period, and that the percentage of nitrogen in the grain varied inversely with the length of this period.

Swanson(47) stated that a climate having dry winters, cool springs with moderate rainfall and hot, fairly dry summers usually produced a hard, strong wheat characterized by a high protein content. It is generally known that immature kernels have a higher protein content than the fully mature kernels. Protein apparently is laid down when the kernel is first set and in the mature kernel the protein content will depend on the degree of dilution as starch is developed to fully develop the kernel. Swanson pointed out that two factors influenced the protein content; (1) concentration of available nitrogen in the soil solution and (2) the amount of this solution. Climate owes its importance to the fact that it is the greatest factor influencing the soil solution.

The greater importance of climate than variety on determining the protein content of wheat was emphasized by the famous tri-local experiments conducted by Le Clerc(31) of the United States Department of Agriculture, cooperating with the Kansas and California stations. Soil was transferred from state to

state in such a way that in each there was soil from each of the two other states alongside the local soil. The soil was dug up in three inch layers and then put down in the original order. This was also done with each local soil so that all would have the same effects of physical handling. The same variety of wheat was grown in all the three states, and the trials were conducted through several seasons so that locally grown seed could be used after the first year. In this way factors of seed and soil were eliminated and climate was the main variable. The results were presented as below:

Protein content %	Grown in Calif. on soil from			Grown in Kan. on soil from			Grown in Md. on soil from		
	Cal.	Kan.	Md.	Cal.	Kan.	Md.	Cal.	Kan.	Md.
	13.2	11.3	14.8	17.5	19.3	19.7	11.8	12.5	12.9

It is apparent from these figures that protein content was influenced both by climate and soil. Protein content was highest in Kansas and lowest in Maryland due to climate. From this experiment, he concluded that wheat of any one variety, from any one source, and absolutely alike in chemical and physical characteristics, when grown in different localities, having different climatic conditions yields crops of very widely different appearance and very different in chemical composition. Further studies were made by Le Clerc and Yoder(32). They concluded that environment rather than heredity was the major factor influencing the physical and chemical properties of the wheat, and that climate was the principal environment factor influencing the protein content of wheat. The soils studied exerted little or no influence, and that soil had very little influence upon the ash content.

Harris et al.(20) studied 56 long - patent flours of hard red spring wheat grown in North Dakota and found that very significant variations in

flour protein content and loaf volume existed between varieties and environment, with the latter exerting the major influence. Mixograms secured at a uniform protein level showed significant variations between varieties and environments for dough development stage, range of dough stability curve height and width.

Rennie(38) studied the influence of fertilizer treatment, soil type and season on the protein content of wheat. He concluded that protein content of the grain was unaffected by nitrogen or phosphorus fertilization but varied greatly as a result of soil or climatic conditions. It is obvious that response to fertilizer will depend on the soil composition and climate. Response to nitrogen fertilizer can be expected only if there is a shortage of nitrogen in the soil and adequate moisture is available.

The distribution of the rainfall during the growing period is important. Limited rainfall in a semi-arid region may produce a high protein content crop if it comes at the time when the plant can use it, especially if it comes so as to favor germination or before and during the time of heading(3). Hopkins(22) indicated that there was a significant negative correlation between the amount of rainfall during the growing season and the nitrogen content of wheat. Early rainfall stimulated tillering and vegetative development, generally. The production of carbohydrate was simultaneously increased by high rainfall resulting in a relatively lower protein content in the mature wheat.

Waldron et al.(50,51) also pointed out that climate had a marked effect upon the protein content of wheat and of the resulting flour. In their study it was found that protein content in varieties differed but little from one year to another through the years but this difference from one year to another

for the 4 years was very marked. This indicated that weather rather than soil had been the determining factor affecting protein differences.

Furthermore, moderate precipitation during major growth periods at frequent intervals usually results in a lower protein percentage than the same amount of precipitation in a few heavy rains. This may be due either to the fact that the run-off from heavy rains is apt to be greater, or to the fact that the nitrates may be leached out of the upper layers of the soil by heavy rains. Light rainfall and large evaporation, which concentrate the nitrates in the upper portion of the soil where they may readily be obtained by the plant, seem to favor the formation of hard, glutinous wheat(4). Soule and Vanatter(45) concluded that the protein content was highest when the rainfall was less than normal during the ripening period. This condition gave a short, quick ripening period which retarded the elaboration and translocation of carbohydrate from the stem to the grains and therefore produced a higher percentage of protein content.

The period from seeding to the formation of the first leaf and the time of blossoming are especially critical periods in the life cycle of the plant. The first period determines the size of the first leaf, which is important for the subsequent growth of the plant; the second determines when the plant begins to mature and dehydrate(4). With regard to the period of flowering, Hooker(21) has shown by statistical analysis that in England cool or rainy weather at the time of flowering is correlated with high yields, and Aamodt(2) has shown experimentally that hot, dry wind at the time of flowering reduces yield and may, indeed, cause crop failure.

Yield and quality are closely correlated with soil moisture. Abundant soil moisture tends to lengthen the growing period, to increase yields, and to lessen protein content of the mature grain. In Thatcher's study(49) protein

content tended to be reduced when wheat was grown in the shade. Probably, the cool conditions under shade prolonged the growing period.

Shutt and Hamilton(43) presented data from two Western and two Maritime Canadian provinces. The comparison, extending from 1912 to 1932, showed the Western stations to have a July and August temperatures of 8°F. above the Maritime but with a precipitation of approximately one-half that of the Maritime provinces. With these differences, the Western wheat had an average protein content of 17.4% versus 13.3% for the Maritime wheats.

From Waldron's et al. (50,51) study of high prevailing temperatures for the 10 day pre-heading period it was shown that both maximum and minimum were associated with greater loaf volume, but only high maximum temperatures were found associated with high protein content of the mature wheat. High day temperatures from 10 days before heading until about the middle of July were found to be conducive to high protein while night temperatures did not modify the amount of protein. In their study the temperatures during the last 2 weeks of maturity had a very minor influence upon protein content of the resulting crop. The effect of temperature during the maturing period on dough mixing time and loaf volume of bread was studied by Finney and Fryer(16). They found that subnormal loaf volumes were consistently associated with high temperatures (above 90°F.) during the last 15 days before harvest. In the absence of high temperatures during the last two weeks before harvest other environmental factors such as rainfall and the chemical and physical composition of the soil appear to have relatively minor effects on protein quality. Protein content accounted for about 95% of the variations in loaf volume if temperature during the fruiting period was not a limiting factor.

In the Great Plains region of the United States and in Western Canada where the summers are hot and the rainfall scanty, the wheats tend to be high

in protein and strong. This may be due in part to the fact that hot weather favors nitrification and the accumulation of an abundant supply of nitrates in the earlier growth stages. Also, hot weather by evaporation at the surface of the soil tends to draw soluble soil constituents to the surface by capillarity. Also, high air temperature increases transpiration and thus may favor absorption of nutrients from the soil(3).

Soil

The soil is the medium through which the climate affects the plant since this is its home during growth and ripening. Wheats of different quality may be grown in adjacent fields where the soil conditions vary. The importance of the soil factors as related to wheat quality have been recognized. Water in the soil is the chief transporting medium for nutrients from the soil into the plant. About 300 pounds of water must pass from the soil to the plant for every pound of dry material constructed(48). As soil become older under constant use, the fertility may be depleted; particularly in nitrogen. The water holding capacity of the soil may be drastically reduced as the organic matter disappears. The protein content of wheat may be expected to decrease under conditions of continuous cropping. The application of nitrogen fertilizer and irrigation water to the soil are normally employed to compensate the soil conditions.

