RELATIONSHIPS OF ENDOSPERM PROTEIN FRACTIONS FROM NORMAL AND HIGH PROTEIN WHEATS TO PROTEIN CONTENT, LYSINE CONTENT, QUALITY, AND NITROGEN FERTILIZATION

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INTRODUCTION

Wheat endosperm proteins can be characterized in many ways. The first comprehensive study of wheat protein was made by Osborne (1907) and was based on protein solubility characteristics. Using this technique of characterization, Orth and Bushuk (1972) reported endosperm protein solubility differences between cultivars. The protein content of the endosperm has been shown to influence solubility fractions in some studies (Bell and Simmonds, 1963; and Pence et al., 1954) while another study (Tanaka and Bushuk, 1972) failed to show that the endosperm protein content had a significant influence on solubility fractions.

In general, low protein wheats contain more lysine (% of protein) than high protein wheats (Johnson, Mattern, Schmidt, and Stroike; 1972). Endosperm protein solubility fractions contain different amounts of lysine (Mattern, Salem, and Volkmer; 1968) which indicates that lysine variation in wheat may be related to differences in protein solubility patterns.

'Atlas 66', a soft red winter wheat containing genes for high protein, has been used in the Nebraska wheat breeding program since 1954. This wheat breeding effort has developed hard red winter wheat lines which have the ability to produce increased amounts of protein (Johnson et al., 1971). Some lines show promise for commercial production in Nebraska and other winter wheat areas of the world (Johnson, Mattern, and Schmidt; 1972). Since protein amino acid composition and several aspects of protein structure are regulated by the genetic code, it is conceivable that the genetic make up of a cultivar could influence the distribution of protein into solubility groups.

Positive responses of wheat to nitrogen fertilization were more frequently observed for protein content than for yield (Schlehuber and Tucker, 1959). An 'Atlas 66' derived line maintained a higher protein content than a commercial cultivar under all levels of nitrogen fertilization (Johnson et al., 1973). The positive response of wheat protein content to nitrogen fertilization was accompanied with increasing percent lysine and decreasing lysine (% of protein) (Hojjali and Maleki, 1972).

The objectives of this study were to evaluate the influence of the 'Atlas 66' high protein trait, protein content, and nitrogen fertilization on the distribution of protein into solubility classes. Possible relationships of the protein fractions with lysine content and quality factors were investigated. Assessments of environmental variation for protein fractions and quality factors were also made.

LITERATURE REVIEW

Breeding for Increased Protein

Breeding to improve wheat protein content was generally thought to be impractical until the early 1950's when Middleton et al. (1954) reported, after 3 years of testing, that five recently developed soft red winter cultivars were consistently higher in protein content than eight other cultivars selected for testing. One cultivar, 'Atlas 66', was 3.2% greater in protein content than the check cultivar as well as higher yielding. 'Atlas 66' and the four other cultivars were developed from crosses with either 'Frontiera' or 'Frondoso' which were introduced from South America.

Most of the research before 1950 indicated that genetic differences in wheat protein content were usually less than 1% actual protein, and that increases in protein would have to be at the expense of yield. It was not only the small genetic difference and the yield-protein relationship, but the environmental influence on protein content that discouraged wheat breeding efforts toward the improvement of protein content. Clark (1926) studied the segregation and inheritance of crude protein content in crosses grown in Montana under environmental conditions favorable for high protein content, and in California under conditions usually producing wheat low in protein. Grain from F_2 plants of a 'Marquis' x 'Hard Federation' cross had an average protein content that was intermediate to the parents, which differed on the average by 0.95% protein. The crude protein contents of the hybrid plants were negatively correlated with their yield. Selections from the F_2's were grown at two locations in Montana. Favorable yield conditions were present at one location

with low yield conditions at the other location. At the high yielding location there was a positive correlation between yield and protein content. At the low yielding location the correlation between yield and protein content in the F_3 was strongly negative. The data on the F_3 failed to show any segrates significantly higher than 'Marquis', but segrates were found lower than 'Hard Federation'. Progeny from a 'Hard Federation' x 'Propo' cross was grown at Davis, California. The F_2 data was similar to that of the low-protein parent, 'Propo', which indicated a dominance effect for low protein content. The average of the F_3 selections was intermediate to the parents which indicated that progress could be made when selecting for higher protein content. Only a few hybrids equalled or exceeded the best check row of 'Hard Federation'. The correlations between yield and protein content in both the F_2 and F_3 were slightly positive. Clark (1926) concluded: a) there was segregation for protein content similar to that for other quantitative characters, b) the inheritance of protein content was complex with environment affecting the results, c) yield and protein were frequently but not always negatively associated, d) higher protein F_3's could be obtained from selected F_2's, and e) to make large increases in protein content yield would have to be sacrificed.

'Atlas 50' and 'Atlas 66' were evaluated in Oklahoma and Texas in 1953, 1954, and 1955 (Reitz, 1964). They were compared with selected hard red winter wheats and were found on the average to be lower yielding but higher in protein content. A significant inverse relationship between yield and protein content was observed. Protein production per acre was lower for the Atlas cultivars. It was proposed by Haunold _et al_. (1962b) that low soil nitrogen may prevent the expression of

genetic differences in protein content. By growing 'Wichita', 'Comanche', 'Atlas 66', and 'Atlas 50' in a greenhouse soil bed under nitrogen fertilized and unfertilized conditions, and at four locations in the southern hard wheat region, 'Atlas 66' was shown to have a significantly higher grain protein content when soil nitrogen was not limiting. At low levels of soil nitrogen availability, grain protein was negatively correlated with yield in all cultivars. Based on the greenhouse data, the operation of a protein threshold was suggested, which represents the maximum level of protein content obtainable independent of yield. Haunold et al. (1962a) used crosses of 'Atlas 66' x 'Comanche' to make genetic measurements of grain protein content. The mean protein contents of the F_2 plants and the F_3 lines were intermediate to the parental cultivars. Heritability estimates as high as 0.65 were obtained and the data suggested a low number of genes to be conditioning protein content in the crosses studied. Stuber et al. (1962) reached a similar conclusion with 'Atlas 66' crosses and indicated the lack of dominant genes for either high or low protein content. Crosses of 'Atlas 66' with 'Chancellor', 'Malakoff-Nittany-3', and 'Leap'; and a cross of 'Leap' x 'Chancellor' were used by Davis et al. (1961) to study the inheritance of protein, texture, and yield in wheat. They concluded: a) protein was the most highly heritable trait studied, b) inferences about different families could be drawn for a wide range of environments based on testing in one year, c) partial dominance was present for low protein content, d) a negative relationship between protein content and yield was observed in three of the four populations, and e) no one family in any of the populations studied combined a maximum expression of all the desired traits. Lofgren et al. (1968) used four crosses ('Atlas 50' and

'Atlas 66' with 'Triumph' and 'Kaw') to obtain estimates of heritability for protein content. They were able to obtain progenies from the 'Atlas 50' x 'Kaw' crosses that contained 1 to 3% more flour protein than 'Kaw' controls. Kuspira and Unrau (1957) reported five different 'Thatcher' chromosomes in 'Chinese' to be responsible for increasing the protein content of 'Chinese'. Kaul and Sosulski (1965), with a cross of 'Selkirk' x 'Gabo', predicted that by selecting the top 5% of the F_2 plants, protein content could be increased 12.4% greater than the F_2 population mean. Chapman and McNeal (1970) studied gene effects for grain protein in five wheat crosses with 'Fronteira'. The crosses were with two cultivars (PI255141 and PI176217) equal to 'Fronteira' in protein content and three cultivars (PI141912, PI174603 and PI181272) 2 to 3% lower in grain protein than 'Fronteira'. They reported significant additive gene effects with all crosses, significant dominance effects in two of the five crosses, and indicated potential for improving grain protein percentage through selection.

Wheat breeding to introduce the genes for high protein from 'Atlas 66' into hard red winter wheats was started at Nebraska in 1954. Similar wheat breeding efforts have been under way in Kansas using 'Atlas 66' and 'Atlas 50'. In 1970, the Crop Research Division, Agricultural Research Service, U. S. Department of Agriculture and the Nebraska Agricultural Experiment Station released 26 advanced germplasm lines that could be traced to crosses of hard winter wheats with 'Atlas 66' made at Lincoln, Nebraska in 1953 (Johnson et al., 1971). All 26 lines have the ability to produce higher grain protein contents than the protein level of their hard red winter parent based on several years of Nebraska field trials. The first and second international winter wheat performance nursery

(IWWPN) included 'Atlas 66' and two high protein selections (Stroike et al., 1971, 1972). One selection was developed in Indiana from a complex cross involving 'Frondoso'. The other high protein selection was developed at Nebraska from an 'Atlas 66' x 'Comanche' cross. Based on 2 years of testing in the IWWPN, 'Atlas 66' and the two high protein selections had the highest grain protein content.

Wheat and Endosperm Lysine

In 1949 McElroy et al. (1949) reported amino acid analysis of nine samples of 'Marquis' wheat. The samples were obtained from an array of environmental conditions with total nitrogen content ranging from 1.94 to 4.03%. The data indicated that lysine (% of protein) decreased with increasing protein content in the grain. The following year Price (1950) reported similar decreases in lysine (% of protein) with increasing wheat protein content. Lawrence et al. (1958) determined the lysine content on 286 samples representing a broad spectrum of wheat varieties and species, related genera, and hybrids. A highly significant inverse relationship was observed between lysine (% of protein) and protein content for the samples ranging up to 13.5% protein. No relationship could be shown for the samples with greater than 13.5% protein. The lysine content was neither affected by the year of growth, nor by the location, except as these affect the total protein level. Comparisons of the amino acid composition were made by Hepburn and Bradley (1965) between different cultivars with a common level of protein and between samples of cultivars which differed widely in protein content. Lysine (mg per g nitrogen) was nearly constant for the samples with the same protein content. The low protein samples had a tendency to contain more lysine (mg per g nitrogen) than the high protein

samples. The data indicated that lysine concentration was influenced more by the protein level than by the cultivars. Robinson and Sageman (1968) studied 22 samples representing six cultivars. The samples ranged in nitrogen content from 2.42 to 3.86%. An inverse relationship between lysine (g lysine nitrogen per 16 g nitrogen) and nitrogen content could not be demonstrated. Seventeen 'Atlas 66' derived lines were evaluated for amino acid content by Mattern, Salem, Johnson, and Schmidt (1968). There was no evidence that lysine (% of protein) decreased with higher protein levels. When lysine was expressed as a percentage of grain the magnitude of the lysine increases were similar to the protein increases.

The first 2,079 samples of the world wheat collection screened for protein and lysine content indicated a positive relationship between protein and lysine when lysine was expressed as a percentage of total dry weight (Mattern, Johnson, Whited, and Schmidt; 1968). When lysine was expressed as a percentage of protein, its relationship with protein was negative. The correlation of protein with percent lysine was +0.81 compared to -0.48 for protein with lysine (% of protein). Regression analysis of the data on 4,100 wheats indicated that 18% of the variation in lysine (% of protein) was attributable to variation in protein as compared to 71% of the variation when lysine was expressed as a percentage of dry weight (Johnson, Whited, Mattern, and Schmidt; 1968 and Johnson et al., 1969). After 7,000 samples had been analyzed, 40% of the variation in lysine (% of protein) was accounted for by variation in protein (Mattern, Johnson, and Schmidt; 1970 and Johnson et al., 1970). An adjustment equation was derived from the data on the 7,000 samples to adjust lysine (% of protein) to 13.5% protein. Correlation analysis of 72 low protein wheat samples indicated a significant

negative relationship between lysine (% of protein) and protein (Johnson, Mattern, and Schmidt; 1972). A significant correlation could not be shown between lysine (% of protein) and protein based on 46 high protein wheat samples. Similar correlations were shown by Johnson, Mattern, Schmidt, and Stroike (1972) with 114 low protein samples and 50 high protein samples. Based on this data the relationship of lysine (% of protein) with protein was considered to be curvilinear.

McDermott and Pace (1960) found that as the protein content of flour increased from 6.7 to 14.0% the lysine content of the protein decreased. Hepburn et al. (1960) indicated that flour contains less lysine than whole wheat and that the offals (red dog, shorts, bran, and germ) contain more lysine than whole wheat.

Wheat Quality

Hehn and Barmore (1965) indicated that it should be possible to breed varieties of wheat exhibiting those various quality factors at the desired level within the limits of available genetic variability. Quality characteristics for which the existance of genotype variation has been established include: protein content, flour yield, flour ash, sedimentation, flour absorption, mixing time, mixing tolerance, loaf volume, kernel hardness, and bran clean-up. Reviews of experimental milling research include the following: Bequette and Barmore (1963), Jones and Ziegler (1964), Shellenberger and Ward (1967), and Ziegler and Greer (1971). Reviews of wheat and flour quality research include the following: Miller and Johnson (1954), Finney (1964), Pratt (1964, 1971), Zeleny (1964, 1971), and Finney and Yamazaki (1967). The subject of breeding wheat for quality has been reviewed by Reitz (1957), and Hehn and Barmore (1965).

Wheat quality can be defined in terms of the properties it imparts to its intended product or use. A quality type represents a combination of characteristics that determine acceptability, usefulness, and value of the products obtained from the grain. The quality of a hard winter wheat cannot be expressed in terms of a single property but depends on several milling, baking, and physical dough characteristics. Finney (1964) has listed the following properties as important to the milling quality of a hard wheat: bolting, hardness, flour ash, flour yield, and protein content. The following properties are considered to be important to the baking quality of a hard wheat: protein content, mixing requirement, mixing tolerance, oxidation requirement, dough handling, water absorption, loaf volume, and crumb grain and color.

Many tests to evaluate quality have been developed. Some require large amounts of sample and others can be used on relatively small amounts of sample, thus, allowing plant breeders to evaluate early generation progeny. Micro-milling procedures have been developed by Shoup et al. (1957) to the point where only 5 g of wheat are required to evaluate the milling quality of a wheat line. Likewise, a mixograph has been developed by Finney and Shogren (1972) that utilizes 10 g of flour. The mixograph measures the combined affects of elasticity, plasticity, and visocosity as functions of continuous mixing. The mixograph can be used to measure mixing requirement (time to peak) and mixing tolerance (slope and width after peak, and stability of mixogram height on either side of peak). The commonly used farinograph measures absorption, optimum mixing time, and mixing tolerance. This instrument takes into account the plasticity and mobility of dough when subjected to prolonged, relatively gentle mixing at constant temperature.

Morris et al. (1944) conducted several quality tests on 29 cultivars representing a wide range in quality. The samples were grown in the eastern soft wheat area. High correlation coefficients were obtained between mixogram areas and several other quality tests including flour protein and loaf volume. Flour water absorption was reported by Finney (1945) to be a linear function of protein content within a cultivar. Differing rates of increasing water absorption with increasing protein were found for flour from different cultivars. An important relationship among wheat quality factors was that loaf volume within a cultivar can be accounted for by protein content (Finney and Barmore, 1948). Loaf volume was found to increase linearly with increasing protein content, with certain cultivars having different relationships (regression lines). It was concluded that the loaf volume-protein content regression lines for cultivars represent differences in protein quality. Miller et al. (1956) found experimental baking mixing time to be more highly correlated with mixograph mixing time than with farinograph mixing time. Variety had a significant effect on the correlation between experimental baking mixing time and mixograph mixing time. Protein content, centrifuge absorption, mixograph development, and loaf volume were measured on 12 cultivars grown at 15 locations in one year and 13 cultivars grown at 13 locations in another year (Miller and Fowler, 1971). A combination of protein, mixograph, and centrifuge absorption gave highly significant multiple correlations with loaf volume. Mixing time, in general, decreases as protein content increases to about 12%, thereafter, remaining approximately constant (Finney and Shogren, 1972; Finney and Yamazaki, 1967). Loaf volume at the 13% protein level increases as mixing time increases from 7/8 to about 3 min (Finney and Shogren, 1972; Finney and

Yamazaki, 1967). Beyond 3 min, loaf volume at 13% protein remains approximately constant with increasing mixing time. With a knowledge of protein content and mixograph mixing time the overall bread baking quality of a wheat can be predicted.

Lofgren et al. (1968) studied crosses of 'Triumph', a cultivar with short to medium mixing time and fair mixing tolerance, with 'Atlas 50' and 'Atlas 66'. They also studied crosses of 'Kaw', a cultivar with medium-long mixing time and good mixing tolerance, with 'Atlas 50' and 'Atlas 66'. 'Atlas 50' and 'Atlas 66' had short mixing time and inferior mixing tolerance. Generally, the progeny of the 'Kaw' crosses had stronger mixing properties and exhibited more mixing tolerance than progeny of the 'Triumph' crosses. For the 'Triumph' crosses, the Atlas type predominated, and no mixogram values approached the 'Kaw' type. For the 'Kaw' by Atlas crosses, about 1/4 of the progenies approached the 'Kaw' type. Several progenies from 'Kaw' x 'Atlas 50' crosses contained 1 to 3% more flour protein than 'Kaw' controls and possessed promising hard wheat milling, baking, and dough mixing properties. Johnson et al. (1963) have summarized some agronomic and quality characteristics of progeny from an 'Atlas 66' x 'Comanche' cross. F_2-derived families from 'Atlas 66' x 'Comanche' crosses were selected for high protein in the F_2 and grain yield in the F_3. All families had higher protein contents and were more productive than 'Comanche'. Some families with the highest protein also were among the more productive families. The 15 F_2-derived families evaluated for quality exhibited a wide range in milling characteristics. Most milled as soft or very soft wheats, with one being intermediate and two milling as hard wheats. There was little or no decrease in flour protein from that in the wheat for some families.

Protein in other families dropped as much as 1.8% from wheat to flour. Water absorption values did not differ from 'Comanche'. Families with mixing time as long as that of 'Comanche' were not recovered. Five families had average mixing times of 2 1/2 min or longer compared with 3 1/3 min for 'Comanche'. Mixing tolerances also differed widely. The families with the longest mixing times were the most tolerant to over mixing. Four families approached and one family was equal to 'Comanche' in loaf volume. It was recognized that none of the families possessed combinations of characteristics necessary in a commerically acceptable hard red winter wheat, but the potential for development of commercially acceptable cultivars was considered to be good. A more recent report (Johnson, Mattern, and Schmidt, 1972) indicated that satisfactory processing quality has been combined with high protein in the second and third breeding cycles with 'Atlas 66' derived lines.

Characterization of Wheat Protein

The characterization of wheat protein began in 1728 when Baccari washed a dough with water and prepared gluten (Baily, 1941). The first comprehensive review of wheat protein research was made by Osborne (1907). Baily (1944) has prepared an excellent review of wheat protein research between 1728 and 1944, including Osborne's work.

The following historical review of wheat protein research was taken from Baily's (1944) book. It was 77 years after Baccari prepared gluten when in 1805 Einhof reported the extraction of a protein substance from flour with alcohol. Einhof assumed the substance to be identical with gluten. Shortly after Einhof's observation Taddai reported the separation of gluten into two protein preparations, one portion dissolved by alcohol and another portion not dissolved by alcohol. The research to

characterize wheat proteins during the nineteenth century was complicated by the use of different terminology to describe what many have been similar extraction methods. Osborne's research was so comprehensive that Baily described it in a separate section of a chapter reviewing early research on wheat proteins. The four major protein fractions prepared by Osborne were designated: albumin, globulin, gliadin, and glutenin. The preparation of albumin consisted of: a) extracting flour with 10% NaCl, b) saturating the extract with $(NH_4)_2SO_4$, c) dissolving the precipitated protein in NaCl solution, d) dialyzing the redissolved protein to precipitate the globulin, and e) heating the dialyzed solution for an hour at 60° to 65° C. The coagulated protein was considered to be albumin. Globulin was prepared by dissolving the precipitate from the dialysis of an albumin preparation in 10% NaCl. Additional purification of the globulin was accomplished by saturating the 10% NaCl solution with $(NH_4)_2SO_4$. Gliadin was prepared from gluten by extracting it with 70% ethanol. When small amounts of gliadin were desired the gluten was prepared by extracting flour with 10% NaCl. When larger amounts of gliadin were desired the gluten was prepared by washing the flour to remove starch and soluble material. Glutenin was prepared by extracting the residue from a gliadin preparation with 0.1% KOH solution. Glutenin could be recovered from the extract by neutralizing with dilute HCl. The dispersion and precipitation was commonly repeated to effect greater purification. Osborne characterized his preparations by elemental and amino acid analysis. The amino acid analysis at that time was not complete, but differences in the fractions were observed. Osborne was interested in obtaining what were considered pure preparations of different wheat proteins; thus, his fractionation procedure

was not considered quantitative. Many attempts have been made to establish quantitative procedures of solvent fractionation. One difficulty encountered with solvent fractionation was that the fractions were not clearly defined, which was illustrated by some of the properties of gliadin. Gliadin was slightly soluble in water and soluble in alcohol-water solution. By increasing the concentration of alcohol to a certain point a maximum amount of gliadin was obtained. Further increases in alcohol concentration reduced the solubility of gliadin until it was no longer soluble in absolute alcohol. Gliadin and glutenin were dispersed by dilute acid or alkali, but glutenin differed from gliadin because it was not soluble in alcohol.

Reviews of wheat protein research made after 1944 include the following: Blish (1945), Sullivan (1954, 1965), Pence, et al. (1956 1964), Pence (1962), Dimler (1965), Wall (1964, 1968, 1971), Kasarda et al. (1971) and Wrigley (1972). Wrigley (1972) reported that the property of solubility still has application as a basis for analysis.

In addition to solvent fractionation, many other methods have been used to characterize endosperm protein. Some of the more commonly used methods are ion-exchange chromatography, gel filtration, and electrophoresis. When these methods are used the protein has to be extracted before it can be applied to the column or gel. The solvents used for extraction could influence the results. The use of new extraction solvents have been reported. Gallus and Jennings (1968), Jennings (1968), and Stanley et al. (1968) reported the use of a phenol-acetic acid-water mixture which used in 3 extractions can solubilize 98% of the protein. Cluskey and Dimler (1967) reported the use of hydrochloric acid 2-chloroethanol to extract the acetic acid-insoluble proteins from wheat

gluten. Meredith and Wren (1966) have reported the use of an aqueous solution of 0.1 M acetic acid, 3 M urea, and 0.01 M cethyltrimethylamnonium bromide to quantitatively extract flour protein including glutenin.

Chen and Bushuk (1970) have recently reported a modified Osborne solvent fractionation procedure. The extraction was accomplished by stirring in a centrifuge bottle on a magnetic stirrer, first with two portions of 0.5 M NaCl followed with one portion of distilled water. The three extracts were combined, dialyzed, and centrifuged to separate the precipitated salt-soluble proteins from the water-soluble proteins which remain in the supernatant. The residue was then extracted with two portions of 70% ethanol followed by extraction with two portions of 0.05 M acetic acid. Five fractions are obtained: water solubles, salt solubles, alcohol solubles, acetic acid solubles, and a residue. With careful procedure it was possible to get 99% recovery of the protein, but due to the large number of stirrings and transfers of solvent fractions certain fractions could easily be underestimated.

