QUALITATIVE AND QUANTITATIVE FACTORS AFFECTING PROTEIN AND LYSINE CONTENT OF WHEAT GRAIN

by

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QUALITATIVE AND QUANTITATIVE FACTORS AFFECTING

PROTEIN AND LYSINE CONTENT OF WHEAT GRAIN

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Analysis of the 12,000 common wheats of the USDA World Wheat Collection indicated significant differences among wheats for grain protein and lysine content (Vogel, Johnson, and Mattern, Nebr. Res. Bull. 285. 1973).

Six hundred wheats were selected from the World Wheat Collection which represented the range of protein and lysine that existed therein to test the validity of the World Collection results and to develop selection criteria for protein and lysine. These wheats were grown at Yuma, Arizona in 1972 and 1973.

Only 10% of the total variation within a nursery could be attributed to yield, test weight, and 1000-kernel weight. Most of the variation for percent protein was due to genetic differences among the wheats tested. Wheats with the highest mean protein percentages were five percentage points higher in protein than the low protein wheats.

Lysine (% of sample) was positively correlated to percent protein but lysine (% of protein) was negatively correlated to percent protein. The correlation between lysine (% of sample) and lysine (% of protein) was not significant. In order to make valid lysine comparisons among wheats differing in protein content, lysine values were adjusted to nursery mean protein percentages. After adjustment, both lysine values

provide the same relative measure of the lysine content of wheat.

Test weight had some influence on lysine content because bran proteins are higher in lysine content than endosperm proteins. Yield and kernel weight had no significant effect on lysine content after accounting for the effect of protein and test weight. Results indicate that significant genetic differences exist among wheats for lysine that are independent of percent protein and test weight. Adjusted lysine values are useful as a selection criterion but unadjusted lysine values are not.

Most societies eat only the ground endosperm or flour of wheat. Almost all of the genetic research involving the protein and lysine content of wheat has been done using whole grain samples. Whole grain and endosperm protein and lysine percentages were compared to determine the validity of this practice.

A modified milling procedure was used to separate endosperm and bran (including the germ) portions of the wheat kernel. The 300 wheats studied were from the USDA World Wheat Collection and the parents and progenies of three crosses of high protein and/or high lysine lines.

Grain protein and endosperm protein percentages are highly correlated. Grain protein content can be used as a selection criterion for endosperm protein content. The combined effects of percent endosperm and bran protein and lysine content on grain lysine (% of protein) was as great as the effect of endosperm lysine (% of protein). Wheats with high grain lysine content did not always have high endosperm lysine content. Significant differences existed among wheats for endosperm protein and lysine content.

On the basis of whole kernel analysis, the variety Atlas 66 had

been identified as a high protein line, Nap Hal, PI 176217, as a high protein/high lysine line, and CI 13449 as a high lysine line.

The high protein of Nap Hal resides in both endosperm and bran. Atlas 66 shows the high protein effect only in its endosperm. Transgressive segregates with endosperm protein values as high as 23% provide evidence that the genes for endosperm protein of Nap Hal are different from those of Atlas 66. The parents averaged 19% protein.

The high grain lysine content of Nap Hal is due to both the high lysine content of its endosperm proteins and to the high protein and lysine content of its bran. The high lysine of CI 13449 is largely due to the high lysine content of its endosperm proteins.

GENERAL INTRODUCTION

Common wheat, Triticum aestivum L. em. Thell, is the main staple food for over one billion people and provides almost 30% of the total food calories for the people of the world (Reitz, 1967). Wheat is also an important source of protein for humans. The total estimated world wheat production for 1972 was 307.2 million metric tons (Agr. Statistics, 1973). In most production areas of the world the protein content of wheat ranges from 10-13%. Using the 10% protein value, the total estimated production of wheat protein for 1972 was 30.72 million metric tons. Considering that the average person of the world's 3.8 billion people requires a minimum of 70g of protein per day, the current annual worldwide protein requirement for man is 97 million metric tons (Levin, 1974). Although not all wheat is used for human consumption, wheat still contributes substantially to the total protein requirements of man even though it is not as high in protein content as meats, fish, and legume seeds.

The nutritive value of a food cannot be determined solely by its protein content because, if calories are deficient, protein will be used as an energy source. In human nutrition, the quality as well as the quantity of protein is important. The quality of a protein is dependent upon its capability to supply amino acids in the same proportion as required by the body. The quality or biological value of a protein is

limited by the amount of essential amino acid in shortest supply. The limiting amino acids in wheat grain protein are lysine, tryptophan, and methionine (Bressani et al. 1960). Isoleucine, valine, and threonine may also be in limited supply. Lysine is the most limiting amino acid in wheat and other cereals.

The lysine requirements of man vary with body size, sex, and age (Clark, 1971; Clark et al., 1960; Hegsted, 1973). Adult men require approximately 800mg of lysine per day while adolescent boys require 1600mg lysine per day (Clark, 1970). Lysine supplementation of diets in which wheat flour protein was the main source of protein, as compared to unfortified diets, significantly improved weight gain and nitrogen retention in small children (Graham, 1969; Bressani et al., 1960). However, evidence exists that wheat, if eaten alone and in sufficient quantity to meet calorie requirements, can meet the protein requirements of adult men (Aykroyd and Doughty, 1970).

Since nutritional diseases affect children more severely than adults, the lysine requirements of children should be used as the reference base for determining the lysine inadequacies of wheat protein. The biological value of whole wheat protein is only 70% that of whole eggs (Aykroyd and Doughty, 1970). The biological value of white flour is 50% that of whole eggs. The mean lysine content of wheat protein of the common wheats in the USDA World Wheat Collection was 3.16% (Vogel, Johnson and Mattern, 1973). Since the biological value of a protein is determined by the amino acid in shortest supply, the biological value of wheat protein indicates that the lysine content of wheat protein should be a minimum of 4.5%.

Wheat with 13% protein and 4.5% lysine (% of protein) would provide 586mg of lysine per 100g whereas wheat of the same protein level with 3% lysine (% of protein) would provide 390mg of lysine per 100g. Lysine increases of this magnitude would greatly improve the nutritive value of wheat and, by doing so, directly improve the diet and health of over one billion people. Lysine content of wheat can be increased by either increasing the protein content or the lysine content of protein or both. Protein and lysine content of diets consisting mainly of wheat can be improved by the addition of higher quality animal and fish protein to the diet, addition of synthetic lysine, or by improving the protein and lysine content of wheat grain by the genetic modification of the wheat plant.

In many of the under-developed nations of the world where wheat is grown, improving the diet by the addition of animal protein is not possible because available food supplies are needed for direct human consumption. The direct fortification of wheat by lysine is possible (Ferrel, Shepard, and Guadagni, 1970) but this requires a technological sophistication that is not available in many of the rural areas of underdeveloped nations. Another disadvantage of direct fortification is the need for fortification of each wheat crop. Improving the quality of wheat by breeding would provide the best solution to the nutritional inadequacy of wheat. High lysine wheat varieties could be grown and utilized without any alternation in production and traditional processing and usage practices by people throughout the world. The main cost involved in producing more nutritious wheat varieties is the cost of the breeding program.

Section I. Re-evaluation of common wheats from the USDA World Wheat Collection for Protein and Lysine Content.

INTRODUCTION

The protein and lysine content of wheat can be altered by breeding only if genetic variability exists for these traits. Results of the protein and lysine analysis of the common wheats of the USDA World Wheat Collection indicated that genetic variability does exist for these traits (Vogel, Johnson, and Mattern, 1973). The purpose of this study was to re-evaluate selected lines from the World Wheat Collection to provide further verification of genetic variability for protein and lysine content in common wheats and to identify high protein and high lysine lines that may be of use to wheat breeders.

LITERATURE REVIEW

Breeding for increased protein content of wheat grain was not practiced on a systematic basis prior to the early 1950's because known genetic differences in protein content of wheat were small in comparison with environmental effects (Johnson et al., 1963). Then Middleton, Bode, and Boyles (1954) reported that the soft red winter variety 'Atlas 66' produced grain significantly higher in protein content than commonly grown cultivars while maintaining yield at a high level.

The genes controlling the high protein trait of Atlas 66 have been transferred to winter wheats adapted to the Great Plains of the U.S. resulting in an improvement in the protein content of the derived lines over the parent winter wheat varieties by as much as three percentage points of protein (Johnson et al., 1968; Johnson et al., 1969). Selections from the second breeding cycle of Atlas 66-derived lines of hard red winter wheats have averaged 20-24% higher in protein than the main commercial varieties in Nebraska (Johnson, Mattern, and Schmidt, 1972b, Johnson et al., 1972c). Some high protein selections have yielded as much as 15% more grain than currently grown commercial varieties. Heritability estimates as high as 0.65 obtained by the regression of F_3 progeny rows on F_2 plants were reported by Haunold, Johnson, and Schmidt (1962) for crosses of Atlas 66 with conventional winter wheats. Stuber, Johnson, and Schmidt (1962) reported heritability estimates ranging from 0.68 to 0.83 depending on the method of computation for the cross 'Atlas 66' x 'Wichita'. This research demonstrates that the nutritive value of wheat can be significantly improved through breeding.

Identification of additional genetic sources of high protein would allow breeders to make additional progress in improving the protein content of wheat.

In addition to improving the protein content, the nutritive value of wheat could also be improved by increasing the lysine content of wheat protein. Genetic variability must exist for a trait before this trait can be altered by breeding. Genes for significantly higher than normal lysine (% of protein) were identified in maize (Mertz, Bates, and Nelson, 1964), sorghum (Singh and Axtell, 1973) and barley (Munch et al., 1970). The common wheats of the USDA World Wheat Collection were analyzed for protein and lysine content to determine the variability that exists for these traits and to identify potential high protein and high lysine lines (Vogel, et al., 1973).

Protein values of the 12,613 wheats of the World Collection ranged from 6.9 to 22% with a mean of 13%; lysine (% of sample) ranged from 0.25 to 0.66% with a mean of 0.40%; lysine (% of protein) ranged from 2.25 to 4.26% with a mean of 3.16% (Vogel et al., 1973). Interpretation of the lysine results was complicated by high positive correlation between lysine (% of sample) and percent protein and by a negative curvilinear relationship between lysine (% of protein) and percent protein. The effect of protein on lysine (% of protein) was strongly negative up to 15% protein but was negligible for wheats with higher protein content. Lawerence et al. (1958) and Villegas, McDonald, and Giles (1970) obtained similar relationships between protein and lysine although their results were based on a much smaller number of wheats. World Wheat Collection lysine (% of protein) values were adjusted to the mean protein level

using the curvilinear relationship between protein and lysine (% of protein) to make valid lysine comparisons among wheats with different protein contents. Adjusted lysine (% of protein) values ranged from 2.28 to 3.71% with a mean of 3.16%.

The high correlation between lysine (% of sample) and percent protein results from lysine being a constituent of protein; as total protein content increases, lysine (% of sample) content also increases. Because of the high correlation of lysine (% of sample) and percent protein Vogel et al. (1973) contended that lysine (% of sample) values per se were more a measure of protein content than protein quality.

The inverse relationship between lysine (% of protein) and percent protein has also been reported in flour samples of different wheats (Simmonds, 1962, McDermott and Pace, 1960). Glutamic acid exhibits a reverse trend, i.e., increasing with increase in protein content.

Proteins of wheat belong to four classes based upon their solubility: albumins, globulins, gliadins, and glutenins (Osborne, 1907). Wheat gluten consists of the gliadens and glutenins. Albumins and globulins are higher in lysine content than the gluten proteins while the gluten proteins are higher in glutamic acid (Simmonds, 1962; Mattern, Salem, and Volkmer, 1968). Pence, Weinstem, and Mecham (1964) reported the albumin and globulin content of the flour of 32 wheats ranged from 13 to 22%. The amount of these proteins increased directly with percent total protein when expressed as a percent of sample but decreased when expressed as a percent of protein. Ulmer (1973) obtained similar results. The inverse relationship between lysine (% of protein) and percent protein can be attributed to differences in the albumin-globulin:gluten ratio

among wheats and flours (Simmonds, 1962; McDermott and Pace, 1960). Wheats with a low protein content have a higher proportion of albumin and globulin proteins and, hence, are higher in lysine (% of protein) than wheats with higher protein content.

Wheats in the World Wheat Collection analysis were grown at Mesa, Arizona but not all were grown during the same year (Vogel et al., 1973). Since environment can affect both protein and lysine content (McElroy et al., 1949), much of the variability for protein and lysine may have been non-genetic in origin. Even so, the large range in protein and lysine values would indicate significant genetic difference among wheats for protein and lysine content. Because of possible genotype x environment interactions, the high protein and high lysine lines from the World Wheat Collection analysis were only tentatively identified as being possible sources of genes for high protein and high lysine.

Environment exerts a strong influence on the protein content of wheat. The correlation of a variety's yield with its protein content for nine varieties grown in an international array of environments varied with variety but was usually slightly negative (Johnson et al., 1973b). Any environmental factor affecting yield can be expected to influence protein content. Protein content of wheat cultivars grown under different levels of nitrogen fertility is lower under higher yielding conditions than under low yielding conditions (Terman et al., 1969). Protein content of wheat can usually be significantly increased by nitrogen fertilization (Hujjati and Maliki, 1972; Hunter and Standford, 1973; Johnson, Dreier, and Grabouski, 1973a). Lodging and high temperatures during the period of grain filling can affect grain size (plumpness) and degree of

shrivelling. Kernel plumpness and degree of shrivelling can affect grain protein content (Johnson et al., 1973b; Johnson et al., 1972c).

Although environment strongly affects protein content, genetically high protein varieties can maintain a protein advantage over similarly adapted conventional lines when grown in the same environment. In a 3-year test in Nebraska, CI14016, an Atlas 66 x Commanche-derived line, maintained a consistent 2% protein advantage over Lancer, a well adapted commercial variety, at 5 different fertility levels (Johnson, Dreier and Grabouski, 1973a). Atlas 66 and Atlas 66- or Frondoso-derived lines maintained their genetic superiority for grain protein content in comparison to conventional varieties in an international array of environments (Stroike and Johnson, 1972). When compared with wheats with similar yields in the International Winter Wheat Performance Nursery, the high protein lines ranged from 0.7 to 3.1 percentage points higher in protein content (Stroike and Johnson, 1972). Because of the effect of environment on protein content, valid comparisons of the protein levels of wheat can be made only with wheats grown in comparable environments.

Since lysine is a constituent of protein, any environmental factor affecting protein can also be expected to influence lysine content. Lawerence et al., (1958) using data from six varieties grown at three locations for three years reported no influence of environment on lysine (% of protein) except as environment affected the percent of protein, i.e., higher lysine content could be attributed to lower protein content. McElroy et al. (1949) reported the nitrogen percent of the variety 'Marquis' ranged from 1.94 to 4.03% over nine locations in Alberta, Canada and lysine content varied inversely with nitrogen content. Other

research demonstrates that as the protein content of a wheat variety increases due to increasing applications of nitrogen fertilizer, the lysine percent of protein decreases (Hujjati and Maleki, 1972; Gunthardt and McGinnis, 1957). Stroike and Johnson (1972) reported an inverse relationship between protein and lysine (% of protein) for wheats grown in an international array of environments.

Since lysine percent of protein varies inversely with protein content, Vogel et al. (1973) suggested the following procedure to identify and select high lysine wheat lines: "For each environment (year-location) the regression of lysine percent of protein on percent protein should be calculated. Lysine values should then be adjusted to the mean protein content for that particular environment with the specific regression equation for that environment. Wheats with adjusted lysine values that are consistently greater than the mean lysine percent over environments could be considered as being genetically superior in lysine content of protein."

The bran, including the aleurone layer and germ of wheat kernels, is higher in both protein and lysine content than the endosperm (Waggle, et al., 1967; MacMasters, Hinton and Bradbury, 1971). Large dense kernels have a larger proportion of endosperm per sample than smaller less dense kernels (Zeleny, 1971). Wheats with small shrivelled seeds could have a higher lysine content than large, plump seeded wheats when measured on a whole kernel basis because of the greater proportion of bran and the higher protein and lysine content of the bran, even if there were no differences in endosperm protein and lysine content. Johnson et al. (1972c) measured the protein and lysine content of large, medium, and small seeds

of four different varieties and could not demonstrate an affect of seed size on protein or lysine content. However, within a size class, wrinkled seeds with open creases were reported to have higher protein contents than plump kernels with closed creases (Johnson et al., 1972c, Johnson et al., 1973b).

Test weight (lbs/bu or kg/hl) and kernel weight are used as indexes for estimating milling yield of wheat (Zeleny, 1971). Optimum milling yield of wheat is determined by the percentage of endosperm of a sample of wheat. Test weight and kernel weight may also be useful in providing an estimate of the effect of seed size and density on the protein and lysine content of wheat grain. The usefulness of test weight and kernel weight in estimating milling yield is dependent upon the class of wheat being measured. Correlations of milling yield with test weight and kernel weight are much higher for hard wheat than for soft wheat classes (Baker and Golumbic, 1970).

Test weight is determined primarily by kernel density and the packing efficiency of the grain in the test container (Hlynka and Bushuk, 1959, Yamazaki and Briggle, 1969). Kernel size has little effect on test weight but kernel shape may affect packing efficiency (Hlynka and Bushuk, 1959). Kernel weight consists of two factors, kernel size and density. Since kernel weight and test weight have only one factor in common, the correlation between the two measurements can not be expected to be high (Hlynke and Bushuk, 1959). Harris and Sibbett (1942) obtained a correlation of $r = .56$ for test weight and 500-kernel weight in hard red spring wheats. Ghaderi and Everson (1971a) obtained an estimate of heritability for test weight on a line mean basis of 0.94. Sun, Shands, and Forsberg

(1972) reported broad sense heritability estimates for kernel weight ranging from 0.51 - 0.85 over crosses and years with one exception of 0.09 for six spring wheat crosses.

MATERIALS AND METHODS

Materials

The hexaploid wheats used in this study were obtained from the World Wheat Collection maintained by the U. S. Department of Agriculture, Agricultural Research Service. Over 600 wheats were selected using the results of the protein and lysine analysis of the World Wheat Collection (Vogel et al., 1973) to represent the range of protein and lysine that exists therein. Spring, winter, and intermediate types and all market classes of common wheat were represented among the wheats chosen. These wheats were grown in the 1971-72 (1972 nursery) and 1972-73 (1973 nursery) growing seasons at the University of Arizona Experiment station, Yuma, Arizona. The Yuma, Arizona site (32°40'N, 114°N'W) was chosen because both spring and winter wheats can be grown in the same nursery from fall seeding. In the 1972 nursery, 641 wheats were seeded and 636 were harvested. Seed from the 636 wheats was planted in the 1973 nursery and 635 were harvested. Plots were not harvested due either to planting error or because the plot was smothered by lodging in an adjacent row. Six check varieties, 'Nap Hal', 'Atlas 66', 'Centurk', 'Inia 66', 'Triumph 64', and 'Scout 66' were included in the 1973 nursery. Some of the World Collection wheats were not pure lines. Variability in plant and kernel characteristics were observed in these lines.

Methods

Nursery Procedures

Wheats were grown in 1.829m (6 ft.) single row plots spaced 51 cm (20 in) apart. Five grams of seed were seeded per plot. The nurseries were planted during the second week of November and harvested the last week of May. Wheats in the 1972 nursery were planted in their CI or PI numerical sequence. Six check varieties replicated four times were included in the 1973 nursery with the World Collection Wheats in an augmented randomized complete block field design (Federer, 1956). The nursery was divided into four blocks; the check varieties were assigned to plots at random within a block; the World Collection Wheats were then assigned to plots at random over the entire nursery. Borderrows were planted around the nurseries. In 1972, 168.2 kg/ha (150 lb/A) and, in 1973, 112.1 kg/ha (100 lb/A) of nitrogen was applied. The nurseries were irrigated.

Many of the World Collection wheats are of poor agronomic type and lodge easily. Lodging was severe in 1972. A lower rate of nitrogen fertilization and better irrigation practices greatly reduced lodging in the 1973 nursery. There was considerable bird damage in 1972 but it was negligible in 1973. The plots were threshed using a Vogel-type plot thresher. Yield was measured as grams of seed per plot.