A. Nitrogen Fertilization

That climate very largely determines soil character has been studied by many workers. Large effects on wheat quality may be brought about by the soil or by special treatment of the soil. Fertilization produces marked effects. Proper fertilization tends to raise grain yields and increase the protein content of the grain.

Humphries and Biffer(24) found that higher protein content could be produced in soft wheat grown in rich soils than in hard wheat grown on lean soils.

As early as the beginning of this century, Snyder(44) found that an increase of nitrogen in the soil gave a higher protein content in the wheat kernel. He maintained that the protein content could be increased by fertilization with nitrogen.

Fajersson(10) made a very complete survey on the effect of nitrogen fertilization on wheat quality for 5 years (1949-53) in Sweden. Five winter wheat varieties, Eroica, Banco, Aros, Ergo, and Ertus were fertilized with nitrogen according to the following plan:

- a. no nitrogen.
- b. 300 kg. $\text{Ca}(\text{NO}_3)_2$ per hectare medium early (Plant height approximately 15 cm.).
- c. 600 kg. $\text{Ca}(\text{NO}_3)_2$ per hectare medium early (Plant height approximately 15 cm.).
- d. 300 kg. $\text{Ca}(\text{NO}_3)_2$ per hectare medium early + 300 at the time of heading.
- e. 300 kg. $\text{Ca}(\text{NO}_3)_2$ per hectare medium early + 150 at heading + 150 at flowering.

The observations obtained were as follows:

1. The total period of growth was slightly increased by nitrogen-fertilization. The temperature of the summer months which influenced the length of the active growing period appeared more important than the nitrogen applications used in these experiments.

2. The 1000-kernel weight was in general slightly decreased by early nitrogen-applications and considerably increased by late applications.

3. The first 300 kg. $\text{Ca}(\text{NO}_3)_2$ increased the protein content of grain from 8.7 to 9.5%, while a further 300 kg. had twice as great an effect (11.0%) when applied medium early. The effect of splitting up 600 kg. nitrate in one earlier and one or two late top-dressings was appreciable, and increased the protein content to 11.8 and 12%, respectively. These effects on protein were visible in each year and in all varieties.

4. a. Without application of bromate to the flour the loaf volume increased on an average for all years and varieties from about 550 (no nitrogen-fertilization) to about 610 cc. when heavily fertilized with nitrogen. Fertilization with two or three dosages of nitrogen yielded larger loaves than the same amount of nitrogen in one dosage only.

b. With optimal bromate application the loaf volume increased on an average from about 600 (no nitrogen-fertilization) to about 740 cc., when fertilized with nitrogen in split applications. When the corresponding amount of nitrogen was given in one heavy dosage the loaf volume remained at 700 cc..

c. There was a practically linear relationship between loaf volume (optimal bromate application) and protein content, taking the average of all varieties and years.

d. The heavy fertilizations with nitrogen when split into two or three dosages increased the dough weight (the amount of water absorption) on an

average from about 160 (no nitrogen) to 165-166g.. The effect of splitting the nitrate fertilization was evident, and was to be seen in all varieties. Erioca and Ertis had a higher dough weight than Aros and Ergo. The difference increased with the protein content.

5. The effect of rate and time of nitrogen-application on 1000-kernel weight, grain protein content and gluten content was in general confirmed in another series of experiments in 1955.

a. The flour protein increased relatively more than the grain protein content with increasing nitrogen-fertilization, and gluten content was favored relatively more than flour and grain protein content.

b. In comparison of different nitrogen-fertilizers, calcium nitrate was superior to urea and calcium cyanamide relative to their effect on protein content.

The literature review of the effect of nitrogen fertilization on wheat characteristics was also well established by Fajersson(10). He concluded that in general it was almost everywhere possible to modify the protein content by nitrogen-fertilization. Late application of nitrogen very often results in higher protein content than early application. Nitrogen fertilization as late as at the time of heading has in many cases had the most favorable effect on the protein content.

B. Soil moisture or Irrigation

In dry climates, irrigation tends to increase yields and lower protein content as compared with wheat grown in the same locality without irrigation.

Alsberg and Griffing(3) pointed out that irrigation acts not merely by supplying the plant with moisture but also may leach out salts or carry them from the surface layers down deep or into the soil where they may not be as accessible to the plant roots. Therefore, in their opinion, irrigation may upset the existing soil equilibrium.

Steward and Hirst(46) of the Utah station found that the protein content of wheat was decreased by irrigation. Greaves and Carter(19) stated that wheat decreased in protein content as the irrigation water was increased. Supplying water by irrigation has an advantage in studying the effects of amount of water on the quality of wheat in that the supply can be controlled as to time and amount.

Widtsøe(52) made studies on the relation of protein content to irrigation, and found that the percentage of protein in wheat kernel increased greatly as the amount of water applied to the soil decreased. Data showed that a plot receiving 30 inches of water produced a wheat with 15.25% protein, and that wheat from a plot receiving 7.7 inches contained 26.72% protein. This indicates that wheat grown under dry farming conditions has higher protein content than irrigated wheat because of a smaller amount of nitrates available in proportion to the yield.

Neidig, and Snyder(35) summarized the effect of moisture and nitrogen fertilizer as follows:

1. A high moisture content in a soil containing sufficient available nitrogen for the maximum growth and development of the wheat plant, results

in high yielding wheat containing a high percentage of protein.

2. A low moisture content in the soil containing an excess of available nitrogen results in a lower yield of wheat but a higher protein content.

A part of the higher protein content may be due to a shriveling of the wheat kernel.

3. A high or optimum moisture content in a soil, which has considerable nitrogen available for the wheat plant in the early periods of growth, but an insufficient amount during the fruiting and ripening periods for maximum growth, resulting in a high yield of wheat of low protein content.

4. A low moisture content in a soil which has sufficient nitrogen available to the wheat plant during the early stages of growth, but an insufficient amount for the fruiting and ripening periods, results in a low yield of wheat, the percentage of protein varying according to the degree to which the wheat is shriveled from moisture deficiency.

Variety

Climate is generally regarded as more important than variety in determining protein content. While this is true, it by no means follows that there is no correlation between wheat variety and quantity. It is still possible to develop varieties that will give relatively high protein wheats in regions where the wheat commonly grown is soft and weak(3). The superiority of one wheat variety as compared with another is principally in adaptation to a certain environment so as to produce high yields of good quality wheat.

Kansas has long been a leading bread-wheat producing state. Varieties of wheat not only affect the quantity of wheat produced in Kansas, but also affect the quality. The varieties of wheat respond differently to the milling

and bread-making processes. The number and extent of varieties grown and their response to the growth environment are the chief contributing factors governing the quality of Kansas wheat as a whole(28).

The quality of Kansas wheat, besides being dependent upon the varieties grown and their response to environment, it also was affected by the extent of varietal distribution. The acreage distribution of varieties changes from one year to the next, depending upon the availability of new and improved varieties.

One of the projects of the Kansas Agricultural Experiment Station is to grow all the standard wheat varieties each year at the various experiment stations and experiment fields in the state, and to compare these with the new crosses that are being studied. Thus, it is possible to evaluate accurately the response of the different wheat varieties to growing conditions at each station.

Shellenberger et al.(41) studied the importance of variety upon the Kansas wheat quality. From the three-year (1950, 1951, and 1952) quality comparison of Kiowa and Ponca (two newest varieties) with Turkey and Comanche, it was evident that the new varieties tended to have higher protein content and lower mineral content than the older varieties. Kiowa and Ponca, when grown beside Comanche, Turkey, or any of the other older varieties, represent improvement both agronomically and in ability to produce satisfactory bread.

