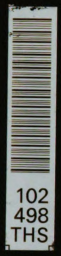

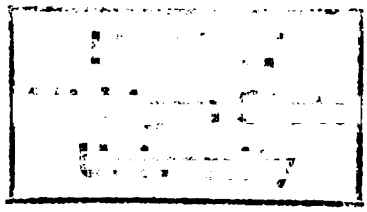

This is to certify that the

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THE LACK OF ASSOCIATION BETWEEN KERNEL
'PROTEIN AND KERNEL TEXTURE IN THIRTY
SOFT WINTER WHEAT GENOTYPES

presented by

James Richard Blakeney, Jr.

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of the requirements for

MS degree in Crop and Soil Sci.

Russell O. Frazel

Major professor

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THE LACK OF ASSOCIATION BETWEEN KERNEL PROTEIN AND KERNEL TEXTURE IN THIRTY SOFT WINTER WHEAT GENOTYPES

By

James Richard Blakeney, Jr.

A THESIS

Submitted to
Michigan State University
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ABSTRACT

THE LACK OF ASSOCIATION BETWEEN KERNEL PROTEIN AND KERNEL TEXTURE IN THIRTY SOFT WINTER WHEAT GENOTYPES

By

James Richard Blakeney, Jr.

Determination of total kernel protein and kernel texture was calculated for 30 soft winter wheat line entries at 8 Michigan locations. A relationship between total kernel protein and kernel texture was not observed within locations or within lines across locations. The major source of variability in kernel protein was due to location effects. The major source of variability in kernel texture was due to line effects.

Determination of kernel water soluble and water insoluble protein and kernel lysine was done for 15 of the above entries at 5 Michigan locations. No relationship between these characters and kernel texture within locations or within lines across locations were observed. A positive relationship between total kernel protein and water insoluble protein and a negative relationship between total kernel protein and the amount of total protein in water soluble form was observed within locations and within lines across locations. A positive relationship between kernel lysine and the amount of total protein in water soluble form was observed within locations and within lines across locations. Negative relationships between kernel lysine and total kernel protein and between kernel lysine and water insoluble protein were seen within locations and within lines across locations.

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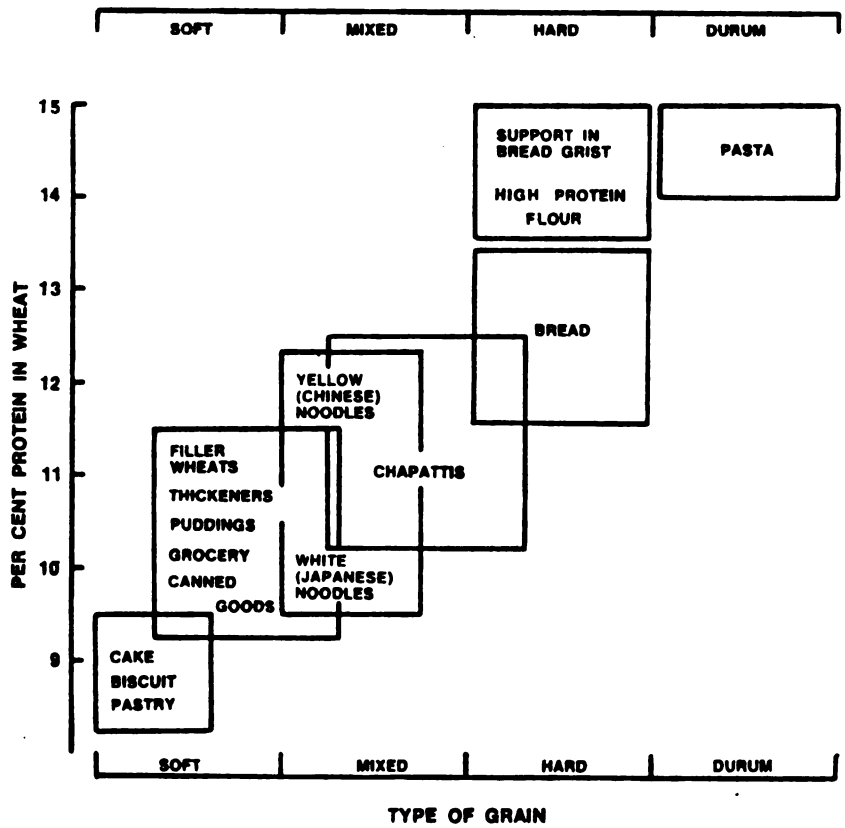
INTRODUCTION

In any discussion of quality components of milled wheat or flour the importance of each component is dependent upon the end-use requirements of the raw material. Wheats grown for bread making have been bred to possess an adequate quantity and quality of gluten proteins. These wheats have a hard kernel texture and exhibit a large degree of damaged starch and water retention capacity when milled. On the other hand, wheats having a soft kernel texture are employed in making cakes, cookies, dougnuts, and similar products. They generally contain less gluten proteins and exhibit less damaged starch and water retention after milling than hard textured wheats.

Although the quantity of crude protein and kernel texture have been shown by many workers to not be intimately associated, it has been the practice of millers to impose protein level limits on wheats intended for specific end products, Figure 1 (Moss, 1973).

In recent years the possible development of soft wheat cultivars with elevated protein levels has been investigated by researchers at Michigan State University. Release of the soft wheat cultivar Tecumseh which generally has a 12.0-12.5 percent protein content and excellent cookie quality was instrumental in this research. The purpose of this study is to analyze the genetic, environmental, and the interaction of these factors in several commercial cultivars and a number of late generation, wheat genotypes. The degree of association, if any, between kernel texture, total kernel protein, water soluble kernel protein, water insoluble kernel protein, and kernel lysine was

Figure 1. Protein content and hardness characteristics of wheats required for different end uses. *



* Moss, 1973

also studied to determine the feasibility of producing a high nutritional quality soft wheat cultivar of superior pastry quality.

LITERATURE REVIEW

The earliest comprehensive investigation of the proteins of the wheat kernel was accomplished by Osborne (1907) who concluded that at least five distinct protein substances are isolatable from the endosperm and bran of milled wheat. Solubility differences of the protein substances were employed to perform the separations. The proteins described by Osborne were: (1) gliadin, distinguishable by its ready solubility in neutral 70 percent alcohol and insolubility in neutral aqueous solutions; (2) glutenin, a protein of similar elementary composition to gliadin but soluble in dilute acid and alkaline solutions; (3) globulin, dialysable from aqueous solutions and readily soluble in dilute salt solutions; (4) leucosin, an albumin-like protein, soluble in pure water and coagulated by heating to 52 degrees F; (5) and one or two proteoses present in very small quantity and soluble in pure water.

The gluten complex of wheat was first described by Becarri in 1728 (Bailey, 1944). This complex is composed of the gliadin and glutenin proteins as described by Osborne (1907, 1924). It is in the form of storage protein of the mature grain and comprises 85-90 percent of the endosperm protein. Because it confers the rheological and mechanical properties of the dough, extensive research has been done ellucidating its deposition in the developing kernel and its behavior during dough formation and baking. The deposition and role of the soluble proteins, the albumins and globulins, have also been studied, in more recent years, due to their possible role in determining the grain hardness of wheat.