Test Weight and 1000-Kernel Weight

Seed was cleaned using a Clipper cleaner with a .17 x 1.27 cm (1/15 x 1/2 in) screen. Bushel weight was measured using a 0.5 pint (.246L) bucket with a standard drop. Bushel weight was converted to kg/hl by

multiplying by the factor of 1.29. Some plots did not produce enough seed for test weight measurement. Seed for 1000-kernel weight measurement was hand cleaned to remove cracked kernels. Thousand-kernel samples were counted using an electronic seed counter. In 1972, some samples had considerable amounts of cracked seed. Because of the excessive amount of time involved in hand cleaning the samples, 1000-kernel weight was measured on only 569 of the 636 samples. In 1973, 1000-kernel weight was measured on all samples.

Laboratory Analyses

Whole kernel samples were analyzed for protein and lysine content. Samples were ground using a Udy Cyclone Sample Mill. Ground samples were brought to uniform moisture levels in a controlled humidity cabinet (Mattern and Bishop, 1973). Samples were then weighed on a dry matter basis for protein and lysine analysis. Macro-Kjeldahl procedure AACC method 46-12 (AACC Approved Methods) was used to determine nitrogen content of the samples. Protein content was calculated as percent N x 5.7. Ion exchange chromatography was used to determine lysine content of the samples (Mattern, Schmidt, and Johnson, 1970). In this procedure, samples are acid hydrolyzed prior to lysine analysis in an automatic amino acid analyzer modified with four short columns. The analyzer is programmed so that only the lysine peak is recorded and integrated. Lysine (% of protein) values were calculated by dividing lysine (% of sample) values by percent protein.

Statistical Analyses

An IBM 360 computor was used for much of the data analysis. The Statistical Analysis System (SAS) programs (Service, 1972) were used extensively. Statistical procedures and interpretation were based on standard methods detailed by Snedecor and Cochran (1967) and Steel and Torrie (1960).

Frequency distributions of percent protein, percent lysine of sample, percent lysine of protein, and kernel weight were tested for normality by plotting the relative cumulative frequencies on probability graph paper (Li, 1964).

Simple correlation coefficients (r values) were calculated each year for percent protein, lysine (% of sample), lysine (% of protein), yield, test weight, and 1000-kernel weight to provide a measure of the degree of association of these factors with each other. The correlation of a line with itself over years provides a measure of the repeatibility of a trait. Correlations over years were calculated for percent protein, lysine (% of sample), lysine (% of protein), adjusted lysine values, yield, test weight, and kernel weight using the results of the original World Wheat Collection analysis and the data from the 1972 and 1973 Yuma nurseries. Plots with yields less than 100 grams were not included in any statistical calculation involving yield. Yields of less than 100 grams were attributed to poor stands within plots.

Regression analysis was used to determine more precisely the relationship of protein content to yield, test weight, and kernel weight. Regression analysis also was used to study the effects of percent protein, yield, test weight, and kernel weight on lysine percent of sample

and lysine percent of protein. Separate regression analyses were calculated from each year's data. First, second, and third degree polynomial models and multiple regression models were tested. The regression model chosen to represent a particular relationship was selected on the basis of a significant F-test, significant partial sum of squares for regression, significant regression coefficients using the "t" test, and, with those criteria met, a maximum coefficient of determination.

Regression lines for the regression of lysine (% of sample) and lysine (% of protein) on percent protein were plotted each year. Lysine (% of sample) and lysine (% of protein) values for each year were adjusted to the mean protein level for that year to permit comparisons among wheats with different protein contents. Lysine values also were adjusted to the mean protein and test weight values for each year for wheats on which test weight was measured. Lysine values were adjusted using the regression coefficients developed by regression analysis. As an example, the adjustment equation used for adjusting lysine (% of protein) for percent protein and test weight was:

$$Y_i \text{ adj} = Y_i - b_1 (X_i - \overline{X}) - b_2 (Z_i - \overline{Z}) - b_3 (Z_i^2 - \overline{Z_i^2}).$$

The b values are the partial regression coefficients; Yi, the observed lysine (% of protein) of the ith sample; Xi, the protein percent of the ith sample, and Zi, the test weight of the ith sample.

Standard partial regression coefficients or path coefficients were calculated for the more pertinent regression equations. Standard partial regression coefficients are the partial regression coefficients when each variable is in standard measure and, as such, give an indication of the relative importance of the independent variables (Steel and Torrie, 1960).

The World Collection wheats were analyzed using analysis of variance procedures in which years were used as replications to determine if significant difference exist among wheats over years for protein and lysine content. The World Collection wheats were not replicated within years. Coefficients of variation (CV) and LSD values were calculated. Analysis of variance procedures were used to analyze the check varieties in the 1973 nursery to determine if there was any significant within-nursery environmental variation for protein and lysine content.

Comparative rankings of wheats using the protein and lysine values of this study with those of the original World Wheat Collection analyses were used along with the correlations over years to determine if adjusted lysine values are a better selection criterion than unadjusted lysine values. The comparative rankings and correlations over years also were used to evaluate the utility of the World Wheat Collection analyses (Vogel et al., 1973) as a guide for selecting lines likely to be genetically superior in protein and lysine content.

RESULTS

Table 1 lists the mean, standard deviations, and range values of percent protein and percent lysine of the wheats selected for growing at Yuma, Arizona and those of the entire World Wheat Collection. Protein and lysine values are from the original World Wheat Collection analyses. The mean and range values of the selected wheats approximate those of the World Collection and indicate that the wheats selected for re-evaluation are representative of the entire World Wheat Collection.

Means, standard deviation, and range values for percent protein, lysine (% of sample), lysine (% of protein), adjusted lysine values, yield, test weight, and 1000-kernel weight for the wheats grown in the 1972 and 1973 Yuma nurseries are listed in Table 2. Values for the check entries in the 1973 nursery are not included. Yield values are not included for plots that produced less than 100 grams of seed.

Table 1. Means, standard deviations, and range values for % protein, lysine (% of sample) and lysine (% of protein) for 12,613 common wheats of the USDA World Wheat Collection and for 635 wheats of the World Wheat Collection grown at Yuma, Arizona in 1972 and 1973.+

Variable	No. of lines	Mean	Standard Deviation	Range
% Protein	12,613	12.97	2.019	6.90-22.00
% Protein	635	12.98	3.65	6.90-22.00
% Lysine (% of sample)	12,613	.40	.049	.25- .66
% Lysine (% of sample)	635	.40	.10	.25- .66
% Lysine (% of protein)	12,613	3.16	.231	2.25- 4.26
% Lysine (% of protein)	635	3.16	.41	2.25- 4.26

+ Protein and lysine values from original World Wheat Collection analyses.

Table 2. Means, standard deviations, and range values for % protein, % lysine (% of sample), % lysine (% of protein), yield, test weight (kg/hl) and 1000 kernel weight for the 1972 and 1973 World Wheat Collection Special Study nursery.

Variable	Year	No. of lines	Mean	Standard Deviation	Range
% Protein	1972	636	17.78	1.411	13.80- 22.10
	1973	635	16.33	1.552	12.10- 21.00
% Lysine (% of sample)	1972	636	.49	.036	.38- .61
	1973	635	.47	.040	.37- .60
% Lysine (% of sample adjusted for % protein)	1972	636	.49	.021	.42- .55
	1973	635	.47	.020	.41- .54
% Lysine (% of protein)	1972	636	2.77	.133	2.40- 3.23
	1973	635	2.89	.136	2.51- 3.27
% Lysine (% of protein adjusted for % protein)	1972	636	2.77	.118	2.40- 3.13
	1973	635	2.89	.120	2.57- 3.31
Yield (on plots ≥ 100g yield)	1972	609	318.19	132.02	100.00-1042.00
	1973	598	360.45	140.73	106.00- 764.00
1000-kernel weight (g)	1972	569	38.14	6.752	21.80- 57.00
	1973	635	40.60	6.655	24.00- 57.90
Test weight (kg/hl)	1972	435	75.69	4.310	61.92- 83.85
	1973	494	77.75	3.305	66.44- 84.50
% Lysine (% of sample adjusted for % protein, kg/hl, and $(kg/hl)^2$)	1972	435	.48	.018	.41- .54
	1973	494	.46	.018	.40- .54
% Lysine (% of sample for above set)	1972	435	.48	.033	.38- .60
	1973	494	.46	.037	.37- .60
% Lysine (% of protein adjusted for % protein, kg/hl, and $(kg/hl)^2$)	1972	435	2.78	.102	2.34- 3.16
	1973	494	2.90	.110	2.54- 3.34
% Lysine (% of protein for above set)	1972	435	2.78	.135	2.44- 3.23
	1973	494	2.90	.137	2.51- 3.27

Mean protein and lysine (% of sample) percentages were higher in 1972 than in 1973. The 1973 lysine (% of protein), yield, 1000-kernel weight, and test weight mean values were higher than in 1972. The percentages of protein and lysine (% of sample) were higher while the lysine percent of protein values were lower for the wheats grown in the Yuma nurseries than for the same wheats in the World Collection analysis. The frequency distribution of percent protein, lysine (% of protein) and 1000-kernel weight approximated normal distributions. The frequency distribution of lysine expressed as a percent of sample only roughly approximated a normal distribution.

Correlation coefficients (r) for yield, test weight, 1000-kernel weight, percent protein, lysine percent of sample, and lysine percent of protein for the 1972 nursery are listed in Table 3. The same correlations for the 1973 nursery are listed in Table 4. The correlation coefficients for the 1972 and 1973 nurseries are similar. Many of these relationships have been reported previously, but not for wheats representing such a large range of agronomic type, seed size, and protein and lysine content, all grown at the same location.

Test weight and 1000-kernel weight were positively correlated with yield although the correlations are small. The correlation of test weight with 1000-kernel weight was positive but low. Protein and lysine (% of sample) are negatively correlated with yield, test weight, and 1000-kernel weight. The correlation coefficients, however, are small. Lysine (% of protein) was not significantly correlated with yield or 1000-kernel weight. Test weight had a small, negative influence on lysine (% of protein). Lysine (% of sample) was highly positively correlated with percent protein.

Table 3. Correlation coefficients (r) for yield, test weight (kg/hl), 1000-kernel weight (g), % protein, % lysine (% of sample), % lysine (% of protein) for the 1972 World Wheat Collection Special Study Nursery grown at Yuma, Arizona. ≠

Variables	Test weight kg/hl	1000-kernel wt (g)	% Protein	Lysine (% of sample	Lysine (% of protein)
Yield +	0.202** 435	0.276** 550	-0.223** 609	-0.184** 609	0.084* 609
Test wt. kg/hl		0.103* 390	-0.170** 435	-0.370** 435	-0.263** 435
1000-Kernel wt. (g)			-0.278** 569	-0.266** 569	0.046 569
% Protein				0.804** 636	-0.458** 636
% lysine (% of sample)					0.154** 636

* Indicates significance at the .05 level of probability.
** Indicates significance at the .01 level of probability.
\+ Yields on lines with ≥100g yield/row.
≠ The number of observations are listed below the correlation coefficients.

Table 4. Correlation coefficients (r) for yield, test weight (kg/hl), 1000-kernel weight (g), % protein, % lysine (% of sample), % lysine (% of protein) for the 1973 World Wheat Collection Special Study Nursery grown at Yuma, Arizona. ≠ ±

Variables	Test weight kg/hl	1000-kernel wt (g)	% Protein	Lysine (% of sample)	Lysine (% of protein)
Yield ≠	0.429** 511	0.384** 621	-0.356** 621	-0.377** 621	0.068 621
Test wt. kg/hl		0.211** 515	-0.181** 515	-0.319** 515	-0.192** 515
1000-kernel wt. (g)			-0.326** 659	-0.379** 659	-0.010 659
% Protein				0.871** 659	-0.474** 659
% lysine (% of sample)					0.013 659

* Indicates significance at the .05 level of probability.
** Indicates significance at the .01 level of probability.
≠ Yields on lines with ≥ 100g yield/row.
≠ The number of observations are listed below the correlation coefficients.
± Six check cultivars replicated 4 times are included in the correlations.

A significant negative relationship existed between lysine (% of protein) and percent protein. Lysine (% of sample) was not correlated with lysine (% of protein) in 1973 but a small positive correlation existed between these traits in 1972.

A summary of the regression models tested for the 1972 nursery is given in Table 5. Table 6 contains a summary of the regression models tested for the 1973 nursery. The coefficient of determination (r^2) value for the multiple regression of protein on kernel weight, yield, and test weight was 0.11 in 1972 and 0.12 in 1973. Coefficient of determination values are the proportion of the sum of squares of the dependent variable that can be attributed to variation of the independent variables. Thus, only about 10% of the total within-year variation for protein in this set of wheats can be attributed to the combined variation of yield, test weight, and kernel weight.

Regression models that provided the best fit for lysine (% of sample) and lysine (% of protein) were the same in 1972 and 1973. Considering only the effect of protein, the linear regression of lysine (% of sample) on percent protein gave the best fit (Models 72-1 and 73-1). The coefficients of determination for the linear regressions of lysine (% of sample) on yield, test weight, and 1000-kernel weight were small. In 1972, test weight was the more important of these factors while in 1973 kernel weight and yield accounted for slightly more variation of lysine (% of sample) than did test weight.

The model (72-32 and 73-29) that gave the best overall fit for lysine (% of sample) was the regression on percent protein, test weight, and test weight squared. The coefficients of determination for these

Table 5. Summary of regression models tested for the World Wheat Collection Special Study Nursery grown at Yuma, Arizona, 1971-1972.

Sample Description	Model Number	Sample Size	Dep. Var.	Independent Variables	Coefficient of determin.
All lines harvested	72- 1	636	LS	P**	0.6459
	72- 2	636	LS	P P^2	0.6469
	72- 3	636	LP	P**	0.2096
	72- 4	636	LP	P** P^2**	0.2206
	72- 5	636	LP	P P^2 P^3	0.2230
Lines on which KWT was measured	72- 6	569	P	KWT**	0.0773
	72- 7	569	LS	KWT**	0.0707
	72- 8	569	LS	P**	0.6328
	72- 9	569	LS	P** KWT	0.6349
	72-10	569	LP	KWT	0.0022
	72-11	569	LP	P**	0.1945
	72-12	569	LP	P** KWT*	0.2007
	72-13	569	LP	P** P^2**	0.2061
	72-14	569	LP	P** P^2* KWT	0.2100
Lines with yields ≥ 100g	72-15	609	P	Yield**	0.0499
	72-16	609	LS	Yield**	0.0345
	72-17	609	LP	Yield*	0.0071
	72-18	609	LS	P**	0.6466
	72-19	609	LS	P** Yield	0.6467
	72-20	609	LP	P**	0.2244
	72-21	609	LP	P** Yield	0.2344
	72-22	609	LP	P** P^2**	0.2344
	72-23	609	LP	P** P^2** Yield	0.2345
Lines on which kg/hl was measured	72-24	435	P	kg/hl**	0.0287
	72-25	435	P	kg/hl* $(kg/hl)^2$*	0.0432
	72-26	435	LS	kg/hl**	0.1367
	72-27	435	LS	kg/hl** $(kg/hl)^2$**	0.2037
	72-28	435	LP	kg/hl**	0.0694
	72-29	435	LP	kg/hl** $(kg/hl)^2$**	0.1016
	72-30	435	LS	P**	0.6128
	72-31	435	LS	P** kg/hl**	0.6706
	72-32	435	LS	P** kg/hl** $(kg/hl)^2$**	0.6999
	72-33	435	LP	P**	0.2366
	72-34	435	LP	P** P^2**	0.2530
	72-35	435	LP	P** kg/hl**	0.3597
	72-36	435	LP	P** kg/hl** $(kg/hl)^2$**	0.4208
	72-37	435	LP	P** P^2 kg/hl**	0.3650
	72-38	435	LP	P** P^2* kg/hl** $(kg/hl)^2$**	0.4272
Lines with yields ≥ 100g and on which KWT and kg/hl were measured	72-39	390	P	Yield kg/hl** KWT**	0.1072
	72-40	390	P	kg/hl** KWT**	0.1030
	72-41	390	P	KWT**	0.0291
	72-42	390	P	kg/hl**	0.0829
	72-43	390	LS	P** Yield kg/hl** KWT	0.6740

Table 5. (cont.)

Sample Description	Model Number	Sample Size	Dep. Var.	Independent Variables	Coefficient of determin.
Lines with yields ≥ 100g and on which KWT and kg/hl were measured	72-44	390	LS	P** kg/hl**	0.6716
	72-45	390	LS	P**	0.5906
	72-46	390	LS	kg/hl**	0.2438
	72-47	390	LP	P** P^2* Yield kg/hl** KWT	0.3838
	72-48	390	LP	P** P^2* kg/hl**	0.3820
	72-49	390	LP	P** kg/hl**	0.3724
	72-50	390	LP	P** P^2**	0.2300
	72-51	390	LP	P**	0.2116
	72-52	390	LP	kg/hl**	0.0633

* Indicates significance at the .05 level of probability of the partial regression coefficient and the partial sum of squares for regression.

** Indicates significance at the .01 level of probability.

/ P = % Protein LS = Lysine (% of sample) LP = Lysine (% of protein) KWT = 1000 kernel weight (g).

Table 6. Summary of regression models tested for the World Wheat Collection Special Study Nursery grown at Yuma, Arizona, 1972-73. +/

Sample Description	Model Number	Sample Size	Dep. Var.	Independent Variables	Coefficient of determin.
All lines harvested	73- 1	659	LS	P**	0.7579
	73- 2	659	LS	P* P^2	0.7580
	73- 3	659	LP	P**	0.2247
	73- 4	659	LP	P** P^2**	0.2335
	73- 5	659	LP	P P^2 P^3	0.2349
Lines on which KWT was measured	73- 6	659	P	KWT**	0.1063
	73- 7	659	LS	KWT**	0.1437
	73- 8	659	LS	P** KWT**	0.7680
	73- 9	659	LP	KWT	0.0001
	73-10	659	LP	P** KWT**	0.2549
	73-11	659	LP	P** P^2** KWT**	0.2635
Lines with yields ≥ 100g	73-12	621	P	Yield**	0.1271
	73-13	621	LS	Yield**	0.1424
	73-14	621	LP	Yield	0.0046
	73-15	621	LS	P**	0.0504
	73-16	621	LS	P** Yield**	0.7557
	73-17	621	LP	P**	0.2392
	73-18	621	LP	P** Yield**	0.2522
	73-19	621	LP	P** P^2**	0.2443
	73-20	621	LP	P** P^2* Yield**	0.2574
Lines on which kg/hl was measured	73-21	515	P	kg/hl**	0.0324
	73-22	515	P	kg/hl $(kg/hl)^2$	0.0367
	73-23	515	LS	kg/hl**	0.1019
	73-24	515	LS	kg/hl* $(kg/hl)^2$**	0.1149
	73-25	515	LP	kg/hl**	0.0369
	73-26	515	LP	kg/hl $(kg/hl)^2$	0.0436
	73-27	515	LS	P**	0.7352
	73-28	515	LS	P** kg/hl**	0.7633
	73-29	515	LS	P** kg/hl** $(kg/hl)^2$**	0.7669
	73-30	515	LP	P**	0.2546
	73-31	515	LP	P** P^2*	0.2609
	73-32	515	LP	P** kg/hl**	0.3374
	73-33	515	LP	P** kg/hl** $(kg/hl)^2$**	0.3514
	73-34	515	LP	P* P^2 kg/hl**	0.3406
	73-35	515	LP	P* P^2 kg/hl** $(kg/hl)^2$**	0.3541
Lines with yields ≥ 100g and on which KWT and kg/hl were measured	73-36	512	P	Yield** kg/hl KWT**	0.1194
	73-37	512	P	Yield** KWT**	0.1173
	73-38	512	P	Yield**	0.0863
	73-39	512	P	KWT**	0.0706
	73-40	512	LS	P** Yield kg/hl** KWT**	0.7689
	73-41	512	LS	P** kg/hl** KWT**	0.7687

Table 6. (cont.)