Variety plays an important role in influencing the bread making characteristics, i.e., absorption, mixing, and loaf volume. As the protein content increased, so does the absorption. However, some varieties consistently have greater absorption capacity than others. Varieties have inherent properties that produce different mixing curves. Some varieties mix to the point of minimum mobility (peak of curve) in a relatively short time, while

others require a longer mixing period. Loaf volume is also affected by variety. Some varieties consistently give greater loaf volume than others for any given protein content.(40,41).

Johnson et al.(27) studied the effect of environment and varieties on Kansas wheat quality from 1958 to 1962. The results were subjected to analysis of variance. They concluded that wheat variety had a significant effect on the quality of Kansas wheat. It was evident also that variety of wheat caused a significant effect on the protein content of the flour and variety of wheat had greater effect on the dough-mixing time than environment. Although environment played a dominant role, variety effect on both loaf volume and quality score was highly significant. They stated that the response of different varieties to locations of growth as measured by quality might be expected to be altered some, depending on conditions prevailing during maturation. However, the response of all varieties to the conditions of growth was generally very similar.

The varieties of wheat grown in Kansas are changing constantly because of introduction of new wheats and because of changes in relative popularity of the established varieties. These changes are a sign of progress in wheat development work which has resulted in making better wheat varieties available to the growers in the state.

MATERIALS AND METHODS

A. Protein content of mature grain and vegetative plant

1. Source of sample. Four hard red winter wheat varieties, Triumph, Ottawa, Kaw, and Bison were selected for this study. The plots were laid out at 9 stations in Kansas, Colby, Tribune, Garden City, Hays, St. John, Hutchinson, Manhattan, Powhattan, and Parsons. Colby and Garden City stations provided both dryland and irrigated plots.
2. Sampling procedure. The plan of the sample procurement was the same at each location. Two different stages of maturity were used. In the early head stage (half bloom) the sample was harvested approximately 1 month prior to maturation. In the second stage, the wheat was mature and harvested in a normal manner.

- a. Early head stage. Wheat plants were cut approximately 4 inches above the ground of a 3-foot section of a single row from each of three replicate plots. The time of cutting was when one half of the heads had reached 50% bloom stage. Air or oven-dried plants were shipped in plastic bags to Manhattan, Kansas. The replicate samples were combined, finely ground to pass a 1 mm. sieve on a Wiley mill and blended before chemical analysis for nitrogen.

- b. Ripe stage. Mature heads from a 3-foot section of a row adjacent to the wheat cut when immature were cut and shipped in plastic bags to Manhattan, Kansas. After the samples arrived, the replicates were threshed and analyzed for nitrogen.

3. Protein analysis. The kjeldahl method was used to determine the nitrogen content of each sample. The nitrogen content was converted to per cent protein by the factor of 5.7(1), and expressed on a 14% moisture basis.

4. Moisture. Moisture content was determined according to A.A.C.C. Air-oven method(1).

B. Environmental study.

1. Origin of samples. Certified wheat seed of the 4 varieties were planted at the experimental fields in approximately 1/16 acre plots in triplicate at each of the 11 locations. When the grain was ripe, it was harvested, threshed, composited and shipped to Manhattan for quality evaluation.

2. Milling procedure. The wheat samples were received in 6-pound lots, cleaned for dockage, blended and scoured. Two thousand grams of each sample were tempered for 24 hours to a 17% moisture level. Milling was performed on the Buhler pneumatic experimental flour mill(1).

3. Wheat and flour analysis. All samples of wheat and flour were analyzed for moisture, ash and protein following the A.A.C.C. methods of analysis(1). Farinograms were made with the 50-gram bowl using a constant flour weight on a 14% moisture basis.

4. Baking procedure. All flours were baked into bread using the straight-dough procedure(34). Flours were weighed on a 14% moisture basis. The absorption of each sample was adjusted to bring all dough out of the mixer with the same consistency. This was predetermined with the Farinograph(1). Optimum amount of mixing for each sample was determined by sight and feel of the dough. The farinogram provided preliminary indication of optimum mixing. The doughs for baking were mixed in the National dough mixer with a 100-gram capacity mixer bowl.

The straight-dough formula was as follows:

STRAIGHT-DOUGH FORMULA

Ingredient	Per Cent	Weight
		grams 100
Flour (14% moisture basis)	100.00	
Sugar	6.00	6
Nonfat dry milk	3.00	3
Shortening	3.00	3
Salt	2.00	2
Malt	0.50	0.5
Potassium bromate	0.002	0.002
Yeast	2.00	2
Water (variable)		

Each dough was given 180 minutes primary fermentation at 85°F. First punch occurred after 110 minutes, with the second punch 50 minutes later. The National hand molder was used. After 20 minutes rest the molding was completed on a Thompson laboratory molder. The loaf was panned in 2 1/8" x 4 7/8" x 2 1/2" pup pans and proofed at 86°F. for 55 minutes. The loaf was baked in a gas-fired reel oven for 25 minutes at 410°F. Loaf volume and weight were recorded immediately when the bread was removed from the oven and subjective quality scores were recorded the day after baking. The loaves were scored for external appearance, grain, texture, and crum color.

C. Fertilizer study

The variety, Ottawa, was chosen from the Manhattan station at early head and ripe stage for the study of the influence of different fertilizer treatment upon the wheat protein content. Both urea and ammonium nitrate were applied at 3 different levels including 33, 66, and 100 pounds of nitrogen

per acre. The fertilizer was applied at 3 different times including early fall, late winter and spring. Samples were analyzed for moisture and protein content following the A.A.C.C. methods of analysis(1).

RESULTS AND DISCUSSION

A. Comparison of the protein content of mature grain and vegetative growth.

The protein content of 4 varieties harvested at green and mature stages are presented in Table 1.

Table 1. Protein content of 4 varieties harvested at 2 different stages.

Location	Triumph		Ottawa		Kaw		Bison	
	Green	Mature	Green	Mature	Green	Mature	Green	Mature
	%	%	%	%	%	%	%	%
Colby (dryland)	7.8	13.2	7.6	14.8	6.6	13.7	7.7	12.4
Colby (irrigated)	7.1	12.2	7.0	10.4	7.0	11.0	7.2	11.1
Garden City (dryland)	9.6	17.3	9.8	17.2	10.3	17.1	9.5	17.5
Garden City (irrigated)	9.1	13.9	8.5	11.2	8.1	12.6	8.8	12.6
Hays	9.5	17.0	9.5	16.7	10.0	16.6	9.5	17.5
Hutchinson	6.3	12.0	6.1	12.1	6.3	10.7	7.2	12.1
Manhattan	9.5	13.0	9.0	11.1	8.4	11.6	9.2	12.1
Parsons	9.8	13.4	11.0	12.6	8.9	11.6	**	**
Manhattan	8.9	13.8	9.4	13.9	8.2	13.9	8.8	14.1
St. John	7.9	14.9	8.3	13.3	7.9	13.8	8.5	15.1
Tribune	<u>7.4</u>	<u>11.3</u>	<u>7.5</u>	<u>11.0</u>	<u>7.4</u>	<u>11.8</u>	<u>7.7</u>	<u>10.8</u>
Mean	8.4	13.9	8.5	13.1	8.1	13.1	8.4	13.6

From the data in Table 1, it is evident that the mature grain has higher protein content than the green plant. It is generally believed that immature kernels have a higher protein content than fully mature wheat. However, in this study the whole plant was used for analysis of the immature state while the whole mature grain was used for comparison. Therefore, the comparison can not be made with Hoseney nor Scott(23,39) who had opposites opinions.