These two broad classes of protein, storage and soluble, are spacially distinct within the wheat kernel (Buttrose 1962; Jennings, et al., 1963; Graham, et al., 1963; Graham and Morton, 1963; and Morton, et al., 1964) and possess independent protein synthesizing machinery (Jennings and Morton, 1964; Graham, et al., 1962; and Donovan, et al., 1976a,b). Incorporation of radioactive isotopes of glycine and sulfate (Graham and Morton, 1964) and proline and sulfate (Morton and Raison, 1964) first led researchers to hypothesize the independence of soluble and storage protein synthesis. Electron microscopy showed proteins to be synthesized by protein-forming plastids adhering to the endoplasmic reticula of the developing endosperm and secreted inwards forming electron dense bodies. These bodies were shown to possess lipoprotein membranes and to be oppressed to starch granules as well as the endoplasmic reticula (Graham, et al., 1964, and Jennings, et al., 1963). Proteins extracted from these bodies form fibrils when hydrated which have proven to be responsible for the macrostructure of dough (Bernardin and Kasarda, 1973; Wrigley, 1972; and Pomeranz, 1971). Outside of this system of endoplasmic reticula and associated storage protein are ribosomes attached to a general reticulum believed responsible for the synthesis of cytoplasmic, soluble, proteins which are soluble in dilute salt and water solutions and function as enzymes concerned with cell maintenance and expansion. Ribosome preparations from this reticulum possess mesenger RNA which codes for the synthesis of the cytoplasmic proteins and is distinct from that carried by the protein forming plastids (Morton, et al., 1964).

Additional evidence of the independence of mechanisms responsible for the synthesis of cytoplasmic and storage proteins is implied from

studies concerned with the rate and longevity of synthesis of these proteins. Ths first study of this type was done by Bishop (1928) in an analysis of barley protein synthesis. Bishop extracted the albumins of barley kernels with dilute salt and water solutions. He extracted hordein, the main storage protein of barley and similar to wheat gliadin, with hot alcohol and calculated the glutenin by subtraction. He found a preferential deposition of hordein within cultivars between differing protein yields of locations and years. Coincident with this deposition, was a concomitant decrease in percent albumin, and interestingly, a constancy of percent glutenin. This 'regulatory principle' as coined by Bishop, appears in wheat protein deposition as well. Wu and McDonald (1976) found an overall increase in percent gluten and decrease in percent soluble protein within cultivars upon increased nitrogen application in field studies. Experimentation by Strbac, et al. (1974) substantiated this phenomenon in addition to the discovery that it is the gliadin portion of the gluten which is preferentially elevated. Jennings and Morton (1964) have shown that as nitrogen continues to be deposited in the developing grain after anthesis, the percent glutamic acid and proline, major constituents of storage protein, increase while the percent lysine, a major amino acid of the cytoplasmic proteins and the first nutritionally limiting, decreases. Jennings, et al. (1963) discovered that the deposition of soluble protein of cultivars studied plateaued at approximately 20 days post anthesis while the deposition of storage protein continued to grain maturity. It thus appears that the longevity of protein deposition dictates the proportions of protein

substances in the mature grain and indirectly affects the ultimate amino acid profile of the total protein.

The interval of protein deposition has proven to be significantly effected by environmental parameters causing variable amounts of soluble and total protein in the mature grain (Donovan, et al., 1976a and 1976b; and Meizan, et al., 1977.) The direct influence of the environment has been shown to be manifested through the substrate inducible enzyme, nitrate reductase. This enzyme regulates the nitrogen uptake by the plant (Eilrich and Hageman, 1973). The level of substrate, available soil nitrogen present as nitrate, nitrite, urea, and ammonium compounds, is affected significantly by nitrogen fertilization (Wu and McDonald, 1976; and Fowler and De La Rache, 1975) and to a lesser extent by soil moisture and temperature (Lawerence, 1976). Compositional changes in grain protein are regulated by genetic components as well as environmental constituents. Donovan, et al., (1976a and 1976b) have shown that the RNA content and ribosome population of a high protein wheat cultivar, Timgalin, were greater than that of a low protein cultivar, Heron, when grown in the same locations and years. Ribonuclease activities were virtually identical throughout the growing season for both cultivars and therefore could not account for the differences in protein levels between cultivars. Ribosome population, however, if considered a measure of protein synthetic capactiy, could account for the observed differences. The ratio of water soluble protein to storage protein proved to decrease throughout grain maturation for both cultivars. However, soluble protein synthesis was shown to plateau at 22 days for Timgalin and 29 days for Heron with Timgalin showing a more rapid incorporation of amino acids into water soluble proteins early in development. The final ratio of

soluble to storage protein was not significantly different between cultivars though the absolute amount of protein were. The authors thus concluded that the high protein cultivar Timgalin possessed a greater ability to synthesize and imcorporate both soluble and storage protein than did Heron. These results suggest a strong grain protein response to genetic factors. Estimates of both environmental and genetic responses to grain protein variation have proven to be significant (Meinze, 1977; and Fowler and De La Roche, 1975).

The amount of water soluble protein of the mature grain has been implicated as a possible cause of grain hardness in wheat. This explanation of cause, though far from proven, stems from the amount of water soluble material in the starch fraction of hard and soft wheats. Scanning electron micrographs reveal starch granules closely packed in a gluten forming protein matrix and illustrate how hard wheats tend to fracture around cell walls and directly through starch granules and the protein matrix when pressure is applied to the grain. Soft wheats, on the other hand, are shown to fracture around individual starch granules affecting a smaller particle size and less damaged starch with applied pressure than exhibited in hard wheat (Simmonds, 1974). The actual hardness of the protein matrix and starch granules are similar between many cultivars and unrelated to the texture of the endosperm as a whole (Barlow, et al., 1973). In addition, electrophoretic examination of storage protein of hard and soft wheats has shown that the composition of such protein is unrelated to kernel texture (Simmonds, 1974; Simmonds, et al., 1973; and Barlow, et al., 1973) as is the total amount of crude and storage protein (Trupp, 1976; and Wrigley, 1972). The association of

starch granules and protein matrix, which together sandwich water soluble material, however, is believed to be different in hard and soft wheats.

Simmonds (1974) has suggested that the degree of vitreousness of wheat cultivars is associated with the nature of the starch granule-protein matrix interface. The opaque nature of many soft wheat cultivars, according to Simmonds, is due to a physical gap between the many strach granule-protein boundries, affecting the scattering and absorption of light in these areas. Hard wheats, because of a tighter association between starch and protein, are unable to effectively scatter light at these boundries and affect a greater vitreousness of grain.

Though an interface gap is indiscernible, indication of a closer association of starch and protein in hard than soft wheats exists. The number of fractures passing through, as opposed to around, starch granules when wheat endosperm is frozen parallels the hardness of wheat as judged by particle size measurements (Barlow, et al., 1973). Because neither the starch nor the protein of these wheats possess different degrees of hardness, the adhesion of starch to protein is implied as the cause of hardness differences. Credence for this implication is seen upon examination of scanning electron micrographs of isolated starch granules from different cultivars. Storage protein is readily visable on the surface of strach granules isolated from hard wheats while soft wheat amyloplasts remain virtually free of adhering protein (Wrigley, 1972; and Simmonds, et al., 1973).

Flourescent antibody staining and extraction of the substance at the starch-protein interface of wheat shows the material to consist of water soluble protein and carbohydrate in a proportion of 1:2 respectively.

No significant differences in this proportion have been noted between hard and soft wheat cultivars. The carbohydrate material was initially believed to yield only glucose upon hydrolysis (Barlow, et al., 1973), but further work revealed the presence of trace amounts of xylose, arabinose, and mannose (Simmonds, et al., 1973). Electrophoretic patterns of the protein surrounding starch granules are the same for hard and soft wheats implicating no specific protein compounds as adhesives at the starch-protein interface. However, the total amount of material adhering to isolated starch granules is much greater in hard than soft wheats.