A method of characterizing wheat protein that combines solvent extraction and column chromatography was first used by Maes (1962). The method consisted of mixing 10 g of flour, 10 g of pumice, and 100 g of fine sand and placing the mixture in a column which was eluted with a sequence of solvents. The following sequence of solvents was used: 1) distilled water, 2) 10% isopropyl alcohol, 3) 30% isopropyl alcohol, 4) 4% lactic acid, and 5) 0.5% KOH. Mattern, Salem, and Volkmer (1968) investigated the feasibility of reducing the sample size requirements of the original Maes method by preparing columns with 1 g, 2.5 g, and 5 g of flour. Recoveries from the columns were in reasonable agreement

when a proportionately smaller column was used for the 1 g sample. After investigating several different solvent sequences and analyzing the extracted protein with starch gel electrophoresis Mattern, Salem, and Volkmer (1968) recommended the following solvent sequence: 1) 40% isopropyl alcohol, 2) 2% NaCl, 3) 3.85% lactic acid, and 4) 0.5% KOH. Williams and Butler (1970) reached a similar conclusion after using several solvent sequences and subjecting the extracted protein to acrylamide gel electrophoresis. The Maes method collects the total of a given solvent fraction from which an aliquot is taken for nitrogen determination, thus avoiding transfer losses.

Factors Related to Protein Fractions

Research efforts to characterize wheat protein are usually accompanied by an attempt to relate the protein fractions to other characteristics of the wheat or endosperm. Some of these characteristics include protein content, amino acid composition, and quality. Several factors related to protein fractions are often included in one literature article; therefore, a review of these has not been arranged in a definite order so that the continuity of individual research efforts can be maintained.

Fractionation and reconstitution techniques (synthetic doughs) have been used by many researchers to study the effect of flour components on baking behavior (Sandstedt _et al._, 1939; Finney, 1943). Pence _et al._ (1951) concluded that the components soluble in 0.1% phosphate buffer (pH 6.8) were required for maximum performance of gluten, with the exception of the gluten from a durum wheat. Additional fractionation of the soluble fraction to obtain what was designated a crude albumin produced a positive loaf volume response and shortened mixing time when

added to gluten-starch doughs. Mattern and Sandstedt (1957) concluded that a water-soluble component was responsible for determining mixing requirement. The mixing requirement was lengthened by removal of the water-solubles. The factor was precipitated with low concentrations of ammonium sulfate, and had a high amide nitrogen content which indicated that it consisted largely of gliadin. Mullen and Smith (1965) separated a short- and a long-mixing flour into salt-soluble, water-soluble, and protein-starch residue fractions. The outstanding difference between the two flours was the greater water solubility of gluten components from the short-mixing flour. Smith and Mullen (1965) concluded that salt-solubles (albumins and globulins) had little affect on mixing characteristics. Protein-starch residues (glutenins) had long-mixing requirements, whereas, the addition of water-solubles (gliadins) markedly shortened the mixing requirements. The short-mixing flour contained more water-soluble protein. Recent research reviewed by Hoseney and Finney (1971) indicated that the albumin and globulin protein fractions did not appear to be involved in bread baking performance except for possible effects of both protein fractions found in the gluten protein complex. The factor responsible for loaf volume potential was located primarily in the gliadin-rich fraction, whereas, the factor responsible for mixing time appeared to be in the glutenin-rich fraction.

Much has been learned about the effect of protein components on quality factors by identifying repeatable protein component differences in samples known to be different in the quality factor of consideration. Baily (1944) reviews many attempts to relate fractionation data to the bread baking quality of a flour. In some cases the amount of protein extracted with a certain solvent was related to quality and in other

cases ratios of the different fractions were thought to give a better indication of quality than would the data on a single fraction. Tsen (1968) states that the quantitative distribution of protain components in flour protein may give a better indication of protein quality and flour quality than other measures that have been proposed. Wrigley (1972) cites historical research on gliadin-to-glutenin ratios. At that time the use of the gliadin-to-glutenin ratios was abandoned largely because of confusion concerning methodology. Currently there has been a renewed interest in the subject because mechanical properties of a dough depend on the relative amounts of weak, extensible gliadin and tough, elastic glutenin.

Bell and Simmonds (1963) fractionated 26 samples by differential extraction with 0.01 M sodium pyrophosphate (pH 7.0) and 0.05 M formic acid (pH 3.5). Both pyrophosphate- and formic acid-soluble nitrogen (% of flour) increased with increasing total nitrogen content. An inverse relationship was observed between pyrophosphate-soluble nitrogen (% of total nitrogen) and total nitrogen compared to a slight upward trend between formic acid-soluble nitrogen (% of total nitrogen) and total nitrogen. There was no significant correlation between pyrophosphate-soluble nitrogen (% of flour) and baking score. The correlation between formic acid-soluble nitrogen (% of flour) and baking score had about the same level of significance as that between total nitrogen and baking score. Pence _et al_. (1954) studied the soluble-protein (albumin and globulin) contents of 32 flours that varied widely in type and baking quality. The amounts of soluble proteins were found to increase directly with total flour protein, but the relationships became inverse when they were expressed as percentages of total protein.

Neither the amounts of the soluble proteins nor the proportions of soluble to gluten protein were correlated with protein quality. The ratio of albumins to globulins was correlated with protein quality as was the total protein content of the flour. When both albumin-to-globulin ratio and protein content were considered the partial correlation of albumin-to-globulin ratio was significant when flour protein was held constant.

Tanaka and Bushuk (1972) used the modified Osborne fractionation procedure of Chen and Bushuk (1970) which fractionated flour protein according to solubility. Five samples of 'Manitou' and 11-463A were compared with one sample of 'Pitic 62', 'Garnet', and 'Talbot'. The 'Manitou' samples varied in protein content from 10.5 to 15.6% and the 11-463A samples varied from 11.6 to 14.2% protein. The samples of 'Manitou' and 11-463A had been grown under the same climatic conditions (same location), but differed in protein content because of soil fertility, as verified by soil nitrogen analysis. The authors concluded that the solubility properties of the proteins of flour milled from similar wheats grown under identical conditions were not affected by protein content. 'Manitou' and 11-463A were considered to have good baking quality, and the other three cultivars to have poor breadmaking quality. Each poor quality cultivar contained less alcohol- and acetic-soluble and more of the insoluble residue protein. Orth and Bushuk (1972) studied 26 cultivars grown at four locations with the modified Osborne procedure of Chen and Bushuk (1970). The cultivars represented a broad spectrum of baking qualities. Statistically significant differences in the proportions of each solubility group were obtained between cultivars and locations, the former differences being larger

than the latter. Significant correlations were found between both glutenin and residue protein, and several breadmaking-quality parameters. The proportions of glutenin in the total flour protein was negatively correlated with loaf volume per unit protein, whereas, residue protein and loaf volume per unit protein were positively correlated. The ratios of gliadin to glutenin and albumin to globulin were also significantly positively correlated with loaf volume per unit protein.

Maes (1966) found a correlation between wheat protein fractions and baking quality. The total protein content of the flour was directly related to the baking quality, and baking quality was highly correlated with the percentage of total protein soluble in 40% isopropyl alcohol. Mattern, Schmidt, Morris, and Johnson (1968) using the modified Maes protein extraction method on flour from chromosome substitution lines observed an association, although not absolute, of high alcohol solubles with inferior dough-mixing properties, and a relationship between loaf quality and the percentage of alkali solubles. Williams and Butler (1970) used the Maes method of solvent fractionation to study flours from wheat species of different and the same chromosome number and different varieties of wheat of the same species. No consistent relation appeared between total protein extracted from flours or amount extracted by a solvent and the baking behavior of the flours, except as protein content itself varied.

Pence et al. (1950) prepared gluten from 17 different flours representing a wide range in wheat types, sources, protein contents, and baking characteristics. The flour protein content ranged from 5.7 to 14.2% (14% moisture basis). The amino acid composition of the glutens were determined and although the authors considered it uniform, the

glutens from three of the flours lowest in protein did contain higher amounts of lysine than the mean of all glutens studied. Gunthardt and McGinnis (1957) have suggested that the differences in lysine contents of high and low protein wheats could be explained by changes in individual protein molecules or by changes in the proportions of the different proteins. Lawrence et al. (1958) indicated that the proportion of total wheat protein in the endosperm in low protein wheats was less than in high protein wheats, but the trend was not large enough to account for the higher percentage of lysine in the total protein of low protein wheats. Lysine (% of protein) was higher in the flour from low protein wheats than in the flour from high protein wheats. The low protein wheats, therefore, not only had relatively more high lysine protein from the bran and germ but the endosperm protein was also high in lysine.

Woychik et al. (1961) isolated a water soluble protein fraction from gluten. The fraction represented 6% of the total gluten and contained four times as much lysine (% of protein) than was contained in other gluten components. The other five gluten components analyzed were essentially constant in lysine with exception of one component that was slightly higher in lysine. The authors were unable to demonstrate any major difference in composition between gluten components which could account for the gross property differences present in these fractions. It appeared likely that molecular or structural variations were responsible for the characteristic differences in solubility of the fractions. Ewart (1967) found glutenin to be higher in lysine than gliadin based on the analysis of both fractions from two strong and two weak wheats. There was no evidence of lysine differences between cultivars in either glutenin or gliadin. Mattern, Salem, and Volkmer (1968)

reported amino acid analysis of solubility fractions from the modified Maes extraction procedure. The lysine content (% of protein) of the fractions differed from the lysine content (% of protein) of the flour as follows: a) the salt fraction contained more than twice as much lysine, b) the acid fraction contained twice as much lysine, c) the alkali fraction contained an equal amount of lysine, and d) the alcohol fraction contained approximately 1/2 as much lysine.

Simmonds (1962) determined the amino acid composition of the whole grain and flour on six samples. The whole grain ranged in nitrogen content from 1.57 to 3.14% and the flour from 1.6 to 2.7%. Lysine (lysine nitrogen as a percent of total nitrogen) had a tendency to decrease with increasing protein in the whole grain and flour. Flour proteins high in lysine were extracted with sodium pyrophosphate. Two of the flours, differing in physical characteristics and nitrogen content, were found to have protein fractions with nearly identical amino acid composition.

Environmental Influences

Much research has been conducted to determine what environmental factors effect plant growth. This research includes the influence of environment on wheat and endosperm (flour) protein, lysine, quality, and protein fractions. Some of the environmental factors studied include: nitrogen fertilization, soil moisture, and air temperature. For some wheat grain and endosperm characteristics the environmental factors have not been identified but measures of environmental variation have been made.

Schlehuber and Tucker (1959) summarized yield and protein content data on 10 nitrogen fertilizer trials conducted in western Oklahoma in 1958. The data indicated a more striking relationship between amounts

of added nitrogen and percent protein than between added nitrogen and wheat yield. More consistent positive responses were observed for protein content than for yield. There was as much difference in protein content between locations as there was due to nitrogen additions at the specific locations. Stickler et al. (1964) grew 'Triumph', 'Pawnee', 'Ottawa', and 'Bison' under different levels of nitrogen fertilization on two soil types in two years. The interaction of nitrogen fertilizer level by cultivar was non-significant for protein content. Wheat protein content increased linearly over the entire range of nitrogen fertilizer levels. Although the differences were small, 'Ottawa', had the inherent ability to utilize soil nitrogen more efficiently than the other cultivars for the production of protein. Johnson et al. (1973) grew CI14016 (NE65307) and CI13547 ('Lancer') at four sites in 1968, three sites in 1969, and three sites in 1970. Different sites were used each year. CI14016, a cross of 'Atlas 66' and 'Comanche', was released as elite high protein germplasm in 1970 (Johnson et al., 1971). Four levels of nitrogen fertilizer were applied in 1968, and six levels were applied in 1969 and 1970 at increments of 22.5 kg per ha. Measurements were made of test weight, grain yield, and grain protein. Significant yield responses to fertilizer occurred at six of the ten sites with one or more sites showing a response each year. A positive response of protein content to fertilizer applications was shown at all sites. Cultivar by fertilizer interaction was present for yield but lacking for protein content. A relationship between yield and protein content was detected.

Hutcheon and Rennie (1960) grew hard red spring wheat in a growth chamber under a range of soil moisture conditions and varying levels of

nitrogen supply. Crop yield and protein content of the grain was strongly influenced by water stress and associated characteristics of available moisture. A single period of stress at any stage of crop growth, even though followed by favorable moisture conditions for the remainder of the growing period, also produced a marked effect on yield and grain protein. The authors concluded that grain protein was more strongly influenced by the level of available moisture than by the supply of available nitrogen. Dubetz (1961) grew 'Thatcher' wheat in the greenhouse on two soil types, under four rates of nitrogen fertilization, and at three moisture levels. Significant decreases in protein percent were obtained with increasing soil moisture on a loam soil but not a loamy sand. Increases in protein percentage with increasing nitrogen fertilizer were obtained on the loam soil only.

Long and Sherbakoff (1951) applied two levels of nitrogen fertilizer at three different times (November 20, March 8, and May 4) to a soft red winter wheat. The wheat and flour protein contents did not differ greatly when topdressings were made in November and March, but were appreciably higher where topdressings were made in May. The higher rate of nitrogen fertilizer increased protein content at each application time. The protein contents from the various treatments ranged from 10.6 to 15.3% (14% moisture basis). The area under the mixogram curve was measured and expressed in square centimeters. The height of the mixogram curve was determined primarily by the protein content of the flour. Thus, higher mixogram areas were associated with the increases in protein content of the flour. McNeal _et al_. (1971) have reported nitrogen fertilization to increase grain and flour protein percentage which produced a corresponding increase in loaf volume, and grain and

texture scores. Farinograph mixing time was reported to have decreased slightly with increasing amounts of nitrogen.

Finney and Fryer (1958) have reported subnormal (reduced) loaf volumes and mixing times to be consistently associated with temperatures above 90° F during the last 15 days before harvest. Mixing time was negatively correlated with heat damage to loaf volume. The extent of damage was at least partially regulated by cultivar because cultivars with longer mixing times were more resistant to the adverse effects of high temperatures than cultivars with relatively short mixing times. Other environmental factors influencing the damage were physical conditions of the soil and relative humidity. High humidities had a tendency to reduce temperature damage of baking properties. Williams (1966) also points out that baking performance of wheat cultivars which have ripened under hot conditions to be usually lower than when the cultivars mature under normal conditions.

Sosulski et al. (1963) grew 'Thatcher' wheat in a growth chamber and studied the effect of air temperature, soil moisture, and nitrogen fertilization on flour quality. Two rates of nitrogen, two moisture regimes, and three temperature regimes were used. The protein content of the flour increased progressively as air temperature was raised. Responses in flour protein to the higher rate of nitrogen fertilization were obtained at each moisture and temperature regime. The effect of moisture supply on protein was dependent on the air temperature. Temperature was the only environmental factor of those studied which had a consistent effect on mixing time. Shorter mixing times were obtained at lower temperatures. The mixing times of two 'Thatcher' field grown samples were longer than any of the growth chamber grown samples. Day

and Barmore (1971) measured several quality factors on samples of flour obtained from 'Maricopa' wheat that received optimum irrigation at Tucson, Arizona and compared it to flour from 'Maricopa' wheat that had been water stressed during a part of the growing season. Three stress periods were studied: jointing stage, flowering stage, and dough stage. Water stress at the jointing and dough stage reduced flour yields compared to optimum irrigation. Moisture stress at flowering increased flour protein content. Stress at the dough stage increased mixing time, while all stress periods increased the mixing curve area.

Johnson, Khan, and Sanchez (1972) measured quality characteristics on flour from seven cultivars grown at eight locations for seven years. Measurements taken were protein content, loaf volume, absorption, farinograph mix time, bakery mixing time, and quality score. Rainfall and maximum temperature during April, May, and June were taken at each location during each growing season. Location and season affected all quality characteristics except bakery mixing time. Cultivars also had significant effects, especially on bakery mixing time. The protein content was influenced by location. Since protein content was highly correlated with loaf volume, absorption, and farinograph mixing time; these quality factors were influenced mainly by location. Warmer temperature in May favored superior breadmaking quality. As more moisture became available greater grain yields resulted and quality decreased. These results suggested that available nitrogen may frequently limit the production of high-quality bread wheat, particularly when moisture was abundant and favorable for large grain yields. Helm et al. (1968) evaluated 177 cultivars and experimental lines of hard red winter wheat grown in the hard red spring wheat region for milling and baking char-

acteristics. The winter wheats were compared with seven commercial spring cultivars and were superior in kernel weight, test weight, and whole kernel ash but inferior in mixogram score, water absorption, and loaf volume. Busch et al. (1969) evaluated protein content, flour yield, flour ash, flour water absorption, mixogram score, and loaf volume on eight cultivars of hard red spring wheat. Data on samples from 57 environments were obtained on seven of the cultivars. Samples from 23 environments were available on the eighth cultivar. A modification of the statistical model proposed by Eberhardt and Russell (1966) was used to determine the response of each cultivar to the environment. The values for each selected cultivar were regressed on an environmental index for each quality characteristic measured. The linear regression coefficients were used for comparison of predicted performance, among cultivars, over a range of environments. The authors proposed a minimum standard system of using check cultivars with known environmental responses to aid in the evaluation of potential cultivars for quality characteristics.

Sosulski et al. (1963) grew 'Thatcher' wheat at three moisture and five nitrogen levels in a growth chamber. The protein content of the grain ranged from 10.2 to 23.0% (14% moisture basis). Soil moisture was the principal factor affecting protein. The largest responses in protein content to increasing rates of nitrogen fertilization were obtained at the medium moisture level. Flour was obtained from wheat samples grown under two levels of nitrogen, two moisture regimes, and three temperature regimes. Flour protein ranged from 11.8 to 16.8% (14% moisture basis). Flour protein increased as air temperature was raised from 62° F to 75° F. Responses in flour protein content to nitrogen

fertilization were obtained at each moisture and temperature regime. The effect of moisture supply on flour protein content was dependent on air temperature during growth. Five wheat grain samples representing the range in protein content were selected and subjected to amino acid analysis. Lysine (lysine nitrogen as a percent of total nitrogen) decreased with increasing protein content in the grain. Four flour samples from the growth chamber and two from the field were subjected to amino acid analysis. The protein contents ranged from 12.0 to 17.3% (14% moisture basis). Whole wheat lysine levels were generally higher than flour lysine levels. Flour lysine (lysine nitrogen as a percent of total nitrogen) decreased with increasing flour protein. Gunthardt and McGinnis (1957) selected high and low protein wheat samples from fertilizer and irrigation experiments. The high protein samples were produced with high rates of nitrogen and several water applications. The low protein samples were obtained with low rates of nitrogen application. The high protein wheats were found to contain lesser amounts of lysine (% of protein) than the low protein wheats. Larsen and Nielsen (1966) grew 'Koga' wheat in a pot experiment with 20 nitrogen fertilizer treatments in order to determine the yield curve to the greatest possible extent. Samples of grain were selected for amino acid analysis from nine treatments representing the entire experimentally accessible yield curve. The supplied fertilizer nitrogen ranged from 0.025 to 5.0 g per pot. The nitrogen and lysine percentages of the grain increased rather sharply from the 0.60 to the 5.0 g per pot treatments. When lysine was expressed as g lysine nitrogen per 100 g total nitrogen no consistent trend could be established between lysine and fertilizer applied until 1.5 g were applied. At this point lysine decreased with

increasing fertilizer up to 5.0 g per pot. The nitrogen content of the grain increased from 1.74 to 3.51% under this same range of fertilizer treatments. Hojjati and Maleki (1972) grew 'Roushan' wheat in the field with nitrogen fertilizer treatments ranging from 0 to 200 kg per ha. The protein and lysine percentages of the grain increased consistently with increasing increments of nitrogen fertilization. Inverse relationships were shown for lysine (% of protein) with nitrogen fertilizer applied and protein percentage of the grain.

With the exception of lysine (% of protein) decreasing with increasing protein resulting from nitrogen fertilizer applications, limited information has been available regarding other environmental influences on the lysine level of wheat and endosperm protein. Based on a limited number of locations and cultivars, Johnson et al. (1969) reported significant genotype by environment interactions for lysine content. Johnson, Mattern, and Schmidt (1972) have reported lysine data from 18 station-years on three potential high lysine wheats and a check cultivar. The range in individual station-year values for lysine (% of protein) was greater than the mean differences between the cultivars. The protein content varied considerably from station-year to station-year, therefore, lysine (% of protein) was adjusted to a common level of protein. The adjustment accounted for a portion of the variation, but the range in individual station-year adjusted lysine values indicated that environment exerted a strong influence on the lysine level of wheat.

Reports in the literature describing environmental influences on protein components have been limited. Lee and Ronalds (1967) analyzed the acetic acid soluble proteins (gliadins) with carboxmethyl-cellulose chromatography and starch-gel electrophoresis. Samples of the following

cultivars with the following protein ranges were obtained from six locations: 'Gabo' (8.1 - 17.9%), 'Spica' (9.6 - 16.0%), 'Javelin' (8.9 - 12.7%), and 'Quadrat' (8.3 - 12.0%). The authors reported that environmental differences in starch-gel and chromatographic patterns were small compared to cultivar differences. The variations between samples of the same cultivars could have resulted from environmental factors or may have been the result of uncontrollable experimental conditions. Koenig et al. (1964) analyzed (with moving boundary electrophoresis) acetic acid extracts of flours from five cultivars of hard red winter wheat grown in six different locations. The following cultivars, differing in mixing and baking properties, were studied: 'Pawnee' (good quality, short mixing time), 'Comanche' (good quality, long mixing time), 'Chiefkan' x 'Tenmarq'--No. 501097 (poor quality, short mixing time), 'Chiefkan' x 'Tenmarq'--No. 501099 (poor quality, very short mixing time), and 'Quivira-Tenmarq' x 'Marquillo-Oro'--CI12995 (good quality, extra long mixing time). The samples were grown in Denton, Texas; Clovis, New Mexico; Goodwell, Oklahoma (under irrigation); Manhattan, Kansas; Alliance, Nebraska; and Akron, Colorado. Relatively small differences in electrophoretic distributions were found for a cultivar grown in different locations. Cultivar differences in electrophoretic patterns were noted along with cultivar differences in amounts of nitrogen extracted by acetic acid, and the amount of nitrogen remaining after dialysis. The authors concluded the differences in the electrophoretic distribution did not parallel the differences in the protein content of the flour or in the amount of protein extracted. 'Comanche' flour showed the greatest difference in distribution of components and also contained the least amounts of acetic acid-extractable protein.

MATERIALS AND METHODS

Samples Evaluated

Ten samples of each of the cultivars listed in Table I were evaluated. The samples of 'Lancer' and NE65307 were obtained from the 0, 45, 90, and 135 kg per ha nitrogen fertilizer treatments of sites 8 and 9; and the 0 and 135 kg per ha nitrogen fertilizer treatments of site 10 (Johnson et al., 1973). Sites 8 and 9 were grown on fallow land in Deuel and Phelps county Nebraska. Site 10 was grown under continuous cropping in Jefferson County Nebraska. Mean yields were 2,345; 3,044; and 3,468 kg per ha in sites 8, 9, and 10 respectively. Samples of the remaining cultivars were obtained from the following Northern Regional Performance Nursery (NRPN) locations: Lethbridge, Alberta; Tetonia, Idaho; St. Paul, Minnesota; Moccasin, Montana; Casselton, North Dakota; Clovis, New Mexico (dryland); Highmore, South Dakota; Sheridan, Wyoming; North Platte, and Sidney, Nebraska.