Sample Description	Model Number	Sample Size	Dep. Var.	Independent Variables	Coefficient of determin.
Lines with yields ≥ 100g and on which KWT and kg/hl were measured	73-42	512	LS	P** KWT**	0.7457
	73-43	512	LS	P** kg/hl**	0.7622
	73-44	512	LS	P**	0.7338
	73-45	512	LS	KWT**	0.0361
	73-46	512	LS	kg/hl**	0.1020
	73-47	512	LP	P** P^2 Yield kg/hl** KWT**	0.3570
	73-48	512	LP	P** P^2*	0.2582
	73-49	512	LP	P** kg/hl** KWT**	0.3519
	73-50	512	LP	P** kg/hl**	0.3350
	73-51	512	LP	P** KWT**	0.2832
	73-52	512	LP	P**	0.2514
	73-53	512	LP	kg/hl**	0.0378
	73-54	512	LP	KWT	0.0015

* Indicates significance at the .05 level of probability of the partial regression coefficient and the partial sum of squares for regression.

** Indicates significance at the .01 level of probability.

≠ P = % Protein LS = Lysine (% of sample) LP = Lysine (% of protein)
KWT = 1000 kernel weight (g)

≠ Samples include 6 check cultivars replicated 4 times.

regression models were $r^2 = 0.70$ and $r^2 = 0.77$ for 1972 and 1973 respectively. On the same set of samples, the coefficients of determination for the linear regression of lysine (% of sample) on percent protein were $r^2 = 0.61$ (Model 72-30) and $r^2 = 0.73$ (Model 73-27). Including test weight in the regression model improved the predictability of lysine (% of sample). After accounting for the variation in lysine (% of sample) attributable to percent protein and test weight, yield had no effect. Kernel weight had no effect in 1972 and only a very slight effect in 1973.

Results of the regression analysis indicate percent protein and test weight affected the lysine (% of protein) content of the wheats analyzed. Kernel weight and yield had no significant effect on lysine (% of protein). First, second, and third degree polynomial models were tested for the regression of lysine (% of protein) on percent protein. The second degree polynomial models (Model 72-4 and Model 73-4) gave the best fit considering only the effect of protein. The models (Model 72-36 and 73-33) for the regression of lysine (% of protein) on percent protein, test weight, and test weight squared gave the best fit of all the models tested. The coefficients of determination for these regressions were $r^2 = 0.42$ and $r^2 = 0.35$ for 1972 and 1973 respectively. The coefficients of determination for the regression of lysine (% of protein) on percent protein and percent protein squared were $r^2 = 0.25$ (Model 72-34) and $r^2 = 0.26$ (Model 73-31) for the same group of samples. These results indicate that between 35-42% of the variability within years for lysine (% of protein) of the wheats studied can be attributed to variation of percent protein and test weight with percent protein having the greater influence.

The means of the independent variables, regression coefficients, 't' values from the 't' test of the regression coefficients, intercept values, and the standard partial regression coefficients for the regression of lysine (% of sample) and lysine (% of protein) on percent protein and on percent protein and test weight are given in Tables 7 and 8. The standard partial regression coefficients for test weight and test weight squared are larger in magnitude than the standard partial regression coefficients for percent protein. However, the standard partial regression coefficients for test weight and test weight squared are opposite in sign. The net effect of test weight on lysine (% of sample) and lysine (% of protein) is less than that of percent protein. The net effect of test weight on both lysine (% of sample) and lysine (% of protein) is negative, i.e., as test weight increases lysine content tends to decrease.

Regression curves for the regression of lysine (% of sample) on percent protein are shown in Figure 1 for the 1972 and 1973 Yuma nurseries and for the entire USDA World Wheat Collection. The World Collection curve is from Vogel et al. (1973). The increase in lysine (% of sample) with increase in protein content is clearly illustrated. The similarity of the curves and their closeness indicates that this relationship is fairly constant over environments. The length of the curves corresponds to the range of protein values for each set of samples. In the World Wheat Collection analyses, the wheats grown at Yuma in 1972 and 1973 represented the entire range of protein values in the World Collection. The mean protein percent of the wheats in the World Collection analyses was 12.97%. The mean protein percentages for the 1972 and 1973 Yuma nurseries were 17.78% and 16.33% respectively. The heights of the

Table 7. Mean, regression coefficients, 't' values for the regression coefficients, and intercept values for the regressions used in algebraic methods for (% of sample) and lysine (% of protein) values for the 1972 Hard World Wheat Collection Special Study Survey

Model	Dependent Variable	Independent Variable	Mean of Independent Variable	Regression Coefficient	't' Value	Intercept	Standardized partial regression coefficient
72- 1	Lysine (% of sample)	% Protein	17.78	b_1 = .020277	34.00	0.12722	
72-32	Lysine (% of sample)	% Protein	17.55	b_1 = .017912	26.69**	- .91459	0.720
		kg/hl	75.69	b_2 = .051396	6.11**		4.054
		$(kg/hl)^2$	5747.80	b_3 = -.000225	- 6.48**		-4.305
72- 4	Lysine (% of protein)	% Protein	17.78	b_1 = -.224173	- 3.70**	5.15102	-2.373
		(% of Protein)	318.195	b_2 = .005049	2.99**		1.918
72-36	Lysine (% of protein)	% Protein	17.55	b_1 = -.058055	-15.41**	-2.52088	-0.578
		kg/hl	75.69	b_2 = .183306	6.35**		5.858
		$(kg/hl)^2$	5747.80	b_3 = -.001315	6.74**		-6.224

** Indicates significance at the .01 level of probability.

Table 8. Means, regression coefficients, 't' values for the regression coefficients, and intercept values for the regression models used in adjusting the lysine (% of sample) and lysine (% of protein) values for the 1973 Yuma World Wheat Collection Special Study Nursery.

Model	Dependent Variable	Independent Variable	Mean of Independent Variable	Regression Coefficient	't' Value	Intercept	Standard partial regression coefficient
73- 1	Lysine (% of sample)	% Protein	16.34	b_1 = .022451	45.35**	.10090	
73-29	Lysine (% of sample)	% Protein	16.10	b_1 = .020898	37.81**	- .64270	0.823
		kg/hl	77.82	b_2 = .021912	2.59**		1.908
		$(kg/hl)^2$	6067.56	b_3 = -.000154	- 2.82**		-2.079
73- 4	Lysine (% of protein)	% Protein	16.34	b_1 = -.167233	- 3.63**	4.59330	-1.922
		$(\%\ Protein)^2$	269.636	b_2 = .003836	2.74**		1.451
73-33	Lysine (% of protein)	% Protein	16.10	b_1 = -.051978	-15.57**	-1.86802	-0.565
		kg/hl	77.82	b_2 = .157792	3.09**		3.794
		$(kg/hl)^2$	6067.56	b_3 = -.001100	- 3.33**		-4.090

** Indicates significance at the .01 level of probability.

regression curves are in inverse order of the mean protein rank.

Regression curves for the regression of lysine (% of protein) on percent protein are shown in Figure 2 for the 1972 and 1973 Yuma nurseries and for the entire USDA World Wheat Collection. The regression curves for the wheats grown in the 1972 and 1973 Yuma nurseries indicate that lysine (% of protein) is negatively related to percent protein for protein levels greater than 15%. The slopes of the regression curves are slightly different for the 1972 and 1973 Yuma nurseries indicating that the magnitude of the inverse relationship between percent protein and lysine (% of protein) varies with environments. The heights of these regression curves also are in inverse order of the mean protein rank.

Since the regression analysis indicated that percent protein and test weight have an effect on lysine content, lysine (% of sample) and lysine (% of protein) values for each Yuma nursery were adjusted to the nursery mean protein and test weight values in order to make valid lysine comparisons among wheats having different protein contents and test weights. Lysine values also were adjusted only for percent protein to make comparisons with the adjusted lysine values of the original World Collection in which lysine values had been adjusted only for percent protein. The mean, range, and standard deviation of the adjusted lysine values are listed in Table 2. Removing the variation attributable to percent protein and test weight reduced the standard deviations. The regression coefficients and mean of the independent variables used in the adjustment equations are listed in Tables 7 and 8.

Six check varieties were grown in an augmented randomized complete block design with the World Collection Wheats in the 1973 Yuma nursery to

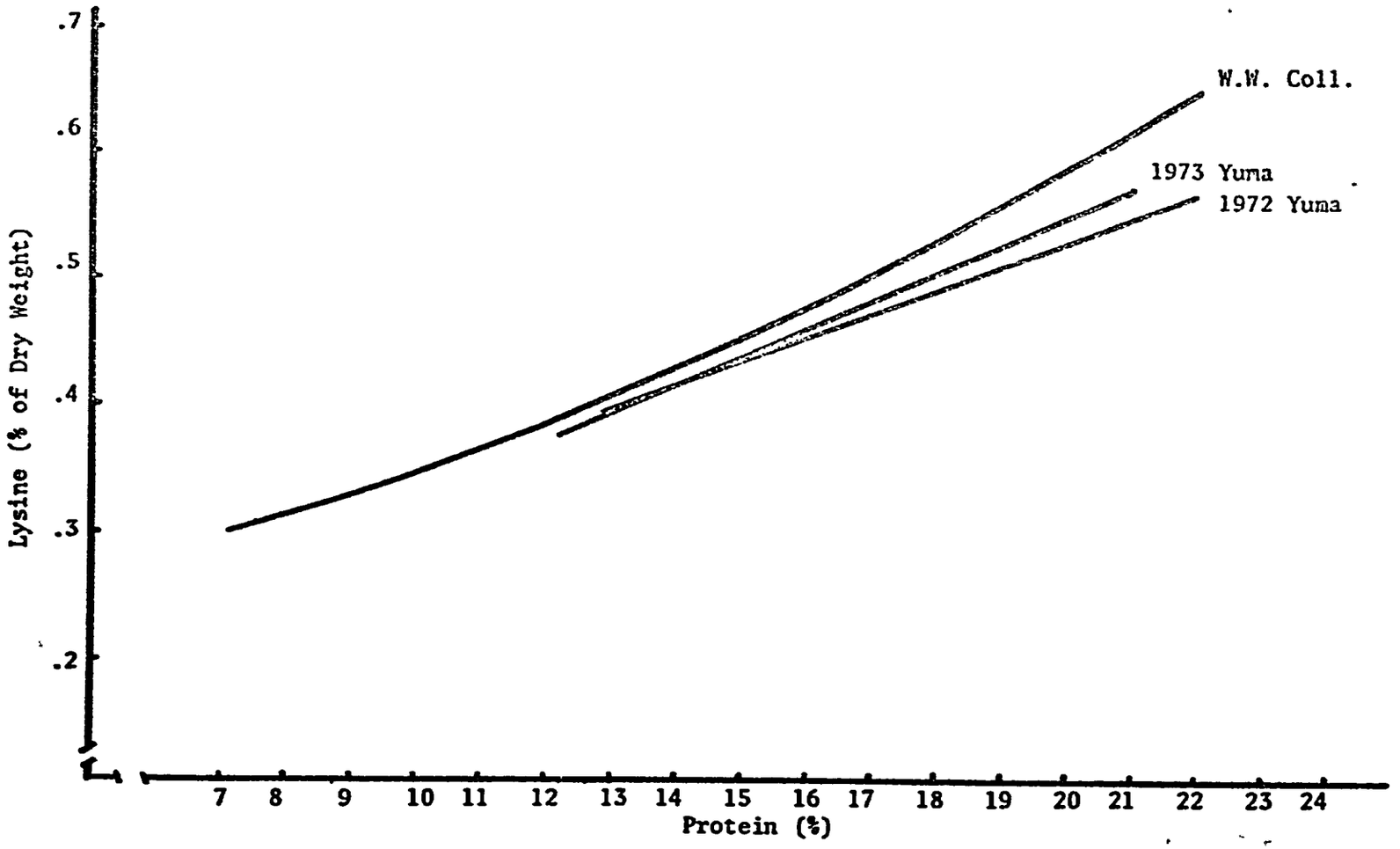

Figure 1. Regression lines for the regression of lysine (% of sample) on % protein for the USDA World Wheat Collection (W.W. Coll.), 1972 Yuma nursery, and the 1973 Yuma nursery.

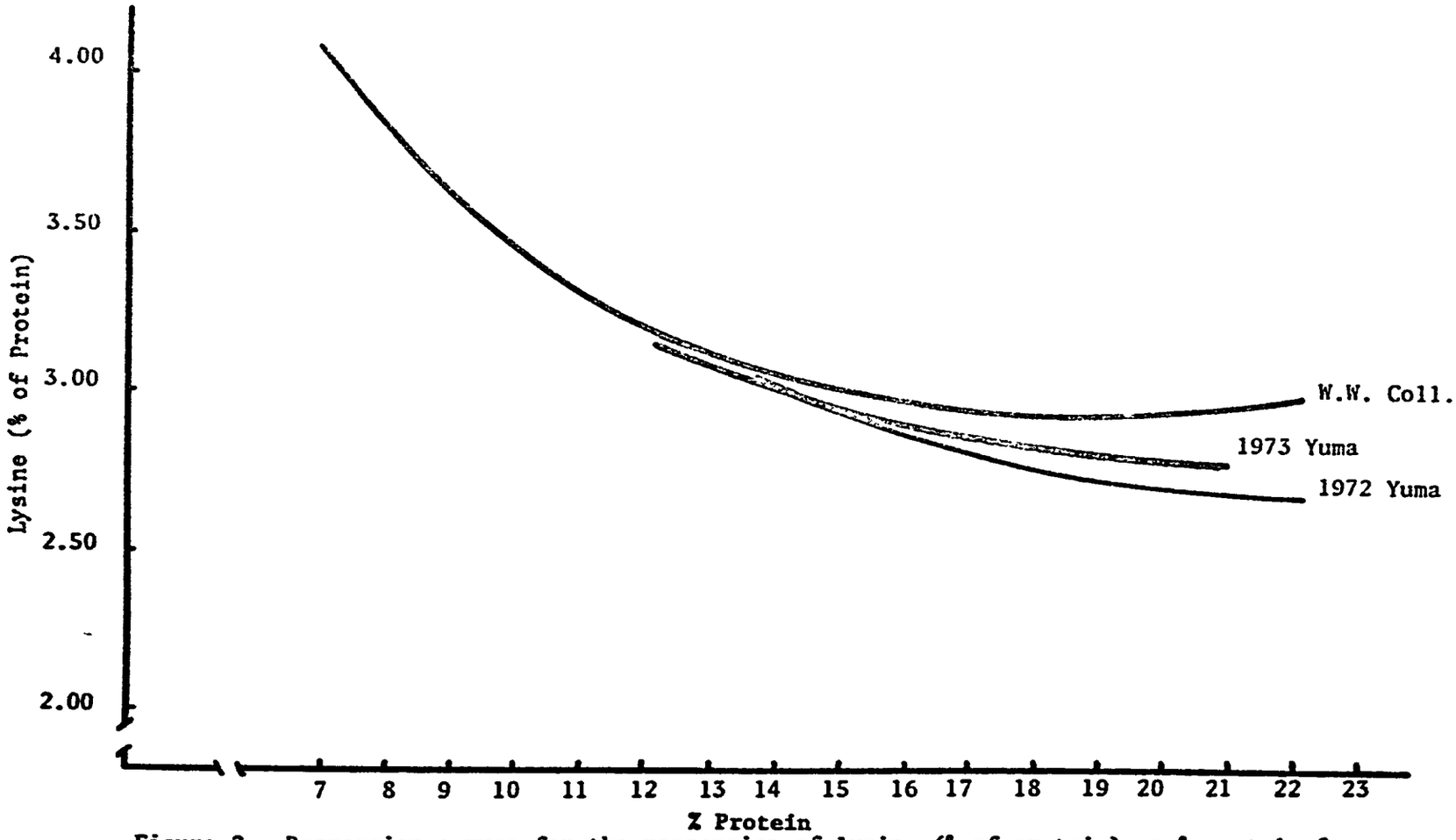

Figure 2. Regression curves for the regression of lysine (% of protein) on % protein for the USDA World Wheat Collection (W.W. Coll.), 1972 Yuma nursery, and the 1973 Yuma nursery.

test for within nursery environmental variation. Protein and lysine contents of the check varieties were analyzed using analysis of variance procedures for the randomized complete vlock design. Means, F ratios, coefficients of variation, and LSD values for the analysis of the 1973 Yuma check varieties are listed in Table 9. Although the check varieties differed significantly for percent protein, lysine (% of sample), lysine (% of protein), and kernel weight; the F ratios for blocks were not significant for protein or lysine content. The F ratio for blocks for kernel weight was significant at the .05 but not at the .01 level of probability. Except for yield, the coefficients of variation are small. Analysis of variance for test weight was not conducted because 3 of the 24 check plots did not produce sufficient seed for test weight measurements.

Means, coefficients of variation, and LSD values from the analysis of variance, considering years as replications, for the World Collection wheats in the 1972 and 1973 Yuma nurseries are listed in Table 10. Range values for the traits studied also are listed. Results of unweighted analysis of variance are given for yield, kernel weight, test weight, and for lysine content adjusted for percent protein and test weight since yield, kernel weight and test weight were not measured on all wheats in both years.

Coefficients of variation were low for all variables except for yield. Yield measurements on single row plots analyzed over years are subject to large experimental error. The low coefficients of variability for percent protein, lysine (% of sample), lysine (% of protein), kernel weight, and test weight indicate that single row plots are suitable for testing these variables in the Yuma environment.

Table 9. Means, LSD values, and coefficients of variation for 1000 kernel weight (g), yield (g/plot), % protein, lysine (% of sample), and lysine (% of protein) for the check varieties in the 1973 World Wheat Collection Special Study nursery

	$\bar{x}$ 1000 kernel weight	$\bar{x}$ yield	$\bar{x}$ % protein	$\bar{x}$ lysine (% of sample)	$\bar{x}$ lysine (% of protein)
CHECK LINES					
Atlas 66	34.7	386 0	19.05	.52	2.76
Triumph 64	40.1	251.5	17.45	.49	2.83
Scout 66	42.2	377.25	14.55	.43	2.99
Inia 66	50.50	427.75	16.40	.46	2.83
Centurk	36.0	466.25	13.92	.43	3.12
Nap Hal	26.4	318.0 ƒ	18.48	.57	3.09
STATISTICS					
'F' ratio for blocks	3.31*	.93	2.81	2.04	2.57
'F' ratio for lines	140.02**	2.61	18.36**	19.35**	12.00 **
Coefficient of variation (%)	3.56	25.20	5.83	3.00	2.91
LSD .05	2.06	142.75	1.463	.04	0.13
LSD .01	2.84	198.13	2.023	.05	.18

*, ** Indicates significance at the .05 and the .01 level respectively.

ƒ Yield measured on 3 plots.

The F ratios from the analysis of variance over years were significant at the .01 level of probability for years and lines for all traits tested. There were significant differences among wheats for lysine (% of sample) and lysine (% of protein) even after lysine values were adjusted to the mean protein and test weight values for each year indicating that genetic differences do exist among the wheats used in this study that are independent of the protein content and test weight of the wheats. Although years had a significant effect, the variability attributable to lines was of much greater magnitude for all traits.

Protein and unadjusted lysine values for the wheats that had the highest and lowest mean protein values over years are listed in Table 11. The high protein wheats are five percentage points higher in percent protein than the low protein wheats.

Wheats with the highest and lowest mean values over years for lysine (% of protein) adjusted for percent protein are listed in Table 12. Wheats that had the highest and lowest lysine (% of protein) values adjusted for percent protein and test weight are listed in Table 13. Including test weight in the adjustment equation changed the rankings of the wheats.