Table 2. Variety mean values of protein content harvested at 2 different stages.

Variety	Green plant	Mature grain
	%	%
Triumph	8.4	13.9
Ottawa	8.5	13.1
Kaw	8.1	13.1
Bison	8.4	13.6
L.S.D.	0.39	0.89

The variation due to the variety is not markedly significant in either the green or mature stage. For example, in the green plant, the mean value of the protein content of four varieties is almost constant at 8%. That the variation of protein content caused mainly by location has been known for many years. The range in protein content is much greater among locations than between varieties (Table 3). This finding agrees well with data of previous workers (27,41) and confirms the importance of environment upon the wheat quality.

Table 3. Location mean values of protein content harvested at 2 different stages.

Location	Green plant	Mature grain
	%	%
Colby(dryland)	7.4	13.5
Colby(irrigated)	7.1	11.2
Garden City(dryland)	9.8	17.3
Garden City(irrigated)	8.6	12.6
Hays	9.6	16.8
Hutchinson	6.5	11.7
Manhattan	9.0	12.0
Parsons	9.9	12.5
Powhattan	8.8	13.9
St. John	8.2	14.4
Tribune	7.5	11.2
L.S.D.	1.25	1.99

Hartley's maximum-F test(18) was applied to determine the relation between variety and protein content in green and mature stage(Table 4 and 5).

Table 4. Hartley's maximum test for the relation between variety and protein content in green stage.

Station	Triumph	Ottawa	Kaw	Bison
	%	%	%	%
Colby(dryland)	7.8	7.6	7.0	7.7
Colby(irrigated)	7.1	7.0	6.6	7.2
Garden City(dryland)	9.6	9.8	10.3	9.5
Garden City(irrigated)	9.1	8.5	8.1	8.8
Hays	9.5	9.5	10.0	9.5
Hutchinson	6.3	6.1	6.3	7.2
Manhattan	9.5	9.0	8.4	9.2
Parsons	9.8	11.0	8.9	*
Powhattan	8.9	9.4	8.2	8.8
St. John	7.9	8.3	7.9	8.5
Tribune	7.4	7.7	7.4	7.5
S_i^2	1.4	2.0	1.6	0.8

$$F_{\max} = (\text{largest } S_i^2) / (\text{smallest } S_i^2) = 2.0 / 0.8 = 2.5$$

$$\omega = F_{\max} > 5.7, \quad 5\% \text{ significant level}$$

$$**S_i^2 = \text{unbiased estimator of variance.}$$

$$\omega = \text{region of rejection.}$$

Table 5. Hartley's maximum test for the relation between variety and protein content in mature stage.

Station	Triumph	Ottawa	Kaw	Bison
	%	%	%	%
Colby (dryland)	13.2	14.8	13.7	12.4
Colby (irrigated)	12.2	10.4	11.0	11.1
Garden City (dryland)	17.3	17.2	17.1	17.5
Garden City (irrigated)	13.9	11.2	12.6	12.6
Hays	17.0	16.7	16.6	17.0
Hutchinson	12.0	12.1	10.7	12.1
Manhattan	13.0	11.1	11.6	12.1
Parsons	13.4	12.6	11.6	**
Powhattan	13.8	13.9	13.9	14.1
St. John	14.9	13.3	13.8	15.7
Tribune	11.8	10.8	11.3	11.0
S_i^2	3.5	5.5	4.8	5.1

$$F_{\max} = (\text{largest } S_i^2) / (\text{smallest } S_i^2) = 5.5 / 3.5 = 1.6$$

$$\omega = F_{\max} \geq 5.7 \quad 5\% \text{ significant level}$$

** S_i^2 = unbiased estimator of variance

ω = region of rejection.

The Hartley's maximum F test shows that the variation in the protein content is almost exclusively accounted for by location. It is recognized that protein content is not related particularly to varieties of wheat when the climatic conditions are critical(26). It is not uncommon to find that the protein content of one variety may vary from 9% in eastern Kansas to more than 16% when grown in the semi-arid conditions of western Kansas(26). In this study, for example, the protein content of the variety, Ottawa, in the green stage varied from 6.1% when grown in Hutchinson to 11.0% when grown in Parsons and from 10.4% when grown in Colby(dryland) to 17.2% when grown in Garden City(dryland) in the mature stage.

The correlation and regression analyses of 4 varieties and 11 locations are illustrated in Figs. 1 to 5.

There was a significant relationship between the protein content of mature grain and the green plant. Among the four varieties, Triumph and Kaw exhibited significant correlations between the protein contents of two stages but for the other two varieties, Ottawa and Bison the correlations were nonsignificant. It was likely due to the small size of sample. If the data from the 4 varieties are combined a significant correlation is obtained (Fig. 6).

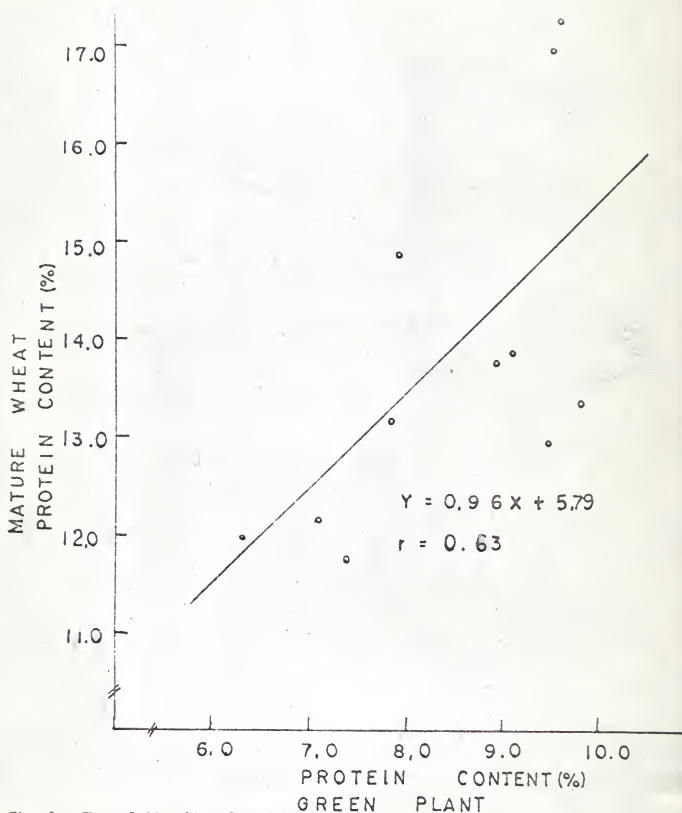


Fig. 1. The relationship of protein content of Triumph in two stages of growth.