The three Nebraska released cultivars represent good milling and baking hard red winter wheats. Johnson et al. (1961) have described the agronomic and quality characteristics of 'Warrior'. 'Kharkof' was introduced from Starobelsk, Kharkof, Russia by M. A. Carleton in 1900 (Clark and Bayles, 1935). 'Bezostaya', a more recently developed Russian cultivar, has proven to be universally adapted and productive based on two years of testing in the IWWPN (Stroike et al.; 1971, 1972). The experimental lines; NE65307, NE68510, and NE68513; are the result of a Nebraska wheat breeding effort which introduced the genes for high protein from 'Atlas 66' into hard red winter wheats. NE65307 was released as an elite high protein germplasm line (Johnson et al., 1971).

Table I. Cultivars evaluated.

Cultivar	CI	Parentage	Origin	Released
Kharkof	1442	Introduction	Russia	1900*
Bezostaya	15158	Lutescens 17 x Skorospekla 2	Russia	1959
Warrior	13190	Pn x Cnn	Nebraska	1960
Lancer	13547	Tk-Cnn x Hope-Cnn	Nebraska	1963
Centurk	15075	Ky 58-Nth-Hope-Tk^2-Cnn x Phr	Nebraska	1971
NE65307	14016	Atl 66 x Cmn	Nebraska	1970†
NE66403		Pn-Cnn-Pn-Ky 58 x Cnn	Nebraska	exp §
NE68510		Wrr-Atl 66-Cmn x Lancer	Nebraska	exp
NE68513	15074	Wrr-Atl 66-Cmn x Cmn-Ott	Nebraska	exp

* Year introduced into U.S. (Clark and Bayles, 1935)

† High-protein germplasm line (Johnson *et al.*, 1971)

§ Experimental

Sample Processing and Quality Evaluation

Seven to ten g of the grain received was ground, blended, and placed in a moisture equilibration cabinet as described by Mattern (1972), and Mattern and Bishop (1973). Forty g of grain was tempered to 15.0% moisture in 70 x 185 mm glass jars for 24 hours and milled on a Brabender Quadrumat Jr. experimental mill. The sifting reel of the mill was removed, therefore, the various mill fractions were collected as a composite in the mill drawer. The milled grain was then placed on a standard sieve No. 70 (65 mesh Tyler equivalent with 210μ openings) and shook mechanically for 90 sec. The portion of the milled grain passing through the sieve will hereafter be referred to as endosperm. Endosperm weights were taken and mill yield was calculated as a percentage of the tempered grain weight. Each bran sample from the milling procedure was compared to a set of five standard bran samples and rated as excellent, good, fair, poor, or very poor. The criterion of evaluation was the amount of endosperm remaining on individual bran particles. Approximately 10 g of endosperm was placed in the moisture equilibration cabinet (Mattern and Bishop, 1973) and sub-sampled for analytical analysis. Ten g of the remaining endosperm was used for a mixograph determination (Finney and Shogren, 1972). Mixing time or requirement was measured as the time in min from the start of the mixogram to the peak. Mixing tolerance was rated on a scale from 0 to 5 taking into account the slope and width after the peak, and the stability of the mixogram height on either side of the peak. A mixing tolerance rating of 0 was considered very poor and a rating of 5 was excellent.

Analytical Procedures

Protein was determined on duplicate 0.5 g (dry weight) samples of

the ground grain and the endosperm with the boric acid modification of the macro-Kjeldahl method (American Association of Cereal Chemists, 1962, method 46-12). The lysine content of the ground grain and endosperm was determined on 100 mg (dry weight) samples (Mattern, Schmidt, and Johnson; 1970).

A modification (Mattern, Salem, and Volkmer; 1968) of the Maes (1962) column extraction procedure was used to extract the endosperm protein according to solubility characteristics. One g (dry weight) of endosperm was placed in a mortar with 2 g of pumice (PHC Italian Laboratory Pumice) and ground gently with a pestle. Twenty-five g of sea sand (Baker and Adamson code 2161 from Allied Chemical) was spread on the center of a paper (350 x 180 mm), along with the endosperm-pumice mixture. The mixture was blended by raising and lowering adjacent corners of the mixing paper, sequentially, until homogeneous. The mixture was transferred, with a gentle continuous flow rate, into a column (200 x 21 mm with a 40 x 6 mm tip) previously prepared with a glass wool plug and a 1 cm layer of sea sand. The mixing paper was carefully brushed to get complete transfer of the sample into the column. Another 1 cm layer of sea sand and a small piece of glass wool were placed on top of the mixture. Normally, 10 columns were prepared for simultaneous fractionation. The columns were sequentially eluted with 40% isopropyl alcohol (v/v), 2% NaCl (w/v), 3.85% lactic acid (w/v), and 0.2% KOH (w/v); hereafter referred to as the alcohol, salt, acid, and alkali fractions respectively. The amount of each solvent used and collected is shown in Table II.

A portion of each extract was used for a nitrogen determination with the micro-Kjeldahl procedure. The digestion flask used was the

Table II. Description of solvent sequence used to elute the modified Maes column.

Fraction	Sequence	Solution	ml Added	ml Collected	ml Portion*
Alcohol	1	40% isopropyl alcohol (v/v)	50	40	8
Salt	2	2% NaCl (w/v)	40	40	20
Acid	3	3.85% lactic acid (w/v)	50	50	20
Alkali	4	0.2% KOH (w/v)	60	60	15

* Portion of the collected extract used for nitrogen determination.

150 ml Kemmerer-Hallett type (K-H type), with a 29/42 ground glass joint on the neck. The digestion was accomplished on a 24 unit electric macro-Kjeldahl digestor by placing an asbestos board, containing 5.8 cm diameter holes, over the digestion units. Glass extension tubes (27 x 190 mm) were placed in the ground glass joints of the flasks in order for the fumes to reach the aspirator. Five ml of digest acid (concentrated H_2SO_4) was used along with 1.6 to 1.8 g of Kel-Pak formulation No. 2P-Gunning Method (Matheson Scientific) which came in packets containing 10 g K_2SO_4, 0.3 $CuSO_4$, and 0.1 g pumice. Before digestion, the salt and alkali portions were placed in dialysis tubing (3/4 inch size, Fisher cat. No. 8 667-1) and allowed to dry overnite in the gentle breeze of a fan. Approximately 190 mm of dialysis tubing was used for a 20 ml aliquot. The alcohol and acid portions were digested as liquid samples. The combination of digest acid, lactic acid, and dialysis tubing caused excess foaming during digestion. The addition of 5 ml of 30% hydrogen peroxide to the digestion mixture reduced the foaming (Bradstreet, 1965). All ingredients of the digestion mixture were added to the flask prior to digestion which was started with low heat and increased to full heat after the digest volume was reduced.

The use of a 40-unit digestor (Tector ab, Kronborgsgatan, Sweden) was found to work successfully with the alcohol and alkali portions. The digester consisted of an aluminum block bored with 40 holes that would hold 25 x 250 mm test tubes. It was difficult to digest liquid samples in a test tube if the total digest solution volume was greater than twice the volume of the digest acid. The combinations of NaCl and lactic acid with dialysis tubing and hydrogen peroxide resulted in excessive foaming that could not be contained within the test tube. For

the alcohol portion, the volume for digestion was reduced to 2 to 3 ml by placing the test tubes in a steam bath for 15 to 20 min before adding the digest acid and Kel-Pak. For the alkali portion, after drying, the dialysis tubing was cut into three or four sections and placed in the test tube. Five ml of digest acid and 2 or 3 drops of 30% hydrogen peroxide were added successively. The reaction of the acid, hydrogen peroxide, and dialysis tubing was instant and violent but effective because with several 2 to 3 drop additions of hydrogen peroxide the dialysis tubing was solubilized into a black solution. Additional portions of hydrogen peroxide (two-0.5 ml and two-1 ml) were required to change the black solution to a reddish brown, which was necessary to minimize foaming during digestion. With both portions, the test tubes were placed in an unheated digester; which required about 1 hour to come to full heat (375° C). During the first hour the water in the sample was gently evaporated from the test tubes. An additional 30 to 45 min was required for complete digestion. Hengar tubes were used as closures for the digestion tubes to reduce the fume problem (Mattern, 1972). After digestion the test tubes were removed from the digester and cooled. After cooling, 7 to 10 ml of water was added to the digest and the mixture transferred to the K-H type flasks for distillation. A difference in nitrogen values could not be detected when equivalent alcohol and alkali portions were taken from the same fraction and digested by either procedure. Two blanks of each fractionation solvent were digested along with each group of fractionations.

The K-H type flask was placed directly on the distillation unit for distillation. The two K-H type distillation units used were connected to an electrically heated 2 liter steam generator. While a

sample was distilling a previously distilled sample was titrated with 0.04 N H_2SO_4 and another sample was prepared for distillation. Twelve ml of 50% NaOH was added to the diluted digest at the start of the distillation. The released ammonia was distilled into 10 ml of 2% boric acid containing bromcresol green-methyl red mixed indicator. The indicator was made by dissolving 60 mg of bromcresol green and 20 mg of methyl red in 5 ml of 0.1 N NaOH, then bringing the volume to 100 ml with 95% ethanol. The indicator solution was added to the boric acid at the rate of 50 ml per 3 liter. The boric acid solution was measured into 125 ml Erlenmeyer receiving flasks with a Calab delivery dispenser. The distillation time was 6 min with the receiving flask lowered during the last min of the distillation.

The nitrogen content of the endosperm was also determined on 100 mg (dry weight) samples with the micro-Kjeldahl procedure described above. A difference in nitrogen values could not be detected when endosperm samples were digested with the K-H type flask or the Tector digester. In both cases 5 ml of digestion acid and 1.6 to 1.8 g of Kel-Pak were used. An average difference of 0.14% nitrogen was found between the micro- and macro-Kjeldahl procedure, therefore, the micro-Kjeldahl values were used with the fractionation data and the macro-Kjeldahl values used with the lysine data. This was necessary in order for the data from the different fractions to be comparable and for the endosperm protein and lysine data to be comparable with the wheat protein and lysine data.

Statistical Methods

In order to make comparisons among cultivar means the NRPN data was subjected to the analysis of variance (ANOV) and Duncan's multiple-range test. The ANOV was calculated as a randomized complete block design

with locations being replications and cultivars being treatments. After ranking the means, Duncan's multiple-range test compared each mean with every other mean taking into account the closeness of the means. For each set of ranked means, letters were placed after each mean to indicate statistical significance. All means in a given grouping (ranking) followed by the same letter were not significantly different at the 5% level. The following NRPN cultivar mean measurements were compared: a) grain and endosperm percent protein, b) grain and endosperm percent lysine, c) grain and endosperm lysine (% of protein), d) the difference between the above grain and endosperm measurements, e) soluble endosperm nitrogen (mg per g endosperm), f) soluble endosperm protein (% of protein), and g) mill yield. Soluble endosperm nitrogen was expressed in mg per g endosperm because 1 g of endosperm was weighed for extraction and nitrogen was measured in mg. When soluble endosperm nitrogen was expressed as a percentage of total endosperm nitrogen (mg per g endosperm) the values obtained were identical to soluble endosperm protein (% of protein).

Linear correlation and regression analysis was calculated with the grain and endosperm data from all 70 NRPN samples to establish the following relationships: a) percent lysine with percent protein, and b) lysine (% of protein) with percent protein. The soluble endosperm nitrogen (protein) and other endosperm data on all 70 NRPN samples was subjected to linear correlation and regression analysis to determine the following relationships: a) soluble nitrogen (mg per g endosperm) with total endosperm nitrogen (mg per g endosperm), b) soluble protein (% of protein) with total endosperm percent protein, c) soluble nitrogen (mg per g endosperm) with percent lysine, d) soluble nitrogen (mg per g

endosperm) with lysine (% of protein), e) soluble protein (% of protein) with percent lysine, and f) soluble protein (% of protein) with lysine (% of protein). The above mentioned relationships also were checked with the data from the 20 nitrogen-fertilizer-trial samples.

The closeness of the linear relationships were evaluated with correlation coefficients (r) and the coefficients of determination (r^2) which also measures the proportion variation in a dependent variable that can be attributed to variation in an independent variable. The following rules of thumb were used to determine the predictive value of regression equations. An r^2 of less than 0.5 indicated the regression equation to be of little predictive value. When r^2 was greater than 0.5 the equation was considered for predictive purposes, except when two or more models were considered for the same measured component, then the model with the largest r^2 value would have the most accurate predictive value.

The analysis of covariance was calculated to compare individual cultivar regression lines when the relationships between variables were of sufficient magnitude. The individual cultivar regression parameters compared with the analysis of covariance are indicated in the following model:

$$Y_{ij} = \alpha_i + \beta_i X_{ij} + \epsilon_{ij}$$

With this model each cultivar (i) had its own regression coefficient (β) and intercept (α) based on j samples. The F test was used to indicate the presence or lack of significant differences in regression parameters among the seven NRPN cultivars. The individual cultivar regression coefficients for the models derived from nitrogen-fertilizer-trial data were compared to the corresponding pooled NRPN regression coefficients with the t test.

An F ratio was calculated between the deviation mean squares from soluble nitrogen regression models and a corresponding statistical measurement of laboratory procedural error. A significant F value indicated greater variation in individual sample soluble nitrogen values than would be expected from the repeated fractionation of portions of one homogenous sample. Six portions of one sample were fractionated, each on a different day, to obtain the estimates of procedural error appearing in Table III. The alcohol soluble fraction was measured with the greatest precision (CV = 1.6) and the salt soluble fraction was measured with the least precision (CV = 9.4). The square of the standard deviations were used as denominators for the F ratios.

The samples were fractionated in a random order to prevent any possible day to day laboratory procedural variation from being associated with cultivar or location differences.

For additional information pertaining to statistical methods see Snedecor and Cochran (1967) or Steel and Torrie (1960).

Table III. Estimates of the repeatability and precision of the extraction procedure. The estimates are based on the fractionation of one sample at six different times.

Fraction	mg Nitrogen/g of endosperm		Fraction as % of protein	
	SD*	CV†	SD*	CV†
Alcohol	0.20	1.6	0.8	1.6
Salt	0.06	9.4	0.2	9.7
Acid	0.10	6.1	0.4	5.8
Alkali	0.28	3.3	1.1	3.3

* Standard deviation
† Coefficient of variation

RESULTS

NRPN Cultivar Protein and Lysine

The regional mean yield[1] for the seven cultivars evaluated from 10 sites of the NRPN was 2,509 kg per ha. Individual cultivar regional mean yields ranged from 2,750 to 2,310 kg per ha for 'Centurk' and 'Kharkof' respectively. The mean protein contents of the grain ranged from 17.2 to 14.6% for NE68513 and NE66403 respectively (Table IV). The regional mean yields of NE66403 and NE68513 were 2,586 and 2,423 kg per ha respectively. The mean grain protein content (Table IV) of NE68513 was significantly higher than the next highest cultivar by 1.2% protein. A group of cultivars (NE68510, 'Kharkof', 'Bezostaya', and 'Warrior') contained equivalent amounts of grain protein. 'Centurk' contained 0.5% less protein than the regional mean and NE66403 was significantly lower than all other cultivars being 1.2% lower than the nursery mean. The ranking of the cultivars according to percent wheat lysine was nearly identical with wheat protein. Ranking according to wheat lysine (% of protein) resulted in rearrangement of the cultivars with NE66403 and NE68510 being higher, and NE68513 and 'Bezostaya' being lower than the regional mean. The protein and lysine contents of the endosperm were lower than that in the whole wheat. With the exception of lysine (% of protein), the cultivars were ranked the same according to wheat and endosperm protein and lysine. 'Kharkof' had an average amount of lysine (% of protein) in the whole grain but had a greater than average amount in the endosperm protein. The mean

[1] Johnson, V. A. 1971. Comparison of winter wheat varieties grown in cooperative nursery experiments in the hard red winter wheat region in 1971. USDA unpublished report. Lincoln, Nebraska.

Table IV. Cultivar mean protein and lysine content (NRPN samples). All data is expressed on a dry weight basis. The means within a group followed by the same letter were not significantly different at the 5% level according to Duncan's multiple-range test (Steel and Torrie, 1960).

Cultivar	Percent protein	
	Wheat	Endosperm
NE68513	17.2a	16.6a
NE68510	16.0 b	15.5 b
Kharkof	15.9 b	15.2 b
Bezostaya	15.9 b	15.7 b
Warrior	15.9 b	15.2 b
Centurk	15.3 c	14.5 c
NE66403	14.6 d	13.9 d
Mean	15.8	15.2
	Percent lysine	
NE68513	0.50a	0.37a
NE68510	0.49ab	0.35 b
Kharkof	0.48 bc	0.35 b
Warrior	0.48 bc	0.34 b
Bezostaya	0.46 cd	0.34 b
Centurk	0.46 cd	0.32 c
NE66403	0.45 d	0.31 c
Mean	0.47	0.34
	Lysine, % of protein	
NE66403	3.11a	2.30a
NE68510	3.10a	2.30a
Centurk	3.05ab	2.25ab
Warrior	3.01 bc	2.27ab
Kharkof	3.00 bcd	2.30a
NE68513	2.93 cd	2.22 bc
Bezostaya	2.92 d	2.18 c
Mean	3.02	2.26

differences between wheat and endosperm protein and lysine are indicated in Table V. The endosperm protein content of 'Bezostaya' contained only 0.2% less protein than the whole grain. A mean reduction of nearly 0.8% protein was measured from samples of NE66403. The mean reduction in percent lysine from whole wheat to endosperm was 0.13% with small differences between cultivars. Cultivar differences between wheat and endosperm lysine (% of protein) ranged from 0.70 to 0.81% for 'Kharkof' and NE66403 respectively.

The correlations (r) of wheat and endosperm lysine with wheat and endosperm protein are shown in Table VI. Highly significant positive correlations occurred between percent lysine and percent protein. Highly significant negative correlations resulted between lysine (% of protein) and percent protein. The coefficients of determination (r^2) were higher for percent lysine than lysine (% of protein). The relationships of wheat and endosperm lysine with wheat and endosperm protein based on all 70 NRPN samples are presented in Figures 1 and 2. The range in protein, 10.4 to 19.8% for wheat and 9.1 to 19.2% for endosperm, is indicated by the vertical lines which cross the regression lines. Positive relationships were indicated for percent lysine with protein for both wheat and endosperm (Figure 1). The grain contained more lysine than the endosperm at all levels of protein. The regression line intercepts were positive. An inverse relationship is shown for lysine (% of protein) with protein (Figure 2).

Statistical comparisons of individual cultivar regression lines for differences in slope and intercept (Table VI) were made with the F test. Differences in slope were not detected. When parallel regression lines can be assumed, the F test for differences in adjusted

Table V. Cultivar comparisons of whole grain and endosperm, protein and lysine (NRPN samples). All data is expressed on a dry weight basis. The means within a group followed by the same letter were not significantly different at the 5% level according to Duncan's multiple-range test (Steel and Torrie, 1960).

Cultivar	Wheat minus endosperm		
	Percent protein	Percent lysine	Lysine, % of protein
Bezostaya	0.20a	0.12a	0.75ab
NE68510	0.52 b	0.14 b	0.80 b
NE68513	0.55 bc	0.13ab	0.71a
Kharkof	0.70 bc	0.13ab	0.70a
Warrior	0.71 bc	0.13ab	0.74ab
Centurk	0.76 bc	0.14 b	0.80 b
NE66403	0.78 c	0.14 b	0.81 b
Mean	0.61	0.13	0.76

Table VI. Statistical comparisons of protein and lysine relationships (NRPN samples). The correlation coefficients (r) and the coefficients of determination (r^2) are based on 70 samples. The F values are for the comparison of individual cultivar regression parameters (percent lysine regressed on percent protein).

Wheat		
	Protein (%)	
Lysine	r	r^2
Percent	0.97**	0.93
% of Protein	-0.89**	0.78

Endosperm		
Percent	0.97**	0.94
% of Protein	-0.82**	0.68

F Value		
Kernel component	Slope	Adjusted mean
Whole grain	0.70	5.02**
Endosperm	0.44	8.56**

** Statistically significant at the 1% level

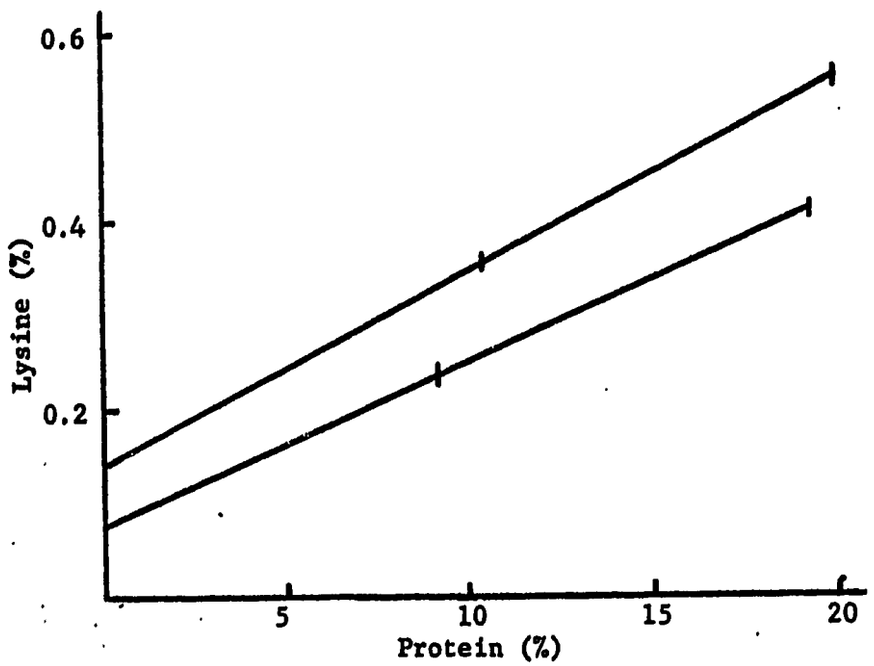

Figure 1. The relationship of lysine (%) to protein (%) based on 70 NRPN samples. The upper line represents wheat and the lower line represents endosperm.

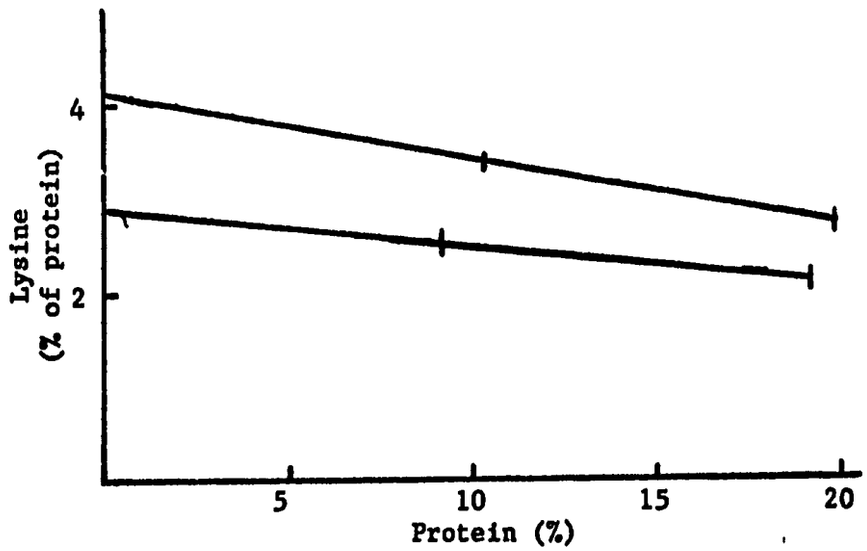

Figure 2. The relationship of lysine (% of protein) to protein (%) based on 70 NRPN samples. The upper line represents wheat and the lower line represents endosperm.

means is identical to an F test for differences in intercepts. Highly significant differences in adjusted means (intercepts) were indicated for wheat and endosperm lysine. The regression coefficients for wheat (Table VII) were greater than for endosperm. Individual cultivar coefficients were nearly identical. The deviation mean squares (Table VII) were small and varied from 0.23×10^{-3} for wheat lysine in 'Kharkof' to 0.06×10^{-3} for endosperm lysine in 'Warrior'.