The variable degree of adhesion between starch and protein matrix of cultivars results in differences in the milling of wheat and baking performances of flour. During the milling of soft wheat, whole starch granules are freed from adhering protein resulting in less damaged starch and smaller particle size than when hard wheat is milled. Though gluten proteins are believed responsible for the network that gives structure to cookies, (Bernardin and Kasadra, 1973; and Yamazaki and Donelson, 1976) it is of minimal importance to soft wheat quality as compared to other flour fractions (Sollars, 1969). This is in contrast to bread quality which is dependent on the extensibility of gliadin and elasticity of glutenin proteins of the dough (Wrigley, 1973). Yamazaki, et al. (1977) have shown that the quality of soft wheat flour is due to the water retentive properties of gluten, starch, and tailings (cell wall material), with starch being the primary determinate of cookie spread. Yamazaki (1954) previously showed that water is held by cohesive and sorptive factors of flour and that cookie and cake quality is a function of the lack of water sorption or retention capabilities of

soft wheat flour. Soft wheat cultivars, unlike hard cultivars, fail to show a positive correlation of water retention and crude protein content. Damaged starch of all wheat flours, however, proved to be highly correlated to water retention capacity, due to the large number of hydration sites of such material, and negatively correlated to flour particle size (Williams, 1967).

Measurements of the inheritance of kernel texture were hampered in earlier work by the absence of a reliable test for grain hardness. Pearling resistance, percentage of bran, ease of milling, and visual inspection were all employed by various workers to distinguish segregating lines. This, of course, led to conflicting opinions in the literature. Aamodt, et al. (1935) proposed that one, two, and multiple gene differences distinguished hard and soft wheat cultivars. Warzella (1942) suggested that texture was inherited quantitatively though relatively few genes were involved. Multigenic inheritance was also suggested by Beard and Poleman (1954). A third explanation was proposed by Nakagawa, et al., (1959) who suggested that texture inheritance was dependent on the epistatic interaction of three sets of alleles with five, four, and three alleles in the respective sets. In more recent years a particle size index, psi, procedure has been developed which reliably measures kernel texture differences (Symen, 1961, 1965, 1969; Williams, 1967; Yamazaki, 1907; and Trupp, 1975). Though psi procedures are commonly varied by individual researchers, each employs a controlled grinding time of whole grain, followed by a controlled sieving time through a uniform meshed screen of a weighed and homogeneous sample. Such controlled conditions result in repeatable measurements and allow for a more exact examination of kernel texture inheritance.

Symes (1965) demonstrated the existence of a single major gene responsible for most of the variation in grain hardness, as measured by psi, of Australian wheats. The occurrence of a second major gene, or an allele of the first, was also demonstrated as well as a relatively few number of modifying genes all effecting kernel texture. Through reciprocal backcrossing methods, Symes was able to produce isogenic hard and soft lines which differed greatly in bread volume and soft wheat quality (Symes, 1969). Employing similar techniques, Baker (1977) found the difference between one hard and soft Canadian cultivar to be governed by two major genes with one or more minor genes modifying their effects. Isogenic hard and soft lines of a third cultivar illucidated a third major gene effecting kernel texture with one or more minor genes modifying its action.

High estimates of heritability of kernel texture have given added credence to the hypothesis of relatively simple inheritance of this trait. For the two sets of crosses performed by Baker (1977), heritability estimates were .85 and .92 respectively. Trupp (1976) computed a correlation of .83 for kernel texture between F_2 and F_3 generations of a single cross. Trupp computed a .19 intergeneration correlation for protein for the same cross which demonstrated a much more complicated mode of inheritance for protein than for kernel texture within these lines.

In many countries a large percentage of human energy and protein intakes are provided by wheat (Maclean, et al., 1975). Although conflicting opinions as to whether wheat should remain primarily an energy supplying food or one of elevated protein ingestion exists, research involving the fortification of wheat flour (Flemming and Solsuski,

1977; Howe, et al., 1965; and Milardi, et al., 1972) and the inheritance of wheat protein (Johnson and Mattern, 1978; Mizian, et al., 1977; and Trupp, 1976) are many. Among wheats of the USDA World Wheat Collection, the percent of lysine, the first limiting amino acid of wheat, has been proven to be negatively correlated with percent protein (Vogel, et. al., 1973). Recent results of genetic studies, however, have shown that protein content of wheat may be increased with concomitant decrease in percent lysine (Vogel, et. al., 1978). Amino acid profiles of various wheat cultivars have indicated that although soft wheats are typically lower in crude protein than hard wheats, the percent lysine of the total protein is greater, affecting a higher biological value of the protein. (Lawerence, et al., 1958; and Waggle et al., 1967).

MATERIALS AND METHODS

Materials for this study were taken from advanced regional yield trial nurseries of the wheat breeding program at Michigan State University. The entries were common to each nursery and consisted of 13 commercial cultivars and 17 late generation lines. These were previously evaluated in preliminary trials and included into the advanced trials only after their superiority in agronomic and quality characteristics were ascertained. Selected late generation lines may be subjected to varietal release or, in addition to selected commercial cultivars, may be used for the hybridization of new genotypes to be evaluated.

The 30 soft winter wheat entries were fall planted in 1975 at a seeding rate of 135 kg/ha in each of 8 Michigan locations chosen to represent the variable environment for Michigan wheat. At each location the entries were planted in a 5 by 6 rectangular triple lattice design. Each entry was planted in three plots consisting of four rows 3.7 m long and 30.5 cm apart. The nurseries were located in Ingham, Monroe, Lenawee, Saranac, Tuscola, Huron, Kalamazoo, and St. Joseph counties. Each nursery was fertilized at a rate of 390 kg/ha with 5-20-20 (N-P-K) just prior to planting and top dressed with 36 kg/ha of nitrogen in the form of urea in the early spring. The nurseries were mechanically harvested in the summer of 1976 and analysed for total kernel protein and kernel particle size index.

All samples for total protein analysis were ground in a Udy Cyclone sample mill and stored in envelopes. The protein determinations were done on a Neotec Grain Quality Analyzer Model 41, of Neotec

Instruments, Inc. This instrument was calibrated to determine both total protein and moisture content of the samples. Each sample analyzed in the GQA 41 was uniformly handled and replicated twice.

The GQA 41 operates on the theory of absorption of near infrared energy as a linear function of the presence of specific molecular combinations. The reflectance spectra of the infrared wavelengths are believed dependent upon various overtone and combination vibrations of C-H, N-H, O-H, H-H, and C=O bonds (Law and Tkachuk, 1977). Constituents such as protein and moisture have well defined absorption bands in the near infrared as a result of these functional groups. For the present study, four wavelengths, where the absorption spectra began to show maximum slope, were determined in the GQA 41 for a small number of samples of known protein and moisture content. Thirty-eight samples of known compliment were then analyzed to determine the relative reflectance, dR/R, of each sample at each of the four wavelengths. The dR/R values were then employed to calculate a multilinear regression equation reflecting the relative importance of the reflectance values for each wavelength. Hence an equation,

$$\% \text{ constituent} = K_0 + K_1 \, dR/R_1 + K_2 \, dR/R_2 + K_3 \, dR/R_3 + K_4 \, dR/R_4$$

for the prediction of moisture or protein content was prepared.

All samples employed in the calibration of the GQA 41 were uniformly ground in a Udy Cyclone sample mill and stored in wax bottles at the time of moisture determination. Sample moisture was determined by drying a 1-2 gram sample for 45 minutes at 130 degrees C in a drying oven. Values were obtained by calculating the amount of water lost during the drying interval. Thirty mg samples were prepared immediately after moisture determination for total nitrogen quantitation on a

Technicon Auto Analyzer. The procedure followed was outlined by Technicon (Technicon Corporation, 1969). Total nitrogen was determined by the digestion of the samples followed by measurement of the amount of ammonia produced. The procedure achieves the quantitation of ammonia with the formation of a blue indolphenol complex which occurs when ammonia is reacted with sodium phenate followed by the addition of sodium hypochlorite. Protein values were calculated on a 5.7 N basis.