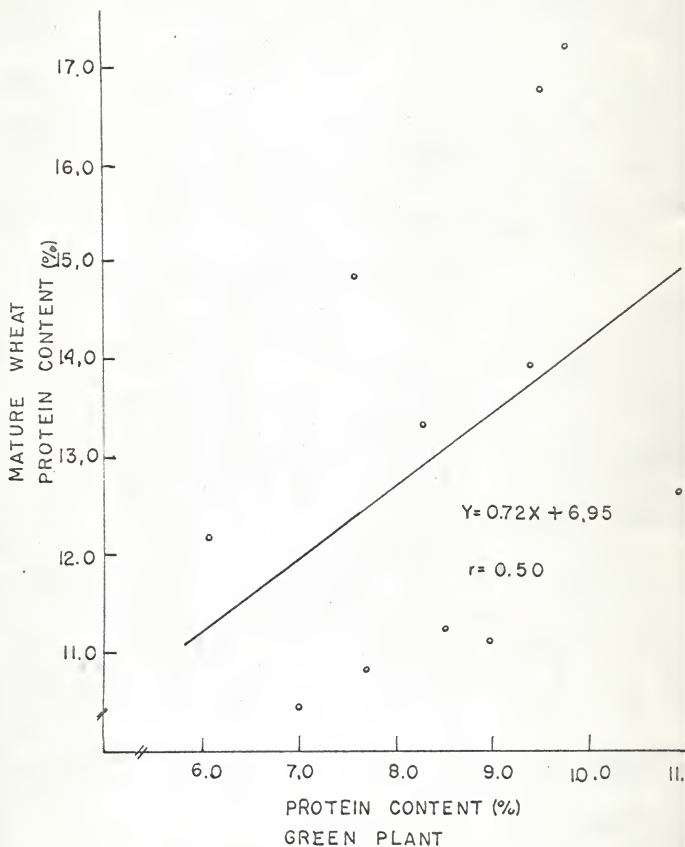


Fig. 2. The relationship of protein content of Ottawa in two stages of growth.

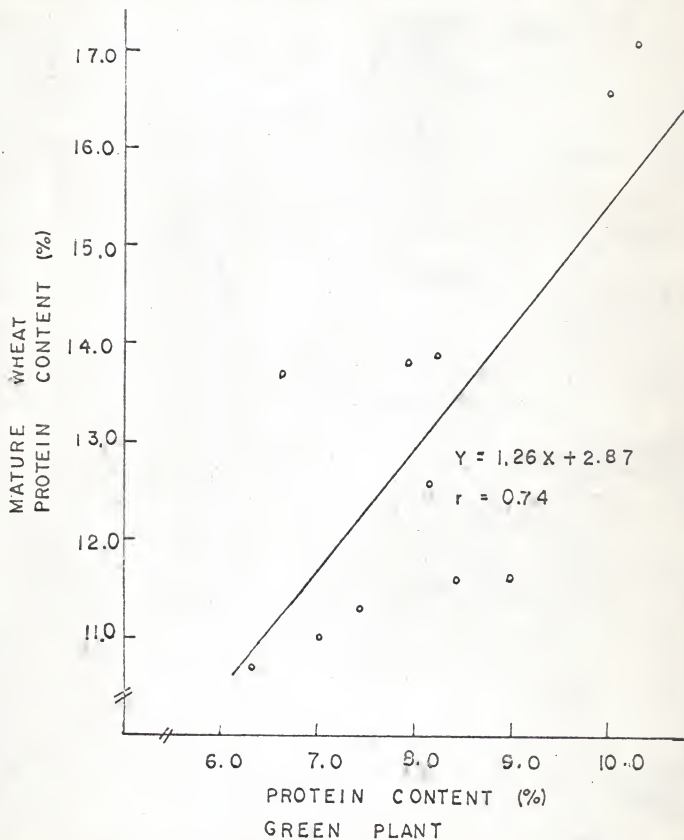


Fig. 3. The relationship of protein content of Kaw in two stages of growth.

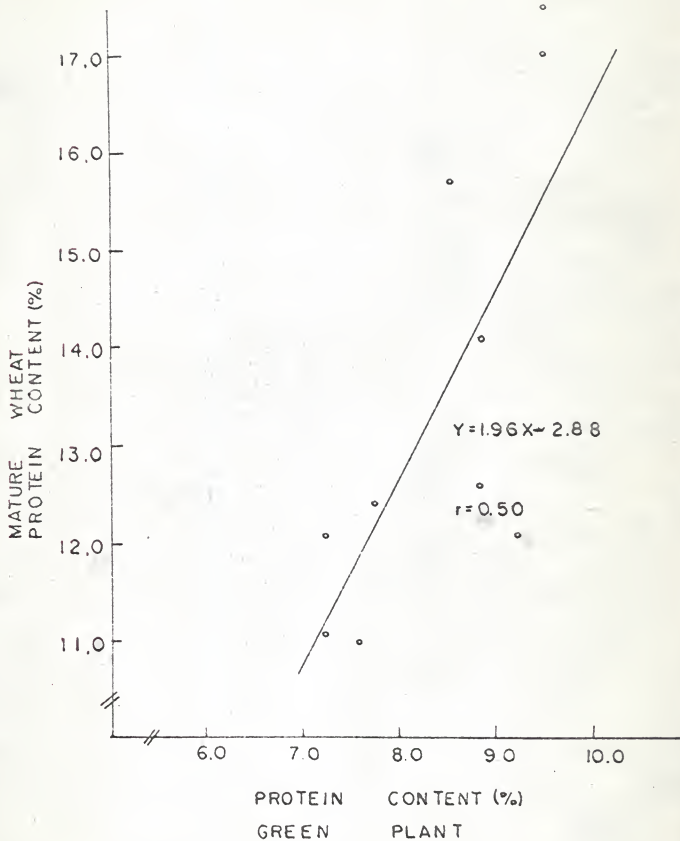


Fig. 4. The relationship of protein content of Bison in two stages of growth.

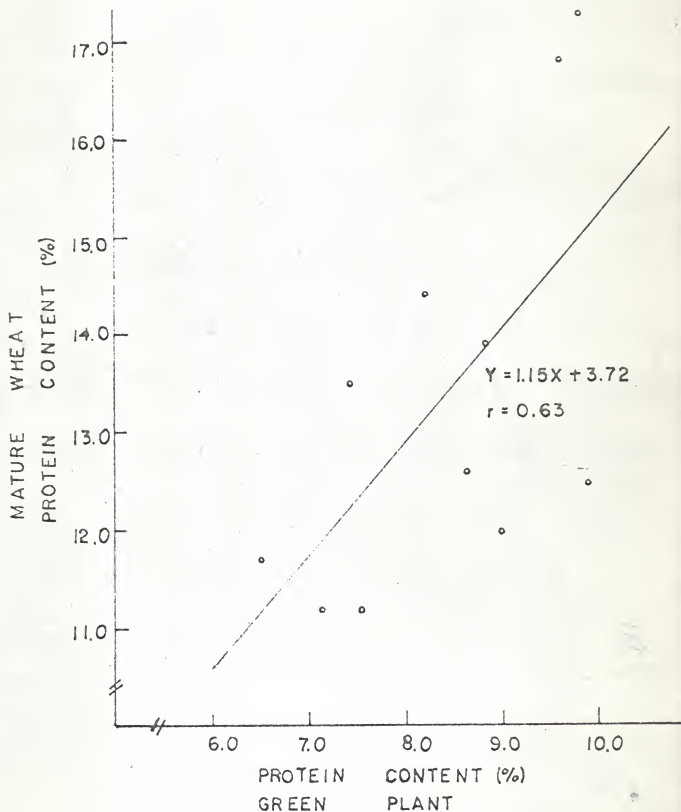


Fig. 5. The relationship of protein content in two stages of growth for 11 locations.