The prediction equations (Figure 1) derived from the data on all 70 NRPN samples were used to adjust individual cultivar percent lysine means to a common protein value. The adjusted percent lysine values appearing in Table VIII are ranked different from the unadjusted percent lysine values in Table IV. The high lysine cultivar (NE68513) and the low lysine cultivar (NE66403), based on percent lysine values, were average or near average in both wheat and endosperm lysine based on adjusted percent lysine values. In order to compare adjusted percent lysine values with lysine (% of protein), adjusted percent lysine was expressed as a percentage of regional mean protein. Although the cultivars were ranked similar, the values for NE68513 and NE66403 were nearer the regional mean in Table VIII than in Table IV. The individual sample percent lysine values for NE68510 and 'Bezostaya' are presented in Figure 3. The differences in wheat lysine were greater than endosperm lysine, but, in both cases the difference occurred over the entire range of protein.

NRPN Cultivar Soluble Nitrogen (Protein)

In general, the nitrogen extracted with a given solvent was dependent upon total endosperm nitrogen (Table IX). The greatest amounts of nitrogen extracted with each solvent, except alkali, were

Table VII. Individual cultivar regression parameters (NRPN samples). The regression coefficients (b) and deviation mean squares (MS) are for the model of percent lysine regressed on protein.

Cultivar	b	MS x 10^{-3}
Wheat		
Kharkof	0.023	0.23
Warrior	0.020	0.08
NE66403	0.020	0.17
NE68513	0.019	0.18
Centurk	0.020	0.13
NE68510	0.022	0.14
Bezostaya	0.022	0.16
Pooled	0.021	0.19
Endosperm		
Kharkof	0.018	0.10
Warrior	0.017	0.06
NE66403	0.016	0.18
NE68513	0.016	0.18
Centurk	0.017	0.14
NE68510	0.018	0.08
Bezostaya	0.017	0.11
Pooled	0.017	0.14

Table VIII. Adjusted cultivar mean percent lysine (NRPN samples). The means from Table IV were adjusted to 15.8% protein for wheat and 15.2% protein for endosperm with the pooled regression equations derived from the NRPN data.

Cultivar	Percent lysine	Lysine, % of mean protein*
Wheat		
NE68510	0.49	3.08
NE66403	0.48	3.00
Centurk	0.47	2.99
Warrior	0.47	2.99
Kharkof	0.47	2.99
NE68513	0.47	2.98
Bezostaya	0.46	2.91
Mean	0.47	2.99
Endosperm		
NE68510	0.35	2.29
Kharkof	0.35	2.28
Warrior	0.34	2.24
NE68513	0.34	2.24
NE66403	0.34	2.22
Centurk	0.34	2.20
Bezostaya	0.33	2.18
Mean	0.34	2.24

* Obtained when adjusted mean percent lysine was expressed as a percentage of regional mean protein (15.8% for wheat and 15.2% for endosperm)

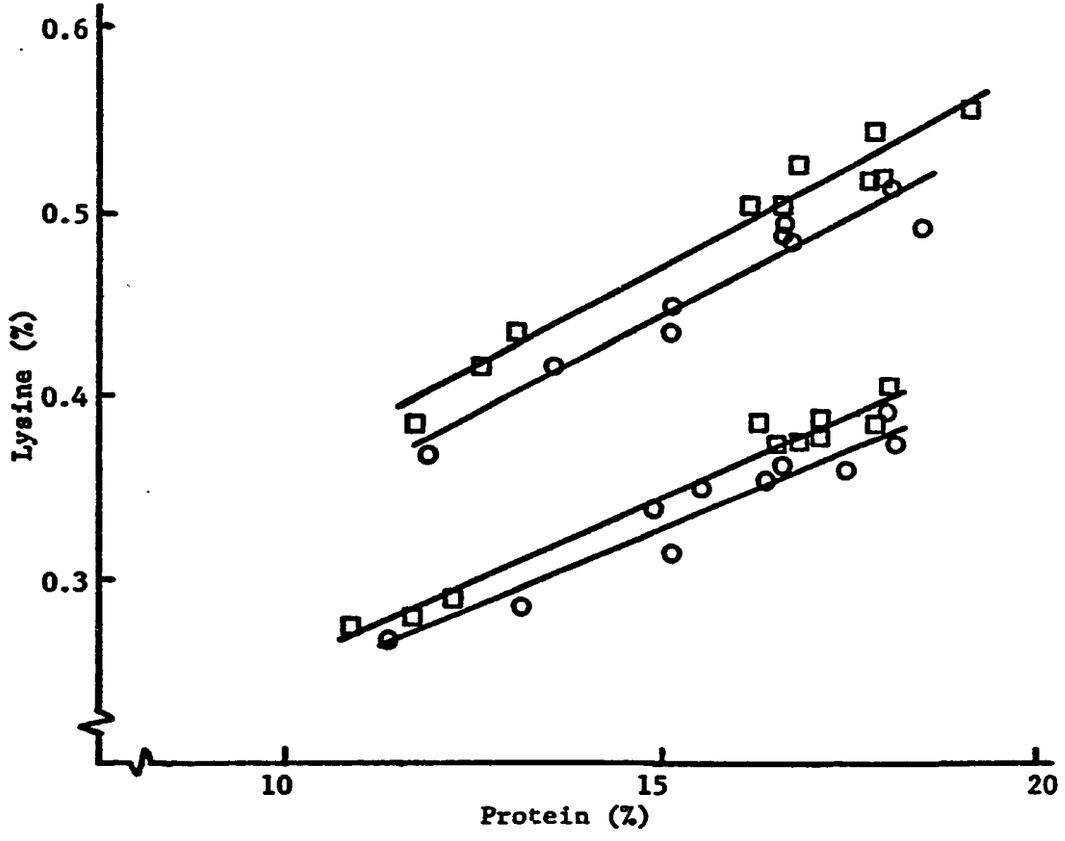

Figure 3. The relationship of lysine (%) to protein (%) in an 'Atlas 66' derived line and 'Bezostaya'. The circles = 'Bezostaya' and the squares = NE68510. The upper set of regression lines represent wheat and the lower set endosperm.

Table IX. Cultivar mean total and soluble endosperm nitrogen (NRPN samples). Nitrogen is expressed as mg per g endosperm (dry weight basis). The means within a group followed by the same letter are not significantly different at the 5% level according to Duncan's multiple-range test (Steel and Torrie, 1960).

Cultivar	Total nitrogen	Soluble nitrogen			
		Alcohol	Salt	Acid	Alkali
NE68513	27.7a	14.9a	0.77a	1.91a	8.6a
Bezostaya	26.0 b	13.6 b	0.70 b	1.71 bc	8.7a
NE68510	25.9 b	12.9 c	0.69 bc	1.89a	8.7a
Kharkof	25.5 b	13.6 b	0.62 cd	1.75 b	7.7 b
Warrior	25.3 b	12.6 cd	0.66 bc	1.74 bc	8.6a
Centurk	24.2 c	12.1 d	0.65 bcd	1.61 cd	8.0 b
NE66403	23.0 d	11.4 e	0.60 d	1.55 d	7.9 b
Mean	25.4	13.0	0.67	1.74	8.3

obtained from samples of NE68513. Samples of NE66403 contained the least amount of alcohol, salt, and acid soluble nitrogen. NE68510, 'Warrior', 'Bezostaya', and 'Kharkof' were statistically identical in total nitrogen, but more nitrogen was extracted with alcohol from 'Bezostaya' and 'Kharkof' than from NE68510 and 'Warrior'. NE68510 contained more acid soluble nitrogen than all other cultivars except NE68513. The cultivars were divided into two groups based on the amount of nitrogen soluble in alkali. The group with the greater amount consisted of: NE68513, 'Bezostaya', NE68510, and 'Warrior'. The group with lesser amounts consisted of: 'Kharkof', 'Centurk', and NE66403. The soluble protein data presented in Table X were obtained by expressing soluble nitrogen (mg per g endosperm) as a percentage of total endosperm nitrogen (mg per g endosperm). By first converting soluble and total endosperm nitrogen to soluble and total endosperm protein with a constant multiplier and then expressing soluble protein as a percentage of total protein, identical percentages are obtained. NE68513, 'Bezostaya', and 'Kharkof' contained greater mean alcohol soluble protein than all other cultivars. Cultivar differences were not present for salt soluble protein. NE68510 contained more acid soluble protein than all other cultivars. NE68513 and 'Kharkof' were lower in alkali soluble protein than all other cultivars.

All soluble nitrogen fractions were positively correlated with total nitrogen (Table XI). When the solubility fractions were expressed as a percentage of total protein the following correlations with total protein were obtained: a) positive for the alcohol fraction, b) negative for the salt and alkali fractions, and c) nonsignificant for the acid fraction. The coefficients of determination (Table XI)

Table X. Cultivar mean total and soluble endosperm protein (NRPN samples). All data is expressed on a dry weight basis. The means within a group followed by the same letter were not significantly different at the 5% level according to Duncan's multiple-range test (Steel and Torrie, 1960).

Cultivar	Total protein (%)	Soluble protein (% of total protein)			
		Alcohol	Salt	Acid	Alkali
NE68513	16.6a	53.4a	2.8a	6.9 b	30.9 b
Bezostaya	15.7 b	52.1 b	2.7a	6.6 b	33.7a
NE68510	15.5 b	49.4 c	2.7a	7.4a	33.5a
Kharkof	15.2 b	52.9ab	2.5a	6.8 b	30.3 b
Warrior	15.2 b	49.7 c	2.7a	6.9 b	34.1a
Centurk	14.5 c	49.6 c	2.7a	6.7 b	33.6a
NE66403	13.9 d	49.1 c	2.7a	6.8 b	34.5a
Mean	15.2	50.9	2.7	6.9	33.0

Table XI. Statistical comparisons of soluble and total endosperm nitrogen (protein) relationships (NRPN samples). The correlation coefficients (r) and the coefficients of determination (r^2) are based on 70 samples. The F values are for the comparison of individual cultivar regression parameters (soluble endosperm nitrogen regressed on total endosperm nitrogen).

Soluble nitrogen (mg/g endosperm)		
	Total nitrogen	
Fraction	r	r^2
Alcohol	0.98**	0.96
Salt	0.71**	0.50
Acid	0.92**	0.85
Alkali	0.91**	0.83
Soluble protein (% of protein)		
Alcohol	0.72**	0.52
Salt	-0.50**	0.25
Acid	-0.16	0.02
Alkali	-0.36**	0.13

	F value	
Fraction	Slope	Adjusted mean
Alcohol	2.01	16.92**
Salt	0.80	1.96
Acid	0.48	2.54*
Alkali	0.50	6.95**

* Statistically significant at the 5% level
** Statistically significant at the 1% level

indicated that greater portions of the variation in solubility fractions were attributed to variation in total nitrogen (protein) when the fractions were expressed as mg per g endosperm rather than as percentages of total nitrogen. Therefore, soluble nitrogen (mg per g endosperm) was regressed on total nitrogen for comparison of individual cultivar regression parameters. The significant F values presented in Table XI point out the regression parameters that are influenced by cultivar. Possible cultivar influences on regression slopes were implied for the alcohol fraction because the F value was between the 5 (F = 2.27) and 10% level (F = 1.88). The slope of other fraction regression lines were not influenced by cultivar as judged by the F test. The F tests indicated cultivar differences in adjusted means (intercepts) of the alcohol, alkali, and acid soluble nitrogen regression lines.

The NRPN data indicated a positive relationship between alcohol soluble protein and total protein (Figure 4). Inverse relationships were shown for the other fractions (Figures 5, 6, and 7). The calculated t value (1.37) for the regression of acid soluble protein on total protein was between the 0.2 and 0.1 probability levels which indicated the slope was not significantly different from zero.

All relationships between soluble nitrogen (mg per g endosperm) and total nitrogen were positive (Figure 8, 9, 10, and 11). Except for salt soluble nitrogen, the high r^2 values (Table XI) for the prediction equations indicated that total nitrogen would be a good predictor of soluble nitrogen. The prediction of alcohol and alkali soluble nitrogen from total nitrogen requires the correction for a constant because of the negative intercept in the former and the positive intercept in the latter case.

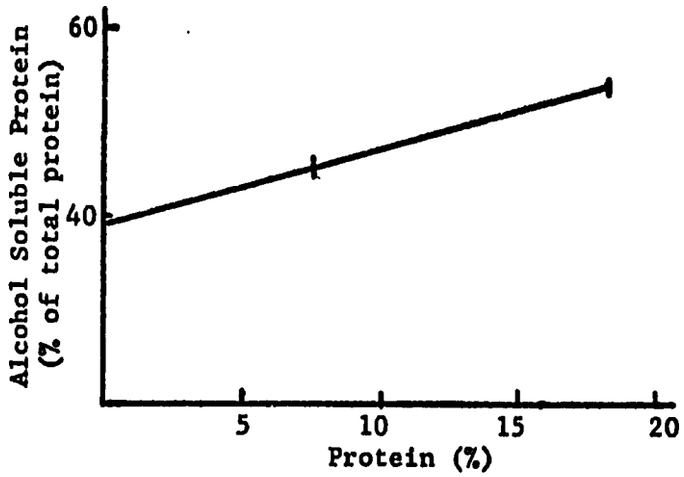

Figure 4. The relationship of percent alcohol soluble endosperm protein to total endosperm protein based on 70 NRPN samples.

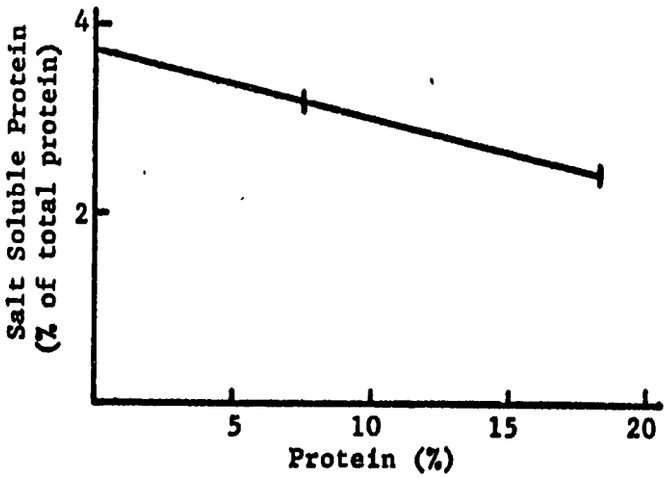

Figure 5. The relationship of percent salt soluble endosperm protein to total endosperm protein based on 70 NRPN samples.

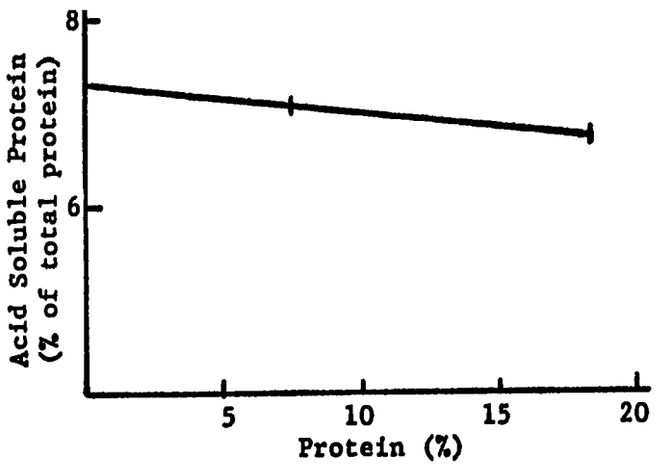

Figure 6. The relationship of percent acid soluble endosperm protein to total endosperm protein based on 70 NRPN samples.

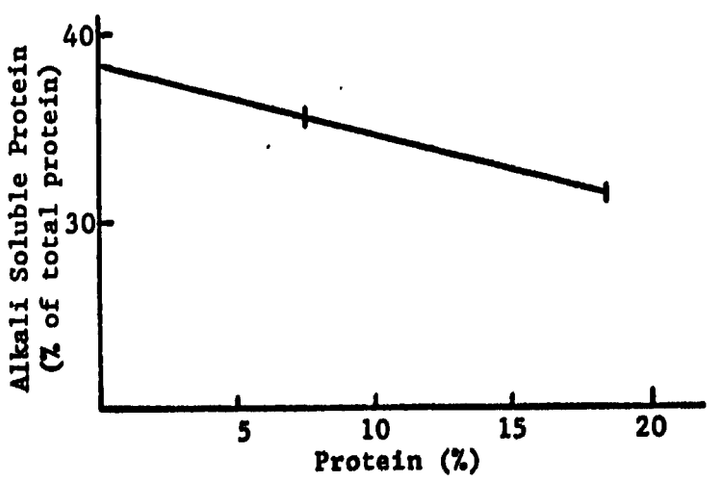

Figure 7. The relationship of percent alkali soluble endosperm protein to total endosperm protein based on 70 NRPN samples.

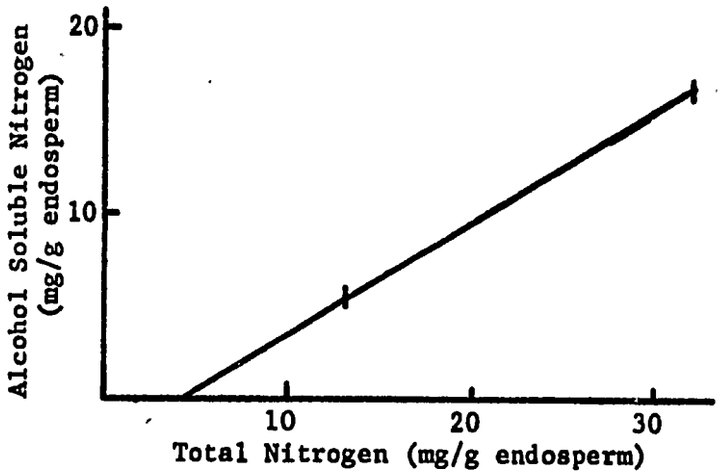

Figure 8. The relationship of alcohol soluble endosperm nitrogen to total endosperm nitrogen based on 70 NRPN samples.

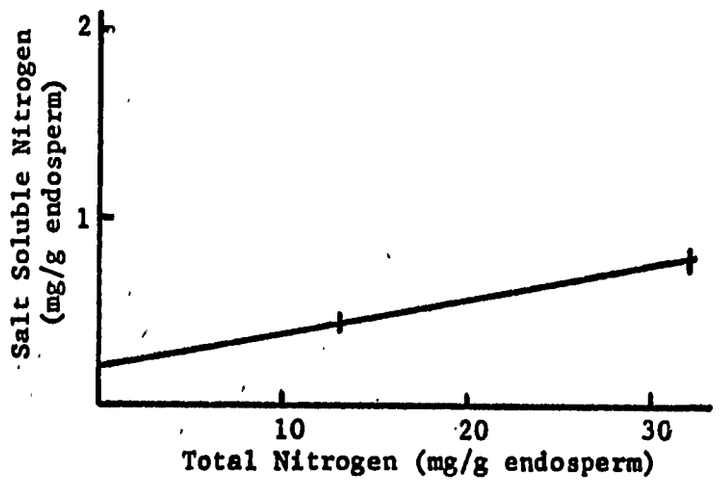

Figure 9. The relationship of salt soluble endosperm nitrogen to total endosperm nitrogen based on 70 NRPN samples.

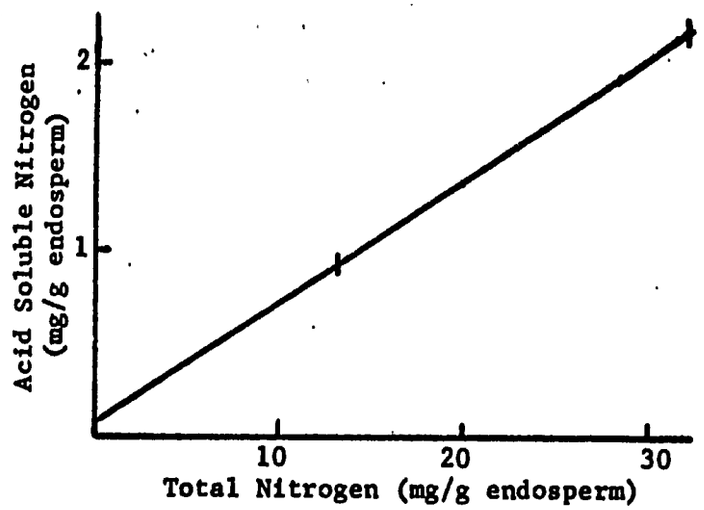

Figure 10. The relationship of acid soluble endosperm nitrogen to total endosperm nitrogen based on 70 NRPN samples.

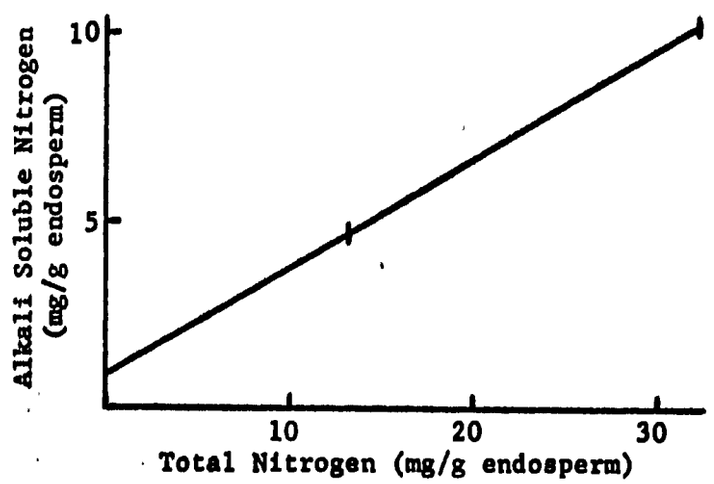

Figure 11. The relationship of alkali soluble endosperm nitrogen to total endosperm nitrogen based on 70 NRPN samples.

Individual cultivar regression coefficients for each soluble nitrogen fraction are given in Table XII. The units for the regression coefficients are mg soluble nitrogen per mg total nitrogen. The coefficients for alcohol soluble nitrogen varied from 0.55 for 'Warrior' to 0.66 for 'Bezostaya'. Salt soluble regression coefficients ranged from 0.028 for NE68513 to 0.011 for 'Kharkof'. The acid soluble regression coefficients were nearly equal. The alkali soluble regression coefficients differed from 0.34 for NE68510 to 0.27 for 'Centurk'.