Kernel texture was determined by particle size index, psi, analysis. The procedure used was a modification of that employed by Trupp (1976). Six grams of whole seed were ground for 60 seconds in a CRC micro mill. All materials contained in the grinding cavity was then brushed from the mill and thoroughly mixed to obtain a homogeneous sample. Two grams of this mixture was sifted through a sieve of 105 micron mesh for 2 minutes on an Allen Bradley sonic sifter. After the sifting of each sample, the top, sieve, and a single spacer under the sieve were removed from the sieve stack assembly and firmly tapped on a flat surface to remove adhering particles from the top and bottom of the sieve which if not removed would cause an underestimation of kernel softness. The sieve, containing the ground meal that did not pass through the mesh, was then weighed and the amount of material that had passed through the mesh was determined by subtraction. The psi values were determined by calculating the percent of the total particles that were small enough to pass through the screen. Softer kernels had a higher proportion of small particles therefore a high psi value. To assure uniformity of results, the grinding interval was controlled with an external timer and the sifting interval by locking the time dial of the sifter at 2.0

minutes. The procedure was conducted twice for each sample and total sittings coincided with complete field replications.

Analysis of variance was conducted within each location to determine the effect of genotype (entry) on total protein content and psi. Pearson correlation coefficients were also obtained within each location and within each entry across locations to ascertain the degree of association between percent total protein and psi. Combined analysis between locations was conducted as repeated lattice designs by the method of Cochran and Cox (1957) in order to determine the overall ranking of entries by their adjusted means. To determine the degree of genotype by environment interaction, a combined analysis as randomized complete blocks was conducted on adjusted location entry means. Combined location correlation of percent total protein and psi was also obtained employing adjusted location entry means.

Materials for water soluble protein analysis and lysine estimation were taken from the advanced nurseries in Ingham, Monroe, Lenawee, Saranac, and Tuscola counties. Of the 30 genotypes at each location, one replicate of the top and bottom 7 entries relative to percent total protein content were analyzed. Other interesting entries showing very high or low total protein or psi values were also analyzed boosting the total number of samples to 15 at each location.

Water soluble proteins were obtained by vigorously shaking 5.0 grams of whole meal in 50 cc of deionized water. The water-meal suspensions were then centrifuged for 10 minutes at 2000 rpm. The supernatants were decanted and total nitrogen was determined by a Kjeldahl procedure employed at the Ohio Agriculture Research and Development Center, Wooser, Ohio. Digestion of the supernatant protein was

accomplished through the addition of prepackaged Kel-Pak Degestion powder, formulation # 2P with pumice, and 20 ml of concentrated sulfuric acid to 800 ml Kjeldahl flasks containing the samples. The flasks were heated on electric burners and digestion continued for 45 minutes or until the liquid in the flasks had become clear. After cooling, 400 ml of water and 80 ml of sodium hydroxide solution were added to each flask for ammonia distillation. Distillation lasted for 30 minutes. The distillate was titrated with hydrochloric acid soultion and protein was calculated on a 5.7 N basis.

Lysine was estimated colourimetrically with a modification of the dye binding capacity, DBC, method outlined by Mossberg (1969). The underlying principle of this procedure involved the quantitative bonding of an anionic dye, Acrilane Orange G, with cationic groups of the basic amino acids, histidine, arginine, and lysine. The procedure used involved the addition of 25 ml of dye, concentration 1.3 grams per liter, to plastic bottles containing enough wheat meal to affect .65 grams of protein. The amount of meal was established from protein determinations on the GQA 41. The samples were then shaken for 60 minutes on an assembly capable of shaking 40 bottles horizontally at a time. After shaking, the samples were placed upright for 5 minutes before being filtered through inert glass fiber filters. The solutions were then passed through perforated discharge screens of 5 mm holes into a spectrophotometer equipped with a flow-through type short-light-path cuvet and a 480 millimicron filter. The optical transmission of each filtrate was recorded and compared to calibration curves prepared from the transmission of dye solutions diluted to known concentrations. The amount of dye bound by each sample was thus established through a

decrease in filtrate dye concentration. The DBC values calculated by determining the decigrams of dye bound per gram of sample protein. Greater per cent lysine therefore affected higher DBC values. Each sample analyzed by this procedure was uniformly handled and replicated twice.

Because samples were collected from only one replication at each location, within location analyses of variance were impossible. Within location, and within entries common to all locations, correlation coefficients of all possible combinations of percent total protein, percent water soluble protein, percent total protein minus water soluble protein, the percent of total protein in water soluble form, psi, and DBC were calculated in order to determine the degree of association, if any, between these characters.

RESULTS AND DISCUSSION

No consistent association between kernel texture and total kernel protein was observed in this study. Table 1 shows the coefficients of determination (r^2) betweel kernel texture and total kernel protein of the 30 wheat lines within each of 8 locations. The + or - signs preceeding the r^2 values in Table 1 and all other tables of this thesis denote the direction of association between characters. The r^2 values of Table 1 ranged from +.01 in two locations to two locations having significant negative values (-.17*, -.29**).

Table 2 shows within genotype coefficients of determination between percent total protein and kernel texture for the 30 wheat cultivars and lines over 8 locations. Significant negative values are shown to occur in 2 of the 30 genotypes evaluated with the remaining entries showing insignificant positive and negative correlations. Although the majority of these latter entries show slight negative relationships between these two characters, 8 entries show positive relationships indicating that no consistent association between percent total protein and kernel texture exists in the 30 soft wheats tested. This lack of association is shown graphically in Figure 2 where the mean values for each entry at each location are plotted against each other. Figure 2 shows 97 percent of the variance of one attribute is not associated with the other. It also shows that many enteries within locations have both high percent total protein soft kernel texture.

Table 1. The relationship between percent total protein and kernel texture (psi) of 30 wheat lines within each of 8 locations. The values shown are the coefficients of determination with a + or - sign denoting the direction of associations.

Location	Kernel texture by Total Protein	Location	Kernel texture by Total Protein
Ingham	+.01	Tuscola	-.05
Monroe	-.09	Huron	-.29**
Lenawee	-.01	Kalamazoo	-.17*
Saranac	+.01	St. Joseph	-.06

* significant at P = .05
** significant at P = .01

Table 2. The relationship between percent total protein and kernel texture (psi) of 30 wheat lines across 8 locations. The values shown are coefficients of determination with a + or - sign denoting the direction of associations.

Wheat line Entry #	Kernel Texture by Total Protein	Wheat line Entry #	Kernel Texture by Total Protein	Wheat line Entry #	Kernel Texture by Total Protein
1	-.01	11	+.04	21	-.12
2	-.18	12	-.17	22	-.04
3	-.12	13	-.10	23	-.21
4	-.26	14	-.57*	24	+.14
5	-.06	15	-.01	25	+.22
6	-.01	16	-.21	26	+.29
7	-.01	17	-.43	27	+.03
8	+.04	18	-.39	28	+.01
9	+.13	19	-.58*	29	-.02
10	-.28	20	-.14	30	-.03