The effect of adjusting lysine (% of protein) values for percent protein was to adjust the lysine values up if protein percent was greater than the mean protein level and down if protein percent was less than the mean. The net effect of the adjustment for test weight was to adjust lysine (% of protein) values up if test weight was greater than the mean test weight and down if test weight was less than the mean. A 0.4 to 0.5 percentage point spread exists between the wheats with the highest and

Table 10. Means, coefficients of variation, LSD values, and range values from the analysis of variance considering years as replications for the 1972 and 1973 Yuma, Arizona World Wheat Collection Special Study Nurseries. ≠

Variable	Mean	CV	LSD .05	LSD .01	Range of Means
% Protein	17.058	6.61	2.21	2.91	14.10- 20.95
Lysine (% of sample)	0.478	5.76	0.05	0.07	.38- .59
Lysine (% of sample) adjusted for % protein	0.478	3.18	0.03	0.04	.43- .54
Lysine (% of protein)	2.832	3.69	0.21	0.27	2.50- 3.13
Lysine (% of protein) adjusted for % protein	2.832	3.11	0.17	0.23	2.54- 3.19
Yield (lines with ≥ 100g yield) +	339.13	36.01	239.8	315.6	127.0 -719.0
1000 kernel weight (g) +	39.43	6.48	5.02	6.61	24.10- 56.85
Test weight (kg/hl) +	76.78	2.22	3.35	4.41	66.76- 84.17
Lysine (% of sample) adjusted for % protein, kg/hl, $(kg/hl)^2$ +	.471	3.13	0.03	0.04	.43- .51
Lysine (% of sample) for above set of wheats +	.471	5.72	0.05	0.07	.41- .56
Lysine (% of protein) adjusted for % protein, kg/hl, $(kg/hl)^2$ +	2.842	2.50	0.14	0.18	2.59- 3.04
Lysine (% of protein) for above set of wheats +	2.842	3.09	0.17	0.22	2.56- 3.06

\+ Results of unweighted ANOVA since all wheats not represented in both years. Range of means values are for wheats represented in both years.

≠ F ratios for years and lines were significant at the .01 level for all variables.

lowest mean adjusted lysine (% of protein) values.

Wheats with the highest and lowest mean values over years for lysine (% of sample) adjusted for percent protein and test weight are listed in Table 14. The effect of adjusting lysine (% of sample) values for percent protein was opposite that of adjusting lysine (% of protein) values. Lysine (% of sample) values were adjusted down when percent protein was greater than the mean and up when less than the mean percent protein. The net effect of the adjustment for test weight on lysine (% of sample) was the same as for lysine (% of protein). The correlation of lysine (% of sample) and lysine (% of protein) after both were adjusted for percent protein and test weight was r = 0.98 for both 1972 and 1973. Thirteen of the 15 lines that had the highest lysine (% of protein) values after adjusting for test weight and percent protein were among the top 15 lines that had the highest lysine (% of sample) values after adjustment for the same factors.

The simple correlation coefficients for protein, lysine, test weight, kernel weight, and yield of individual lines correlated over years are listed in Table 15. The simple correlation coefficient for a trait over years provides a measure of the repeatability of that trait. A correlation of r = 1.0 would indicate a completely heritable trait, i.e., not influenced by year to year environmental variation.

Adjusting lysine (% of sample) for percent protein slightly decreased the correlations over years as compared to unadjusted lysine (% of sample). Adjusting lysine (% of protein) improved the correlation of lysine (% of protein) over years. The correlation coefficients over years of lysine (% of sample) and lysine (% of protein) adjusted for

Table 11. Protein, lysine, and 1000 kernel weight mean values for the wheats that had the highest and lowest mean protein values for the common wheats of the World Wheat Collection Special Study.*

Rank	CI/PI	1000-kernel wt. g	P	LS	LP
1	6225	25.50	20.95	.58	2.80
2	11028	24.75	20.20	.54	2.70
3	173438	24.50	20.20	.55	2.76
4	8681	28.20	20.15	.56	2.78
5	174680	31.75	20.10	.56	2.84
6	298580	43.65	20.05	.52	2.62
7	286000	29.20	19.95	.54	2.70
8	284666	34.70	19.90	.54	2.76
9	166788	28.50	19.85	.54	2.75
10	268542	40.60	19.75	.52	2.66
11	7695	32.05	19.75	.52	2.64
12	185349	49.20	19.75	.52	2.66
13	9178	28.75	19.75	.52	2.66
14	285880	40.35	19.70	.55	2.80
15	192014	52.85	19.70	.54	2.76
⋮					
632	231308	37.70	14.40	.41	2.90
633	304390	39.10	14.30	.44	3.09
634	166182	39.50	14.30	.43	3.00
635	6836	41.10	14.20	.43	3.06
636	131276	50.95	14.10	.42	3.06

*P = % Protein
LS = % Lysine (% of sample)
LP = % Lysine (% of protein)
CI = Cereal Investigation number
PI = Plant Introduction number

Table 12. Protein, lysine, and test weight mean values for wheats that had the highest and lowest mean lysine (% of protein) values adjusted for percent protein for the common wheats of the World Wheat Collection Special Study.*

Rank	CI/PI	Test weight kg/hl	P	LS	LP	Adjusted LP
1	176217	74.8	19.4	.60	3.10	3.18
2	245604	----	17.8	.54	3.12	3.15
3	162008	71.0	17.3	.54	3.12	3.14
4	6616	71.0	17.1	.53	3.13	3.14
5	174702	72.2	17.0	.52	3.08	3.09
6	166859	72.2	16.8	.51	3.06	3.05
7	254829	76.8	17.2	.52	3.04	3.04
8	192701	73.5	16.8	.51	3.06	3.04
9	298587	75.1	16.8	.51	3.06	3.04
10	13563	----	18.4	.54	2.98	3.04
11	166946	78.4	16.8	.51	3.04	3.04
12	306513	76.4	16.2	.50	3.05	3.03
13	254077	----	19.2	.56	2.94	3.02
14	272427	78.0	18.1	.53	2.96	3.02
15	298568	71.0	17.5	.52	2.99	3.01
⋮						
632	164501	81.9	16.7	.42	2.58	2.58
633	9041	80.6	18.8	.47	2.50	2.57
634	9019	81.9	16.4	.42	2.59	2.57
635	9050	82.6	14.6	.38	2.68	2.55
636	9047	81.5	14.7	.38	2.66	2.54

P = % Protein
LS = % Lysine (% of sample)
LP = % Lysine (% of protein)

Table 13. Protein, lysine, and test weight (kg/hl) values for the wheats that had the highest and lowest mean lysine (% of protein) values adjusted for % protein and test weight for the common wheats of the World Wheat Collection Special Study.+

Rank	CI/PI	Kg/hl	P	LS	LP	LSAD	LPAD
1	168946	78.37	16.85	.51	3.04	.51	3.05
2	254[illegible]	76.43	17.20	.52	3.04	.51	3.03
3	192736	78.60	18.25	.53	2.94	.50	3.03
4	10937	79.01	17.55	.52	2.97	.50	3.03
5	185453	80.62	15.55	.47	3.04	.50	3.02
6	203587	75.14	16.80	.50	3.06	.50	3.01
7	15546	78.37	15.50	.48	3.08	.50	3.02
8	176300	77.08	17.50	.52	2.98	.50	3.00
9	165380	75.21	19.15	.56	2.92	.50	2.99
10	204053	80.50	14.70	.44	3.06	.49	2.99
11	305523	76.43	16.25	.50	3.05	.50	2.99
12	165[illegible]	79.65	18.25	.52	2.88	.50	2.99
13	11875	76.43	16.10	.49	3.05	.50	2.99
14	124744	78.04	17.25	.51	2.96	.50	2.99
15	109568	79.01	15.70	.47	3.02	.50	2.99
⋮							
354	9041	80.52	18.85	.47	2.50	.44	2.66
355	9040	81.92	16.45	.42	2.59	.44	2.66
356	9047	81.59	14.70	.38	2.66	.44	2.62
357	6477	74.82	18.55	.47	2.56	.43	2.62
358	225223	68.05	16.95	.44	2.64	.43	2.59

+ P = % Protein LS = Lysine (% of sample)
LP = Lysine (% of protein)
LSAD = Lysine (% of sample) adjusted for % protein, kg/hl, $(kg/hl)^2$
LPAD = Lysine (% of protein) adjusted for % protein, kg/hl, $(kg/hl)^2$

Table 14. Protein, lysine, and test weight (kg/hl) values for the wheats that had the highest and lowest mean lysine (% of sample) values adjusted for % protein and test weight for the common wheats of the World Wheat Collection Special Study.+

Rank	CI/PI	kg/hl	P	LS	LP	LSAD	LPAD
1	[illegible]829	76.43	17.20	.52	3.035	.51	3.04
2	168916	78.37	15.85	.51	3.04	.51	3.05
3	170900	77.08	17.50	.52	2.98	.50	3.00
4	192758	78.69	18.25	.53	2.94	.50	3.03
5	155433	80.52	15.55	.47	3.04	.50	3.02
6	10907	79.01	17.55	.52	2.97	.50	3.03
7	306513	76.43	16.25	.50	3.05	.50	2.99
8	15846	78.37	15.50	.48	3.08	.50	3.02
9	185380	73.21	19.15	.56	2.92	.50	2.99
10	124744	78.04	17.25	.51	2.96	.50	2.99
11	298587	75.14	16.80	.50	3.06	.50	3.01
12	11875	76.43	16.10	.49	3.05	.50	2.99
13	185343	79.66	18.25	.52	2.88	.50	2.99
14	11986	76.10	16.70	.50	3.02	.50	2.98
15	176223	72.56	18.4	.54	2.94	.50	2.97
⋮							
354	164501	81.92	16.70	.42	2.58	.44	2.66
355	169629	75.14	18.30	.48	2.64	.44	2.69
356	9041	80.62	18.85	.47	2.50	.44	2.66
357	6177	74.82	18.55	.47	2.56	.43	2.61
358	225223	68.05	16.95	.44	2.64	.43	2.59

+ P = % Protein
LS = % Lysine (% of sample)
LP = % Lysine (% of protein)
LSAD = % Lysine (% of sample) adjusted for % protein, kg/hl, $(kg/hl)^2$
LPAD = % Lysine (% of protein) adjusted for % protein, kg/hl, $(kg/hl)^2$

percent protein are of similar magnitude. The coefficients for the correlation of the 1972 and 1973 Yuma protein and adjusted lysine values with the protein and adjusted lysine values of the World Wheat Collection analyses are approximately the same as the coefficients for the correlation of the 1972 and 1973 Yuma data. The size and similarity of the correlations over years for protein and lysine adjusted for percent protein suggests that the rank relationship among wheats for these traits over environments are reasonably similar.

Test weight and thousand-kernel weight were not measured on the wheat samples used in the World Wheat Collection analysis. The correlations over years for test weight and kernel weight are high indicating that these traits were highly heritable under the Yuma conditions. Removal of the variation attributable to test weight and percent protein by adjusting the lysine values decreased the correlation over years for both lysine (% of sample) and lysine (% of protein).

The correlations over years for yield was low. This was probably due in part to the experimental error of measurement for the single row plots and partly because yield is a trait that is considerably influenced by year to year environmental variation.

The protein values and rank of the wheats in the 1972 and 1973 Yuma nurseries with the highest and lowest mean values and the protein values and rank of the same 636 wheats using World Wheat Collection analysis, results are listed in Table 16. With the exception of PI173438 and CI8661, wheats with high and low protein content of grain in the Yuma nurseries ranked similarly in the World Wheat Collection analyses even though the protein range was much greater in the World Collection analysis.

Table 15. Correlation coefficients (r) for percent protein, lysine (% of sample), lysine (% of protein), Yield, 1000 kernel weight, and test weight (kg/hl) over years for wheats grown in the World Wheat Collection Special Study at Yuma, Arizona in 1972 and 1973 and for the initial USDA World Wheat Collection analysis results.+

Variable	W. W. Collection analysis ≠ vs Yuma 1972	W. W. Collection analysis ≠ vs Yuma 1973	Yuma 72 vs Yuma 73
Percent Protein	.439**	.330**	.424**
	635	634	635
Lysine (% of sample)	.492**	.449**	.475**
	635	634	635
Lysine (% of sample) adjusted for % protein	.404**	.246**	.450**
	635	634	635
Lysine (% of protein)	.349**	.096*	.398**
	635	634	635
Lysine (% of protein) adjusted for % protein	.413**	.252**	.448**
	635	634	635
Yield (lines with ≥ 100g yield)			.194**
			575
1000 kernel weight (g)			.853**
			568
Test weight (kg/hl)			.707**
			358
Lysine (% of sample) adjusted for % protein, kg/hl, (kg/hl)^2			.263**
			358
Lysine (% of sample) for above set of wheats			.432**
			358
Lysine (% of protein) adjusted for % protein, kg/hl, (kg/hl)^2			.253**
			358
Lysine (% of protein) for above set of wheats			.407**
			358

\+ Number of lines correlated are listed below the r values.

*, ** Indicates significance at the .05 and .01 levels of probability, respectively.

≠ Adjusted lysine values for the World Wheat Collection analysis data adjusted using regression coefficients in: Vogel, Johnson, Mattern, 1973.

Wheats grown at Yuma with the highest and lowest mean lysine (% of protein) and lysine (% of protein) adjusted for percent protein values and the lysine values and rank of the same wheats using World Collection analysis results are given in Tables 17 and 18. The adjusted lysine values and their relative rank are in much better agreement than the unadjusted lysine values. On the basis of the lysine values obtained at Yuma, lysine (% of protein) values adjusted for percent protein would be the better selection criterion for selecting wheats genetically superior for lysine content from the World Wheat Collection.

Table 16. Protein values of the wheats in the 1972-73 Yuma tests with the highest and lowest mean values and the protein values and rank of the same 636 wheats using World Wheat Collection analysis results.

CI/PI number	Yuma rank	Yuma % Protein	W. W. Collection % Protein	W. W. Collection rank
6225	1	20.95	19.5	26
11028	2	20.20	18.5	67
175438	3	20.20	9.0	535
8681	4	20.15	13.20	297
174680	5	20.10	20.6	9
231[illegible]8	632	14.40	9.1	514
[illegible]0	633	14.30	8.8	559
166182	634	14.30	13.9	231
6836	635	14.20	9.12	513
131276	636	14.10	8.8	574

Mean protein % = 17.058
LSD .05 = 2.21

Table 17 Lysine values (% of protein) of the wheats in the 1972-1973 Yuma tests with the highest and lowest mean values and the lysine values and rank of the same 636 wheats using World Wheat Collection analysis results.

CI/PI number	Yuma rank	Yuma % Lysine	W. W. Collection % Lysine	W. W. Collection rank
6616	1	3.13	2.98	411
162008	2	3.125	3.15	259
245604	3	3.120	3.04	356
263849	4	3.115	3.97	8
142521	5	3.105	3.29	206
6177	632	2.56	2.44	628
9053	633	2.56	2.71	548
9393	634	2.56	2.78	528
9045	635	2.56	2.75	536
9041	636	2.50	2.60	606

LSD .05 = .21
Lysine (% of Protein) $\bar{X}$ = 2.832

Table 18. Lysine (% of protein) values adjusted for percent protein in the 1972-73 Yuma tests with the highest and lowest mean values in the adjusted lysine (% of protein) values of the same 636 wheats using World Wheat Collection analysis results.

CI/PI	Yuma rank	Yuma % Lysine (adjusted)	W. W Collection % Lysine (adjusted)	W. W. Collection % Lysine	W. W. Collection rank
[illegible]	1	3.18	3.27	3.06	150
[illegible]	2	3.15	3.23	3.04	208
[illegible]	3	3.14	3.36	3.15	51
[illegible]	4	3.14	3.19	2.98	267
174702	5	3.09	3.25	3.04	180
164501	632	2.58	2.71	3.29	528
[illegible]	633	2.57	2.66	2.60	553
[illegible]	634	2.57	2.56	2.69	601
[illegible]	635	2.66	2.28	2.60	636
[illegible]	636	2.54	2.51	2.99	620

1972-73 Adjusted lysine $\bar{X}$ = 2.832
LSD .05 = .21

DISCUSSION

The 1972 Yuma mean protein percent was higher than the mean 1973 Yuma protein percent. The higher rate of nitrogen fertilization used in 1972 contributed to the higher protein content in 1972. This effect of nitrogen fertilization upon protein content agrees with the results of Hoppati and Malaki (1973), Hunter and Standford (1973), and Johnson et al. (1973a).

The high rate of nitrogen fertilization in 1972 resulted in prolific vegetative growth. Severe lodging resulted when the wheats were irrigated late in the growing season. Because lodging hindered normal grain filling, the yield, test weight, and kernel weight were lower in 1972 than in 1973. The small negative correlation between percent protein and yield, test weight, and kernel weight obtained in this study indicate that these factors also contributed slightly to the higher protein content for the 1972 nursery. This conclusion is supported by the results of Terman et al. (1969) and Johnson et al. (1973b) who reported that the protein content of wheat grain tends to be lower under high yielding conditions than under low yielding conditions. Johnson et al. (1973b, 1972c) reported that plump kernels tend to be higher in protein content than shriveled kernels. Ghaderi et al. (1971b) reported a low positive correlation ($r = 0.11$) of protein content and test weight. This positive correlation, however, was obtained using low protein soft winter wheats that did not differ greatly in protein content.

The average protein content of the wheats in the Yuma nurseries was much higher than the average protein content of the same group of

wheats in the World Collection analysis but the range in protein values was smaller. The high protein percentages of the Yuma nurseries can be attributed to fertilization, absence of diseases, and irrigation. The range in protein values was larger for the World Collection analyses even so not all wheats had been grown during the same year. Much of the variation in protein content reported by Vogel et al. (1973) for the World Collection, thus was non-genotic in origin.

The multiple regression of percent protein with yield, kernel weight, and test weight indicated that only about 10% of the variability for protein among wheats within a nursery could be attributed to variation among wheats for these factors. This indicates that variation for protein content among wheats in the same environment is not influenced to any great extent by seed size or percent endosperm of samples. A variation for protein content for a given wheat over environments is probably influenced by these factors.

In both the 1972 and 1973 Yuma nurseries, high positive correlations existed between lysine (% of sample) and percent protein. This relationship was reported by Vogel et al. (1973) for the entire World Wheat Collection and is to be expected since lysine is a constituent of protein. The mean for lysine (% of sample) was higher in the 1972 nursery than for the 1973 nursery and the mean lysine (% of sample) of both nurseries were much higher than for the same wheats in the World Collection analyses. The mean lysine (% of sample) values ranked in the same order as the protein means for the three sets of data indicating that the lysine (% of sample) values of wheats will be higher in high protein than in low protein environments. However, the ordinates of the regression

curves at common protein levels are in inverse order of the mean protein levels. This means that the average lysine (% of sample) for wheats that had 18% protein was lower for the wheats of the 1972 Yuma nursery than for wheats of the 1973 Yuma nursery that had 18% protein. This inconsistency can be explained if proportionately more gluten proteins are produced in high protein environments than in low protein environments. The results of Ulmer (1973) show that more gluten proteins are produced under high protein than under low protein environments by the same wheats.

The inverse relationship between protein and lysine (% of protein) among wheats of the Yuma nurseries was similar to that of the entire World Wheat Collection. This relationship was also reported by Lawrence et al. (1958), Villegas et al. (1970), Simmonds (1962), and McDermott and Pace (1960) and probably exists among wheats in all environments. The Yuma results differed from those of the World Wheat Collection in that the curvilinearity was not as pronounced and the negative relationship between protein and lysine (% of protein) existed for wheats with more than 15% protein. These differences were probably due to the high mean protein levels of the Yuma nurseries and to the narrower protein range.

The inverse relationship between lysine percent of protein and percent protein for wheats within a nursery can be attributed to high protein wheats having proportionally more gluten proteins on the average than low protein wheats, (Simmonds, 1962; Mattern et al., 1968; Pence et al., 1964; Ulmer, 1973; McDermott and Pace, 1960). The relative heights and slopes of the regression curves for the regression of lysine (% of protein) vary with environment indicating that there is an environmental

effect on the albumin-globulin:gluten ratio of wheat proteins. The ordinates for the regression curves at common protein levels are in inverse order of the mean protein levels for the regression curves in Figure 2. The mean lysine (% of protein) values are also in inverse order of the mean protein levels. McElroy et al., (1972), Hojjati and Waleki (1972), Gunthardt and McGinnis (1957) and Stroike and Johnson (1972) have reported an inverse relationship between percent protein and lysine (% of protein) for a given variety over environments. This suggests that proportionally more gluten proteins are produced by a wheat variety in a high protein environment than in a low protein environment. The inverse effect of protein on lysine (% of protein) cannot be attributed to different proportions of bran and endosperm. Higher protein contents due to shrivelled seeds would result in higher, not lower, lysine (% of protein) values because the non-endosperm proteins are much higher in lysine content than the endosperm protein.