The deviation mean squares from the pooled regression models (Table XII) indicated that the variation in alcohol and alkali soluble nitrogen was greater than the procedural error. Individual cultivar regression models with variation of this magnitude were shown for: a) alcohol soluble nitrogen with 'Bezostaya', 'Warrior', and NE68510; b) salt soluble nitrogen with 'Bezostaya'; and c) alkali soluble nitrogen with 'Bezostaya'.

The adjusted soluble nitrogen means in Table XIII were not greatly different, but significant cultivar influences were suggested with the F test (Table XI) for all fractions except the salt soluble fraction. The rankings of the cultivars according to adjusted soluble nitrogen were in agreement with the arrangements of the cultivars in Table X, therefore, the multiple-range test used in Table X would give an indication of cultivar differences in adjusted soluble nitrogen.

The largest cultivar differences in adjusted alcohol soluble nitrogen were shown between 'Kharkof' and NE68510. These differences were expressed more so in high protein samples than in low protein samples (Figure 12). The 'Kharkof' and NE68510 regression lines were nearly equal in slope but appeared to be different in elevation. Based

Table XII. Individual cultivar regression parameters (soluble nitrogen, NRPN samples). The regression coefficients (b) and the deviation mean squares (MS) are for the models of soluble nitrogen (mg/g endosperm) regressed on total nitrogen.

Cultivar	b	MS
Alcohol soluble		
Kharkof	0.62	0.13
NE68513	0.64	0.13
Bezostaya	0.66	0.20*
Centurk	0.62	0.06
NE66403	0.56	0.10
Warrior	0.55	0.23*
NE68510	0.57	0.20*
Pooled	0.61	0.28*
Salt soluble		
NE68513	0.028	0.009
Bezostaya	0.017	0.018*
NE68510	0.018	0.003
Centurk	0.016	0.004
Warrior	0.017	0.002
NE66403	0.012	0.004
Kharkof	0.011	0.005
Pooled	0.018	0.007
Acid soluble		
NE68510	0.06	0.005
NE68513	0.07	0.009
Kharkof	0.07	0.006
Warrior	0.07	0.025
NE66403	0.06	0.010
Centurk	0.06	0.009
Bezostaya	0.06	0.042
Pooled	0.06	0.017
Alkali soluble		
Warrior	0.33	0.16
NE66403	0.30	0.22
NE68510	0.34	0.17
Bezostaya	0.28	0.48*
Centurk	0.27	0.33
NE68513	0.30	0.16
Kharkof	0.28	0.24
Pooled	0.29	0.35*

* Significantly greater than the square of the appropriate standard deviation given in Table III (5% probability level).

Table XIII. Adjusted cultivar mean soluble nitrogen (NRPN samples). The data is expressed on a dry weight basis. The means in Table IX were adjusted to 25.4 mg nitrogen per g endosperm (14.5% protein).

Cultivar	mg nitrogen/ g endosperm	% of mean total nitrogen
	Alcohol soluble	
Kharkof	13.5	53.2
NE68513	13.4	53.0
Bezostaya	13.2	52.2
Centurk	12.8	50.6
NE66403	12.8	50.5
Warrior	12.7	50.0
NE68510	12.6	49.5
Mean	13.0	51.3
	Salt soluble	
NE68513	0.73	2.9
Bezostaya	0.69	2.7
NE68510	0.68	2.7
Centurk	0.68	2.7
Warrior	0.66	2.6
NE66403	0.65	2.5
Kharkof	0.62	2.4
Mean	0.67	2.6
	Acid soluble	
NE68510	1.86	7.3
NE68513	1.75	6.9
Kharkof	1.74	6.8
Warrior	1.74	6.8
NE66403	1.71	6.7
Centurk	1.68	6.6
Bezostaya	1.67	6.6
Mean	1.74	6.9
	Alkali soluble	
Warrior	8.6	34.0
NE66403	8.6	33.9
Bezostaya	8.5	33.7
NE68510	8.5	33.6
Centurk	8.4	33.1
NE68513	7.9	31.0
Kharkof	7.7	30.2
Mean	8.3	32.8

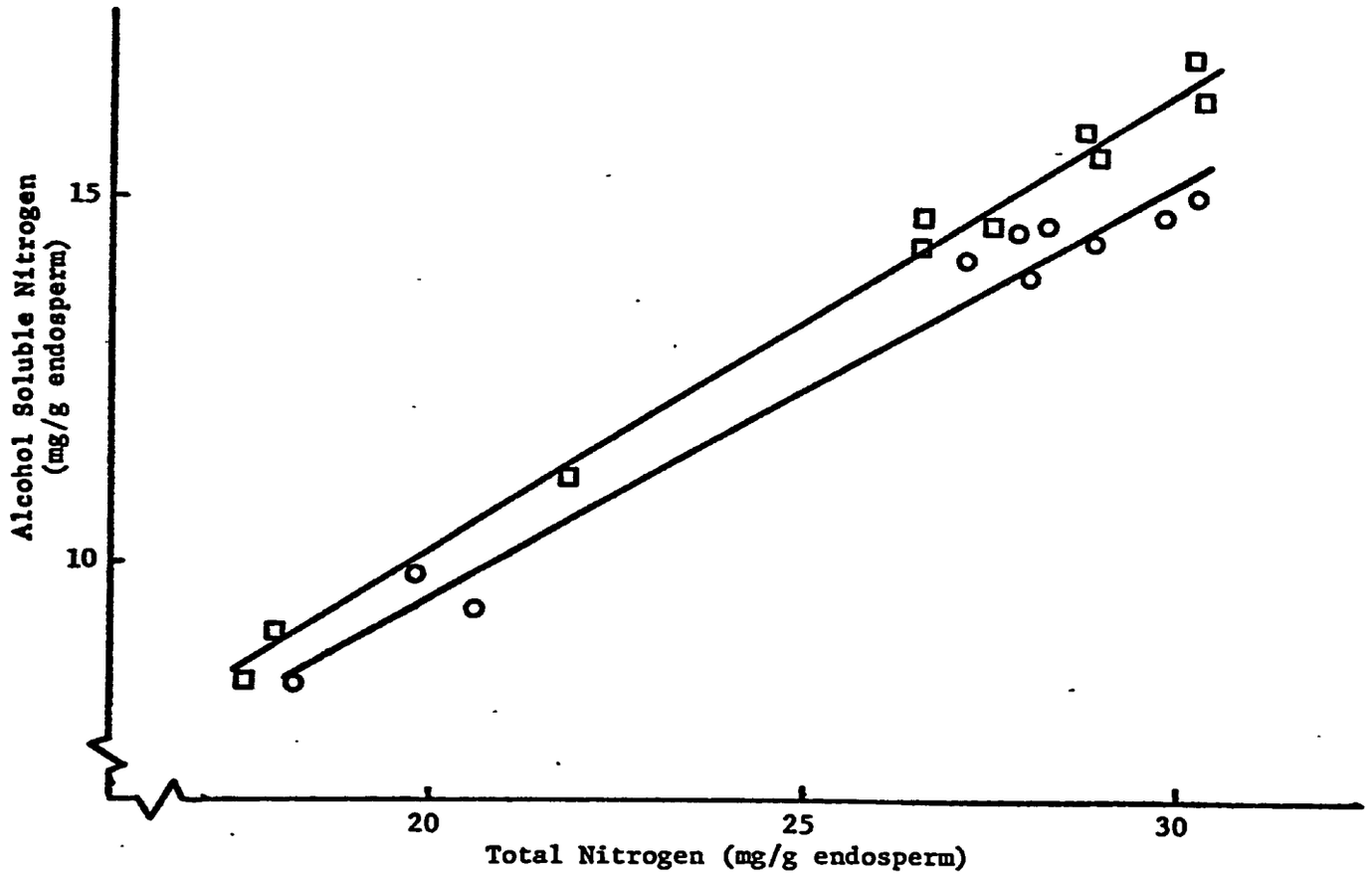

Figure 12. The relationship of alcohol soluble endosperm nitrogen to total endosperm nitrogen in an 'Atlas 66' derived line and 'Kharkof'. The circles = NE68510 and the squares = 'Kharkof'.

on adjusted alcohol soluble nitrogen, the difference between 'Warrior' and 'Kharkof' was nearly as large as the difference between NE68510 and 'Kharkof', but a plot of individual sample values (Figure 13) indicated that at low protein levels they were nearly identical. The regression slope for 'Warrior' was less than that of any other cultivar (Table XII). There was a tendency for the two 'Atlas 66' derived lines to differ in alcohol soluble nitrogen at high protein levels but they were nearly equal at low protein levels (Figure 14). 'Warrior' and NE68513 tend to be different based on high protein samples but the regression slopes had a tendency to converge at low protein levels (Figure 15). In general, the cultivar differences in alcohol soluble nitrogen were expressed in high protein samples but not in low protein samples.

The differences in acid soluble nitrogen between NE68510 and 'Centurk' were consistent in low protein samples but individual sample variation in high protein samples indicated only a trend for the cultivars to be different (Figure 16).

The cultivar comparisons for alkali soluble nitrogen in Figures 17 and 18 indicated that the elevation of the regression line for NE68513 was less than the regression lines for NE68510 and 'Warrior'. Likewise, the regression line for 'Kharkof' was lower than for NE68510 and 'Warrior' (Figures 19 and 20). The F tests for cultivar differences in regression slope and elevation (Table XI) substantiated the facts that, within the seven NRPN cultivar alkali soluble nitrogen regression lines, there were significant differences in elevation but not in slope. The comparisons of 'Kharkof' and 'Warrior' represent the extremes (Figure 20) and with the exception of one sample the 10 values of each cultivar did not overlap. Other comparisons indicated that

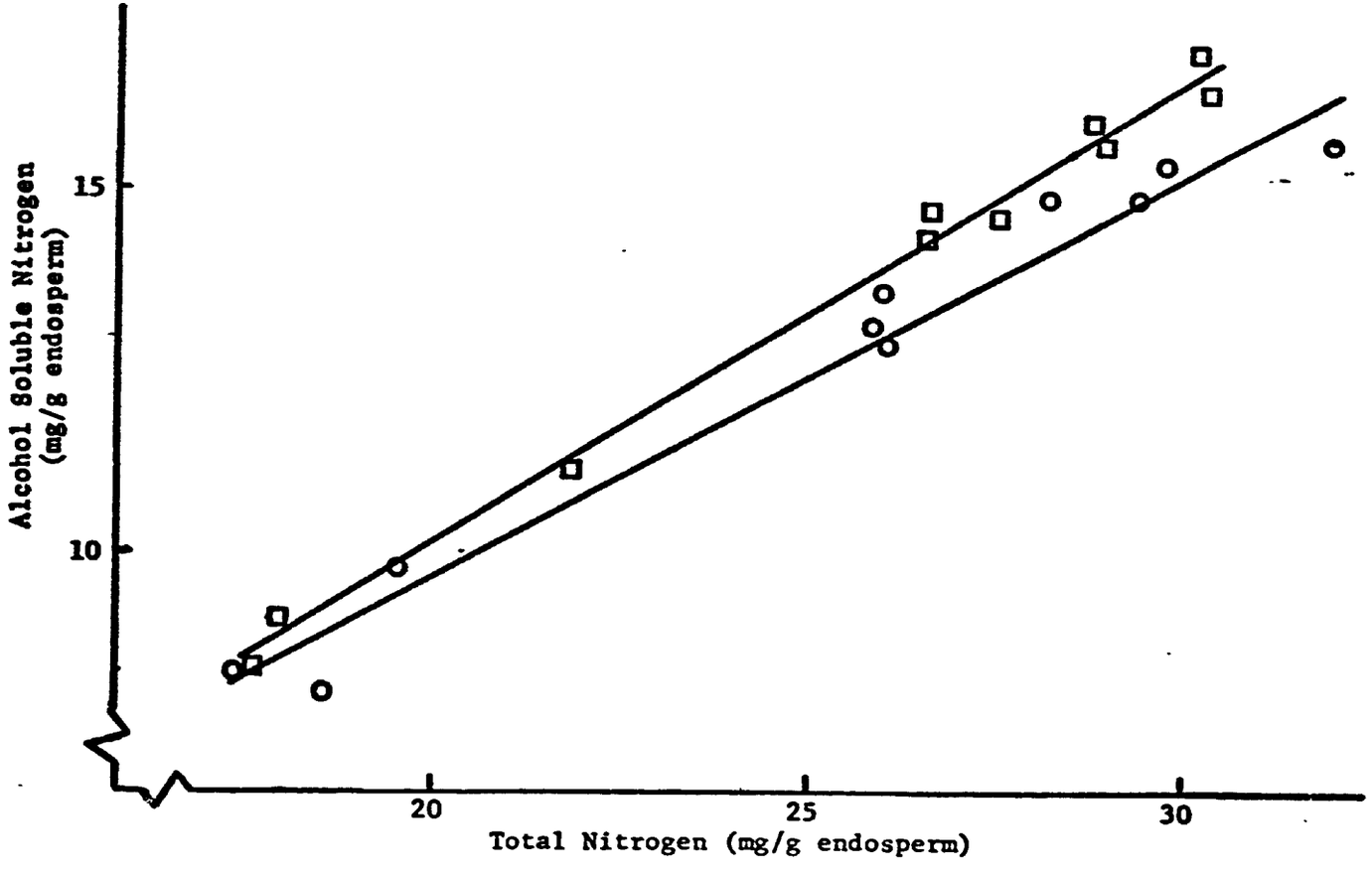

Figure 13. The relationship of alcohol soluble endosperm nitrogen to total endosperm nitrogen in 'Kharkof' and 'Warrior'. The circles = 'Warrior' and the squares = 'Kharkof'.

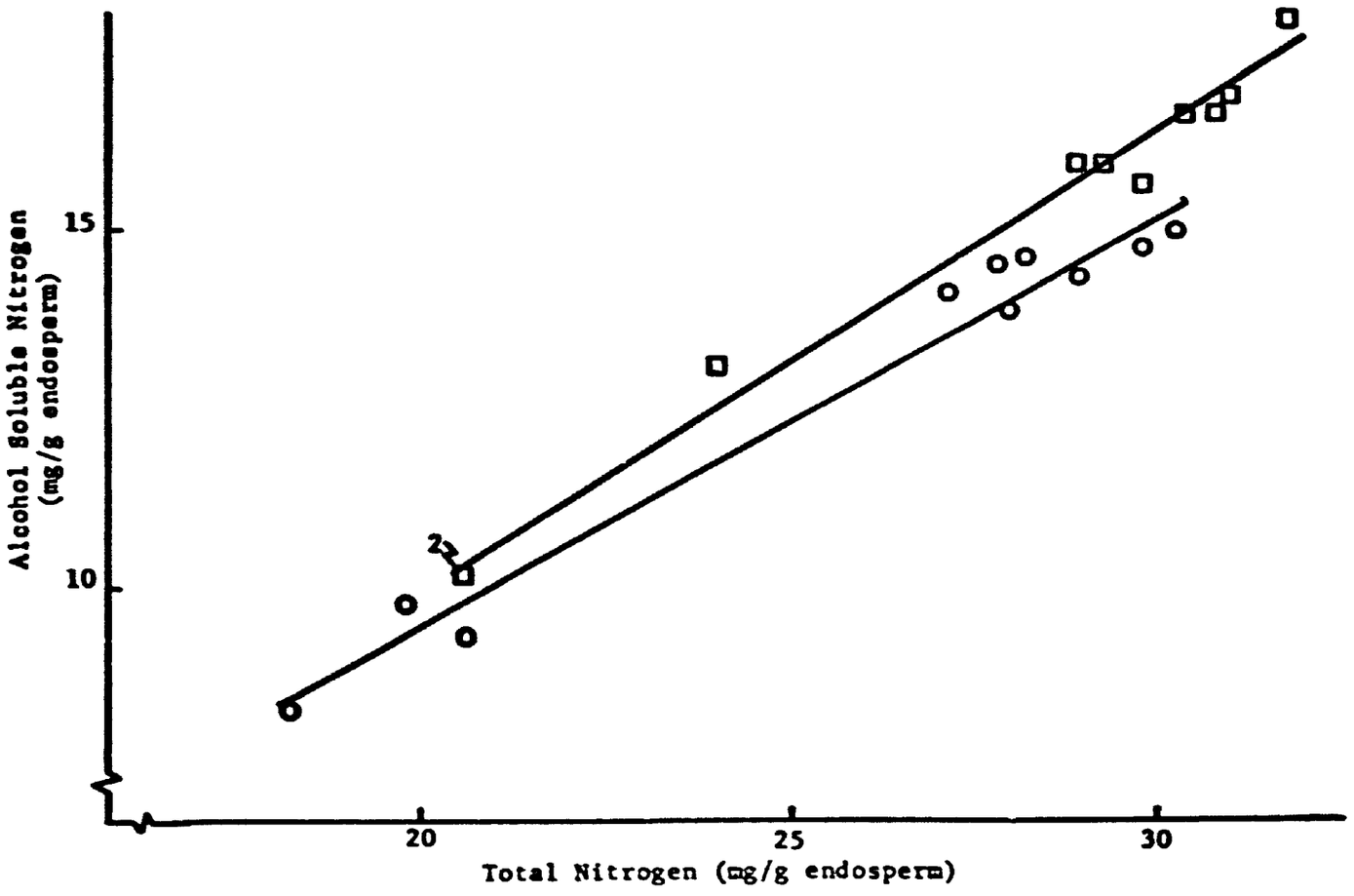

Figure 14. The relationship of alcohol soluble endosperm nitrogen to total endosperm nitrogen in two 'Atlas 66' derived lines. The circles = NE68510 and the squares = NE68513.

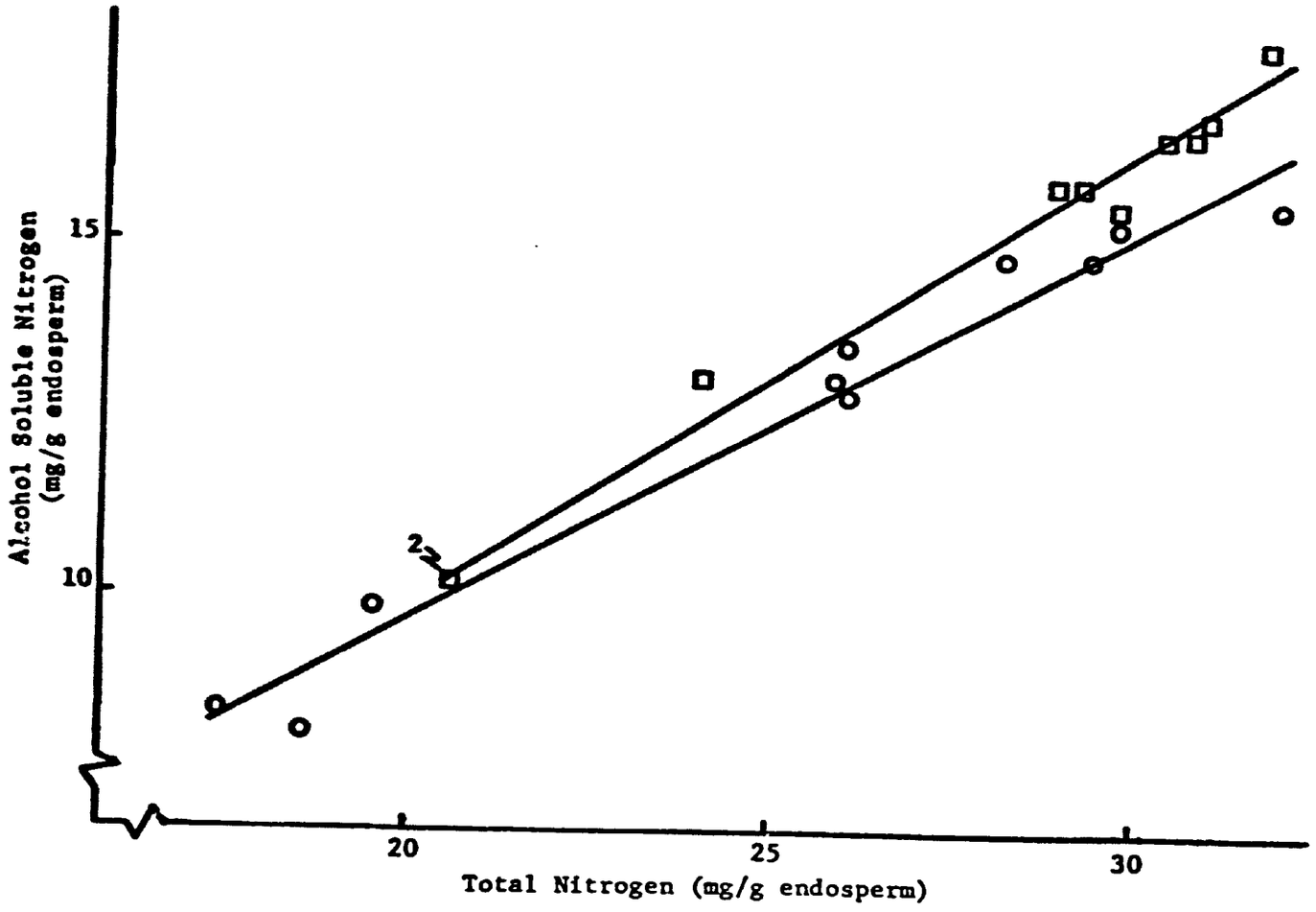

Figure 15. The relationship of alcohol soluble endosperm nitrogen to total endosperm nitrogen in an 'Atlas 66' derived line and 'Warrior'. The circles = 'Warrior' and the squares = NE68513.

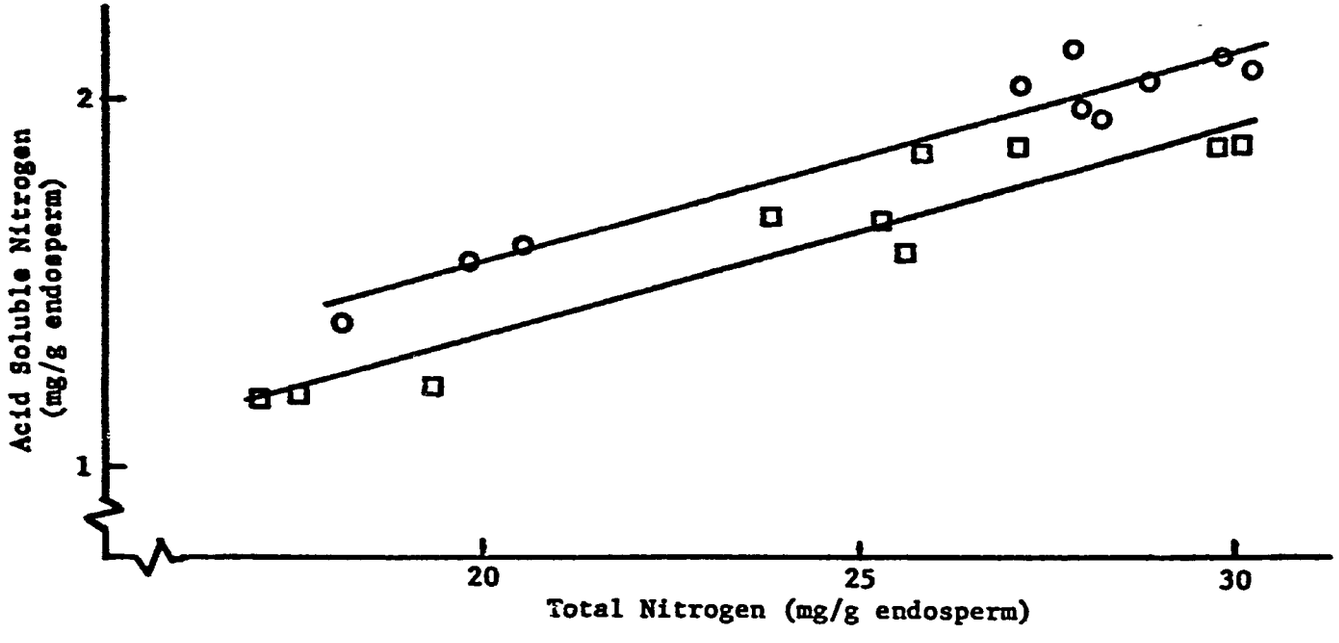

Figure 16. The relationship of acid soluble endosperm nitrogen to total endosperm nitrogen in an 'Atlas 66' derived line and 'Centurk'. The circles = NE68510 and the squares = 'Centurk'.