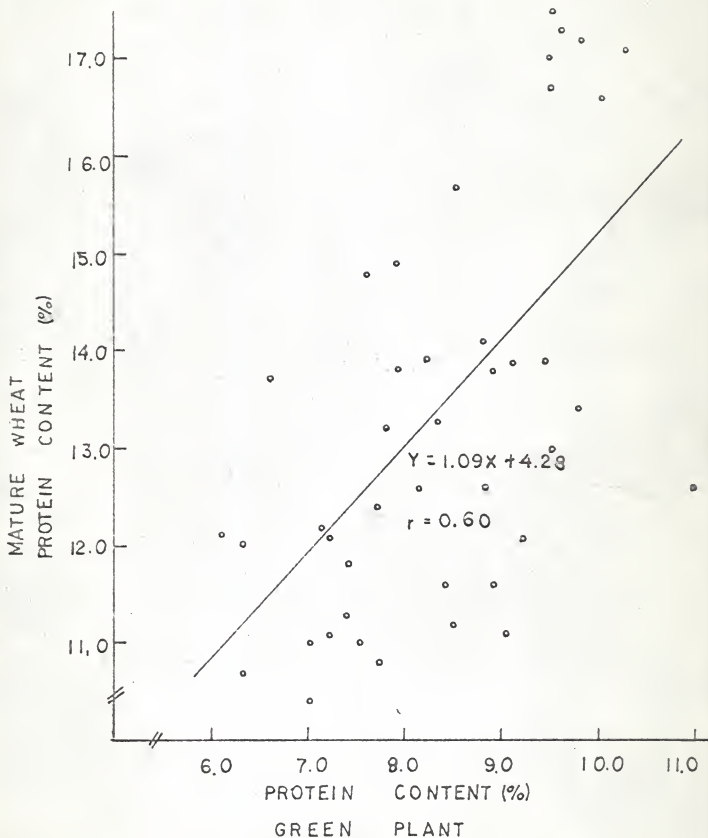


Fig. 6. The relationship of protein content in two stages of growth for all samples.

B. Environmental study.

The wheat and flour analyses and the baking data representing the effect of variety and location on quality are presented in Tables 6 to 9. Each table represents a given variety grown at the 11 locations. The analysis of variance for protein content, mixing time, loaf volume and quality score are presented in Table 10. The average values and the least significant mean differences representing varieties and locations are presented in Tables 11 and 12, respectively. The correlation analysis between flour protein content and loaf volume calculated from Table 11 is shown in Fig. 7.

Table 6. Quality comparison of Triumph grown at 11 locations.

Location	Wheat	Flour Analysis				Baking Test		
	Pro- tein %	Pro- tein %	Mix- time min.	Absorp- tion %	Valori- meter	Mix- time min.	Loaf Vol. cc.	Total score %
Colby (dryland)	14.7	13.7	7.0	70.0	63.0	3.0	855	79.0
Colby (irrigated)	12.9	11.6	4.0	66.0	45.0	2.0	750	70.5
Garden City (dryland)	18.3	17.3	6.0	70.0	58.0	2.3	910	83.5
Garden City (irrigated)	14.3	12.8	4.5	67.0	50.0	2.3	870	82.0
Hays	18.0	16.7	5.5	70.6	60.0	2.3	965	90.5
Hutchinson	12.2	10.5	5.5	61.4	54.0	3.5	740	60.0
Manhattan	14.2	12.6	7.5	65.0	64.0	3.8	930	79.0
Parsons	13.6	12.7	7.0	68.8	62.5	3.5	785	69.0
Powhattan	14.8	12.7	6.0	66.0	57.5	3.0	835	68.0
St. John	16.6	15.3	6.0	68.0	57.0	2.5	910	76.0
Tribune	12.1	10.6	4.0	65.2	45.0	2.0	740	66.5
AVERAGE	14.7	13.3	5.7	67.1	56.0	2.7	845	74.9

Table 7. Quality comparison of Ottawa grown at 11 locations.

location	Wheat	Flour Analysis				Baking Test		
	pro- tein	Pro- tein	Mix- time	Absorp- tion	Valori- meter	Mix- time	Loaf Vol.	Total Score
	%	%	min.	%		min.	cc.	%
Colby (dryland)	15.6	13.7	10.0	72.6	74.0	3.8	940	81.0
Colby (irrigated)	11.3	10.0	3.5	67.8	40.0	2.0	765	72.5
Garden City (dryland)	18.6	17.1	6.0	73.0	58.0	2.5	1000	85.5
Garden City (irrigated)	13.6	12.1	5.5	69.6	55.0	2.8	920	87.5
Hays	16.3	14.6	9.0	73.0	74.0	4.5	1005	92.5
Hutchinson	12.3	10.4	5.0	66.2	52.0	4.5	785	64.5
Manhattan	13.4	11.4	5.5	67.0	54.0	3.3	910	78.5
Parsons	13.8	13.1	5.0	74.0	52.0	3.0	900	60.0
Powhattan	14.8	12.7	5.0	72.2	52.0	2.5	915	76.0
St. John	15.4	14.2	11.5	72.2	79.0	4.0	930	79.0
Tribune	11.3	9.6	3.0	67.2	37.0	1.5	750	64.5
AVERAGE	14.2	12.6	6.3	70.4	56.4	3.2	893	76.5

Table 8. Quality comparison of Kaw grown at 11 locations.

location	Wheat		Flour Analysis			Baking Test		
	Pro- tein %	Pro- tein %	Mix- time min.	Absorp- tion %	Valori- meter	Mix- time min.	Loaf Vol. cc.	Total score %
Colby (dryland)	11.2	10.5	5.5	65.0	55.0	3.0	810	67.0
Colby (irrigated)	11.7	10.3	6.0	63.8	57.5	3.3	710	66.5
Garden City (dryland)	18.1	17.0	9.0	70.2	71.0	2.3	1000	85.0
Garden City (irrigated)	14.7	13.1	8.0	65.4	66.0	4.0	805	73.5
Hays	16.7	15.0	10.0	67.6	76.0	5.0	1010	89.5
Hutchinson	11.0	9.6	2.5	59.6	30.0	7.0	665	46.0
Manhattan	12.4	11.1	9.0	61.0	71.0	5.0	770	63.0
Parsons	12.8	12.1	8.0	69.2	67.0	4.8	760	65.0
Powhattan	13.4	11.9	5.0	66.0	52.0	4.5	830	67.5
St. John	15.4	14.3	11.5	67.0	78.0	4.5	830	77.5
Tribune	11.6	9.9	5.5	63.0	56.0	2.8	710	58.0
AVERAGE	13.5	12.3	7.3	65.3	61.8	4.2	809	69.0

Table 9. Quality comparison of Bison grown at 11 locations.

location	Wheat		Flour Analysis			Baking Test		
	Pro- tein %	Pro- tein %	Mix- time min.	Absorp- tion %	Valori- meter	Mix- time min.	Loaf Vol. cc.	Total Score %
Colby (dryland)	12.6	12.1	10.0	68.2	74.0	4.0	835	74.5
Colby (irrigated)	11.4	10.8	5.0	66.5	52.0	2.5	785	72.5
Garden City (dryland)	18.3	17.5	9.0	70.8	70.5	3.0	1020	86.0
Garden City (irrigated)	13.0	12.2	6.0	66.6	57.0	3.0	830	77.0
Hays	16.8	15.8	10.0	70.2	78.0	5.0	1040	92.0
Hutchinson	11.4	10.4	7.5	63.0	65.0	4.0	775	59.0
Manhattan	12.9	11.8	7.5	64.5	65.0	3.8	895	75.5
Parsons	14.4	13.7	8.5	72.0	69.0	3.5	785	69.0
Powhattan	15.3	13.4	5.5	69.0	55.0	2.8	940	74.0
St. John	16.5	15.7	12.0	70.8	79.0	4.0	1020	82.0
Tribune	11.1	10.0	4.0	66.0	45.0	2.0	700	59.5
AVERAGE	13.9	13.0	7.7	68.0	64.5	3.4	875	74.6

Table 10. Analysis of variance for 1966 wheat flour baking quality.