Although yield and kernel weight were negatively correlated with lysine percent of sample, the regression analysis indicated that these factors did not have any significant effect on either lysine (% of sample) or lysine (% of protein) among wheats within a nursery after accounting for the variation attributable to percent protein and test weight. Yield probably indirectly influences lysine content by its effect on percent protein and test weight.

MacMasters et al. (1971) have reported that the endosperm contains over 70% of the protein of the wheat kernel but only 50% of the lysine. On the basis of these data, percent endosperm of sample would be expected to influence the lysine content of wheat. Test weight and kernel weight

can both be used as indexes in estimating endosperm percent of a sample of wheat. Test weight probably provided a better measure of the percent endosperm of sample than kernel weight for the wheats analyzed and consequently had more influence on lysine content than kernel weight, particularly when used with protein in a multiple regression analysis. The simple correlation coefficients and standard partial regression coefficients indicate that although test weight, i.e., percent endosperm of sample, contributes to the variability among wheats for lysine content, its effect is much less than that of percent protein.

Adjusting lysine values to the mean protein content of each nursery permits comparisons to be made among wheats differing in protein content. It is not possible to make valid comparisons among wheats differing in percent protein for lysine content because of the strong influence of protein on lysine. Adjusting lysine values to the mean protein percent and test weight allows valid lysine comparisons to be made among wheats differing for both protein content and test weight. Because environment affects the relationship of protein to lysine, lysine values for each year were adjusted using regression coefficients and means from the regression analysis for that year. Using a common adjustment equation to adjust lysine values of wheats grown in different environments is statistically unsound and may result in distorted lysine values for wheats in some years.

Unadjusted lysine (% of sample) and lysine (% of protein) values were not correlated in 1973 and only slightly correlated in 1972. The use of lysine (% of sample) as a selection criterion would produce results inconsistent with the use of lysine (% of protein) as a selection

criterion. The use of either lysine (% of sample) or lysine (% of protein) adjusted for percent protein and test weight would produce the same results because, after adjustment, their correlation is r = +.98 and they both provide the same relative measurement of lysine content of wheat grain. However, adjusted lysine expressed as a percent of protein provides a more magnified display of lysine differences among wheats.

The results of the analysis of variance for the protein and lysine content of the check varieties in the 1973 nursery indicate that there was no significant within nursery environmental variation for these traits in 1973. Within-nursery environmental variation also was probably not significant in 1972 because the nursery did not cover a large land area and was fertilized and irrigated uniformly. Consequently, within-nursery environmental variation probably did not contribute significantly to the differences found for protein and lysine content among wheats within a nursery.

Only 10% of the total variation for percent protein within a nursery was attributable to variation in yield, test weight, and kernel weight. Since the within-nursery environmental variation for percent protein was not significant, within-nursery variation for percent protein must have been due largely to genotype and genotype x environment interaction effects.

Approximately 40% of the variability for lysine (% of protein) and 70% of the variability for lysine (% of sample) within a Yuma nursery could be attributed to variation in protein content and test weight. Adjusting the lysine values for protein content and test weight removed this variability. Within-nursery variation for adjusted lysine also must have been largely due to genotype and genotype x environment interaction effects.

The analysis of variance over years for protein and adjusted lysine showed that, even though the effect of years was significant, most of the variation for protein and lysine content was due to differences among the lines tested. This indicates that significant genetic differences exist among the wheats tested for protein and lysine content. Part of the variability among wheats for protein and lysine content is due probably to the different degree of adaptability of the wheats to the Yuma environment (genotype x location interaction effects).

The genetic sources for high protein that have been identified to date, i.e., the Atlas 66 materials, are capable of maintaining their protein superiority over years and locations (Johnson et al., 1968, 1969, 1973a, Stroike and Johnson, 1972). The wheats with the highest mean protein levels in the Yuma World Collection nurseries had protein levels equivalent to that of Atlas 66 and they maintained these protein levels over a two-year period. Although testing in different locations may indicate that some of the wheats are high in protein content because of specific adaptability to the Yuma environment, it seems reasonable to assume that at least some of the wheats may possess genes for high protein content of wheat grain and these may be different from those of Atlas 66. If so, considerable improvement in the protein content of wheat can be expected to be made by wheat breeders.

The wheats with the highest adjusted lysine values also maintained their superiority over the two years of the test. The wheats with the highest adjusted lysine values were two LSD values higher in lysine content than the wheats with the lowest adjusted lysine values. This strongly suggests that wheat breeders should be able to use these lines

for improving the lysine content of wheat. These wheats are diverse in origin and they may contain different genes for high lysine. In that case, high lysine x high lysine crosses should result in transgressive segregates that have higher lysine content than either parent. Johnson et al. (1975b) have reported evidence of transgressive segregation for lysine.

If the high lysine effect is due to an alteration of the albumin-globulin:gluten ratios, only small numbers of genes may be involved. However, if the high lysine effect is due to the gluten proteins having more lysine residues than normal, then many genes would be involved, each having a very small effect. Both situations probably exist. Because wheat is a heterogamic it is doubtful that single genes difference for lysine content exist as in corn (Mertz et al., 1964), sorghum (Singh and Axtell, 1973), and barley (Munch et al., 1970).

The correlation of a homozygous line with itself provides a measure of the repeatability of a trait such as protein content. The correlations over years for percent protein were fairly high considering the effect environment can have on protein content.

Correlation coefficients for unadjusted lysine (% of protein) are lower than for lysine (% of protein) adjusted for percent protein. Adjusting for percent protein removed some of the environmental variation affecting lysine (% of protein). Adjusting lysine (% of sample) values for percent protein decreased the correlation over years. After adjusting for percent protein, lysine (% of sample) correlations over years are of the same magnitude as the correlation for adjusted lysine (% of protein). Lysine (% of sample) measures both lysine (% of protein) and

percent protein. Adjusting lysine (% of sample) values removed the effect of protein and provided a more valid estimate of the lysine content of the sample that was independent of percent protein and this caused the reduction in the correlation over years.

The correlation coefficients for lysine adjusted for percent protein are either slightly lower or of the same size as the correlation coefficients over years for percent protein. This indicates that the effect of environment on adjusted lysine is as large or slightly larger than its effect on protein. Heritability values for adjusted lysine, would be expected to be at most as large as for percent protein. The genetic studies of Diehl (1974) and Kuhr (1974) support this contention. Their results show that for seven different crosses the heritability of adjusted lysine (% of protein) was almost as high as for percent protein.

The correlations over years for test weight and kernel weight were very high indicating that they are highly heritable traits. The heritability estimates of Ghaderi and Everson (1971a) for test weight and Sun et al. (1972) for kernel weight were high. The low correlation obtained in this study between kernel weight and test weight indicates that these traits are largely independent of each other. Ghaderi and Everson (1971d) obtained no genetic correlation between these traits. A low genetic correlation between these traits would be expected because density is the only factor they have in common.

The correlation over years for lysine adjusted for percent protein and test weight were lower than the unadjusted lysine values. Both lysine (% of sample) and lysine (% of protein) are influenced by test weight. Test weight in the Yuma environment was highly heritable.

Removal of the lysine variation due to this heritable trait would be expected to lower the correlation coefficient between years and this is what happened. Adjusting for test weight did not remove all of the lysine variability among wheats due to different percentages of endosperm because the utility of test weight as an index for estimating percent of endosperm varies with wheats. Because most wheat-eating societies eat the endosperm portion of the kernel, protein and lysine analysis of endosperm fractions should be tested to determine the relationship between whole kernel and endosperm protein and lysine content.

The size and similarity of the correlations over years for protein and lysine adjusted for percent protein suggest that the rank relationship among the wheats in the World Wheat Collection analyses are similar to the rank relationship of the same wheats grown at Yuma over a two year period. Examination of Tables 16 and 17 show that with a few exceptions the wheats that had high and low protein and adjusted lysine values in the Yuma nurseries ranked in the top and bottom one-third respectively in the World Collection analyses. Because of the large effectof protein on unadjusted lysine (% of protein), there is no similarity in the rank relationship of the unadjusted lysine (% of protein) values of the Yuma nurseries with those of the World Collection analyses. Unadjusted lysine values are not usuable as a selection criteria for identifying high lysine wheats.

Most of the wheats that had high protein and adjusted lysine values in the World Wheat Collection analysis were not included in this study. The favorable comparison of protein and adjusted lysine values of the wheats grown at Yuma with the protein and adjusted lysine values of

the same wheats in the World Collection analysis indicates that wheat breeders can use the results of the World Collection analyses with reasonable assurance of selecting at least some lines genetically superior for protein content and for lysine content if adjusted lysine values are used. However, some of the high lysine lines may be high in lysine due to low test weights.

Several hundred of the wheats that had high protein and adjusted lysine values in the World Wheat Collection analyses which were not included in this study should be regrown to specifically identify those wheats that are genetically superior for protein and lysine content and to eliminate those that are not before they are used in any breeders program. The high protein and high lysine lines of the Yuma tests should supply wheat breeders with usable germ plasm for improving the nutritive quality of wheat.

SUMMARY

The analysis of the 12,000 common wheats of the USDA World Wheat Collection indicated significant differences among wheats for grain protein and lysine content (Vogel et al., 1973). Lysine is the limiting amino acid in wheat protein. Much of the variation for protein and lysine content was non-genetic in origin since the wheats analyzed were not all grown during the same year.

In order to test the validity of the World Wheat Collection results and to develop selection criteria to use in selecting high protein and lysine lines in a breeding program, over 600 wheats were selected from the World Wheat Collection which represented the range of protein and lysine that existed among the common wheats. These wheats were grown at Yuma, Arizona in 1972 and 1973. The Yuma nursery was used because spring and winter wheats could be grown in the same nursery.

Protein content was slightly influenced by yield, test weight, and 1000-kernel weight. In a multiple regression analysis only 10% of the total variation for percent protein could be attributed to those factors. The mean protein content of the 1972 nursery was higher than the mean protein content of the 1973 nursery. Although the effect of years was significant, most of the variation in percent protein was due to genetic differences among the wheats tested. The wheats with the highest mean protein percentages were five percentage points higher in protein than the low protein wheats. Wheats that should be useful to wheat breeders as genetic sources for high protein were identified.

Lysine expressed as a percent of sample was positively correlated

to percent protein but lysine expressed as a percent of protein was negatively correlated to percent protein. Selection for lysine using both criteria would produce conflicting results.

Results of other researchers indicate that the complex relationship between lysine and protein is due to high protein wheats having more gluten proteins which are low in lysine than low protein wheats. In order to make valid lysine comparisons among wheats differing in protein content, lysine values were adjusted to nursery mean protein values using regression procedures. After adjustment for percent protein the correlation of lysine (% of sample) and lysine (% of protein) was r = .50 indicating that both adjusted lysine values provide the same relative measure of lysine content of wheat protein.

Test weight had some influence on lysine content. This is due to bran proteins being much richer in lysine than endosperm proteins. Yield and kernel weight had no significant effect on lysine content after accounting for the effect of protein and test weight. Lysine values were adjusted for percent protein and test weight and compared over years. Much of the variation for adjusted lysine was due to genetic differences among the lines tested. Results indicate that significant genetic differences exist among wheats that are independent of percent protein and test weight. Comparison of the results of the Yuma nurseries with the results of the World Wheat Collection analyses indicate that adjusted lysine values are a useful selection criteria but unadjusted lysine values are not.

Section II. Protein and Lysine Content of Whole Grain, Endosperm, and Bran of *Triticum aestivum* L. em. Thell Lines Differing in Protein and Lysine Content.

INTRODUCTION

Wheat bran and germ are higher in protein and lysine content than the endosperm (MacMasters, Hinton, and Bradbury, 1971). Variability for protein and lysine content among wheats could be due to some wheats having a greater proportion of bran in their seed than other wheats. The results of Section I of this study, "Re-evaluation of Common Wheats of the USDA World Wheat Collection for Protein and Lysine Content", show that test weight influences the lysine content of whole grain samples. This indicates that variability for percent endosperm does contribute to lysine variability among wheats.

Many societies eat only the endosperm of wheat. It is necessary to determine if lysine variability among wheats is due to variation in the amount of endosperm or to variation in the lysine content of the endosperm. Most of the breeding and genetic research involving the protein and lysine content of wheat has been done using whole grain samples. Comparisons of the protein and lysine content of whole grain and endosperm samples would determine the validity of this practice. Wheats genetically superior in endosperm protein and lysine content would be most useful to wheat breeders in improving the nutritive quality of wheat.

Wheat endosperm is separated commercially from the bran and germ by milling. White flour is finely ground endosperm. It is impossible to obtain a complete separation of the endosperm from the bran by milling due to the anatomical structure of the wheat kernel. It is also difficult to obtain a uniform separation of bran and endosperm by milling in wheats varying in hardness especially where small lots of seed are involved. Wheats varying in kernel hardness mill differently. Hard wheats usually have higher milling yields than soft wheats.

Uniform separation of bran and endosperm is essential for endosperm protein and lysine analyses. The outer portion of wheat endosperm is much higher in protein than the interior of the kernel (McDermott and Pace, 1968). Protein and lysine variation in flour milled from different wheats could be due to differences in milling and not to differences among wheats for protein and lysine content of endosperm.

Various methods can be used to separate the endosperm from the bran (Mecklenburg et al., 1971). All have some disadvantages. The most precise method is to manually dissect the wheat kernel. This is extremely tedious and only small lots of seed can be analyzed in any reasonable period of time. The method used in this study is a modified milling procedure. After milling, the endosperm remnants adhering to the bran are removed by washing with a 80:20 (v/v) ethanol:acetone solution, dried, and added to the mill flour to reconstitute the endosperm.

Endosperm and bran samples of wheats differing in whole grain protein and lysine content were analyzed for protein and lysine. The wheats studied were from the USDA World Wheat Collection and the parents and progenies of three crosses of high protein and/or high lysine lines.

The purpose of this study was to identify the within-kernel site of protein and lysine variation among wheats and to determine if the protein and lysine content of wheat grain and endosperm can be improved by breeding.

LITERATURE REVIEW

The structure and composition of the wheat kernel has been studied extensively and the literature on the subject is voluminous. Comprehensive reviews are available. The most recent is by MacMasters, Hinton, and Bradbury. Bradbury et al. (1956a, b, c, d) made an extensive study of the anatomy of the wheat kernel. The wheat kernel is botanically classified as a caryopsis. The fruit coat or pericarp of the wheat caryopsis surrounds the seed. The seed consists of the endosperm and embryo or germ which are enclosed by the nucellar epidermis and seed coat. The endosperm consists of the aleurone layer and the starchy endosperm. The embryonic axis and the scuttelum (cotelydon) comprise the embryo. The bran of the flour miller consists of the pericarp, seed coat, nucellar epidermis, and the aleurone layer. The aleurone layer in wheat is one cell thick. Some starchy endosperm tissue adheres to the aleurone layer and forms part of the mill bran. Germ forms part of the bran in the initial milling steps but is usually separated from the bran in later steps of commercial milling operations (Kent, 1966a; Ziegler and Greer, 1971).

Milling applies a crushing and tearing stress to the wheat kernel (Ziegler and Greer, 1971). The bran (including the aleurone layer), because of its toughness and resiliancy, largely withstands the crushing and shearing force of the milling rolls. Because of its friability, the endosperm is sheared away from the bran and crushed. The germ is also more tough than the endosperm and is flattened by the milling rolls (Swanson, 1938, Ziegler and Greer, 1971). Bran and germ flakes are

removed from the crushed endosperm by sifting.

There is no natural cleavage line between the aleurone layer and the starchy endosperm (MacMasters et al., 1971). The amount of starchy endosperm removed with the bran in the milling process depends upon the friability of the endosperm. The endosperm of hard wheats is more brittle and less resilient than the endosperm of soft wheats. More endosperm is sheared away from the bran of hard wheats than soft wheats. There are natural lines of cleavage between the germ and starchy endosperm and germ and bran (MacMasters, et al., 1971). The germ breaks away cleanly from the endosperm in milling. Particles of germ may break off and be mixed into the flour by the milling process (Kent, 1966a).

Various methods have been used to separate the structural parts of the wheat kernel for chemical analyses. They include manual dissection of the untreated kernel, dissection after the kernel has been soaked in water, removal of unwanted parts by insect feeding, and by a combination of mechanical and chemical treatment of whole kernels and mill products (MacMasters et al., 1971). For methods other than manual dissection, there is some unavoidable contamination of one structural part with another. For methods involving chemical reagents there is also some alteration in the chemical composition. It is possible to analyze more numerous and larger lots of seed with non-dissection methods, thus reducing the sampling error. The results obtained depend on the method used, the varieties examined, and the environmental conditions under which they were grown. Reasonable agreement does exist among the published data.

The reported range values for percent of kernel contributed by the

main kernel components on hand-dissected samples are: bran (including aleurone layer), 10.4 to 21.4%, starchy endosperm, 74.9 to 86.5%, and germ, 2.5 to 3.8% (Hinton, 1953, 1959; MacMasters et al., 1971; Farrand and Hinton, 1974). The aleurone layer comprises slightly less than 50% of the bran. The results of the non-dissection methods are similar (MacMasters et al., 1971). Median range values would be: bran 13 to 17%, endosperm 73 to 84%, and germ 2.5 to 3.5%.

Part of the variation in bran and endosperm percent can be attributed to differences among wheats in the thickness of the bran layers. Bates (1943) found differences among hard red winter wheat varieties grown in the same nursery for bran layer thickness. He also reported that the percent of the cross-sectional area of wheat kernels occupied by the starchy endosperm varied with variety. The cross-sectional area occupied by starchy endosperm was 77.5% for the variety 'Tenmarq' and 69.9% for the variety 'Blackhull'. Tenmarq had large kernels and thin bran while Blackhull had small kernels and thick bran. Larkin et al. (1951) reported significant differences among Pacific Northwest wheat varieties for aleurone layer thickness. Differences in pericarp layer thickness were not as apparent. Crewe and Jones (1951), however, found no difference in mean bran thickness in nine wheats which represented a variety of kernel types.

Aleurone cells within the wheat kernel vary in thickness and the inner surface of the aleurone can be irregular. This irregularity may contribute to the difficulty of obtaining clean separation of the starchy endosperm from the bran by milling (MacMasters, et al., 1971; Crewe and Jones, 1951). Variation also exists among wheats in the thickness of the

aleurone cell walls and the amount of space occupied by the aleurone cell contents (Cobb, 1905). Cobb (1905) reported that the amount of space occupied by the aleurone cell contents varied from about 55 to 78% of the area of a radial section among 50 Australian wheat varieties. This variation in aleurone cell space does not effect the proportion of the kernel that is bran but it probably contributes to differences among wheats in the chemical composition of wheat bran.

The percentage of endosperm of wheats is influenced by the environment in which they are grown. Evidence for this is based on milling yields, test weight, and kernel weights of wheats grown in different environments. Literature pertaining to the relationship of endosperm percent of sample and milling yield, test weight, and kernel weight is discussed in Section I of this study. Some of the environmental factors that can influence test weight, kernel weight, and milling yield are the presence or absence of diseases, lodging, variation in available moisture, the time period between the formation and ripening of the kernels, and the temperature during ripening (Leonard and Martin, 1963). Although kernel weight and test weight of a wheat variety are influenced by environment, wheat varieties do differ genetically for kernel size (Ausemus, McNeal, and Schmidt, 1967) and, thus, also for percent endosperm.

The protein and lysine content of the bran and germ are higher in both protein and lysine content than the endosperm. This has been established by protein and lysine analysis of the products of milling and by analyses of dissected samples.

The protein and lysine content of the mill products of the major commercial classes of wheat grown in the United States are reported in

the Millfeed Manual (1967). The ranges in protein percentages are: whole wheat 9.2 to 13.8%, bran 13.3 to 16.9%, germ 23.9 to 27.0%, and white flour 8.35 to 12.65%. The reported range values for lysine (% of protein) are: whole wheat, 2.25 to 3.17%, bran, 3.55 to 4.44%; germ, 5.37 to 7.40%, and flour, 1.63 to 2.29%. Bran and germ protein percentages were calculated as %N x 6.25; wheat and flour protein percentages were calculated as %N x 5.7. The extraction rate for the wheats studied ranged from 72.5% to 77.0%. Similar results have been reported elsewhere (Baumgarten, Mather, and Stone, 1946; Waggle et al., 1967) for the protein and lysine content of the bran and germ. White flour is lower in protein and lysine content than the wheat from which it was milled.