r^2 of all means = -.03*
* significant at P = .05

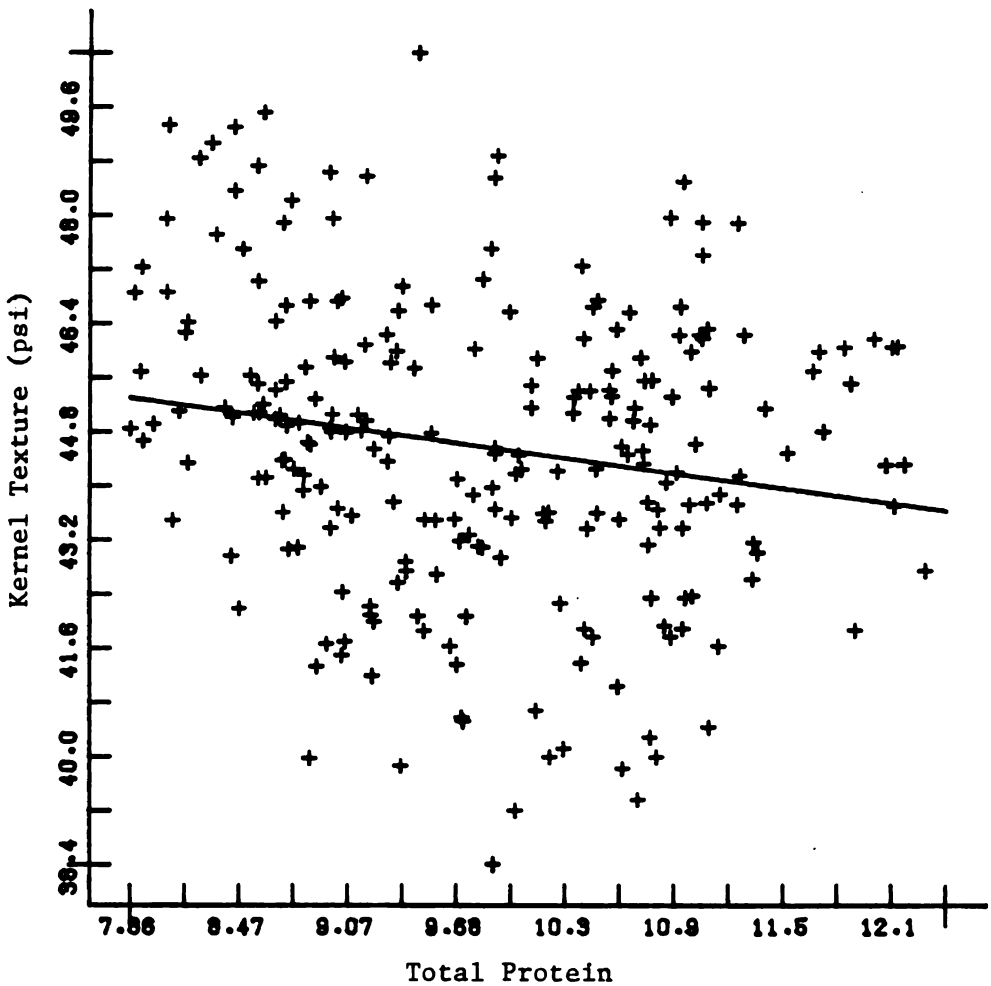


Figure 2. Relationship of total kernel protein with kernel texture for 30 wheat lines in each of 8 locations. $r^2 = .03$.

Appendices A and B show the combined analyses of variance performed as repeating lattice designs over locations for percent total protein and kernel texture (psi) repsectively. These analyses show that wheat genotype has a highly significant effect on both percent total protein and kernel texture.

Mean values of the 30 wheat entries with respect to yield, percent total protein, and kernel texture across locations are given in Appendix C. Appendix D gives the entry numbers and pedigrees of the 30 entries used in the tests. The average percent total protein of the entries varied from 8.83 to 10.77, kernel texture from 41.08 to 47.23, and yield from 2991 kg/ha to 3756 kg/ha. The LSD values given in Appendix C show that significant difference between entry means are common for each attribute.

In order to determine the effects of location and location by genotype interaction on the variability of percent total protein and kernel texture the greater efficienty of a repeated lattice design was abandoned and the data was analysed as if belonging to a randomized complete block design. This type of analyses with percent total protein and kernel texture is given in Appendices E and F respectively. The results show the effects of location and location by genotype interaction on both attributes to be highly significant.

The relative amounts of variation due to the effects of location, replications within location, genotype, and location by genotype interaction for percent total protein and kernel texture are computable from the mean square values of Appendices E and F and are shown in Table 3.

Table 3. Percent of total variance associated with location, replications within locations, genotype, and location by genotype interaction effects with respect to percent total protein and kernel texture (psi) for the 30 entries analysed.

Component of Variation	Total Protein % of Variation	Kernel Texture (psi) % of Variation
Location	75.03	30.13
R(L)	6.78	9.89
Genotype	16.34	42.20
L by G	1.84	17.59

Table 3 shows that the majority of variation in percent total protein is due primarily to location effects and secondarily to genotype effects. Location differences are associated with 75.03 percent of the variance of percent total protein while the variance associated with genoty differences is 16.34 percent of the total variance. The replications within location and location by genotype interaction effects account for very little of the variance of this attribute.

The important components of variance in kernel texture, as shown in Table 3, are genotype, location, and location by genotype interaction with replications within location of significance but secondary importance. The greatest amount of variance in kernel texture resided in genotypic effects with 42.20 percent of the total. The contribution of the effects of location and location by genotype interaction on the variance in kernel texture are in contrast to those effects on percent total protein. The effect of location on kernel texture is lesser and the effect of location by genotype interaction greater than that seen for kernel protein.

Relationships of percent total protein and kernel texture with yield and test weight within locations and within genotypes were insignificant in the study and are therefore not shown.

Location means for percent total protein, percent water soluble protein, percent water insoluble protein, the percent of total protein in water soluble form, kernel lysine (DBC), and kernel texture of the 15 entries evaluated for these characters at each location are given in Appendix G. The range of values between protein fraction attributes is of interest and is also given in the appendix. The largest ranges between location means is seen in percent water insoluble protein, percent total protein, and the percent of total protein in water soluble form. The smallest protein fraction range occurred in percent water soluble protein which indicates that, among the genotypes tested, location least effected a change in this protein fraction. The data also suggests that changes in percent total protein between locations coincided with changes in the water insoluble protein fraction.

Coefficients of determination between kernel texture and percent total protein, percent water soluble protein, percent water insoluble protein, and kernel lysine within locations for the 15 entries analysed for these characters are given in Table 4. None of the r^2 values of Table 4 are significant and no clear pattern of association between kernel texture and any of the protein fractions or kernel lysine is apparent. This lack of significant correlations supports the earlier thesis of a general lack of important association between kernel protein and kernel texture within locations.

Table 4. Within location r^2 values between psi and percent total protein, percent water soluble protein, percent water insoluble protein, and DBC for the 15 lines evaluated at each location.

Location	psi by % Total Protein	psi by % Water Soluble Protein	psi by % Water Insoluble Protein	psi by DBC
Ingham	+.06	+.02	+.04	-.07
Monroe	-.37	+.05	-.40	+.25
Lenawee	+.08	+.03	+.07	-.06
Saranac	-.02	-.16	-.01	+.01
Tuscola	-.01	+.01	-.01	-.09

Means of entries common to all locations and analysed for percent total protein, percent water soluble protein, precent water insoluble protein, the percent of total protein in water soluble form, kernel lysine (DBC), and kernel texture are given in Appendix H. As with the location means for these variables, the ranges within the protein attributes is of interest. For the genotypes tested the largest range of means occur for the percent of total protein in water soluble form, the percent of water insoluble protein, and the percent total protein. The smallest range of mean values exists for percent water soluble protein which shows that, among the genotypes analysed, variability of this protein fraction is slight and suggests that differences in percent total protein between entries is coincident with changes in the percent water insoluble protein.

Within entry coefficients of determination between kernel texture and percent total protein, percent water soluble protein, percent water

insoluble protein, and kernel lysing (DBC) for entries analysed and common to all locations are listed in Table 5.

Table 5. Within entry r^2 values between psi and percent total protein, percent water soluble protein, percent water insoluble protein, and DBC for lines common to all locations.

Entry	psi by % Total Protein	psi by % Water Soluble Protein	psi by % Water Insoluble Protein	psi by DBC
3	-.90*	-.23	-.83*	-.67*
9	-.08	+.09	-.09	-.01
10	-.13	+.59	-.19	+.01
14	-.33	+.02	-.33	+.06
16	-.16	+.05	-.19	+.01
18	-.67*	-.01	-.58	+.80*
22	-.16	+.73*	-.25	+.01
30	-.78*	+.01	-.11	+.02
r^2 of all Genotypes	-.11	+.01	-.11	+.02

* significant at P = .05

Significant r^2 values are uncommon in Table 5 thus showing a lack of close assocation between kernel texture and any protein fraction assayed or between kernel texture and kernel lysine. Both positive and negative correlations between kernel texture and the protein fractions occur in different genotypes studied. This shows that there is not an important correlation nor general direction of association among the soft wheats tested for these characters.