Source of variance	Degree								
	of	Mean	F	Mean	F	Mean	F	Mean	F
	freedom	squares		squares		squares		squares	
Varieties	3	2.39	6.46*	4.46	11.44*	14812.7	10.23*	119.8	8.21*
Location	10	20.60	55.68*	2.58	6.62*	35614.5	24.60*	391.0	26.78*
Error	30	0.37		0.39		1447.9		14.6	

* significant at 5% level.

Table 11. Variety mean values of certain quality factors.

Variety	Flour Protein %	Mixing Time min.	Loaf Volume c.c.	Total Score 100
Triumph	13.3	2.7	845	75
Bison	13.0	3.4	875	75
Ottawa	12.6	3.2	893	77
Kaw	12.3	4.2	809	69
L.S.D.	1.0	1.4	82.8	7.4

Table 12. Location mean values of certain quality factors

Location	Flour Protein %	Mixing Time min.	Loaf Volume c.c.	Total Score 100
Hays	15.5	4.3	1005	91
Garden City	14.9	2.8	919	83
St. John	14.9	3.8	923	79
Parsons	12.9	3.7	836	68
Powhattan	12.7	3.2	880	71
Manhattan	11.7	3.9	876	74
Colby	11.6	2.9	806	73
Hutchinson	10.2	4.8	741	57
Tribune	10.0	2.1	725	62
L.S.D.	2.2	0.9	97.8	11.1

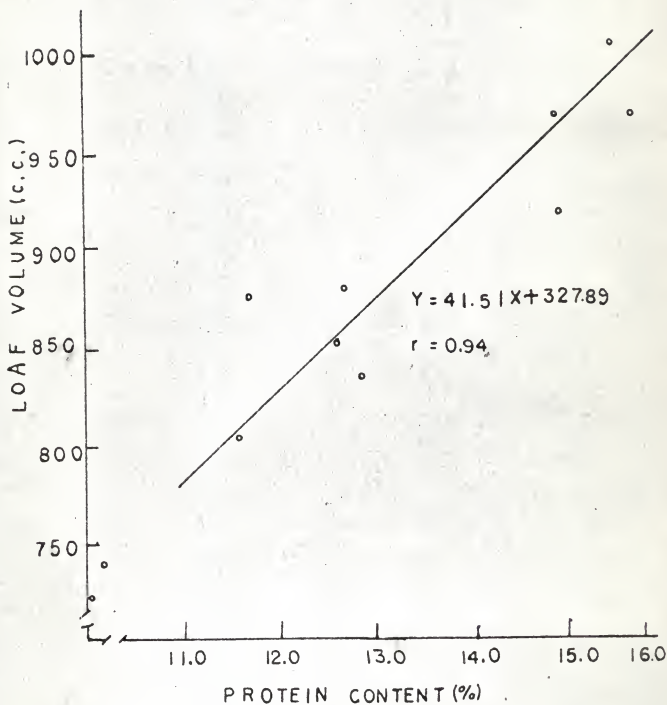


Fig. 7. The correlation of protein content and loaf volume representing 11 locations.

The protein content of the wheat, as a whole, was high in 1966. At the 9 stations, the average protein content of the wheat was 12.7%. Since wheat protein content is so highly correlated with flour protein content(30), the discussion can as well be based on the latter. Flour protein content is one of the most important quality factors affecting bread-making properties. From the analysis of variance (Table 10), it is evident that variety of wheat caused a significant effect on the protein content of the flour. However, a much more important factor affecting protein content was the environment. This agrees well with the work of Johnson et al., on Kansas wheat in 1958-62 (27). The range in protein content of the flour by varieties was 1% (Table 11) while the range representing the effect of location was 5.5% (Table 12).

Mixing characteristics of the flours are shown in the Tables 6 to 9. In general, the varietal effects are evident in the mixing times required to reach a peak and the rate of decline after reaching the peak consistency. However, station effects are even more significant. The analysis of variance of the dough mixing time (Table 10) indicates that variety of wheat has greater effect than environment, although both factors are highly significant. As for the varieties, Kaw had the longest dough-mixing requirement, followed by Bison, Ottawa and Triumph.

The baking value of wheat for bread production is a summation of many factors including loaf volume, break and shred, symmetry, grain, texture and crumb color. These factors have been weighted and are summarized in a total quality score. Of all the measurements used to determine quality of bread, only loaf volume is objective(5,6,12,36). The baking data (Tables 6 to 9) indicate large variation in loaf volume as well as in the total quality score. The analysis of variance (Table 10) suggests that most of the

variation in loaf volume and quality score can be attributed to the effect of location, although variety effect is also highly significant. The close relation of loaf volume and protein content has been established.(33).

Barmore et al., (14,15) have shown that these two factors were essentially linear between the limits of protein encountered, i.e., 8 to 18%. The correlation analysis between flour protein content and loaf volume for the 1966 crop are shown in Fig. 7. It is noted that correlation coefficient is 0.94. This agrees with the results of Johnson et al., (26). No significant correlation ($r = 0.29$) between loaf volume and protein content existed when the effect of environment was eliminated.

The influence of irrigation upon the protein content is shown by the limited data from the Colby and Garden City stations. The protein content decreased markedly when the irrigation was applied.

C. Fertilizer study.

The effects of the fertilizer treatment in the protein content of the head stage are presented in Table 13. The analysis of variance of these data is summarized in Table 14.

Table 13. Effect of fertilizer treatment on the protein content of wheat in the early head stage.

Time	Urea *			NH ₄ NO ₃ *		
	33N	66N	100N	33N	66N	100N
	%	%	%	%	%	%
Early Fall	10.3	11.2	11.1	10.4	10.8	10.9
	10.3	11.2	11.2	10.5	10.8	10.9
Late Winter	9.9	10.9	11.3	11.2	9.6	12.0
	9.9	11.0	11.3	11.3	9.7	12.0
Spring	9.5	9.9	11.6	9.7	10.4	11.0
	9.6	9.7	11.6	9.6	10.5	11.0

* pounds of nitrogen/ acre.

** no nitrogen 9.6%

Table 14. Analysis of variance of data on the effect of the fertilizer treatment on protein content of the early head stage.

Source of variance	Degree of freedom	Mean squares	F
Fertilizer concentration (C)	2	4.23	4.74*
Kind of fertilizer (K)	1	0.02	0.02
Time of application (T)	2	0.93	1.04
C x K	2	0.61	0.68
C x T	4	0.69	0.77
K x T	2	0.13	0.14
Error	22	0.893	

*Significant at 5% level.

Fertilizer concentration

Conc.	Mean	Conclusion
100N	11.33 <*	
66N	10.48 <*	
33N	10.18	100N > 66N > 33N

< * Indicates means are significantly different at the 5% level of significance as judged by Fisher's L.S.D. at 5% level.

From Table 13, it is noted that nitrogen fertilizer increased the protein content. This is further exemplified by the Fisher F test (Table 14). The higher the concentration of nitrogen fertilizer, the higher the protein content of wheat in the green stage. In this case, the application of 100 pounds of nitrogen per acre produced the largest protein content. This finding agrees well with many previous workers(10,53) who showed that protein content is increased by large dosages of nitrogen fertilizer. In the case of these data, wheat from replicate plots was not available. Therefore, the triple interaction was combined with the error of duplicate determinations of the nitrogen content for use in calculation of the fractions. It is likely that had replicate plots been available, other conclusions might have been made.