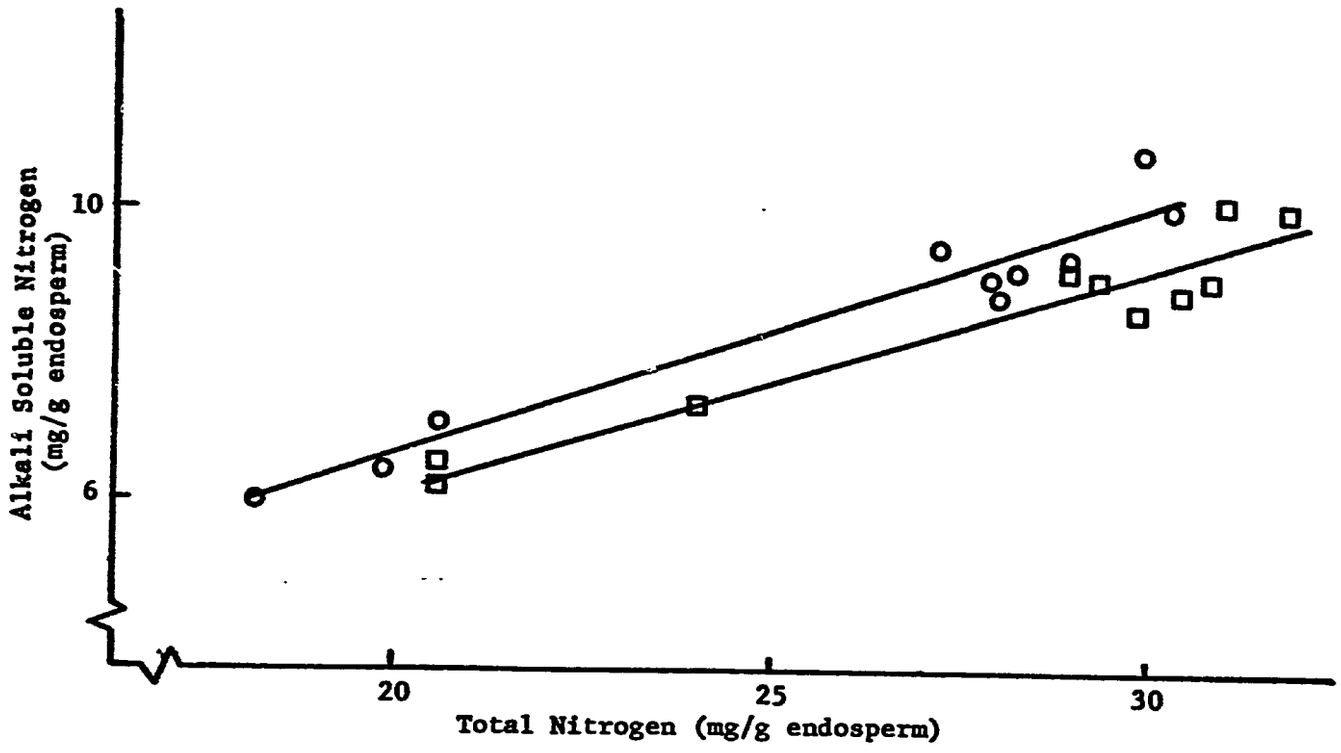

Figure 17. The relationship of alkali soluble endosperm nitrogen to total endosperm nitrogen in two 'Atlas 66' derived lines. The circles = NE68510 and the squares = NE68513.

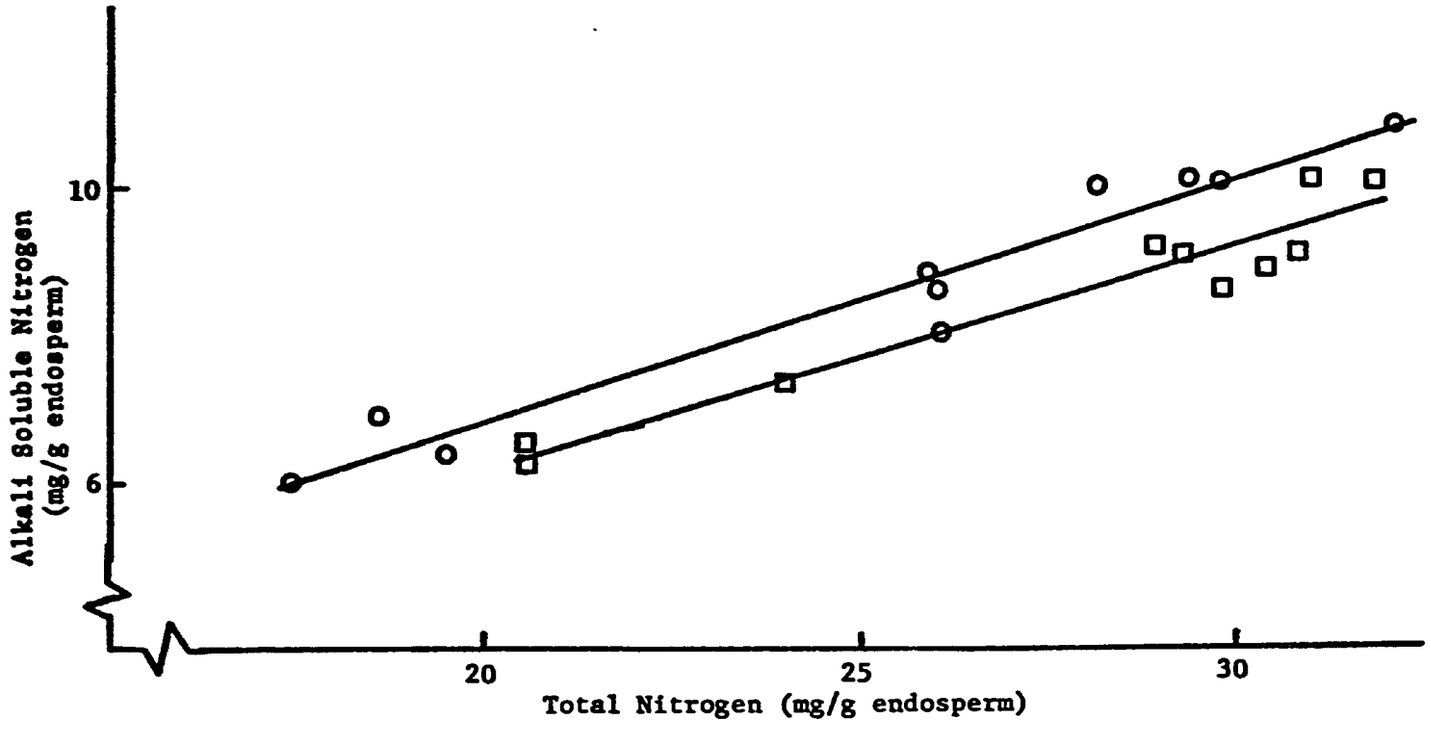

Figure 18. The relationship of alkali soluble endosperm nitrogen to total endosperm nitrogen in an 'Atlas 66' derived line and 'Warrior'. The circles = 'Warrior' and the squares = NE68513.

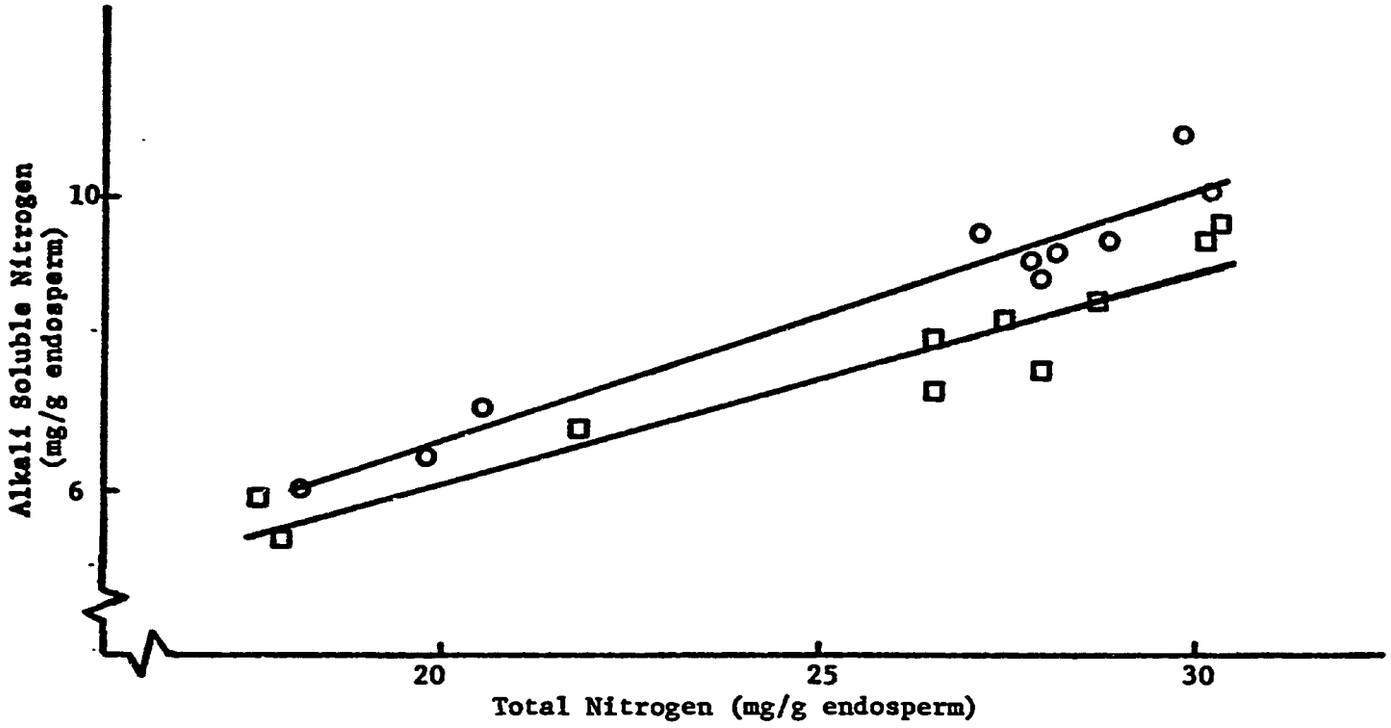

Figure 19. The relationship of alkali soluble endosperm nitrogen to total endosperm nitrogen in an 'Atlas 66' derived line and 'Kharkof'. The circles = NE68510 and the squares = 'Kharkof'.

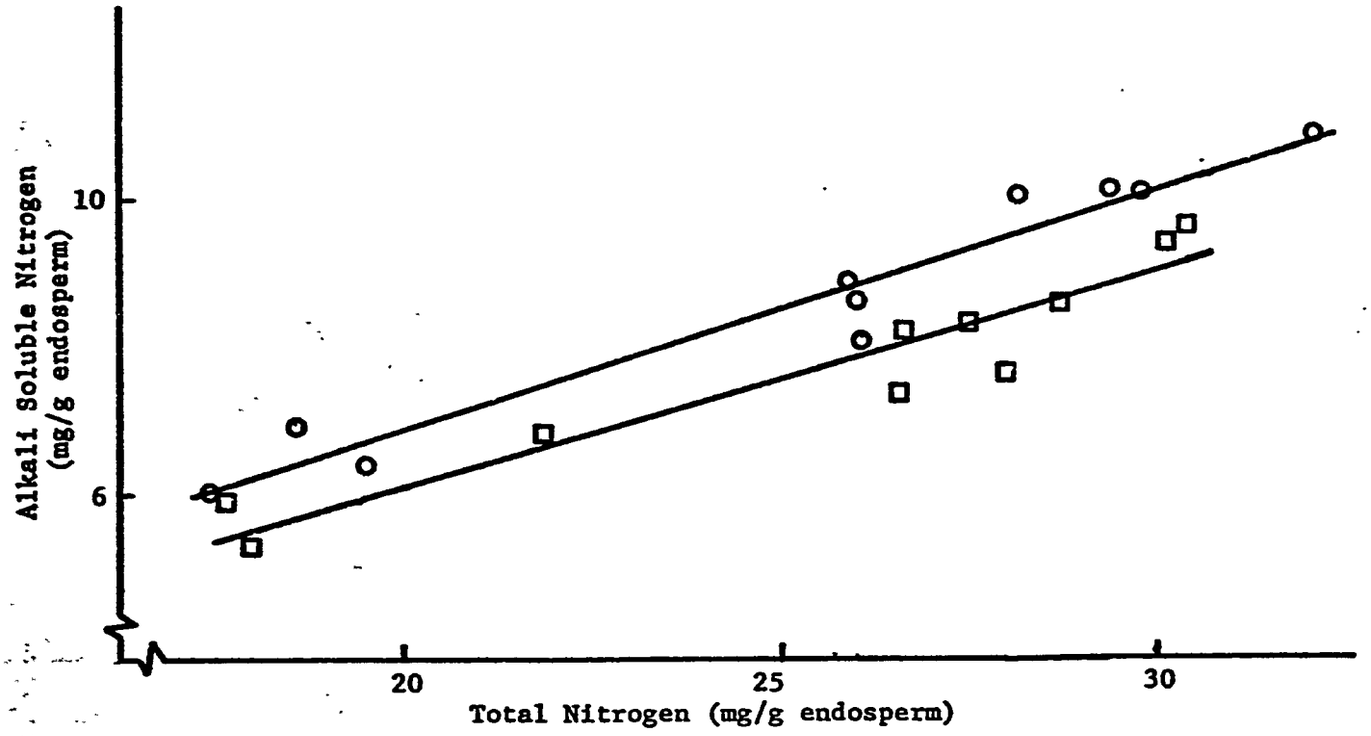

Figure 20. The relationship of alkali soluble endosperm nitrogen to total endosperm nitrogen in 'Warrior' and 'Kharkof'. The circles = 'Warrior' and the squares = 'Kharkof'.

NE68513 was similar to 'Kharkof' and that all other cultivars were similar to 'Warrior'.

The correlations between soluble endosperm nitrogen and endosperm lysine were highly significant and positive when both soluble nitrogen and lysine were expressed on a weight of endosperm basis (Table XIV). The correlations were negative and highly significant when lysine (% of protein) was related to soluble nitrogen (mg per g endosperm). The alcohol fraction (% of protein) was positively correlated with percent lysine and negatively correlated with lysine (% of protein). The reverse was found for the salt fraction (% of protein), a negative correlation with percent lysine and a positive correlation with lysine (% of protein). The acid fraction (% of protein) was not significantly correlated with percent lysine but was positively correlated with lysine (% of protein). The alkali fraction (% of protein) was negatively correlated with percent lysine and not significantly correlated with lysine (% of protein).

A relationship between cultivar endosperm lysine differences and cultivar solubility fraction differences was lacking. The NRPN cultivars high in alcohol soluble nitrogen (Table XIII); 'Kharkof', NE68513, and 'Bezostaya'; were above average, average, and below average in lysine (Table VIII). The cultivar high in acid soluble nitrogen, NE68510, was also high in endosperm lysine but not different from 'Kharkof' in endosperm lysine. The high and low endosperm lysine cultivars (Figure 3), NE68510 and 'Bezostaya', were equal in alkali soluble protein.

The Influence of Nitrogen Fertilizer

Tables XV thru XXII and Figure 21 summarize data obtained from wheat nitrogen fertilizer trials. The data from the Jefferson County

Table XIV. Correlation of endosperm lysine with endosperm solubility fractions (NRPN samples). The correlations are based on 70 samples.

Soluble nitrogen	Lysine	
	%	% of protein
(mg/g endosperm)		
Alcohol	0.96**	-0.81**
Salt	0.69**	-0.57**
Acid	0.91**	-0.70**
Alkali	0.86**	-0.80**
(% of total nitrogen)		
Alcohol	0.69**	-0.65**
Salt	-0.48**	0.42**
Acid	-0.11	0.24*
Alkali	-0.41**	0.18

* Statistically significant at the 5% level
** Statistically significant at the 1% level

samples were not included in the fertilizer treatment means because only the 0 and 135 kg per ha treatments were analyzed and the response to nitrogen fertilization in Jefferson County was of a different magnitude than in the other counties. The Jefferson County samples were included in correlation and regression analysis.

Whole wheat and endosperm protein increased with increasing increments of nitrogen fertilizer (Table XV). Average differences of 2.1 and 2.6% protein were measured between NE65307 and 'Lancer' in the grain and endosperm respectively. Cultivar grain and endosperm protein content differences were present at all levels of applied nitrogen. In both cultivars there was a tendency for the differences between wheat and endosperm protein content to become less when nitrogen fertilizer was increased from 0 to 90 kg per ha. A smaller average difference between wheat and endosperm protein, over fertilizer treatments, was indicated for NE65307 (0.1%) in comparison with 'Lancer' (0.6%). A positive relationship was indicated between wheat and endosperm percent lysine and applied nitrogen fertilizer. NE65307 contained a higher percent lysine in both the wheat and endosperm at each level of fertilizer. Wheat and endosperm lysine (% of protein) decreased with increasing increments of nitrogen fertilizer. The average cultivar differences in lysine (% of protein), over fertilizer treatments, were approximately 0.2 and 0.1% in the grain and endosperm respectively.

Total endosperm nitrogen and all soluble nitrogen fractions increased with increasing nitrogen fertilizer applications (Table XVI). At each level of applied fertilizer the samples of endosperm from NE65307 contained more soluble nitrogen than the samples of 'Lancer'. NE65307 was higher in alcohol soluble protein (% of protein) and lower

Table XV. Fertilizer treatment means (protein and lysine). All data is expressed on a dry weight basis. All means are based on the data from the Phelps and Deuel County samples except as footnoted.

Cultivar	Nitrogen fertilizer applied (kg/ha) 0	45	90	135	Cultivar Mean	Cultivar Mean*
Wheat protein (%)						
NE65307	11.3	13.5	15.7	17.5	14.5	15.3
Lancer	9.7	11.3	13.5	15.2	12.4	13.0
Endosperm protein (%)						
NE65307	10.9	13.4	15.9	17.6	14.4	15.2
Lancer	8.9	10.6	13.1	14.6	11.8	12.4
Protein (wheat-endosperm)						
NE65307	0.46	0.10	-0.16	-0.09	0.08	0.02
Lancer	0.82	0.66	0.41	0.50	0.60	0.59
Wheat lysine (%)						
NE65307	0.36	0.40	0.45	0.49	0.43	0.44
Lancer	0.33	0.36	0.41	0.45	0.39	0.40
Endosperm lysine (%)						
NE65307	0.26	0.29	0.34	0.38	0.32	0.32
Lancer	0.22	0.25	0.29	0.32	0.27	0.28
Wheat lysine (% of protein)						
NE65307	3.18	2.96	2.88	2.84	2.98	2.91
Lancer	3.42	3.23	3.04	2.98	3.17	3.13
Endosperm lysine (% of protein)						
NE65307	2.40	2.20	2.12	2.14	2.21	2.16
Lancer	2.50	2.39	2.24	2.16	2.33	2.27

* Including the Jefferson County samples

Table XVI. Fertilizer treatment means (total and soluble endosperm nitrogen). Nitrogen is expressed as mg per g endosperm (dry weight basis). All means are based on the data from the Phelps and Deuel County samples except as footnoted.

Cultivar	Nitrogen fertilizer applied (kg/ha) 0	45	90	135	Cultivar Mean	Cultivar Mean*
Total endosperm nitrogen						
NE65307	17.90	21.96	25.94	29.30	23.78	24.93
Lancer	14.24	17.22	21.29	24.06	19.20	20.17
Alcohol soluble nitrogen						
NE65307	8.91	11.50	14.61	16.55	12.89	13.68
Lancer	6.58	8.27	11.08	12.71	9.66	10.14
Salt soluble nitrogen						
NE65307	0.57	0.60	0.66	0.78	0.65	0.67
Lancer	0.49	0.50	0.60	0.60	0.55	0.59
Acid soluble nitrogen						
NE65307	1.29	1.54	1.80	2.06	1.67	1.76
Lancer	1.06	1.26	1.55	1.69	1.39	1.46
Alkali soluble nitrogen						
NE65307	5.90	6.84	7.93	8.66	7.33	7.73
Lancer	5.30	6.21	7.34	8.12	6.74	6.70

* Including the Jefferson County samples

in alkali soluble protein (% of protein) at all fertilizer levels (Table XVII). NE65307 was slightly lower in salt and acid soluble (% of protein), but not consistently over fertilizer treatments. With both cultivars, increases in nitrogen fertilization were associated with positive responses in alcohol soluble protein (% of protein) and negative responses in alkali soluble protein (% of protein). The salt and acid fractions were less responsive to fertilizer than the alcohol and alkali fractions but tendencies for both fractions to decrease with increasing nitrogen were suggested by the data.

The slopes of individual cultivar soluble nitrogen and lysine regression lines were compared with the corresponding slopes of the pooled NRPN regression lines (Figures 1, 8, 9, 10, and 11). The calculated t values were not significant at the 5% level except for the regression of alcohol soluble nitrogen on total nitrogen in NE65307 (Table XVIII). The slope of the regression (0.68 mg alcohol soluble nitrogen per mg total endosperm nitrogen) for NE65307, was greater than the corresponding slope for 'Lancer' (0.59 mg alcohol soluble nitrogen per mg total endosperm nitrogen). All other regression models were not significantly different in slope. Soluble nitrogen deviation mean squares from regression were within the variation expected for the extraction procedure (Table XVIII). The deviation mean squares for wheat and endosperm lysine in 'Lancer' were smaller than any recorded for the NRPN cultivars (Table VII). The deviation mean square for wheat lysine in NE65307 was similar to the NRPN cultivars but the deviation mean squares for endosperm lysine in NE65307 was greater than twice the pooled NRPN value. The relationships between alcohol soluble nitrogen and total nitrogen for NE65307 and 'Lancer' endosperms are shown in

Table XVII. Fertilizer treatment means (soluble protein, % of total protein). All means are based on the data from the Phelps and Deuel County samples except as footnoted.