Within location coefficients of determination between percent total protein and percent water insoluble protein and between percent total protein and the percent of total protein in water soluble form are shown in Appendix I. A highly significant positive correlation is seen to exist between percent total protein and percent water insoluble protein with all locations showing high significance. A clear negative correlation is seen between percent total protein and the percent of total protein in water soluble form with 4 of 5 locations showing significance.

Within entry coefficients of determination between percent total protein and percent water insoluble protein and between percent total protein and the percent of total protein in water soluble form for genotypes evaluated and common to all locations are given in Table 6. The common lines are seen to possess highly significant positive correlations between percent total protein and percent water insoluble protein and a general negative correlation between percent total protein and the percent of total protein in water soluble form. The associations of these characters with all analysed entries are depicted graphically in Figure 3. The capacity of these entries to raise their total kernel protein by selectively elevating water insoluble protein is indicated. That is, 98 percent of the variance in percent total protein is positively associated with the variance in percent water insoluble protein and 66 percent negatively associated with the percent of total protein in water soluble form.

Within location coefficients of determination between kernel lysine and percent total protein, percent water insoluble protein, and the percent of total protein in water soluble form are given in Appendix J. All locations show a negative association between kernel lysine and percent

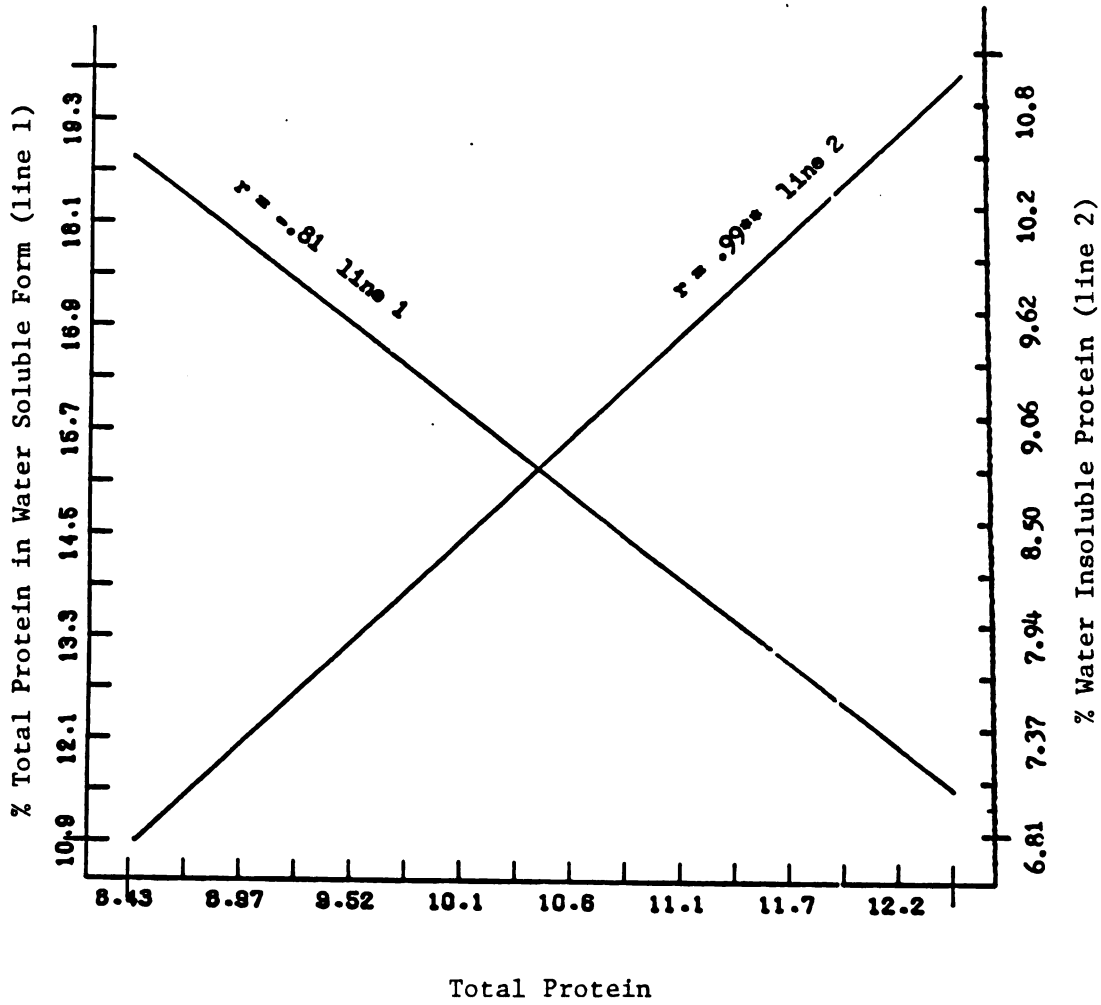


Figure 3. Relationship of total kernel protein with the amount of total protein in water soluble form and the amount of water insoluble protein on 15 wheat lines in each of 5 locations.

** significance at P = .01

total protein and between kernel lysine and percent water insoluble protein with 4 or 5 correlations significant for each. All locations show a positive association between kernel lysine and the percent of total proetin in water soluble form though none of the correcations are signigicant.

Table 6. Within entry r^2 values between percent total protein and percent water insoluble protein and between percent total protein and the percent of total protein in water soluble form for lines common to all locations.

	3	9	10	14	16	18	22	30
% Total Protein by % Water Insoluble Protein	+.97**	+.99**	+.99**	+.99**	+.94**	+.98**	+.98**	+.99**
% Total Protein by % Water Soluble of Total Protein	-.28	-.94**	-.96**	-.76*	-.28	-.79*	-.64	-.80*

* significant at P = .05
** significant at P = .01

Within entry coefficients of determination between kernel lysine and percent total protein, percent water insoluble poretin, and the percent of total protein in water soluble form of genotypes common to all locations are given in Table 7. The values of Table 7 show that a clear positive association exists between kernel lysine and the percent of total protein in water soluble form for the common entries. A clear negative association is shown between kernel lysine and both percent total protein and percent water insoluble protein for these same entries. Graphical representations of these association with all evaluated genotypes are shown in Figure 4.

The interpretation reached when Table 6 and 7 of the text are viewed together is that a selective increase in percent water insoluble protein,