The results from the study of the effect of fertilizer treatment on the protein content of mature wheat are presented in Table 15. Table 16 presents the analysis of variance.

Table 15. Effect of fertilizer treatment on protein content of mature wheat.

	Urea *			NH ₄ NO ₃ *		
	33N %	66N %	100N %	33N %	66N %	100N %
	14.4	14.3	14.6	13.4	14.1	14.6
Early fall	14.3	14.3	14.7	13.3	13.9	14.6
	13.0	14.6	14.8	13.5	14.4	16.0
Late winter	13.2	14.6	15.0	13.5	14.4	16.1
	13.7	14.4	16.1	13.7	15.1	15.1
Spring	13.9	14.7	16.2	13.7	15.1	15.1

* pounds of nitrogen / acre.

** no nitrogen 13.0%

Table 16. Analysis of variance of the effect on protein content of fertilizer treatments.

Source of variance	Degree of freedom	Mean squares	F
Fertilizer Concentration (C)	2	7.85	12.66*
Kind of Fertilizer (K)	1	0.04	0.07
Time of application (T)	2	0.87	1.40
C x K	2	0.07	0.01
C x T	4	0.57	0.92
K x T	2	0.64	1.03
Error	22	0.628	

* significant at 5% level.

Fertilizer concentration

Conc.	Mean	Conclusion
100N	15.250 *	100N 66N 33N
66N	14.492 *	
33N	13.633	
L.S.D.	0.076	

* Indicates means are significantly different at the 5% level of significance as judged by Fisher's L.S.D. at 5% level.

The effect of fertilizer treatment on protein content of mature wheat was about the same as that found in the green stage. The higher the concentration of fertilizer, the higher the protein content produced in the mature wheat. Effect of fertilizer application time was nonsignificant. Kind of fertilizer used had no significant effect on the protein content.

From the above results it is evident that late application of nitrogen fertilizer resulted in higher protein content. The larger concentration of nitrogen fertilizer caused higher protein content in the wheat.

The data from Tables 13 and 15 were subjected to regression analysis and are summarized in Fig. 8. From this figure, again, the significant correlations between protein content of green plant and mature grain may be observed.

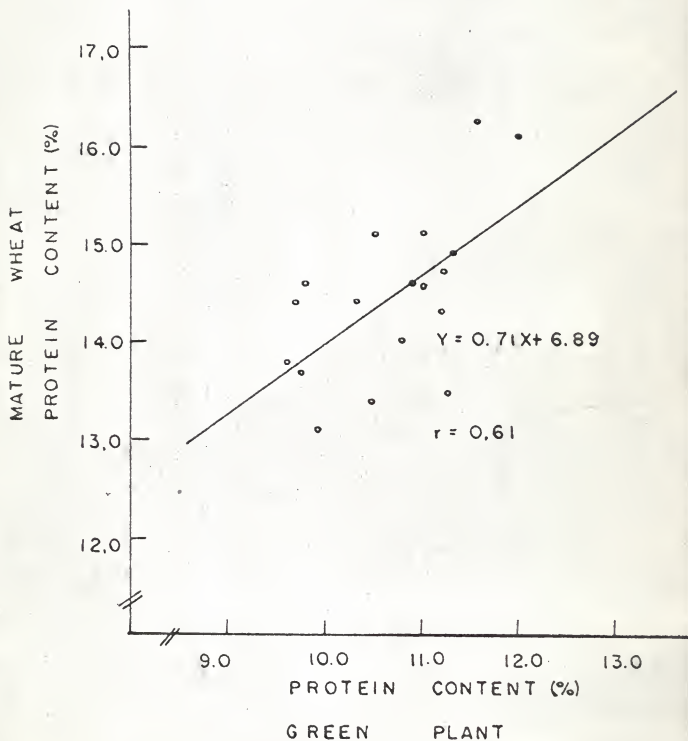


Fig. 8. The relationship of protein content in the fertilizer series.

SUMMARY AND CONCLUSION

The importance of the environment upon the protein content of wheat has been recognized for years. Many studies have been made of the effect of environment but not including the study of the relationship of protein content of green wheat to mature wheat. Four hard red winter wheat varieties from 11 locations were selected at two different stages of maturity, i.e., early head and mature stages, for the nitrogen analysis to determine whether whole wheat plant analysis of nitrogen could be used to predict the protein content of the mature grain. The mature grain was tested also for physical dough properties of the flour and bread baking to observe the influence of environment upon the wheat quality. In addition, one variety from one station was chosen to study the effect of nitrogen fertilization on protein content. This included 33, 66, and 100 pounds of nitrogen per acre of urea and ammonium nitrate, respectively, applied at 3 different times which included fall, late winter and spring. The following conclusions can be made:

1. There was a significant correlation in the protein content of wheat between the two stages of maturity but this correlation was not sufficiently high for purpose of accurate prediction of the protein content of the mature grain from the green plant.

2. In both the immature plant and mature grain, the protein content was affected mostly by the environment.

3. The influence of variety on the protein content was relatively not as significant as the environment.

4. Baking quality characteristics were influenced significantly by the protein content.

5. Protein content of the wheat was influenced by the nitrogen

fertilizer treatment resulting in higher protein content. The higher concentration of nitrogen application produced the highest protein content. Urea or ammonium nitrate or time of application had about the same effect on the protein content of the wheat.

SUGGESTION FOR FUTURE RESEARCH

This study has shown that protein content of mature wheat can be estimated from the nitrogen content of the immature plant but that the correlation is too low for accurate and practical prediction. It is recommended that further research be devoted to determine what stage of maturity will most highly correlate with the protein content of the mature grain. In addition, the study of the protein content which is highly correlated with baking characteristics, studies should be devoted to the direct correlation of the nitrogen content of certain parts of the immature plant to the baking characteristics. The use of triplicate plots is recommended.

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RELATIONSHIP OF PROTEIN CONTENT
OF MATURE WHEAT AND VEGETATIVE GROWTH

by

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The importance of the environment upon the protein content of wheat has been recognized for years. Much effort has been devoted to this area; but, not including the study of the relationship of protein content of green wheat to mature wheat. Four hard red winter wheat varieties from 11 locations were selected at two different stages of maturity, i.e., early head and mature stages, for the nitrogen analysis to determine whether whole wheat plant analysis of nitrogen could be used to predict the protein content of the mature grain. The mature grain was tested also for physical dough properties of the flour and bread baking to observe the influence of environment upon the wheat quality. In addition, one variety from one station was chosen to study the effect of fertilizer treatment. Three different levels of nitrogen fertilization on protein content, this included 33, 66, 100 pounds of nitrogen per acre of urea and ammonium nitrate, respectively, applied at 3 different times, which included fall, late winter and spring. The following conclusions can be made:

1. There was a significant correlation in the protein content of wheat between the two stages of maturity but this correlation was not sufficiently high for purpose of accurate prediction of the protein content of the mature grain from the green plant.
2. In both the immature plant and mature grain, the protein content was affected mostly by the environment.
3. The influence of variety on the protein content was relatively not as significant as the environment.
4. Baking quality characteristics were influenced significantly by the protein content.
5. Protein content of the wheat was influenced by the nitrogen fertilization treatment. The higher concentration of nitrogen application produced

the highest protein content. Urea or ammonium nitrate or time of application had about the same effect on the protein content of the wheat.