Cultivar	Nitrogen fertilizer applied (kg/ha) 0	45	90	135	Cultivar Mean	Cultivar Mean*
Alcohol soluble						
NE65307	49.7	52.3	56.2	56.5	53.7	54.4
Lancer	46.2	48.0	52.0	52.8	49.8	49.9
Salt soluble						
NE65307	3.22	2.74	2.54	2.66	2.79	2.74
Lancer	3.51	2.87	2.83	2.49	2.93	2.97
Acid soluble						
NE65307	7.19	7.00	6.93	7.04	7.04	7.07
Lancer	7.46	7.32	7.30	7.04	7.28	7.26
Alkali soluble						
NE65307	33.0	31.2	30.6	29.5	31.1	31.2
Lancer	37.2	36.0	34.6	33.8	35.4	34.6

* Including the Jefferson County samples.

Table XVIII. Statistical comparisons of individual cultivar regression models (fertilizer treatment samples). The t test is for H_o: β = pooled NRPN b. Soluble nitrogen was regressed on total nitrogen and percent lysine was regressed on protein.

Cultivar	t	b	MS
	Alcohol soluble nitrogen		
Lancer	-0.59	0.59	0.138
NE65307	3.95**	0.68	0.059
	Salt soluble nitrogen		
Lancer	0.16	0.019	0.005
NE65307	-0.16	0.018	0.004
	Acid soluble nitrogen		
Lancer	-0.10	0.06	0.005
NE65307	0.80	0.07	0.014
	Alkali soluble nitrogen		
Lancer	-1.11	0.25	0.144
NE65307	-0.86	0.26	0.150
	Wheat lysine (%)		
Lancer	1.68	0.022	0.00001
NE65307	-0.95	0.020	0.00013
	Endosperm lysine (%)		
Lancer	-1.83	0.016	0.00003
NE65307	-1.25	0.015	0.00032

** Statistically significant at the 1% level

Figure 21. The low protein samples of NE65307 were not different from 'Lancer' in alcohol soluble nitrogen but the high protein samples of NE65307 contained more alcohol soluble nitrogen than 'Lancer' and were beyond the range of the 'Lancer' samples in protein content.

The pooled NRPN regression equations were used to adjust the fertilizer treatment means (Table XIX) of all soluble nitrogen fractions except for the alcohol soluble fraction from NE65307, which was adjusted with the regression equation derived from the NE65307 samples. Soluble nitrogen, based on adjusted soluble nitrogen values, was not influenced by applied fertilizer nitrogen except for alkali soluble nitrogen in NE65307 which decreased with increasing increments of fertilizer. Cultivar differences in soluble nitrogen, after adjustment, were smaller than the unadjusted differences in Table XVI. Cultivar mean adjusted soluble nitrogen values indicated the following cultivar differences: a) NE65307 greater than 'Lancer' in alcohol soluble nitrogen; b) NE65307 less than 'Lancer' in alkali soluble nitrogen; c) NE65307 equal to 'Lancer' in salt and acid soluble nitrogen. The adjusted means in Table XIX were expressed as a percentage of mean endosperm nitrogen (Table XX) and when compared with the unadjusted percentages in Table XVII indicated that the influence of nitrogen fertilization on protein solubility fractions could be attributed to the influence of nitrogen fertilization on protein content.

The pooled NRPN regression equations were used to adjust the wheat and endosperm percent lysine data in Table XV to a common level of protein. The adjusted values (Table XXI) were nearly constant at all levels of nitrogen fertilizer applied and the cultivars were not different in lysine.

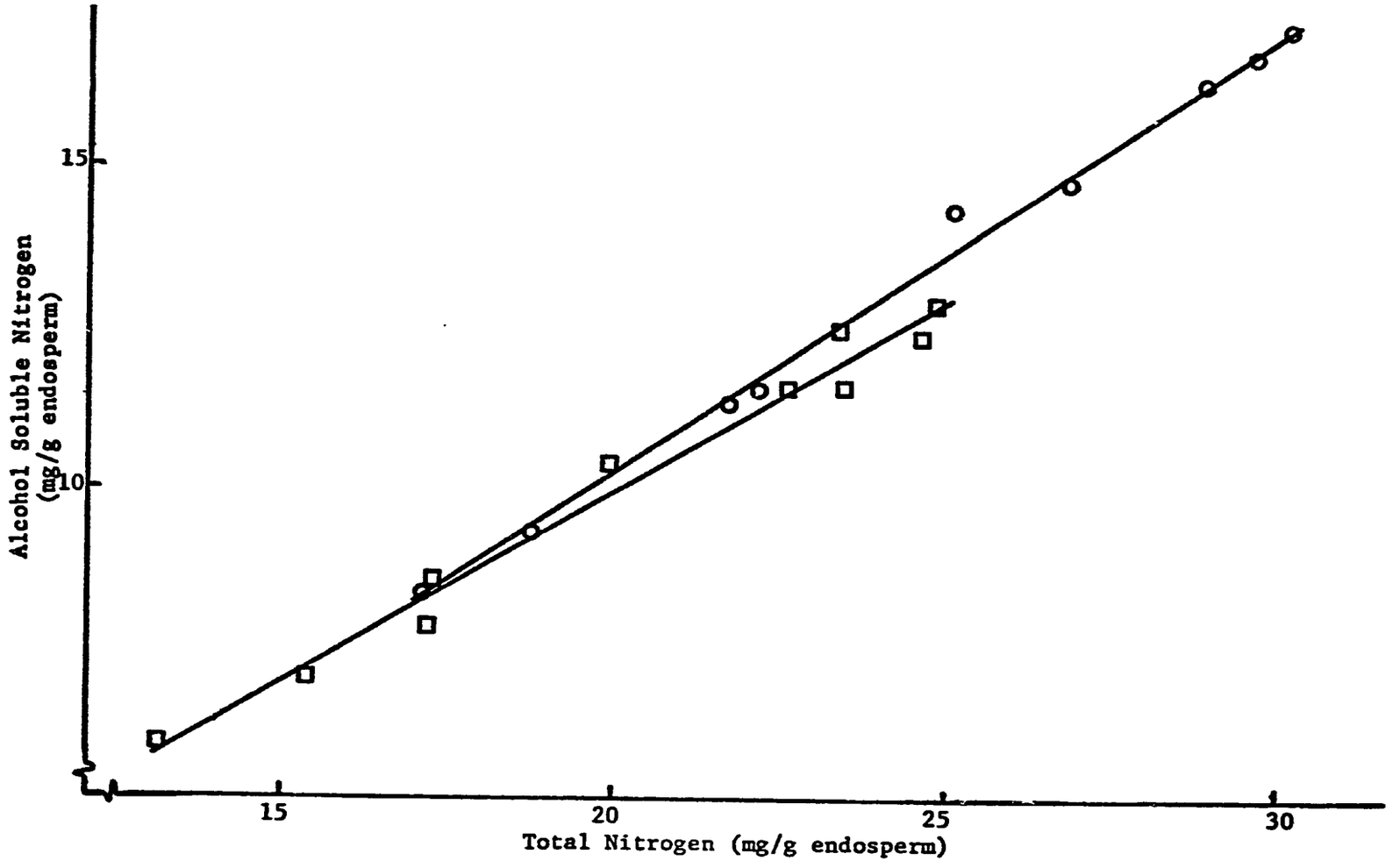

Figure 21. The relationship of alcohol soluble endosperm nitrogen and total endosperm nitrogen in an 'Atlas 66' derived line and 'Lancer'. The circles = NE65307 and the squares = 'Lancer'.

Table XIX. Adjusted fertilizer treatment means (soluble endosperm nitrogen). Soluble nitrogen is expressed as mg per g endosperm. The means in Table XVI were adjusted to 22.5 mg nitrogen per g endosperm. All means are based on the data from the Phelps and Deuel County samples except as footnoted.

Cultivar	Nitrogen fertilizer applied (kg/ha) 0	45	90	135	Cultivar Mean	Cultivar Mean*
Alcohol soluble nitrogen						
NE65307	12.03	11.87	12.27	11.93	12.89	12.03
Lancer	11.49	11.41	11.80	11.78	11.62	11.52
Salt soluble nitrogen						
NE65307	0.68	0.61	0.60	0.65	0.63	0.63
Lancer	0.64	0.59	0.62	0.57	0.61	0.64
Acid soluble nitrogen						
NE65307	1.59	1.57	1.58	1.62	1.59	1.60
Lancer	1.60	1.61	1.63	1.59	1.61	1.61
Alkali soluble nitrogen						
NE65307	7.23	7.00	6.94	6.69	6.96	7.03
Lancer	7.69	7.74	7.69	7.67	7.69	7.57

* Including the Jefferson County samples

Table XX. Adjusted fertilizer treatment means (soluble endosperm protein, % of mean protein). The adjusted means from Table XIX were expressed as a percentage of 22.5 mg nitrogen per g endosperm (12.8% protein). Soluble nitrogen was adjusted to this total nitrogen value which represents the mean of all fertilizer treatment samples evaluated. All means are based on the data from the Phelps and Deuel County samples except as footnoted.

Cultivar	Nitrogen fertilizer applied (kg/ha) 0	45	90	135	Cultivar Mean	Cultivar Mean*
Alcohol soluble						
NE65307	53.5	52.7	54.5	53.0	54.4	53.5
Lancer	51.0	50.7	52.4	52.4	51.6	51.2
Salt soluble						
NE65307	2.92	2.73	2.65	2.90	2.79	2.79
Lancer	2.87	2.63	2.76	2.54	2.69	2.85
Acid soluble						
NE65307	7.06	6.99	7.02	7.19	7.06	7.13
Lancer	7.09	7.14	7.26	7.08	7.14	7.15
Alkali soluble						
NE65307	32.1	31.1	30.8	29.8	30.9	31.2
Lancer	34.3	34.4	34.2	34.1	34.2	33.6

* Including the Jefferson County samples

Table XXI. Adjusted fertilizer treatment means (wheat and endosperm lysine). All data is expressed on a dry weight basis. The percent lysine means from Table XV were adjusted to the mean protein of all fertilizer treatment samples (14.1% for wheat and 13.8% for endosperm). The adjusted percent lysine values also were expressed as a percentage of mean protein. All means are based on the data from the Phelps and Deuel County samples except as footnoted.

Cultivar	Nitrogen fertilizer applied (kg/ha) 0	45	90	135	Cultivar Mean	Cultivar Mean*
	Wheat lysine (%)					
NE65307	0.42	0.42	0.42	0.42	0.42	0.42
Lancer	0.42	0.42	0.42	0.43	0.43	0.42
	Wheat lysine (% of mean protein)					
NE65307	2.96	2.95	2.97	2.97	2.97	2.94
Lancer	2.99	3.00	2.98	3.04	3.00	3.01
	Endosperm lysine (%)					
NE65307	0.31	0.30	0.30	0.31	0.31	0.30
Lancer	0.31	0.31	0.31	0.30	0.31	0.30
	Endosperm lysine (% of mean protein)					
NE65307	2.24	2.18	2.18	2.25	2.22	2.18
Lancer	2.22	2.24	2.22	2.19	2.21	2.20

* Including the Jefferson County samples

Table XXII shows the correlations of endosperm lysine with soluble endosperm nitrogen based on the 20 samples from nitrogen fertilizer trials. Highly significant positive correlations were obtained when soluble nitrogen and lysine were expressed on an endosperm weight basis. Highly significant negative correlations occurred for lysine (% of protein) with soluble nitrogen (mg per g endosperm). Alcohol soluble protein (% of protein) was positively correlated with percent lysine and negatively correlated with lysine (% of protein). The salt and alkali fractions (% of protein) were negatively correlated with percent lysine and positively correlated with lysine (% of protein). The acid fraction was not significantly correlated with percent lysine but positively correlated with lysine (% of protein).

Quality Evaluation

Table XXIII gives the endosperm mill yield and bran clean-up of all samples. Greater yields of endosperm were obtained from 'Lancer', 'Warrior', and NE66403. 'Centurk', 'Kharkof', and NE65307 yielded less endosperm than all other cultivars except NE68513, which was not different from NE68510. With the exception of 'Kharkof' all cultivars had eight or more samples with a good bran clean-up rating. Only one sample of bran from 'Kharkof' was rated as good and nine were rated as fair. Therefore, based on both factors, mill yield and bran clean-up, all cultivars studied were better milling wheats than 'Kharkof'.

The mixing time and tolerance of all samples are presented in Table XXIV. The cultivars are arranged in the table according to increasing mean mixing time. Endosperm with less than 12% protein (14% moisture basis) was considered to have a pseudo mixing time and tolerance (Finney and Shogren, 1972), therefore, two cultivar means were

Table XXII. Correlation of endosperm lysine with endosperm solubility fractions (fertilizer treatment samples). The correlations are based on 20 samples.

Soluble nitrogen	Lysine	
	%	% of protein
(mg/g endosperm)		
Alcohol	0.97**	-0.84**
Salt	0.83**	-0.62**
Acid	0.97**	-0.76**
Alkali	0.90**	-0.88**
(% of total nitrogen)		
Alcohol	0.88**	-0.76**
Salt	-0.60**	0.72**
Acid	-0.18	0.46*
Alkali	-0.78**	0.53*

* Statistically significant at the 5% level
** Statistically significant at the 1% level

Table XXIII. Mill yield and bran clean-up. The numbers listed under the bran rating refer to the number of samples given that rating. The mill yields are cultivar means. The means within a group followed by the same letter were not significantly different at the 5% level according to Duncan's multiple range test (Steel and Torrie, 1960). 'Lancer' and NE65307 were not included in the multiple range test.

Cultivar	Mill Yield	Bran rating	
		Good	Fair
Warrior	62.7a	9	1
NE66403	62.5a	10	—
Besostaya	61.4 b	8	2
NE68510	61.2 bc	8	2
NE68513	60.2 cd	10	—
Centurk	59.9 d	9	1
Kharkof	59.7 d	1	9
NRPN Mean	61.1		
Lancer	65.9	10	—
NE65307	59.2	9	1

Table XXIV. Endosperm protein, mixing time and tolerance. Protein is expressed on a 14% moisture basis. Mixing time is expressed in min and tolerance is rated on a 0 to 5 rating.

NE65307

Location	Protein	Mixing time	Mixing tolerance
Deuel	8.8	3.67	2
Phelps	9.9	3.33	2
Deuel	11.5	2.00	2
Phelps	11.6	2.33	2
Phelps	13.5	2.00	2
Deuel	13.8	1.33	1
Deuel	15.0	1.33	1
Phelps	15.2	2.00	0
Jefferson	15.6	2.33	1
Jefferson	16.2	2.33	0
Mean N=10	13.1	2.26	1.0
Mean N=6	14.9	1.88	0.8

Kharkof

Sheridan	9.0	2.67	4
St. Paul	9.1	2.33	4
Casselton	10.9	2.67	3
Lethridge	13.7	2.00	1
Highmore	13.9	2.00	2
N. Platte	14.1	2.67	2
Tetonia	14.5	2.00	1
Moccasin	14.8	2.00	2
Clovis	15.3	2.67	2
Sidney	15.7	2.00	2
Mean N=10	13.1	2.30	2.3
Mean N=7	14.6	2.19	1.7

Lancer

Deuel	7.2	5.33	2
Phelps	8.0	4.33	2
Deuel	9.0	3.00	4
Phelps	9.2	3.00	2
Phelps	10.7	2.33	2
Deuel	11.8	2.33	3
Phelps	12.3	2.33	3
Jefferson	12.3	2.33	3
Deuel	12.9	2.33	3
Jefferson	12.9	2.33	2
Mean N=10	10.6	2.96	2.6
Mean N=5	12.4	2.33	2.8

Table XXIV. (Continued)

Location	Protein	Mixing time	Mixing tolerance
	NE68513		
Sheridan	10.5	3.33	3
St. Paul	10.8	2.33	4
Casselton	12.0	2.00	1
Highmore	15.0	2.00	2
N. Platte	15.1	2.67	2
Lethbridge	15.1	2.00	2
Tetonia	15.9	2.33	2
Clovis	16.0	3.00	1
Sidney	16.0	3.00	2
Moccasin	16.5	2.33	2
Mean N=10	14.3	2.50	2.1
Mean N=8	15.2	2.42	1.9
	NE68510		
Sheridan	9.3	3.33	4
Casselton	10.1	2.00	3
St. Paul	10.5	3.33	4
Lethbridge	14.0	2.67	3
Highmore	14.2	2.67	3
Moccasin	14.5	3.67	4
Tetonia	14.7	2.67	2
N. Platte	14.8	3.67	4
Clovis	15.3	3.33	3
Sidney	15.5	3.33	4
Mean N=10	13.3	3.07	3.4
Mean N=7	14.7	3.14	3.3
	Warrior		
St. Paul	9.2	3.67	3
Sheridan	9.2	3.33	4
Casselton	9.9	3.00	3
Highmore	13.2	2.67	2
N. Platte	13.5	4.33	3
Lethbridge	13.6	3.00	2
Tetonia	14.7	3.67	2
Sidney	15.2	4.00	4
Moccasin	15.5	3.67	3
Clovis	16.5	5.00	2
Mean N=10	13.0	3.63	2.8
Mean N=7	14.6	3.76	2.6

Table XXIV. (Continued)

Location	Protein	Mixing time	Mixing tolerance
		Bezostaya	
Sheridan	9.7	3.33	5
St. Paul	11.3	4.00	5
Lethbridge	12.8	3.00	2
Casselton	13.0	3.67	4
Highmore	13.8	3.67	3
Tetonia	14.1	3.33	2
N. Platte	14.2	4.33	4
Moccasin	15.0	3.33	3
Sidney	15.4	3.67	3
Clovis	15.6	6.67	2
Mean N=10	13.5	3.90	3.3
Mean N=8	14.3	3.96	2.9
		NE66403	
Sheridan	7.8	4.33	3
St. Paul	8.3	4.00	3
Casselton	9.8	3.00	1
Highmore	12.0	2.33	1
Lethbridge	12.4	4.00	2
N. Platte	12.6	4.33	3
Moccasin	13.7	4.00	3
Tetonia	13.8	4.33	2
Clovis	14.3	4.33	1
Sidney	14.4	5.33	2
Mean N=10	11.9	4.00	2.1
Mean N=7	13.3	4.09	2.0
		Centurk	
Sheridan	8.6	5.00	5
St. Paul	9.2	3.67	5
Casselton	9.5	3.33	3
Lethbridge	12.3	3.67	3
Tetonia	13.0	4.00	3
N. Platte	13.2	5.33	5
Highmore	13.4	3.33	4
Moccasin	14.3	3.33	4
Sidney	15.4	4.33	4
Clovis	15.7	6.33	3
Mean N=10	12.5	4.23	3.9
Mean N=7	13.9	4.33	3.7

calculated, one based on all 10 samples and the other based on samples at 11.8% protein or greater. Conclusions about mixogram characteristics were confined to endosperm samples with 11.8% protein (14% moisture basis) or greater. The cultivars studied exhibited a considerable range in mean mixing time varying from 1.9 min for NE65307 to 4.3 min for 'Centurk'. An amazingly stable mixing time and tolerance was observed with the samples of 'Lancer'. All higher protein samples of 'Lancer' had a mixing time of 2.3 min and most samples had a tolerance rating of three. In general, the shorter mixing cultivars tend to show less variation in the mixograms from location to location. The shorter mixing cultivars were NE65307, 'Kharkof', 'Lancer', NE68513, and NE68510. The four longer mixing cultivars were 'Warrior', 'Bezostaya', NE66403, and 'Centurk'.

DISCUSSION

Cultivar Protein and Lysine Differences

Cereal crops make up a large part of the worlds food supply. Wheat is one of the leading cereals on a world wide basis, therefore, its nutritional improvement would have a world wide impact. The improvement of nutritional quality is being included in many cereal breeding programs. The main nutritional objectives in wheat breeding programs are: a) improving protein content, and b) improving essential amino acid balance. Since lysine is the most limiting amino acid in wheat protein, the latter objective of improving essential amino acid balance is usually manipulated by increasing the lysine content of the protein.

High protein wheat cultivars have been defined as cultivars which produce higher levels of protein than other cultivars grown under the same environmental conditions (Johnson, Whited, Mattern, and Schmidt; 1968). The description of a high protein wheat cultivar does not imply a fixed level of protein, but refers to a measured increase in protein content based on uniform testing under several growing conditions (Johnson _et al_. 1971). A higher protein content accompanied by a decrease in yield would be of little value, at least from the practical production point of view. The environment in which a wheat cultivar is grown has a greater influence on the measured protein content than the genetic potential of the cultivar. Therefore, Johnson _et al_. (1969) have restricted protein comparisons to experimental lines of comparable productiveness. When mean protein contents and yields from several growing conditions have been obtained, a high protein cultivar would have a greater mean protein content with a mean yield, equivalent to or

above other cultivars. This was essentially the criterion set forth when Middleton *et al.* (1954) reported the high protein trait in 'Atlas 66'.

The cultivars studied from the 1971 NRPN possessed differing genetic abilities for the production of protein. Based on the cultivar mean protein contents, NE68513 contained the high protein trait of 'Atlas 66'. The grain and endosperm of NE68513 contained 1.4% more protein than the regional mean. Protein content in the grain and endosperm of NE68510 was not different from 'Kharkof', 'Berostaya', and 'Warrior', indicating that NE68510 does not contain the 'Atlas 66' high protein trait. A small number of genes are thought to be involved in the 'Atlas 66' high protein trait and a genetic linkage between high protein and leaf rust resistance has been indicated (Johnson, Schmidt, and Mattern; 1968). NE68513 and NE68510 were both resistant to leaf rust and high in protein prior to 1971, but were reported[1] as susceptible to leaf rust in 1971. NE68513 produced an increased amount of protein in the presence of leaf rust and NE68510 did not. Therefore, the two 'Atlas 66' derived lines must have different genetic mechanisms for increased protein production. One high protein mechanism operates in the presence of leaf rust and the other does not.

NE66403 contained 1.2% less protein than the regional mean. A relationship between yield and protein was lacking in the cultivars studied. The high protein line, NE68513, and the low protein line, NE66403, were nearly equal in mean yield as well as being nearly equal to the overall regional mean yield. The fact that NE66403 contains a

[1] Johnson, V. A. 1971. Comparison of winter wheat varieties grown in cooperative nursery experiments in the hard red winter wheat region in 1971. USDA unpublished report. Lincoln, Nebraska.

lesser amount of protein at an equal yield level indicates that NE66403 may possess a genetic mechanism for increased starch production.

It has been generally accepted that the endosperm usually contains 1% less protein than the whole grain. In fact, McNeal et al. (1971) converted endosperm protein percentages to wheat protein percentages based on this assumption. The difference between wheat and endosperm protein in 'Atlas 66' derived lines has been variable (Johnson et al. 1963). The wheat and endosperm protein contents of 'Bezostaya' differed on the average by 0.2% protein. In fact, some samples of endosperm from 'Bezostaya' had higher protein contents than the whole grain. The mean reduction in protein content from wheat to endosperm in the 'Atlas 66' derived lines was approximately 0.5%. The difference between wheat and endosperm protein in the other cultivars was approximately 0.75% protein. Apparently certain cultivars possess genetic abilities to distribute greater amounts of protein into the endosperm.