Hepburn, Calhoun and Bradley (1960) have reported that there is less lysine, glycine, arginine, alanine, and aspartic acid in flour protein but more in the millfeed protein than in whole wheat protein. There is more glutamic acid, proline, and phenylalanine in flour protein but less in the millfeed protein than in wheat protein. Pomeranz, Finney, and Rubenthaler (1966) and Hepburn, Lewis, and Elvehjem (1957) reported similar results. These results can be easily explained if the endosperm and non-endosperm portions of the wheat kernel contain different proteins or different proportions of the same proteins. Teller and Teller (1932) have shown that there are relatively more albumins, globulins, and glutenins in bran than in flour. Gliaden nitrogen often comprises half of the total nitrogen in the endosperm, but, in the bran, it is not more than one-eighth of the total nitrogen (Teller and Teller, 1932). Albumins and globulins are higher in lysine content than the gliadens and glutenins (Simmonds, 1962, Mattern, Salem, and Volkmer, 1968). Gliadens have

lower lysine contents than the other wheat proteins.

Farrand and Hinton (1974) hand dissected two varieties of wheat and determined the nitrogen content of each component. The ranges in protein content (%N x 5.7) for the kernel components were: embryo, 33.2 to 34.0%, scuttlelum, 27.4 to 28.9%; pericarp-testa, 4.7 to 6.2%; aleurone layer, 23.1 to 25.5%, starchy endosperm, 7.1 to 11.8%. The range values for nitrogen expressed as percent of total nitrogen in grain were: embryo, 3.0 to 4.6%; scuttlelum, 3.7 to 4.7%; pericarp-testa, 3.1 to 4.2%; aleurone layer, 10.2 to 13.9%; and starchy endosperm 72.6 to 79.2%. Morris, Alexander, and Pascoe (1946) have reported similar results for the protein content of bran and endosperm. The protein values for germ reported by Morris et al. (1946) ranged from 24.3 to 26.8%. The above authors did not determine the lysine content of the dissected kernel components.

Kernel component lysine values from hand-dissected samples are not available. Stevens, McDermott, and Pace (1963) isolated aleurone cell contents using the techniques of air classification and differential centrifugation in non-aqueous media. The isolated material had 5.45% nitrogen (31.5% protein) and 5.0% lysine expressed as a percent of nitrogen. Those values are probably high because only the aleurone cell contents and not the entire cells were analyzed. These results and the values of the dissected samples indicate that the high protein and lysine content of wheat bran (excluding the germ) is due to the high protein and lysine content of the aleurone layer. MacMasters et al. (1971) have estimated that about 50% of the total lysine in the wheat kernel is located in the starchy endosperm and about 30% is located in the aleurone layer.

There is a protein gradient in the endosperm of wheat. The outer portions of the endosperm are higher in protein content than the interior of the kernel. This has been demonstrated by researchers using a variety of methods. Farrand and Hinton (1974) peripherally dissected the endosperm of two varieties of wheat. The outer layers were significantly higher in protein than the inner layers. Farrand and Hinton (1974) reported that the protein gradient between the inner and outer endosperm was correlated with the protein content of the wheat. The higher the protein content of the wheat, the steeper was the gradient. McDermott and Pace (1960), Morris et al. (1946, 1945) have obtained similar results using dissection procedures. Normand, Hogan, and Diebold (1965) using a tangential abrasion process showed that the protein content of the endosperm decreased as the outer layers were ground off. Pomeranz and Shellenberger (1961) used histochemical methods to map the distribution of protein within the wheat kernel. The outer endosperm portions showed a higher dye-binding capacity, indicating a higher protein content than the inner portions of the kernel. In flour milling, the protein content usually increases from the first to the last break flour reflecting the protein gradient within the endosperm (Ziegler and Greer, 1971).

Since lysine is a component of protein, it would be expected that there is also a lysine gradient within the wheat endosperm. McDermott and Pace (1960) used a micro drilling technique to obtain samples of vitreous, intermediate, and mealy endosperm from single grains of the same wheat variety, 'Hybrid 46'. Mealy portions were located in the interior of the kernels and vitreous portions in the exterior of the kernels. The vitreous endosperm had the lowest lysine content while the

mealy endosperm had the highest lysine content expressed as a percent of protein. Endosperm intermediate in hardness was intermediate in protein and lysine content. These results indicate that the gradient for lysine (% of protein) is opposite that of percent protein.

Subaleurone endosperm or peripheral endosperm cells are a single row of cells just inside the aleurone layer (MacMasters et al., 1971). Kent and Evers (1969) obtained particles of these subaleurone cells by differential sedimentation in mixtures of carbon tetrachloride and benzene. The protein content of these subaleurone cell particles was 55.5% and the lysine (% of protein) content was 1.59%. The protein content of cell particles from the inner endosperm was 9.5% and the lysine (% of protein) content was 2.51%. Although whole cells were not analyzed, these results suggest that the proteins in the extreme exterior of wheat endosperm are lower in lysine content than the proteins in the interior of the endosperm.

Fulcher, O'Brien, and Simmonds, (1972) used the Sakaguchi reaction to map the distribution of proteins rich in arginine in the seeds of wheat and other cereals. In the wheat, the protein bodies of the aleurone layer, scutellar parenchma, and scutellar epithelium were intensely stained by the Sakaguchi reaction. Wheat endosperm was largely unstained but did contain a few distinct regions intensely stained by the Sakaguchi reaction particularly in the subaleurone layer. The inclusions in the subaleurone cells that stained intensely for arginine resembled small protein bodies or were thin strands of material resembling the endoplasmic reticulum in appearance. Large areas of the subaleurone cells were not stained by Sakaguchi reaction.

The proteins of wheat that are high in arginine content are also high in lysine content. These are the albumins and globulins (Simmonds, 1962, Mattern et al., 1968). The protein bodies of the aleurone layer, scutellum, and the inclusions of the subaleurone layer that are intensely stained by the Sakaguchi reaction probably consist mostly of albumin and globulin proteins. Teller and Teller (1932) have demonstrated that bran proteins are higher in albumins and globulins than endosperm proteins. The inclusions in the subaleurone cells that stain positively for arginine also are probably high in lysine content.

Horn et al. (1958) reported a higher protein and lysine (% of sample) content in an 84.9% extraction flour than in a 71.5% extraction flour. Both flours were milled from the same sample of wheat. Although not reported, lysine (% of protein) values can be computed using the protein and lysine (% of sample) values of Horn et al. (1958). Lysine (% of protein) values are higher for the 84.9% than for the 71.5% extraction flour. These results indicate that either the outer portions of the starchy endosperm contain proteins higher in lysine content than the proteins found in the interior of the kernel or that the 84.9% extraction flour contains some bran. Ziegler and Greer (1971) state that if the flour yield exceeds 80% in ordinary milling, that the flour contains some non-endosperm particles.

The effect of protein content on the lysine content of wheats and flours differing in protein content is discussed in Section I. In brief, lysine (% of sample) is positively correlated and lysine (% of protein) is negatively correlated with percent protein. The results of MacDermott and Pace (1960) and Kent and Evers (1969) indicate that this relationship

also exists within the endosperm of a kernel of wheat. The results of Fulcher et al. (1972) and Horn et al. (1958), however, suggest that the subaleurone cells may contain proteins high in lysine content.

Some of the mechanical and chemical methods that have been used to separate and isolate components of the wheat kernel have been mentioned (Pomeranz and Evers, 1969, Stevens et al., 1963). Shetlar et al. (1947) used mechanical and chemical treatments to separate the bran layers of wheat. In some respects, their methods are similar to the method used in this study. They removed the epidermis by suspending the wheat in water and stirring with a blender. The dried peeled wheat was soaked in a 95% ethanol solution nearly saturated with sodium hydroxide. After soaking, the supernatant liquid was poured off. The testa layer was then removed by stirring the grain in a 95% ethanol solution with a blender. The debranned wheat was dried and then milled to obtain samples of endosperm and aleurone layer. No attempt was made to remove the endosperm adhering to the aleurone layer. Instead, corrections were made to the results of the chemical analyses of the aleurone layer based upon its starch content.

The method used in this study to separate endosperm from mill bran involved washing bran with a 80:20 (v/v) ethanol:acetone solution. Wheat gliadens are the only wheat proteins that are soluble in strong alcohol solutions (Osborne, 1907). Gliadens dissolve in dilute alcohol solutions. Their solubility in alcohol solutions increases with increasing concentration of alcohol until a maximum solubility is reached at about 70% alcohol, then solubility decreases (Osborne, 1907). Wheat gliaden is wholly insoluble in absolute ethanol.

Lipoproteins are present in small amounts in wheat flour and they

are soluble in acetone. The amount of ether-extractable lipoproteins in flour of common wheats ranges from 20 to 78 mg per 100 g of flour (Mecham, 1971). The lipid content of mill bran ranges from 3.1 to 6.25% depending on the solvent used (MacMasters et al., 1971). Mill bran contains six percent acetone extractable lipids (MacMasters et al., 1971).

Parents and progenies of three crosses were analyzed in this study. The crosses were 'Nap Hal' x 'Atlas 66', 'Nap Hal' x 'CI 13449', and 'Nap Hal' x 'CIMMYT 8156'. Nap Hal, PI 176217, is a soft red wheat of spring type. It is an old Indian variety that was first identified as a high protein wheat by Watson et al. (1966) and as a high lysine line by Johnson, Mattern and Schmidt (1971). Atlas 66, CI 12561, is a high protein soft red winter wheat that was developed at North Carolina State University where it was first identified as a high protein line (Middleton, Bode, and Bayles, 1954). It has been used extensively in breeding programs as a high protein parent (see Literature Review in Section I). CI 13449 had the highest adjusted lysine value of any wheat in the USDA World Wheat Collection (Vogel et al., 1973). It is a low protein, soft white semi-dwarf wheat selected from a Norin 10-Brevor x Brevar sib cross (Johnson and Mattern, 1972a). CIMMYT 8156 is a semi-dwarf red spring wheat from the CIMMYT program in Mexico. It is neither a high protein or high lysine line. It was crossed with Nap Hal because of superior agronomic traits.

Whole grain protein and lysine content of the parents and progenies of the cross Nap Hal x Atlas 66 have been reported (Johnson et al., 1972a, c, 1973b). F_2 progeny bulk rows were grown in the F_3 and F_4 generation at Yuma, Arizona in 1971 and 1972 respectively. Significant transgressive

segregation existed for both low and high protein which was attributed to different genes in Nap Hal and Atlas 66 for high protein. The Nap Hal x Atlas 66 progeny segregated for lysine (% of protein) but there was no evidence of transgressive segregation. The progeny population mean in each year approximated the mid-parent value. Some of the progeny bulk rows were equal to Nap Hal in lysine content.

Grain protein and lysine (% of protein) values for the parents and F_2 progeny bulk rows of the Nap Hal x CI 13449 cross have also been reported (Johnson et al., 1973b). F_2 progeny bulk rows were grown in the F_3 generation at Yuma, Arizona in 1972. Nap Hal had means of 20.2% protein and 3.23% adjusted lysine (% of protein). CI 13449 had means of 15.5% protein and 3.35% adjusted lysine (% of protein). The progeny bulk population mean for percent protein was 16.7% which suggest partial dominance for low protein. No progeny row had a protein value greater than the Nap Hal mean protein percent. Eleven percent of the progeny bulk rows had adjusted lysine levels outside the parent range indicating transgressive segregation for lysine (% of protein). The F_2 progeny bulk population mean for adjusted lysine (% of protein) approximated the mid-parent value.

MATERIALS AND METHODS

Materials

The wheats used in this study were grown at the University of Arizona experimental station at Yuma, Arizona in the 1972-1973 (1973 nursery) growing season. The World Collection wheats analyzed were the wheats in the 1973 World Wheat Collection Special Study nursery that were in either the top or bottom 50 for percent protein, lysine (% of protein), lysine (% of protein) adjusted for percent protein, or for lysine (% of protein) adjusted for percent protein and test weight. Some of the wheats were in more than one of these categories. Including the check entries, 155 samples were analyzed for whole grain, starchy endosperm, and bran protein and lysine content. There were six check varieties replicated four times. The 1973 World Wheat Collection Special Study nursery is described in detail in Section I.

Heads were selected from Nap Hal x Atlas 66 F_2 progeny bulk rows grown in the F_4 generation and from Nap Hal x CI 13449 and Nap Hal x CIMMYT 6156 F_2 progeny bulk rows grown in the F_3 generation at Yuma, Arizona in 1972. Heads were selected at random from the bulk rows. Seed from the selected heads was planted in 1.37m (4.5 ft) rows spaced 51cm (20 in.) apart in the 1973 Yuma head row nursery. Head rows from a progeny bulk were planted in the same block. Parents and the check variety 'Centurk' were planted at the ends of each block of rows. The head row nursery was fertilized with 112.1 kg/hl (100lb/A) of nitrogen and was irrigated.

Head rows were selected for harvest on the basis of agronomic type

and the protein and lysine content of their parent F_2 progeny bulk row in the previous year. Selected head rows were cut by hand and threshed using a Vogel-type plot thresher. Whole grain protein and lysine content was determined on all the harvested rows.

Wheats that represented the range of grain protein and lysine values existing among the head rows for a particular cross were selected for endosperm and bran protein and lysine analyses. Ninety-four rows were selected from the Nap Hal/Atlas 66 population, including three rows each of the parent and check varieties. Sixty-four rows were selected from the Nap Hal/CI 13449 population and 18 from the Nap Hal/CIMMYT 8156 population. Two rows each of the parent and check varieties were included in the latter groups. CI 13449 rows in the head row nursery contained a mixture of wheats due to accidental contamination of the seed source used for planting. These rows were not analyzed. CI 13449 seed from another plot in the 1973 Yuma nursery was used instead.

Large field plots of Atlas 66, Nap Hal, Centurk, and Bezostayia were grown at Yuma in 1973 for nutrition studies. Large lots of these wheats were milled on the Kansas State University pilot mill. Centurk and Bezostayia were milled at a 70% extraction rate. The soft wheats, Atlas 66 and Nap Hal, did not yield 70% white flour. The millfeeds, excluding the mill bran, of Nap Hal and Atlas 66 were pin milled. Flour from the pin milled samples was added to the mill flour in sufficient quantity to obtain 70% extraction flour samples for these wheats. Grain 70% extraction flour, and mill bran protein and lysine data for Atlas 66, Nap Hal, Centurk, and Bezostayia and samples of their mill bran were obtained from Professor P. J. Mattern for use in this study.

Methods

Laboratory Procedures

A modified milling procedure was used in this study to obtain endosperm samples. Average moisture content of the whole grain samples was determined. Twenty gram samples of wheat were then tempered to 14.5% H_2O 24 hours before milling. The tempered wheat samples were milled on a Brabender Quadraplex experimental mill. This mill and its operation is described by Shellenberger and Ward (1967). The sifter on the mill was removed. Bran and ground endosperm were collected in the same tray. The bran was then sifted from the mill flour by sifting for 90 seconds on a mechanical shaker using standard sieves.

A U.S. Standard Testing Sieve No. 35 was used to sift bran from flour for the head row samples and a No. 50 sieve was used for the World Wheat Collection samples. Mesh openings for the No. 35 and No. 50 sieves are 500 and 297 microns respectively. The head row samples were processed first. There was a small amount of bran contamination in the flours of the hard wheats, Centurk and CIMMYT 8156, after sifting with the No. 35 sieve. The other head row samples were relatively soft wheats and there was little bran contamination of the flours of these wheats. The smaller mesh sieve was used for the World Collection wheats because of the possibility of bran contamination of flours of hard wheats sifted on the larger mesh sieve. The mill flour and mill bran were weighed after sifting. Milling yield was calculated as follows: Milling yield = (mill flour wt./mill flour wt. + mill bran wt.) x 100.

The method of washing the bran with an alcohol:acetone solution was developed by Gene W. Lenser of Con Agra, Inc. It was obtained from

him by personal communication via Prof. P. J. Mattern. The method used in this study is basically Lenser's procedure with some minor modifications.

The washing solution was a 80:20 (v./v.) absolute ethanol:acetone solution. All of the bran obtained from milling a 20g sample of wheat was placed in a small Waring blender jar. Fifty ml. of the washing solution was added and the mixture was stirred for one minute at slow speed on a Waring blender. The endosperm adhering to the bran is washed off and is suspended in the washing solution. After stirring the flour suspension was decanted into a beaker. A piece of a 70GG silk sifting screen (screen opening = 236 microns) was fitted over the top of the beaker to strain out bran particles. Fifty ml. of the washing solution was added to the bran in the blender jar and the process was repeated. After decanting the flour suspension, the bran was washed out of the blender jar with the washing solution onto the 70GG screen.

The screen and washed bran were placed on a watch glass and dried in a 55°C oven for 12 hours. Suspended endosperm particles were filtered from the washing solution using vacuum filtration. A Gilman type E fiber glass filter was used. The bran flour and filter were placed on a watch glass and dried for 12 hours in a 55°C oven.

After drying, the bran flour was removed from the filter with a small spatula and then ground with mortar and pestle. The ground bran flour and mill flour samples were placed in a humidity controlled cabinet for 72 hours to bring all the samples to a uniform moisture level. The samples were then weighed and their average moisture content determined. Sample dry weights were calculated using the average moisture percentage.

After weighing, the bran flour was added to the mill flour to reconstitute the endosperm. Samples were thoroughly blended by shaking.

After drying, the head row nursery bran samples were ground using a Udy Cyclone Sample Mill. The ground bran samples were then placed in the humidity cabinet for 72 hours and weighed. Sample dry weights were calculated using the average moisture content of the samples. Some loss of bran sample occurred during grinding. Because of the grinding loss, the procedure was altered for the bran samples of the World Collection wheats. These bran samples were placed in a humidity controlled cabinet for 72 hours, weighed and then ground.

The reconstituted endosperm samples and the ground bran samples were then analyzed for protein and lysine content using the procedures described in Section I. The bran samples from the wheats milled at Kansas State University also were washed. The washed bran and bran flour obtained from these bran samples were analyzed for protein and lysine content. All protein percentages were calculated as %N x 5.7. Laboratory variability for percent protein is ±0.2 of a percentage point. Laboratory variability for lysine (% of sample) is ±.01 of a percentage point.

Endosperm percentages, sample recovery percentages, and other pertinent data were calculated using the equations listed below. Bran refers to washed bran samples and the abbreviation, dwt, indicates dry weight. Kernel component weights, and protein and lysine (% of sample) percentages were used to calculate the amount (grams) of protein and lysine in the endosperm and bran.

1. Endosperm (dwt) = mill flour (dwt) + bran flour (dwt)

2. Sample recovery % = $\frac{\text{endosperm (dwt) + bran (dwt)}}{\text{grain (dwt)}} \times 100$

3. % endosperm of sample = $\frac{\text{endosperm (dwt)}}{\text{endosperm (dwt) + bran (dwt)}} \times 100$

4. Calculated grain protein % = $\frac{\text{(endosperm protein (g)+bran protein (g))}}{\text{endosperm (dwt) + bran (dwt)}} \times 100$

5. Calculated grain lysine (% of sample) = $\frac{\text{(endosperm lysine (g) + bran lysine (g))}}{\text{endosperm (dwt) + bran (dwt)}} \times 100$

6. % of kernel protein in endosperm = $\frac{\text{(endosperm protein (g)}}{\text{endosperm protein (g) + bran protein (g)}}) \times 100$

7. % of kernel lysine in endosperm = $\frac{\text{(endosperm lysine (g)}}{\text{endosperm lysine (g) + bran lysine (g)}}) \times 100$

Statistical Methods

Statistical procedures and interpretations are based on standard methods detailed by Snedecor and Cochran (1967) and Steel and Torrie (1960). Computer analyses using the Statistical Analysis System (SAS) programs (Service, 1972) were used extensively.

Means, ranges, and standard deviations were calculated for most of the variables. Simple correlation coefficients were calculated for grain % protein, endosperm % protein, bran % protein, grain lysine, endosperm lysine, bran lysine, and % endosperm to provide a measure of the degree of association of these variables with each other. Correlations involving other variables also were calculated.