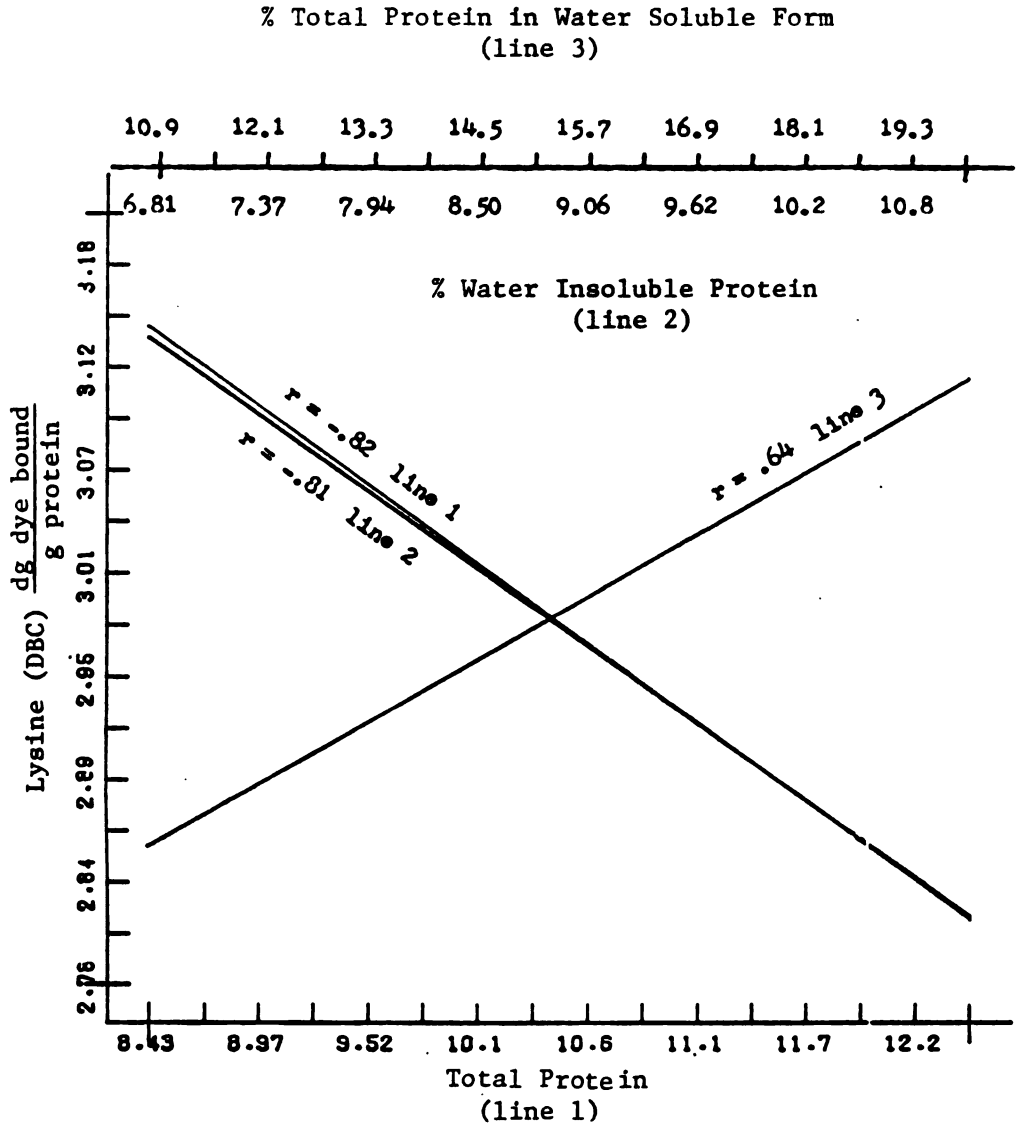


Figure 4. Relationship of kernel lysine with total protein, water insoluble protein, and the amount of total protein in water soluble form of 15 wheat lines in each of 5 locations.

Table 7. Within entry r^2 values between DBC and percent total protein, percent water insoluble proetin, and percent of total protein in water soluble form for lines common to all locations.

	Entry							
	3	9	10	14	16	18	22	30
DBC by % Total Protein	-.77*	-.57	-.81*	-.80*	+.06	-.77*	-.73*	-.62
DBC by % Water Insoluble Protein	-.76*	-.62	-.79*	-.73*	+.04	-.72*	-.62	-.58
DBC by % Water Soluble of Total Protein	+.26	+.71*	+.74*	+.39	+.03	+.48	+.18	+.39

* significant at P = .05

resulting in an overall increase in percent total protein of the genotypes studied, causes a decrease in percent lysine of the total protein and therefore a lowering of the nutritive quality of these wheats. No predictable change in kernel texture is possible with changes in the protein fractions of the wheats studied because of the lack of association between kernel texture and the protein attributes.

SUMMARY AND CONCLUSIONS

Very little association between kernel texture and kernel protein was observed during the study. Significant negative correlations did occur between kernel texture and total kernel protein in 2 of 8 locations and only 2 of 30 genotypes tested. Slight positive relationships, however, also occurred and the great majority of correlations between the two characters were insignificant. When genotypic means of all entries at every location were analysed together a coefficient of determination of .03 was observed and many genotypes had both high percent total protein and soft kernel texture at given locations.

Location, genotype, and the interaction of these effects significantly effected total kernel protein and kernel texture of the 30 soft winter wheat lines analysed. The magnitude of these effects, however, were different for the two characters. Location was the primary determinant in the observed variability of total protein contributing 75 percent of the total variance. In contrast, location was seen to effect kernel texture to a lesser extent by contributing 30 percent of the total variance. Genotype and location by genotype interaction effects had a more meaningful role in determining kernel texture than in determining total kernel protein. The percent of total variance for genotype approximated 42 percent in kernel texture and 16 percent in total kernel protein.

For the 15 lines evaluated for protein fraction distribution no clear pattern of association between kernel texture and any of the protein fractions or kernel lysine was apparant. However, significant

correlations between the different protein fractions and between protein fractions and kernel lysine were seen. A significant positive relationship appeared between percent total protein and percent water insoluble protein while a negative relationship of similar magnitude was seen between percent total protein and the percent of total protein in water soluble form. Significant negative correlations were also seen between kernel lysine and percent total protein and between kernel lysine and percent water insoluble protein. A significant positive relationship was seen between kernel lysine and the percent of total protein in water soluble form.

Because little, if any, association between kernel texture and protein fraction levels or kernel lysine was seen in the study it seems realistic that a nutritionally improved soft wheat of superior pastry quality could be produced through a genetic increase in percent water soluble protein assuming appropriate variability of this character can be found. This same effect could also be realized by a genetic increase in the percent lysine of the water insoluble proteins.

APPENDICES

Appendix A. Combined analysis of variance of 30 entries at 8 locations for percent total protein as a repeating lattice design.

Percent Total Protein

Source of Variance	Deg. of Freedom	Sums of Squares	Mean Squares	F Statistic
Replications	23	928.8824	40.3862	
Entries				
Unadjusted	29	211.3959	7.2895	29.8469**
Adjusted	29	211.1078	7.6244	38.6683**
Blocks Within Replications	120	70.7484	.5896	
Component (A)	105	65.6483	.6252	
Component (B)	15	5.1001	.3400	
Error				
RCB	667	162.9014	.2442	
Effective	547	107.8545	.1972	
Total	719	1303.1796		

Least Significant Differences:
P = .05 LSD = .2205
P = .01 LSD = .2866

Coefficient of Variation = 3.8877
Efficiency of Lattice over RCB = 123.86

** significant at P = .01

Appendix B. Combined analysis of variance of 30 entries at 8 locations for kernel texture as a repeating lattice design.

Kernel Texture

Source of Variance	Degs. of Freedom	Sums of Squares	Mean Squares	F Statistic
Replications	23	1241.3569	53.9720	
Entries				
Unadjusted	29	1484.8746	51.2026	12.9200**
Adjusted	29	1485.5058	51.2243	14.3422**
Blocks Within Replication	120	918.2552	7.6521	
Component (A)	105	837.7082	7.9782	
Component (B)	15	80.5471	5.3698	
Error				
RCB	667	2643.3554	3.9641	
Effective	547	1953.6568	3.5716	
Total	719	5369.5863		

Least Significant Differences:
P = .05 LSD = 1.0911
P = .01 LSD = 1.4184

Coefficient of Variation = 4.2390
Efficiency of Lattice over RCB = 110.96

** significant at P = .01

Appendix C. Average yield, protein content, and kernel texture (psi) of 30 wheat lines at 8 locations.

Wheat line Entry #	Yield (Kg/Ha)	Percent Total Protein	Kernel Texture psi
1	3374	9.89	42.51
2	3530	9.89	41.08
3	3440	9.29	46.34
4	3471	10.06	43.18
5	2991	9.76	42.15
6	3477	9.60	45.60
7	3255	10.77	44.15
8	3280	10.39	45.10
9	3333	10.59	44.59
10	3264	10.67	44.16
11	3220	10.03	45.28
12	3239	9.54	44.89
13	3297	9.35	45.05
14	3437	9.18	45.06
15	3687	9.40	45.05
16	3628	9.40	45.05
17	3154	9.90	43.20
18	3756	8.83	44.20
19	3466	9.33	47.23
20	3369	9.31	46.24
21	3217	9.60	46.24
22	3184	10.09	46.97
23	3145	9.83	43.28

Appendix C. Continued.