The cultivars contained, although small from a practical point of view, statistically different amounts of lysine. NE66403, the low protein cultivar, was low in percent lysine but high in lysine (% of protein). NE68513, the high protein cultivar, was high in percent lysine but low in lysine (% of protein). In both the grain and endosperm and based on all NRPN samples, percent lysine was positively correlated and lysine (% of protein) was negatively correlated with protein. Several researchers have observed similar relationships (see Literature Review). Although genetic differences in grain and endosperm protein have been shown, most of the measured protein content differences from location to location are due to environmental influences. Therefore, accounting for differences in lysine that are related to protein content would for

the most part be removing environmental variation in lysine. Lysine values, unadjusted for protein variation, would be misleading from a genetic standpoint (Johnson, Mattern, and Schmidt; 1972). In order to evaluate genetic differences a lysine adjustment equation should account for as much variation as possible.

Two possible lysine adjustment models were derived from the NRPN grain and endosperm data. The coefficients of determination indicated that the models of percent lysine regressed on protein accounted for more lysine variation than the models of lysine (% of protein) regressed on protein. Therefore, the percent lysine models were used to adjust mean grain and endosperm percent lysine to a common level of protein. After adjustment, NE68510 and 'Bezostaya' represented the range in percent lysine rather than NE68513 and NE66403. The cultivar lysine differences, although small from a practical point of view, were expressed over the entire range of protein content and were more pronounced in the whole grain than in the endosperm. The increased lysine in NE68510 was preferentially contained in portions of the grain other than the endosperm which necessitates the consumption of the whole grain in order for the increased lysine to have a nutritional impact.

Endosperm Protein Components

Endosperm proteins can be characterized in many ways. Although electrophoresis, ion-exchange chromatography, and gel filtration have shown solubility fractions to be heterogeneous mixtures of protein, the classic fractions described by Osborne (1907), based on protein solubility, continue to be used and accepted (Pomeranz, 1971). Tsen (1967) suggested that differences in protein quality could likely be due to the variation in the quantitative distribution of protein components.

Protein solubility methods, although in some cases require several extractions (Chen and Bushuk, 1970; Bell and Simmonds, 1963), can be quantitated by determining the amount of protein (nitrogen) extracted with each solvent. The Maes (1962) column extraction procedure replaces the step wise batch extractions with a continuous sequential extraction, thus, eliminating the error due to transferring and batching of extracts.

In the past, some researchers have used similar terminologies to describe what may have been different fractionation procedures, while other workers have used different terminologies to describe the same procedure (Baily, 1944; Wrigley, 1972). More recent uses of protein solubility have either been described independently or relative to the fractions from the Osborne procedure. In this discussion the terms alcohol, salt, acid, and alkali were used to describe the fractions rather than using the Osborne terms because the extraction procedure was greatly different from the Osborne method. The use of Osborne protein fraction definitions should be restricted to fractionation methods that have been shown to resemble the Osborne method. In keeping with the classical fractionation definitions, Mattern, Schmidt, Morris, and Johnson (1968) classify the alcohol soluble protein from the modified Maes extraction procedure as gliadin and the alkali soluble protein as primarily glutenin.

Relationships between soluble protein fractions and total endosperm protein have been reported by Bell and Simmonds (1963), and Pence _et al_. (1954). The five protein solubility groups studied by Tanaka and Bushuk (1972) were not influenced by the protein content of the endosperm. The lack of such an influence could have been due to an insufficient range in endosperm protein content or insufficient pre-

cision of the extraction procedure. The protein solubility patterns obtained in this study were definitely related to the endosperm protein content. The relationships of all solubility fractions with protein were positive when the fractions were expressed on a weight of endosperm basis. The acid fraction was not influenced by protein when it was represented as a percentage of total protein; but the alcohol fraction increased, and the salt and alkali fractions decreased when endosperm protein content increased.

Cultivar differences in solubility fractions (% of protein) were indicated. NE68513, 'Kharkof', and 'Bezostaya' were higher in alcohol soluble protein than the other cultivars. Cultivar differences were not detected for salt soluble protein. NE68513 was higher in acid soluble protein than all other cultivars. NE68513 and 'Kharkof' contained less alkali soluble protein than all other cultivars.

Individual cultivar regression equations were derived from the NRPN data in order to determine if the influences of protein content on protein solubility groups were common to all or specific for certain cultivars. The coefficients of determination indicated that 52% of the variation in the alcohol fraction, 25% of the variation in the salt fraction, and only 13% of the variation in the alkali fraction could be accounted for when soluble protein (% of protein) was regressed on total endosperm protein. When soluble nitrogen (mg per g endosperm) was regressed on total nitrogen, the following portions of variation in soluble nitrogen were accounted for: 96% for alcohol, 50% for salt, 85% for acid, and 83% for alkali. Therefore, individual cultivar regression models were derived with the solubility fractions expressed as mg nitrogen per g endosperm.

Significant cultivar differences in regression slope were not detected with the possible exception of the alcohol fraction which had an F value between the 5 and 10% probability levels. This indicated a tendency for the genetic mechanism of a cultivar to influence the rate at which alcohol soluble protein changes with changing total endosperm protein content.

Significant differences in regression line elevations were indicated for all fractions except the salt fraction. It was necessary to assume parallel lines in order for differences in elevation to have any meaning, therefore, the interpretation of individual cultivar alcohol soluble nitrogen regression line elevations was of limited value because of possible differences in regression slope. The regression slopes for the alcohol fraction varied from 0.55 for 'Warrior' to 0.66 for 'Bezostaya'.

The influence of the 'Atlas 66' genotype on the distribution of endosperm protein into solubility groups was evaluated by comparing the 'Atlas 66' derived lines with the NRPN check cultivars, 'Kharkof' and 'Warrior'. Relative to 'Warrior', NE68513 appeared to preferentially increase alcohol soluble protein in environments favorable for high protein content. 'Warrior' and NE68513 were not different in alcohol soluble protein at low endosperm protein levels, but NE68513 consistently contained more alcohol soluble protein than 'Warrior' at high protein levels. For 'Warrior' and NE68513, the amounts of alcohol soluble protein synthesized were divergent under conditions of increasing total endosperm protein synthesis. The regression lines for 'Kharkof' and NE68510 were different in elevation and appeared parallel which implied a constant difference in alcohol soluble protein at all

endosperm protein contents. The genetic mechanisms controlling alcohol soluble protein synthesis apparently operated at a higher capacity in 'Kharkof' than in NE68510 with the difference between the mechanisms being measured as a constant.

The regression coefficients for the alkali fraction varied from 0.27 for 'Centurk' to 0.34 for NE68510. Parallel regression lines were assumed and significant cultivar differences in regression line elevation were indicated for the alkali fraction. The two NRPN check cultivars represented the range in types encountered. The 'Atlas 66' derived line, NE68513, was more like 'Kharkof' and the remaining cultivars resembled 'Warrior' in alkali soluble protein. The parallel cultivar relationships in alkali soluble protein implied a genetic mechanism that was not differentially influenced by the environmental factors which influence protein content. The genetic mechanism producing alkali soluble protein appeared to give a cultivar a certain proportion of alkali soluble protein for a given protein content.

The 'Atlas 66' trait for high protein appeared to be associated with a preferential increase in alcohol soluble protein and a decrease in alkali soluble protein. Such protein fraction shifts were measured in samples of NE68513, but not in samples of NE68510. The 'Atlas 66' high protein trait was expressed by NE68513, but not by NE68510. The 'Atlas 66' trait for high protein did not cause the protein fraction shifts, because similar trends were observed in 'Kharkof' which was average in protein content. The substitution of alcohol soluble protein for alkali soluble protein may be linked to the 'Atlas 66' trait for high protein.

Adjustment of the solubility fractions to a common level of protein did not change the grouping of the cultivars as determined by mean unadjusted soluble protein (% of protein). The adjustment equations were not necessary because the samples were obtained from a uniform nursery and a similar range in protein content was represented for each cultivar. When only two or three samples of a cultivar are used for fractionation, the data should be interpreted in accordance with the general relationships established between soluble and total protein. For example, the relationship shown by the data indicated that a low protein sample from a cultivar with average amounts of alkali soluble protein would have more alkali soluble protein (% of protein) than a high protein sample from the same cultivar. Differences in protein solubility fractions have been reported by Orth and Bushuk (1972) within a group of 26 cultivars grown at one location. The protein content of the samples ranged from 10.9 to 15.5% (14% moisture basis) with most samples being between 13.0 and 15.0% protein; therefore, the influence of protein content on the solubility fractions was minimal. Tanaka and Bushuk (1972) also have reported cultivar differences in protein solubility fractions. Although they indicated solubility properties were not affected by protein content, they restricted cultivar comparisons to samples with nearly equal protein contents.

The cultivar differences in genetic abilities to produce protein have been indicated. The protein differences in individual samples were due, for the most part, to an environmental influence. Therefore, the regression analysis of each solubility fraction with protein content removed a large amount of the environmental variation from the solubility fraction data. When considering the data on all 70 samples in a

pooled regression analysis, part of the deviations from regression were attributed to individual cultivar differences as indicated by significant differences in adjusted means. The sample deviations from an individual cultivar model, if greater than laboratory error, can be considered an environmentally influenced variation for the trait of consideration. The deviation mean squares from regression with such magnitude were pointed out. 'Bezostaya' showed the greatest divergence from regression for solubility fractions with significant deviations in the alcohol, salt, and alkali fractions. Significant environmental variations were not revealed for the acid fraction and the only other significant variations were for the alcohol fraction with 'Warrior' and NE68510.

Certain cultivars may not have as stable a genetic mechanism for the production of alcohol soluble protein as other cultivars. Orth and Bushuk (1972) have reported the gliadins (alcohol solubles), glutenins (acetic acid solubles) and the insoluble residue to be less sensitive to environment than the albumins (water solubles) and globulins (salt solubles). This does not agree with the present observations for the alcohol solubles, but Orth and Bushuk (1972) did not take into account the influence of protein content on the solubility fractions and used an entirely different fractionation procedure.

Relationships between solubility fractions and protein have been established as have relationships between lysine and protein. Although several authors have suggested relationships between protein solubility fractions and lysine, such relationships have not been reported. Amino acid analysis of the fractions from the modified Maes column (Mattern, Salem, and Volkmer; 1968) showed the salt and acid fractions to be

higher and the alcohol fraction to be lower in lysine than the alkali fraction. Therefore, decreases in endosperm lysine (% of protein) with increasing protein can be explained by shifts in solubility fractions with changing protein content. Primarily the increase in alcohol soluble protein and the decrease in salt soluble protein accounted for the decreasing lysine with increasing protein content. The acid and alkali fractions may have some influence on the relationship between lysine (% of protein) and protein, but to a much lesser extent than the alcohol and salt fractions.

Quality

Under the conditions of milling imposed, 'Centurk', 'Kharkof', and NE65307 were in a poor milling group according to endosperm yield, but 'Kharkof' was the only cultivar studied that had a significantly lower bran clean-up rating. Therefore, based on both factors, mill yield and bran clean-up, all cultivar evaluated were better milling wheats than 'Kharkof'.

The cultivars exhibited a considerable range in mean mixing time varying from 1.9 min for NE65307 to 4.3 min for 'Centurk'. The longer mixing time cultivars were less stable over environments than the shorter mixing time cultivars. If stability of quality over environments were to be included in plant breeding objectives, mixing time should not be any longer than absolutely required by industry. NE68510 had an acceptable mixing time in combination with stability of mixing time over environments.

With the exception of 'Bezostaya' the cultivars high in alcohol soluble protein from the NRPN were shorter in mixing time than the cultivars low in alcohol soluble protein. The longer mixing time NRPN

cultivars were higher in alkali soluble protein.

With the exception of NE66403, the samples of the long mixing time cultivars from Clovis were much longer than average. The sample of 'Bezostaya' from Clovis, had a mixing time approaching 7 min in length, when most other 'Bezostaya' samples were near 4 min in mixing time. The growing conditions reported[1] from Clovis included low seasonal rainfall, which produced low grain yields. The samples of 'Centurk', 'Warrior', and NE66403 (cultivars with long mixing time requirements) from Highmore were shorter than average in mixing time. The sample of NE66403 from Highmore, for example, had a mixing time of 2.33 min compared to the NE66403 mean of 4.1 min. The growing conditions reported from Highmore were severe drought in early spring and abundant precipitation in June and July. The environmental influence on mixing time at Clovis agrees with the increase in mixing time obtained by Day and Barmore (1971) when wheat was moisture stressed during the dough stage. The shorter mixing times at Highmore agrees with the decreased mixing time obtained with moisture stress during jointing stage (Day and Barmore, 1971).

The Influence of Nitrogen Fertilizer

Environmental factors affecting plant growth can be divided into soil factors and climatic factors. Other factors influencing the crop plants are weeds, diseases, and pests which also are influenced by the environment. Soil factors include the availability of the essential plant nutrients and the water relations in the soil. The climatic factors of greatest importance are rainfall, temperature, wind, and

[1] Johnson, V. A. 1971. Comparison of winter wheat varieties grown in cooperative nursery experiments in the hard red winter wheat region in 1971. USDA unpublished report. Lincoln, Nebraska.

humidity. The addition of fertilizers could be considered a man induced soil factor and the addition of irrigation water a man induced climatic factor. Whenever a factor can be added to the environment, experiments can be designed to test the response of some plant component to additional increments of the added factor. Whenever the environmental factor cannot be controlled observations can be made when differences in the factor occur. When several environments have been sampled, estimates of environmental variation for a trait can be made even if the reason for such variation cannot be identified. Studies of environmental influences can be classed as: a) controllable, b) identifiable, or c) random.

The research reported by Johnson et al. (1973) was a controllable study of an environmental influence. Several increments of nitrogen fertilizer were applied to two cultivars of wheat at several sites in three different years. The two cultivars, 'Lancer' and NE65307, differed inherently in their ability to produce protein. Significant protein responses to nitrogen fertilizer occurred in all trials. The responses of both cultivars were linear and positive. The high protein cultivar, NE65307, maintained a 2% protein advantage over 'Lancer' at all levels of fertilizer.

The wheat protein data in this study indicated that the samples selected for fractionation were representative of the response to nitrogen fertilizer reported by Johnson et al. (1973). The endosperm protein advantage of NE65307 was greater than the whole grain protein advantage. The reduction in protein content from whole grain to endosperm in both cultivars decreased with increasing applications of fertilizer. At high rates of fertilizer the endosperm from NE65307

contained more protein than the whole grain. NE65307 possesses the 'Atlas 66' high protein trait in the whole grain and can distribute the increased protein into the endosperm.

The protein responses to nitrogen fertilization were accompanied with positive responses in percent lysine which was reasonable since lysine is a constituent of protein. The response of lysine (% of protein) to nitrogen fertilize was inverse. The decrease in lysine (% of protein) for both whole grain and endosperm, and for both cultivars was very slight from the 90 kg per ha treatment to the 135 kg per ha treatment compared to the decrease from the 0 treatment to the 90 kg per ha treatment. The decreasing lysine (% of protein) was accompanied with a linear increase in protein for both whole grain and endosperm. The relationship of lysine (% of protein) with protein was in agreement with the data of Lawrence et al. (1958); Robinson and Sageman (1968); and Mattern, Salem, Johnson, and Schmidt (1968). All of the above failed to show an inverse relationship between lysine (% of protein) and protein at high levels of protein. An inverse relationship at low protein levels was shown by Lawrence et al. (1958).

When the pooled regression equations derived from the NRPN samples were used to adjust the wheat and endosperm percent lysine fertilizer treatment means to a common level of protein, nearly 100% of the variation in lysine was accounted for by the relationship of percent lysine with protein. The cultivar differences in lysine (% of protein) shown in this study were non existent after adjustment. NE65307 and 'Lancer' did not possess differing genetic abilities for lysine (% of protein), but did contain an average difference in lysine (% of protein) as a result of their differing abilities to produce

protein. The average protein of NE65307 was higher than the average protein of 'Lancer'; therefore, the mean lysine (% of protein) for NE65307 was located on a lower portion of the regression line than the mean lysine (% of protein) for 'Lancer'.

This study showed that the endosperm protein solubility fractions were influenced by nitrogen fertilization. Although no definite relationship could be established between the salt and acid fraction, and nitrogen fertilization, a positive response in the alcohol fraction and a negative response in the alkali fraction were clearly present. The genetic make-up of the cultivars also had an influence on the alcohol and the alkali fractions. NE65307 on the average was higher in alcohol solubles and lower in alkali solubles than 'Lancer'. After adjustment the mean cultivar differences in the alcohol and alkali fractions were reduced but still present. NE65307 possessed the ability to respond to favorable protein environments with increased alcohol solubles.

When the appropriate adjustment equations were used, nearly all of the influence of nitrogen fertilization on protein solubility fractions was attributed to the influence of nitrogen fertilization on the protein content of the endosperm.

The inverse relationship between endosperm lysine (% of protein) and protein in the NRPN samples was explained primarily by shifts in the alcohol and salt fractions with changing protein content. The same relationships were found to hold true for the samples obtained from nitrogen fertilizer treatments. The alkali fraction also appeared to have an influence on lysine (% of protein) as indicated by the positive correlation between the alkali fraction (% of protein) and lysine (% of protein).

SUMMARY

The NRPN cultivars possessed differing genetic abilities to produce protein. The protein data indicated that NE66403 is genetically low in protein and that NE68513 has the 'Atlas 66' high protein trait. NE68510 appeared to lack the 'Atlas 66' high protein trait. Both 'Atlas 66' derived lines were high in protein and resistant to leaf rust prior to 1971, but were susceptible to leaf rust in 1971. The 'Atlas 66' high protein trait is linked to leaf rust resistance; therefore, the high protein trait in NE68510 must be associated with the leaf rust resistance compared with the high protein trait in NE68513 which was expressed in the presence of leaf rust.

The endosperm usually contains 1% less protein than the whole grain. The reduction in protein content from whole grain to endosperm was 0.5% in the 'Atlas 66' derived lines. The differences between whole grain and endosperm protein in 'Bezostaya' was only 0.2% protein.

The regional mean grain lysine (% of protein) was 3.0% compared to the regional mean endosperm lysine (% of protein) of 2.3%. The cultivars had different mean lysine (% of protein) contents in both the grain and endosperm with greater differences in the whole grain. Since indications are that lysine values, unadjusted for protein variation would be misleading from a genetic standpoint, the following linear regression models were derived from the data: a) percent lysine regressed on protein and b) lysine (% of protein) regressed on protein. The regression of percent lysine on protein accounted for 93% and 94% of the lysine variation in the whole grain and endosperm respectively. Based on adjusted lysine values, NE68510 was higher than the other cultivars in

whole grain lysine but equal in endosperm lysine. The increased lysine in NE68510 was apparently in parts of the grain other than the endosperm. The increased lysine in NE68510 was consistent over environments and protein levels.

The endosperm protein content influenced the distribution of protein into solubility fractions. As endosperm protein content increased: a) alcohol fractions increased, b) salt and alkali fractions decreased, and c) acid fractions remained constant. Increases in lysine (% of protein) were explained primarily by decreases in the alcohol fraction and increases in the salt fraction with possible although not consistent, influences of the acid and alkali fractions. When studying solubility fractions in samples grown under identical conditions the magnitude of the protein content influence on solubility fractions normally would not be great enough to mask cultivar differences. When sample protein differences are large, as can occur in different environments, the cultivar fractionation differences may be confounded with fractionation differences due to protein content.

Cultivar also influenced the distribution of protein into solubility fractions. 'Warrior', 'Centurk', and NE66403 had identical fractionation patterns. NE68513, 'Kharkof', and 'Bezostaya' were higher in alcohol soluble protein. NE68513 and 'Kharkof' were lower in alkali soluble protein and NE68510 was high in acid soluble protein. The trait for increased alcohol solubles and decreased alkali solubles may be linked to the 'Atlas 66' trait for high protein. Such protein fraction shifts were observed in NE68513 which is an 'Atlas 66' derived line expressing the high protein trait, but not in NE68510, an 'Atlas 66' derived line not expressing the high protein trait.

The cultivar differences in solubility fractions were not related to lysine differences, but with one exception ('Bezostaya'), were related to the mixing requirement of the endosperm protein. Longer mixing requirement was associated with decreased alcohol solubles and increased alkali solubles.

The alcohol fraction was subject to more environmental variation than the other fractions. The distribution of 'Bezostaya' endosperm protein into solubility fractions was subject to more environmental variation than any of the other cultivars studied. The NRPN cultivars with longer mixing time requirements appeared to have less stability over environments for mixing requirement than did the cultivar with shorter mixing time requirement.

Grain samples of 'Lancer' and NE65307 (an 'Atlas 66' derived line) were obtained from three nitrogen fertilizer trials. Both cultivars produced positive linear responses of grain and endosperm protein content to increasing increments of nitrogen fertilizer. The cultivars differed inherently in their ability to produce protein at all levels of applied fertilizer. NE65307 had an average grain protein advantage of 2.3% protein and an average endosperm protein advantage of 2.8% protein. The differences between grain and endosperm protein contents were less at higher rates of fertilizer. The endosperm from NE65307 contained even more protein than the grain when grown under high rates of nitrogen fertilizer. The combination of genetically higher protein and nitrogen fertilization would have a significant protein nutritional impact on societies using wheat endosperm in their diets.

The protein response to nitrogen fertilization in the grain and endosperm was accompanied with a positive response in percent lysine

and a negative response in lysine (% of protein). The inverse relationships of grain and endosperm lysine (% of protein) to nitrogen fertilization were greatest from the 0 to the 90 kg per ha treatment and very slight from the 90 to the 135 kg per ha treatment. The slight inverse response of lysine (% of protein) to nitrogen fertilizer occurred within the 13 to 18% grain and endosperm protein range. This was in agreement with other research indicating the lack of a relationship between lysine (% of protein) and protein at higher grain protein levels.

The cultivars also differed in grain and endosperm lysine (% of protein), but when the lysine data were adjusted with models derived from the NRPN samples to a common level of protein, the cultivar lysine differences were non-existent. Differences in grain and endosperm lysine (% of protein) due to nitrogen fertilization also were accounted for by differences in protein content.

A positive response in the alcohol fraction and a negative response in the alkali fraction resulted with increasing nitrogen fertilizer. The influence of nitrogen fertilization on protein solubility fractions was attributed to the influence of nitrogen fertilization on protein content.

This study has important implications relating to the nutritional and processing quality of wheat. For example, endosperm from high protein wheat was higher in alcohol soluble protein and lower in lysine (% of protein) compared with that from lower protein cultivars. This implies that in order to maintain protein nutritional quality a high protein cultivar should be low in alcohol soluble protein. The 'Atlas 66' derived lines which expressed the high protein trait had a tendency to be high in alcohol soluble protein, but fortunately the differences

were not large enough to greatly influence the overall endosperm lysine content. Cultivars with short mixing times were high in alcohol soluble protein. Since the 'Atlas 66' genotype tends to shorten mixing time, the selection of a genetic source for higher protein should be evaluated in view of the relationships between mixing time and protein solubility patterns. The kernel components that are used for human consumption determine the actual nutritional impact of increased protein and lysine. The data from this study indicated differential distribution of protein and lysine into kernel components. Therefore, genetic sources of higher protein and lysine should be evaluated in light of these differences. A knowledge of kernel components and protein fractionation differences enables the plant breeder to evaluate the potential for employing specific genetic sources for improving the nutritional potential of wheat and still maintain adequate quality for conventional processing.

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