Regression analysis was used to determine more precisely the relationship of protein and lysine in whole grain, endosperm, and bran samples. Separate regression analyses were calculated for the World Collection wheats, the Nap Hal/Atlas 66, Nap Hal/CI 13449, and the Nap Hal/CIMMYT 8156 head rows. Multiple regression analyses were

used to determine the effect of endosperm and bran protein and lysine content and percent endosperm on whole grain protein and lysine content. The regression model chosen to represent a particular relationship was selected on the basis of a significant F test, significant regression coefficients using the "t" test, and with those criteria met, a maximum coefficient of determination.

World Wheat Collection grain, endosperm, and bran lysine values were adjusted to population mean protein levels. Lysine values of the Nap Hal/Atlas 66 and Nap Hal/CI 13449 samples were adjusted to mid-parent protein levels using the regression coefficients calculated in the regression analysis. As an example, the equation used for adjusting endosperm lysine (% of protein) to the mid-parent protein level was:

$$Y_i \text{ adj} = Y_i - b\ (X_i - MPX).$$

The b value is the regression coefficient, Yi, the measured lysine (% of protein) of the ith sample, Xi, the protein content of the ith sample, and MPX, the mid-parent protein value.

World Wheat Collection grain lysine (% of protein) values were also adjusted for percent protein and % endosperm of sample and for percent protein and test weight. The latter adjustment was calculated using the adjustment equation developed in Section I for the 1973 Yuma World Wheat Collection nursery. Correlations between the various adjusted lysine values also were calculated.

Standard partial regression coefficients were calculated for some of the regression equations. Standard partial regression coefficients give an indication of the relative importance of the independent variables (Steel and Torrie, 1960).

Most of the wheats analyzed were not replicated. The parent and check varieties were replicated in the head row nursery. The 1973 World Wheat Collection nursery was an augmented randomized complete block design with four blocks. Check varieties were planted in each block. The parent and check varieties of the head row nursery and the check varieties of the World Collection Special Study nursery were analyzed using analysis of variance procedures to obtain estimates of experimental error and the standard error of a treatment mean. The World Collection check varieties were analyzed using analysis of variance procedures for the randomized complete block design. The head row nursery checks were analyzed using procedures for a completely randomized design.

RESULTS

Kernel Fractionation

Four wheat varieties were grown in large field plots at Yuma, Arizona in 1973. Grain from these wheats was milled on the Kansas State University pilot mill. Grain, 70% extraction flour, mill bran, washed bran, and bran flour protein and lysine (% of protein) values for these wheats are listed in Table 1. Mill bran is higher in protein and lysine content than whole grain samples. Grain samples are higher in protein and lysine than 70% extraction flour. Lysine differences between grain and flour samples are of greater magnitude than the protein differences. The bran flour samples are much higher in protein content than the washed bran samples, but they are lower in lysine (% of protein) content. The bran flour samples are considerably higher in both protein and lysine content than the 70% extraction flours.

Atlas 66 and Nap Hal have significantly higher protein content than Centurk or Bezostayra in both grain and 70% extraction flour. The grain lysine (% of protein) percentage of Nap Hal is higher than the grain lysine (% of protein) values of the other wheats. Lysine (% of protein) of Nap Hal 70% extraction flour is only slightly higher than the flour lysine (% of protein) percentages of the other wheats.

Miller's terminology is used in the results and discussion. The term endosperm refers to the starchy endosperm. Morphologically, the aleurone layer is also endosperm tissue. The term bran refers to the non-starchy endosperm portions of the wheat kernel.

Table 1. Whole grain, 70% extraction flour, bran, bran flour, and washed bran protein and lysine (% of protein) percentages for four cultivars grown at Yuma, Arizona in 1973.

Variables	Cultivars			
	Atlas 66	Centurk	Nap Hal	Bezostaya
Whole grain % protein	17.2	15.0	18.5	13.2
Whole grain lysine (% of protein)	2.86	2.90	3.05	2.80
70% extraction flour				
" % protein	17.2	12.4	16.5	11.1
" lysine (% of protein)	2.12	2.18	2.20	1.99
Bran Flour % protein	26.6	26.4	28.3	23.2
Bran Flour % lysine (% of protein)	3.31	4.22	3.12	4.12
Bran % protein	19.3	18.9	23.9	16.10
Bran lysine (% of protein)	4.00	4.46	4.04	4.57
Washed bran % protein	18.0	17.8	23.3	14.2
Washed bran lysine (% of protein)	4.38	4.67	4.71	4.85

Figure 1 is a photograph of the bran of the hard red winter wheat, Scout 66, before and after washing. Figure 2 is a photograph of the bran of the soft winter wheat Atlas 66. The bran samples were obtained by milling 20g samples from check rows in the World Collection nursery. There is more endosperm adhering to the mill bran of Atlas 66 than to the mill bran of Scout 66. After washing there is no visible difference between the brans of the two wheats for endosperm content. Although the washed bran samples of both wheats still contain some endosperm, both are cleaner than before washing. The average endosperm content in the World Collection nursery on a weight basis was 40.0% for Atlas 66 bran and 30.4% for Scout 66 bran. These photographs show that it is possible to obtain a more uniform separation of bran and endosperm among wheats differing in milling quality by using the bran washing procedure than by simply milling the wheats.

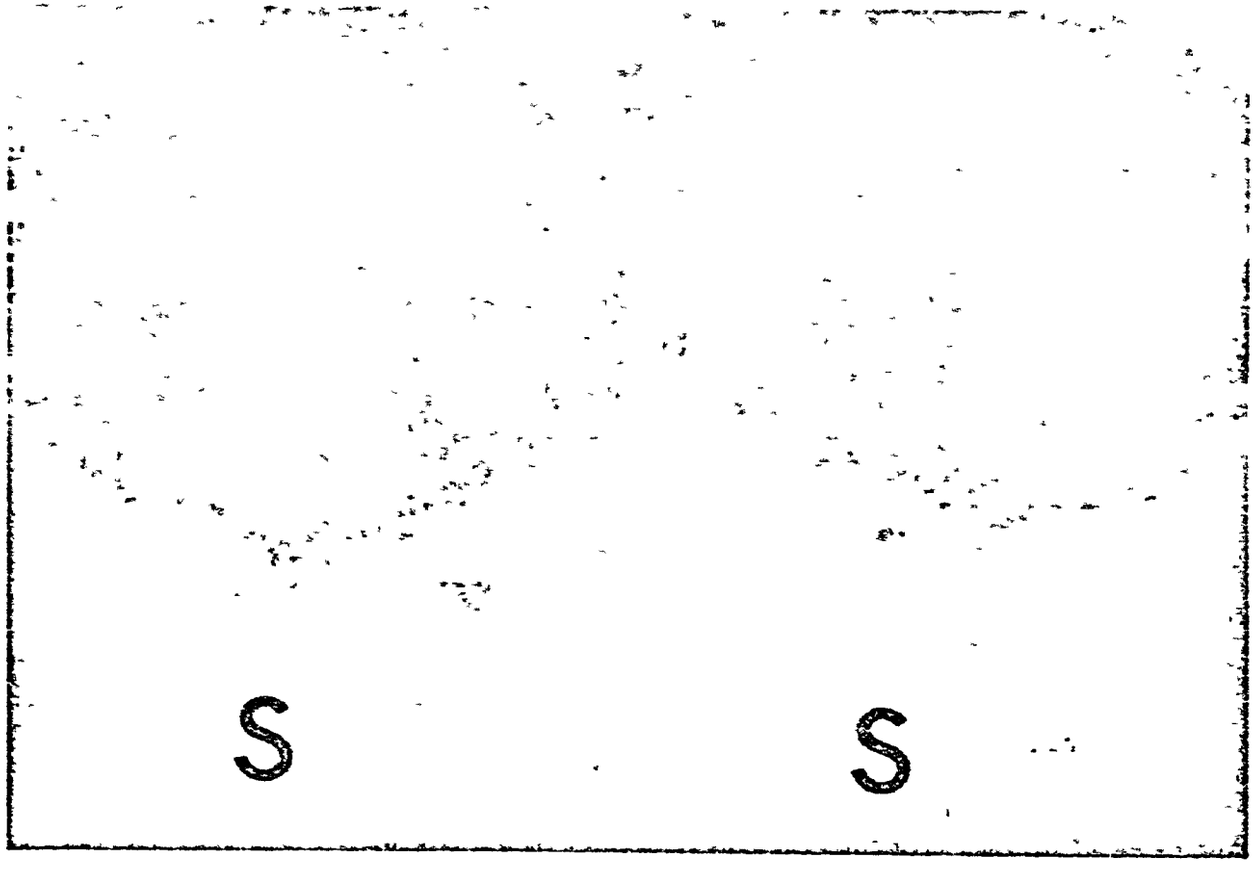

Figure 1. Bran of the hard red winter wheat 'Scout 66' before (left) and after (right) washing with an 80:20 ethanol:acetone solution.

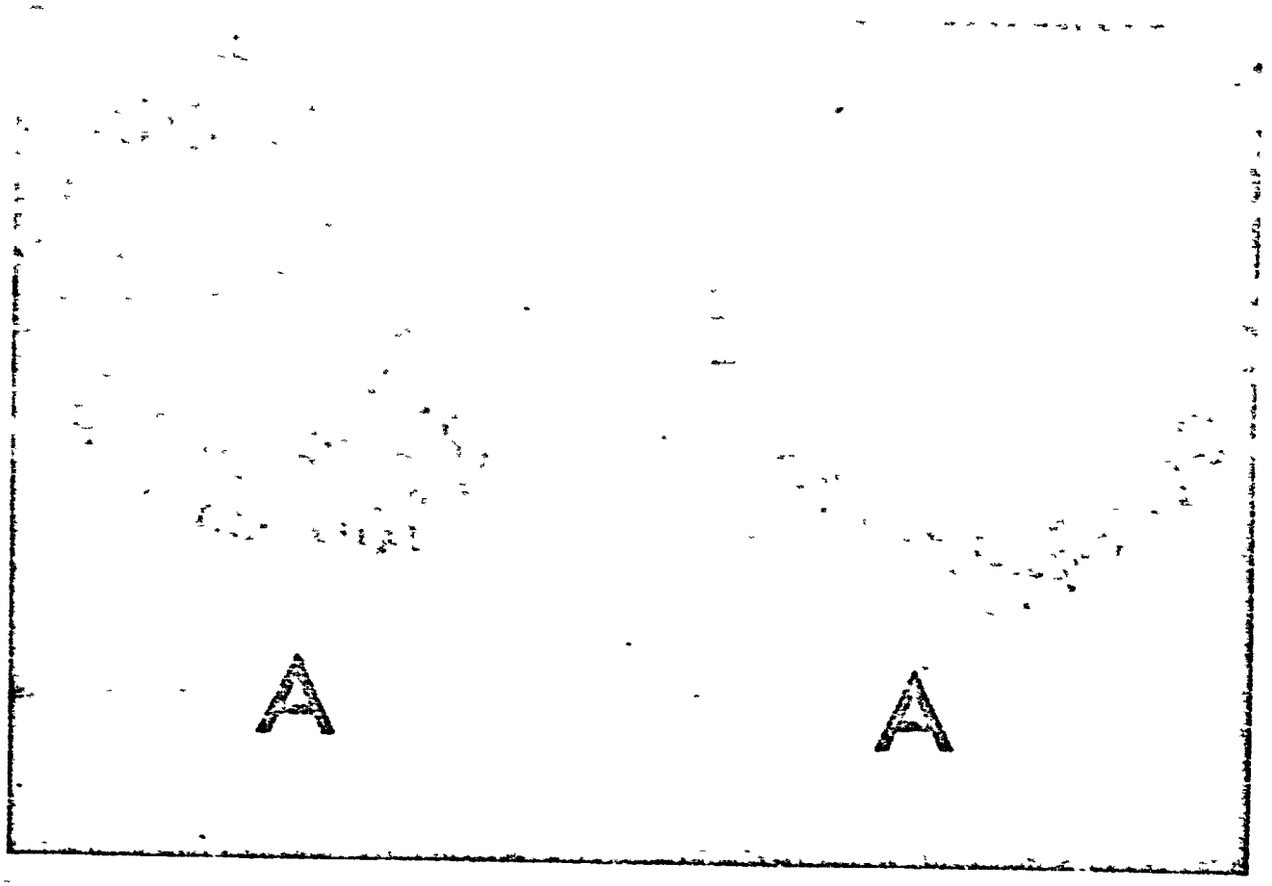

Figure 2. Bran of the soft red winter wheat 'Atlas 66' before (left) and after (right) washing with an 80:20 ethanol:acetone solution.

Examination of the washed bran samples with a dissecting microscope revealed that the bran, including the aleurone layer, was still largely intact even though pieces of washed bran are smaller than pieces of mill bran. It is possible that very small pieces of bran or germ broke off in the washing process and went into suspension with the endosperm particles and were separated with the endosperm. If this did occur, it was to a very limited extent only. The bran flour did not contain any visible bran contamination. In a simple milling and sifting operation such as the one used in this study, almost all of the germ remains with the bran.

Means, ranges, and standard deviations for milling yield, flour weight, bran flour weight, bran weight, and endosperm and sample recovery percentages are listed in Table 2 for the wheat samples fractionated from the head row and World Collection nurseries. The mean milling yield was higher for the head row nursery than for the World Collection nursery but the amount of flour removed from the bran was smaller. These differences can be attributed to the different sieve sizes used in sifting flour for the two groups of wheats from the bran.

The mean percent endosperm of sample for the head row samples was larger than that of the World Collection wheats. The mean sample recovery percent of the World Collection wheats was higher than that of the head rows. The head row bran samples were ground before weighing and some grinding loss occurred. This reduced the sample recovery percentage and resulted in the higher endosperm percentage for the head row samples. In comparison to the World Collection fractionation results, the endosperm percentages of the head row samples are inflated about 2 to 3 percentage

Table 2. Mean, range, and standard deviations for kernel components and kernel recovery percentages from the fractionation of 20g samples of 176 rows from the 1973 Yuma head row nursery and for 153 rows from the 1973 World Wheat Collection Special Study nursery.

Variables	Mean	Range	Standard deviation
1973 Yuma head row nursery*			
100 kernel wt (g)	3.04	2.17- 3.95	.405
Milling yield (%)	75.1	70.7 -83.2	2.60
Flour wt (g) +	13.73	12.82-15.89	.517
Bran flour wt (g) +	1.26	.52- 1.77	.232
Washed bran wt (g) +	2.80	2.06- 3.54	.266
% endosperm	84.2	80.0 -88.5	1.54
Sample recovery % ≠	96.8	94.3 -99.2	1.16
1973 World Wheat Collection Special Study nursery**			
Test weight (g/hl)	77.89	70.95-83.85	3.279
1000 kernel wt (g)	40.63	24.95-57.94	7.351
Milling yield (%)	71.2	63.1 -78.8	3.18
Flour wt (g) +	1.60	.92- 2.50	.390
Washed bran wt (g) +	3.15	2.56- 4.77	.309
% endosperm	81.9	72.8 -85.5	1.77
Sample recovery %	98.1	96.3 -99.2	.50

* Average dry weight of whole grain samples = 18.38g

** Average dry weight of whole grain samples = 17.79g

\+ Dry weight basis

≠ Excluding rows 15,812 and 19,046 which had sample recovery percentages of 107.0 and 102.2% respectively.

points. The two sample recovery percentages of more than 100% are due to more than 20g of sample having been milled.

The ranges and standard deviations for percent endosperm of sample are smaller than those of milling yield. The range in percent endosperm of sample for the World Collection wheats was 72.8% to 85.5%.

Means and the results of the analysis of variance of the parent and check rows of the head row nursery and the checks of the World Collection nursery for kernel weight, milling yield, flour weight, bran flour weight, washed bran weight, sample recovery percent and percent endosperm are listed in Tables 3 and 4 respectively. The parent and check varieties differ significantly for all factors except for sample recovery percent. In the World Collection nursery, replications had a small effect on all variables except for sample recovery percent. Sample recovery percent was independent of the wheat variety processed. Coefficients of variation were small for all variables.

The parent and check varieties Centurk, Nap Hal, and Atlas 66 were in both nurseries. The endosperm percentages for these wheats are higher in the head row nursery than in the World Collection nursery for the reason explained previously. However, the relationship of these varieties to each other within a nursery is the same. Centurk is 3 to 4 percentage points higher than Atlas 66 and Nap Hal is about one percentage point higher than Atlas 66 in endosperm percent of sample.

There are large differences among the wheats for milling yield that are almost entirely due to kernel texture. In the World Collection nursery, the mean milling yield of Triumph 64, a hard red winter wheat, is eight percentage points greater than that of Atlas 66. Triumph 64,

Table 3. Means, coefficients of variation, F ratios, and LSD values for kernel components and kernel recovery percentages for check cultivars in the 1973 World Wheat collection special study nursery.+

Variable	Mean of check cultivars [illegible]						CV %	F ratio for cultivars	F ratio for replications	LSD.05
	Atlas 66	[illegible]	[illegible] Scout 66	[illegible] 66	Centurk	[illegible]				
1000-kernel wt.	34.7	40.1	42.2	50.5	36.0	26.4	3.56	140.0**	3.31*	2.06
Milling yield %	65.8	74.2	76.3	75.9	74.5	68.8	.57	419.0[illegible]**	4.47*	0.63
Flour wt. (g)	11.74	13.22	13.62	13.57	13.25	12.27	.79	225.41**	3.21*	0.15
Bran Flour wt (g)	2.26	1.19	1.18	1.08	1.30	1.86	4.69	185.43**	11.20**	0.10
Washed bran wt (g)	3.37	3.04	2.70	2.87	2.88	3.27	2.65	40.52**	3.20*	0.12
% endosperm	80.59	82.56	84.56	83.63	83.44	81.23	.55	44.42**	3.17*	0.68
Sample recovery %	97.67	98.11	98.43	98.50	98.02	97.82	.54	1.53	1.31	0.80

*,** Indicates significance at the .05 and .01 levels respectively.
+ Average dry weight of whole grain samples fractionated = 17.79 g.

Table 4. Means, coefficients of variation, F ratios, and error variances from the analysis of variance for kernel components and kernel recovery percentages for parent and check cultivars in the 1973 Yield Head Row nursery

Variable	Mean of parent and check cultivars					Coefficient of variation %	F ratio	Error mean square
	Centurk n=7	Atlas 66 n=3	Nap Hal n=7	CI 13[illegible]9 n=2	CI [illegible] 8156 n=2			
100 kernel wt.	3.61	3.63	2.62	2.21	3.92	3.90	113.6**	.0154
Milling yield %	81.8	74.3	75.5	72.0	82.1	1.43	58.3**	1.2380
Flour wt. (g)	14.96	13.57	13.75	13.15	15.47	1.94	39.4**	.0764
Bran flour wt.	.68	1.20	1.20	1.63	.55	9.78	60.1**	.0097
Washed bran wt.	2.25	3.01	2.76	3.02	2.32	5.76	22.73**	.0226
Sample recovery %	97.37	96.53	96.41	97.06	98.02	.78	2.74	.5539
% endosperm	87.4	83.1	84.4	83.0	87.4	1.03	22.73**	.7766

*,** Indicates significance at the .05 and .01 levels respectively.
+ Average dry weight of whole grain sample fractionated = 18.38 g.

however, is only 2 percentage points higher in endosperm percent. Atlas 66 had almost twice as much endosperm adhering to the bran as Triumph 64.

Whole grain protein and lysine percentages were calculated using endosperm and bran weights and protein and lysine percentages. The means, ranges, and standard deviations for measured and calculated protein and lysine (% of sample) percentages are listed in Table 5. Calculated protein and lysine mean, range, and standard deviation values are almost identical to the measured values. The correlations between the calculated and measured protein and lysine values are very high.

The wheats analyzed in this study, particularly those from the World Collection, represent a large range of kernel types and differ widely for kernel weight, test weight, milling quality, and endosperm percent. The correlations for these traits are listed in Table 6. In the head[illegible] nursery 100-kernel weight was not correlated to percent endosperm. A low but significant correlation existed between 100-kernel weight and milling yield. In the World Collection nursery, the correlations of test weight with both milling yield and percent endosperm were higher than those of 1000-kernel weight. The correlation of both test weight and 1000-kernel weight with percent endosperm were low but significant. Test weight provides a better index of milling yield and percent endosperm than 1000-kernel weight. The correlation between test weight and kernel weight is low.