Entry #	Yield (Kg/Ha)	Percent Total Protein	psi
24	3371	9.86	45.27
25	3264	9.78	44.62
26	3380	9.86	45.15
27	3270	10.21	44.11
28	3295	10.16	42.67
29	3260	9.71	45.75
30	3158	10.70	45.84
Mean	3341	9.82	44.58
LSD P = .05	315	.22	1.09
P = .01	410	.29	1.42

Appendix D. Michigan accession numbers and pedigrees of the 30 entries included in the advanced regional yield trial nurseries.

Entry #	Michigan Accession #	Pedigree
1	M0001	Yorkwin/Honor/Forward
2	M0260	Genesee*3/Redcoat
3	M0202	Genesee*3/3/Yorkwin//Brevor/Norin 10
4	M0205	Avon sib.//Heins VII/cornell
5	M0273	Genesee/4/82a1-2-4-7(NY wheat-rye)/3/Genesee// Caldwell 8/cornell 595/5/Heins Vii/6/Genesee*2// Brevor/Norin 10/3/Avon sib. 9NY 4848-2
6	M0266	Washington sel. 101/Genesee//C.D. 6707
7	M0280	Minhardin/Wabash/5/Fultz sel./Hungarian/2/W38/3 Wabash/4/Fairfield/6/Redcoat sib./Wisc. CI012633/ 7/Vigo/4/Trumbull/2/Hope/Hussar/3/Fulhio/Purkof (Purdue 427a1-1-3)*3/5/Kenya Farmer
8	M0201	Minhardi/Wabash/5/Fultz sel./Hungarian/2/W38/3/ Wabash/4/Fairfield/6/Redcoat sib./Wisc. CI012633/ 7/Vigo/4/Trumbull/2/Hope/Hwssar/3/Fulhio/Purkof/5/ Kenya Farmer
9	M0263	Arthur*4/3/Purdue 6028A2-15-2/2/Riley sib.*2/Riley 67
10	M0274	Arthur 71/5/Arthur*3/3/Ribox/2/Riley*2/Riley 67 (Purdue 6559 sel.)*2/4/Arthur*2/3/Riley67*2/2/ Riley/Bulgaria 88 (PI94407)
11	M0256	Vermillion/Lucas
12	M0276	Ohio-TN1542
13	B0272	Genesee/Redcoat//Genesee*2/Redcoat/4/Norin 10/ Brevor//Yorkwin/3/2*Genesee
14	B0246	Genesee*5/Redcoat//Norin 10/2*Genesee
15	B2218	Norin 10/2*Genesee/3/Genesee*3/Redcoat
16	B2219	ibid.
17	B2006	Purdue 5216//Genesee/Redcoat

Appendix D. Continued.

Entry #	Michigan Accession #	Pedigree
18	B2201	Genesee*3/3/Yorkwin//Brevor/Norin 10/4/Genesee*3/ Redcoat
19	B2035	Genesee*3/3/Yorkwin//Brevor/Norin 10/4/Genesee/ Redcoat
20	B2036	ibid.
21	B0253	Genesee*2/Redcoat/3/Suwon 92/Brevor//5*Genesee
22	B0223	Purdue 5517-a1/3/Suwon 92/Brevor//5*Genesee
23	A7055	Genesee*2/Redcoat
24	A9051	Ac4835/4*Genesee
25	B0240	ibid.
26	B0254	German MI/3*Genesee
27	B0270	Asosan/3*Genesee/4/Norin 10/Brevor//Yorkwin/3/2* Genesee
28	B0215	Asosan/4*Genesee
29	B0216	Asosan/3*Genesee//Genesee/Redcoat
30	M0250	Arthur*5/3/Purdue 6028A2-15-9-2/2/Riley sib.*2/ Riley 67

Appendix E. Combined analysis of variance for percent total protein as a randomized complete block design.

Percent Total Protein

Source of Variance	Degs. of Freedom	Sums of Squares	Mean Squares	Sig. of F Statistic
Location	7	867.5226	123.9318	.01
Reps Within Locations	16	61.3597	3.8350	.01
Genotype	29	211.3959	7.2895	.01
Location by Genotype	203	63.4754	.3127	.01
Error	464	99.4259	.2143	
Total	719	1303.1796		

Appendix F. Combined analysis of variance for kernel texture (psi) as a randomized complete block design.

Kernel Texture

Source of Variance	Degs. of Freedom	Sums of Squares	Mean Squares	Sig. of F Statistic
Location	7	975.7440	139.3920	.01
Reps Within Location	16	265.6124	16.6008	.01
Genotype	29	1484.8716	51.2026	.01
Location by Genotype	203	1139.9549	5.6155	.01
Error	464	1503.4005	3.2401	.01
Total	719	5369.5863		

Appendix G. Location means for percent total protein, percent water soluble protein, percent water insoluble protein, the percent of total protein in water soluble form, kernel texture (psi), and kernel lysine (DBC) for 15 lines at each location.

Location	% Total Protein	% Water Soluble Protein	% Water Insoluble Protein	% Water Soluble of Total Protein	psi	DBC
Ingham	10.80	1.50	9.30	13.81	44.23	3.00
Monroe	10.78	1.50	9.28	13.91	46.12	2.93
Lenawee	10.45	1.59	8.86	15.22	42.65	2.97
Saranac	11.05	1.61	9.43	14.57	45.61	2.96
Tuscola	9.26	1.66	7.60	17.93	48.78	3.05
Range	9.26-11.05	1.50-1.66	7.60-9.43	13.81-17.93		
Difference	1.79	.16	1.83	4.12		

Appendix H. Entry means for percent total protein, percent water soluble protein, percent water insoluble protein, the percent of total protein in water soluble form, kernel texture (psi), and kernel lysine (DBC) for lines common to all locations.

Entry #	% Water Protein	% Water Soluble Protein	% Water Insoluble Protein	% Water Soluble of Total Protein	psi	DBC
3	9.72	1.52	8.20	15.64	48.30	3.03
9	11.37	1.49	9.88	13.10	45.90	2.84
10	11.12	1.49	9.63	13.40	45.00	2.89
14	9.89	1.60	8.22	16.29	45.05	3.04
16	10.21	1.62	8.59	15.87	45.45	2.99
18	10.09	1.64	8.45	16.29	44.35	3.01
22	10.48	1.65	8.83	15.74	47.80	3.02
30	11.29	1.50	9.79	13.29	46.50	2.88
Range	9.72-11.37	1.49-1.64	8.20-9.88	13.10-16.29		
Difference	1.65	.16	1.68	3.19		

Appendix I. Within location r^2 values between percent total protein and percent water insoluble protein and between percent total protein and the percent of total protein in water soluble form for 15 entries at each location.

Location	% Total Protein by % Water Insoluble Protein	% Total Protein by % Water Soluble of Total Protein
Ingham	+.98**	-.55*
Monroe	+.98**	-.61*
Lenawee	+.98**	-.52*
Saranca	+.97**	-.53*
Tuscola	+.95**	-.24

* significant at P = .05
** significant at P = .01

Appendix J. Within location r^2 values between kernel lysine (DBC) and percent total protein, percent water insoluble protein, and the percent of total protein in water soluble form for 15 entries at each location.

Location	DBC by % Total Protein	DBC by % Water Insoluble Protein	DBC by % Water of Total Protein
Ingham	-.83**	-.76*	+.32
Monroe	-.67*	-.71*	+.58
Lenawee	-.70*	-.65*	+.25
Saranac	-.85**	-.84**	+.49
Tuscola	-.54	-.54	+.18

* significant at P = .05
** significant at P = .01

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