In both nurseries, milling yield was strongly correlated with percent endosperm. In both nurseries, there was a high negative correlation between milling yield and bran flour weight. As milling yield increased the amount of endosperm remaining on the bran decreased.

Table 5. Means, ranges, and standard deviations for % protein and lysine (% of sample) for grain samples obtained by whole kernel analysis and by calculation using endosperm and bran protein and lysine percentage for 176 rows from the 1973 Yuma head row nursery and for 153 rows from the 1973 World Wheat Collection Special Study nursery.

Variable	Mean	Range	Standard deviation
1973 Yuma head row nursery			
Grain % protein	17.98	11.60-24.20	3.15
Calculated grain % protein +	18.11	11.75-23.98	3.09
Grain lysine (% of sample)	0.55	0.38- 0.68	0.068
Calculated grain lysine (% of sample) ≠	0.54	0.38- 0.68	0.065
1973 World Wheat Collection Special Study Nursery			
Grain (% protein)	16.39	11.20-21.00	2.29
Calculated grain (% protein) +	16.60	11.36-21.16	2.32
Grain lysine (% of sample)	0.47	0.37- 0.60	0.059
Calculated grain lysine (% of sample) ≠	0.47	0.36- 0.59	0.056

+ The correlation of calculated grain % protein and grain % protein = r = .9[illegible] for the head row samples and r = .98** for the World Wheat Collection samples.

≠ The correlation of calculated grain lysine (% of sample) and grain lysine (% of sample) = r = 0.96** for the head row samples and r = 0.95** for the World Wheat Collection samples.

Table 6. Correlation coefficients for kernel traits for 176 rows from the 1973 Yuma head row nursery and for 153 rows from the 1973 World Wheat Collection Special Study nursery.

Traits correlated		Correlation coefficient r
1973 Yuma head row nursery		
100 kernel wt (g)	Milling yield	.21**
100 kernel wt	% endosperm of sample	.06
Milling yield	% endosperm	.89**
Milling yield	Bran flour wt (g)	-.83**
1973 World Wheat Collection Special Study Nursery		
Test weight (kg/hl)	Milling yield	.41**
Test weight (kg/hl)	% endosperm of sample	.23**
1000 kernel wt. (g)	Milling yield	.26**
1000 kernel wt (g)	% endosperm of sample	.20*
Milling yield	% endosperm of sample	.70**
Milling yield	Bran flour wt (g)	-.83**
Test weight (kg/hl)	1000 kernel wt (g)	.26**

*, ** Indicates significance at the .05 and .01 levels respectively.

On the average, 80% of the protein in wheat grain is endosperm protein while only an average 68 to 71% of the total lysine resides in endosperm proteins. The means, ranges, and standard deviations for these variables are listed in Table 7. The percentages of kernel protein and lysine in the endosperm are slightly larger for the head row nursery than for the World Collection nursery. This can be attributed to the larger endosperm percentages of the head row samples (Table 2). The range values are large for percentages of both protein and lysine that are endosperm in origin.

Table 7. Means, ranges, and standard deviations for percentage of whole kernel protein and lysine that is endosperm protein and lysine for 176 rows from the 1973 Yuma head row nursery and for 153 rows from the 1973 World Wheat Collection Special Study nursery./ ≠

Variable	Mean	Range	Standard Deviation
1973 Yuma head row nursery			
% of kernel protein in endosperm	80.5	75.5-85.9	2.12
% of kernel lysine in endosperm	70.7	64.7-77.9	2.55
1973 World Wheat Collection Special Study nursery			
% of kernel protein in endosperm	79.3	70.5-84.9	2.47
% of kernel lysine in endosperm	68.0	58.8-74.8	2.94

/ % of kernel protein in endosperm = (endosperm protein (g)/total protein (g)) x 100.

≠ % of kernel lysine in endosperm = (endosperm lysine (g)/total lysine (g)) x 100.

World Collection Wheats

The mean protein, lysine (% of protein), and lysine (% of sample) percentages of the bran samples of the World Collection wheats were considerably higher than those of the endosperm and whole wheat samples (Table 8). Whole grain samples were higher in protein and lysine content than the endosperm samples because of the high protein and lysine content of the bran. The mean difference of whole grain and endosperm protein percentages is small. The lysine mean differences of grain and endosperm were relatively larger than the protein mean differences.

Correlation coefficients for grain, endosperm, and bran protein and lysine values for the World Collection wheats are listed in Table 9. Endosperm percent protein was highly correlated with whole grain percent protein. Grain and endosperm protein percentages were correlated with bran percent protein but these correlations were lower than the correlation of grain and endosperm protein. The correlation of grain percent protein with percent endosperm of sample was negative, i.e., as percent endosperm of sample increased the protein content of the whole grain sample decreased.

Grain lysine percentages were positively correlated with endosperm and bran lysine percentages. The correlations of grain lysine percentages with the endosperm lysine percentages were higher than the correlations with the bran lysine percentages. The correlations of grain lysine values with endosperm lysine values are lower than the correlation for percent protein. Grain lysine (% of sample) was negatively correlated with percent endosperm. Grain lysine (% of protein) was not correlated with percent endosperm.

Table 8. Means, range values and standard deviations for whole grain, endosperm, and bran protein and lysine values of wheats grown in the World Wheat Collection Special Study nursery at Yuma, Arizona in 1973. + ≠

Variable	Mean	Range	Standard deviation
% Protein			
Whole grain	16.4	11.2 -21.0	2.29
Endosperm	16.1	10.8 -21.0	2.43
Bran	19.0	13.1 -25.1	2.40
Lysine (% of sample)			
Whole grain	.47	.37- .60	.059
Endosperm	.39	.29- .50	.050
Bran	.83	.46- 1.14	.107
Lysine (% of protein)			
Whole grain	2.90	2.52- 3.37	.214
Endosperm	2.45	2.14- 3.08	.166
Bran	4.40	3.23- 4.97	.286

\+ Bran includes the germ

≠ Results are for 129 World Collection Wheats plus 6 checks replicated 4 times.

Table 9. Correlation coefficients for whole grain, endosperm, and bran protein and lysine percentages for 135 wheats grown in the World Wheat Collection Special Study nursery at Yuma, Ariz. in 1973 + #

	Whole kernel		Endosperm			Bran			%
	LS	LP	P	LS	LP	P	LS	LP	END
Whole kernel									
% protein	.85**	-.49**	.98**	.89**	-.54**	.75**	.55**	-.34**	-.35**
Lysine (% of sample)		.04	.82**	.91**	-.15**	.72**	.59**	-.22**	-.41**
Lysine (% of protein)			-.48**	-.17*	.78**	-.23**	-.07	.28**	-.03
Endosperm									
% protein				.90**	-.55**	.67**	.49**	-.32**	
Lysine (% of sample)					-.14	.64**	.48**	-.30**	
Lysine (% of protein)						-.31**	-.23**	.14	
Bran									
% protein							.87**	-.21**	
Lysine (% of sample)								.29**	

*,** Indicates significance at the .05 and .01 levels of probability respectively.
+ Results are for 129 World Collection wheats plus 6 checks replicated 4 times.
# Abbreviations: P = % protein; LS = lysine (% of sample); LP = lysine (% of protein); % End = percent endosperm.

Within each group of samples, i.e., whole grain, endosperm, and bran, lysine (% of sample) was positively correlated with percent protein and lysine (% of protein) was negatively correlated to percent protein. Within each group of samples, the correlation of lysine (% of sample) and lysine (% of protein) was either not significant or low.

Table 10 contains a summary of the regression models tested for the regression of lysine on percent protein for the whole grain, endosperm, and bran samples of the World Collection wheats. Models for the regression of grain protein and lysine on percent endosperm also are listed. Within each group of samples, i.e., grain, endosperm, and bran, the linear models for the regression of lysine (% of sample) and lysine (% of protein) on percent protein gave the best fit. The coefficients of determination for the regression of lysine (% of sample) on percent protein were higher than those for lysine (% of protein). Eighty-one percent of the variability that existed among wheats for endosperm lysine (% of sample) could be attributed to variation for endosperm protein while 31% of the variation for endosperm lysine (% of protein) could be attributed to endosperm protein variation. The coefficient of determination for the regression of bran lysine (% of protein) on bran percent protein is very low.

The coefficient of determination for the regression of grain lysine (% of sample) on grain percent protein and percent endosperm is slightly larger than the coefficient of determination for its linear regression on grain percent protein. Percent endosperm has a small but significant effect on grain lysine (% of sample) after taking into account variability due to variation in percent protein. Although the correlation of percent endosperm and grain lysine (% of protein) was

Table 10. Summary of regressions models tested for whole grain endosperm and bran protein and lysine content for wheats grown in the World Wheat Collection Special Study nursery at Yuma, Arizona in 1973. Results are for 129 World Collection Wheats plus 6 check varieties replicated 4 times. +

Model number	Sample size	Dependent variable	Independent variable	Coefficient of determination
C73- 1	153	ELS	EP**	.8098
C73- 2	153	ELS	EP EP^2	.8099
C73- 3	153	ELP	EP**	.3088
C73- 4	153	ELP	EP* EP^2	.3199
C73- 5	153	BLS	BP**	.7570
C73- 6	153	BLS	BP** BP^2	.7586
C73- 7	153	BLP	BP**	.0464
C73- 8	153	BLP	BP BP^2	.0559
C73- 9	153	GP	% End**	.1227
C73-10	153	GLS	% End**	.1670
C73-11	153	GLS	GP**	.7206
C73-12	153	GLS	GP GP^2	.7209
C73-13	153	GLS	GP** % End**	.7348
C73-14	153	GLP	% End	.0009
C73-15	153	GLP	P**	.2364
C73-16	153	GLP	P* P^2	.2471
C73-17	153	GLP	P** % End**	.2823

*,** Indicates significance at the .05 and .01 levels of probability respectively of the partial regression coefficient.

Abbreviations.
G = Whole grain
E = Endosperm
B = Bran
% End = % Endosperm
P = % Protein
LS = Lysine (% of sample)
LP = Lysine (% of protein)

not significant, percent endosperm did have a significant effect on grain lysine (% of protein) when included in a multiple regression model with grain percent protein.

The regression lines for the linear regression of lysine (% of protein) on percent protein for whole grain, endosperm and bran samples of the World Collection wheats are shown in Figure 3. The lengths of the regression lines correspond to the range in protein values for each group of samples. There was more variation among wheats for bran protein content than for endosperm or grain protein content. The slope of the bran regression line is slightly less than the slopes of the grain or endosperm lines. The gentle slope of the bran regression line illustrates that bran protein content did not have much effect on bran lysine (% of protein). The slope of the grain regression line is slightly steeper than the slope of the endosperm regression line. The similarity between the curves reflects the very high correlation of grain and endosperm percent protein and their reasonably high lysine (% of protein) correlation.

Standard partial regression coefficients are the partial regression coefficients when each variable is in standard measure and, as such, give an indication of the relative importance of the independent variables. The standard partial regression coefficients for the regression of grain percent protein on endosperm and bran percent protein and percent endosperm are listed in Table 11. Percent endosperm has very little effect on grain protein content. In comparison with endosperm protein content, the effect of bran percent protein on grain protein content is small.

The standard partial regression coefficients for the regression of grain lysine (% of sample) on endosperm and bran percent protein and

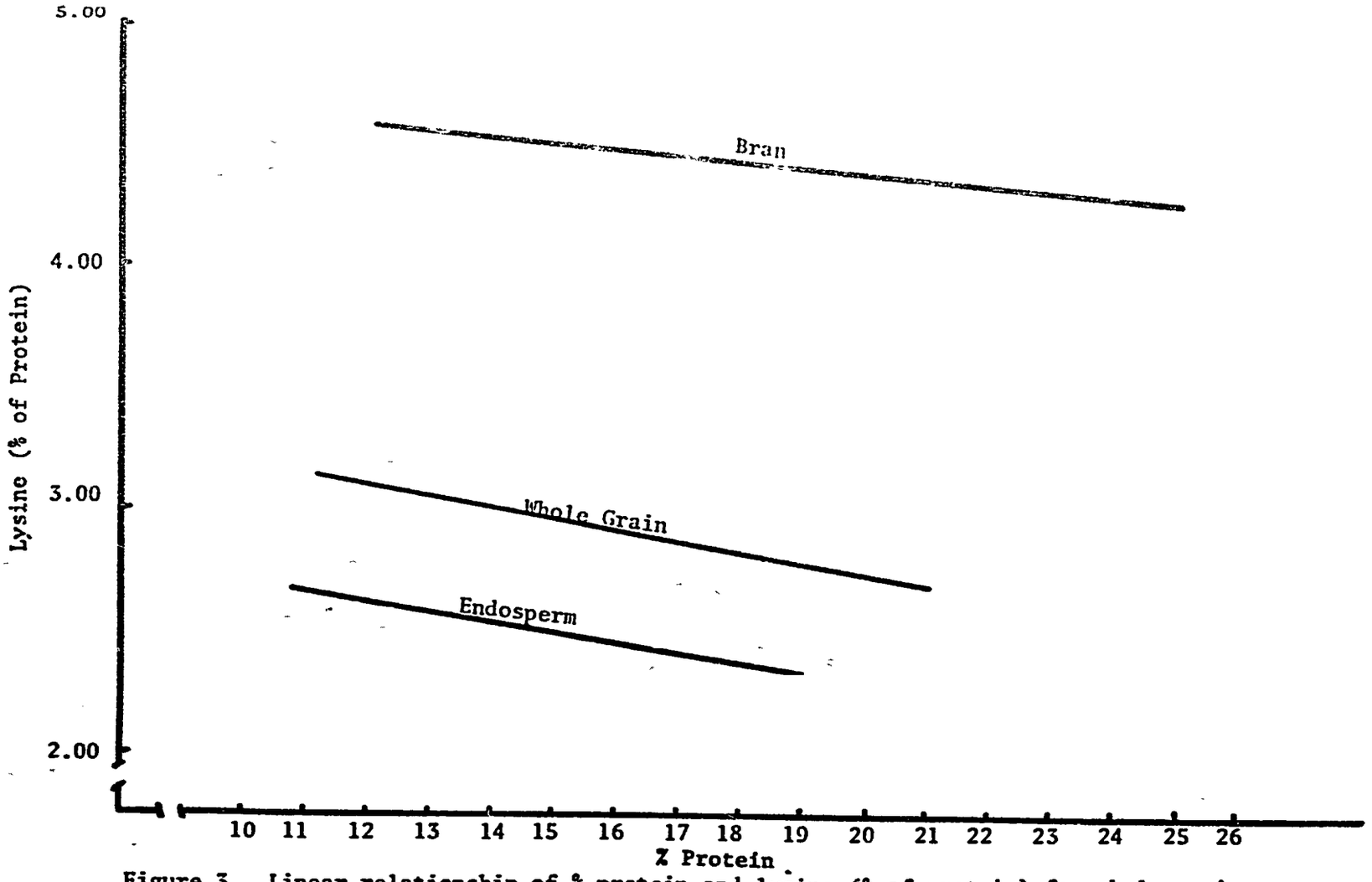

Figure 3. Linear relationship of % protein and lysine (% of protein) for whole grain endosperm, and bran of wheats from the 1973 World Wheat Collection Special Study nursery.

Table 11. Standardized partial regression coefficients for whole grain protein and lysine content from the regression analysis of wheats grown in the 1973 Yuma World Wheat Collection Special Study nursery. Results are for 129 World Collection wheats plus 6 check varieties replicated 4 times.

Dependent variables	Independent variables	Standardized partial regression coefficients	Coefficient of determination for regression model
Whole grain % Protein	1. Endosperm % protein	.8717**	.9792
	2. Bran % protein	.1599**	
	3. % Endosperm	-.0099	
Whole grain lysine (% of sample)	1. Endosperm % protein	.8866**	.8679
	2. Endosperm lysine (% of protein)	.3961**	
	3. Bran % protein	.2458**	
	4. Bran lysine (% of protein)	.1333**	
	5. % Endosperm	-.1161	

*,** Indicates significance of the partial regression coefficients at the .05 and .01 levels of probability respectively.

lysine (% of protein) and on percent endosperm (Table 11) show that endosperm protein content has as much effect on grain lysine content as all the other factors combined. The effect of endosperm lysine (% of protein) on grain lysine (% of sample) is greater than the effects of bran protein or bran lysine (% of protein). Percent endosperm has the smallest effect on grain lysine (% of sample) of the factors tested.

Grain, endosperm, and bran lysine (% of sample) and lysine (% of protein) values were adjusted to their respective mean protein levels so that valid grain, endosperm, and bran lysine comparisons could be made among wheats differing in protein content. Grain lysine values also were

adjusted for grain percent protein and grain percent endosperm. Independent variable means and the regression coefficients used in these adjustment equations are given in Table 12. Some of the regression models listed in Table 12 were summarized in Table 10.

Correlation coefficients of adjusted grain, endosperm, and bran lysine values for the World Collection wheats are listed in Table 13. The grain lysine values adjusted for percent protein and test weight were adjusted using the adjustment equations from Section I for the 1973 Yuma nursery. The correlation of adjusted grain lysine (% of sample) with adjusted grain lysine (% of protein) was high (r = .99) for all three grain lysine adjustment equations. Coefficients for the correlation of adjusted endosperm and bran lysine (% of sample) values with their respective lysine (% of protein) values were r = .99. After adjustment, lysine (% of sample) and lysine (% of protein) provide the same relative measure of protein quality.

The coefficients for the correlation of adjusted endosperm lysine (% of protein) with grain lysine (% of protein) adjusted for percent protein and test weight, percent protein and percent endosperm, and percent protein were r = 0.64, r = 0.66, and r = 0.70 respectively. The coefficient for the correlation of unadjusted grain lysine (% of protein) with unadjusted endosperm lysine (% of protein) was r = 0.78 (Table 9). Removal of the effect of percent protein on lysine content by adjusting lysine values for percent protein decreased the correlation between grain and endosperm lysine percentages. Including percent endosperm or test weight in the grain lysine adjustment equations with percent protein resulted in an additional decrease in correlation of grain and endosperm

Table 12. Means, regression coefficients, 't' values for the regression coefficients, and intercept values for the regression of lysine (% of sample) and lysine (% of protein) for whole grain, endosperm, and bran samples for the wheats fractionated from the World Wheat Collection Special Study Nursery grown at Yuma, Arizona in 1973.+

Model number	Dependent variable	Independent variable	Independent variable mean	Regression Coefficient	't' value	Intercept	Dependent variable mean	Coefficient of determination
C 73-1	ELS	EP	16.09	.0186	25.35**	.0880	.388	.8098
C 73-3	ELP	EP	16.09	-.0380	-8.21**	3.0658	2.454	.3088
C 73-5	BLS	BP	18.96	.0386	21.68**	.0976	.830	.7579
C 73-7	BLP	BP	18.96	-.0256	-2.71**	4.8914	4.406	.0464
C 73-11	GLS	GP	16.39	.0218	19.74**	.1096	.468	.7206
C 73-13	GLS	GP	16.39	.0207	17.92**	.4743	.468	.7348
		% End	81.93	-.0042	-2.83**			
C 73-15	GLP	GP	16.39	-.0455	-6.84**	3.6434	2.898	.2364
C 73-17	GLP	GP	16.39	-.0530	-7.67**	6.0288	2.898	.2823
		% End	81.93	-.0276	-3.09**			

** Indicates significance at the .01 level of probability.
+ Abbreviations: G = whole grain; E = Endosperm; B = Bran; % End = % Endosperm; P = % Protein; LS = lysine (% of sample) ; LP = Lysine (% of protein).

Table 13. Correlation coefficients of whole grain, endosperm, and bran adjusted lysine values for wheats grown in the World Wheat Collection Special Study nursery at Yuma, Arizona in 1973. Results are for 129 World Collection Wheats plus 6 check varieties replicated 4 times.

	GLP (GP KG/HL) 73-33 Section I	GLS (GP % End) C73-13	GLP (GP % End) C73-17	GLS (GP) C73-11	GLP (GP) C73-15	ELS (EP) C73-1	ELP (EP) C73-3	BLS (BP) C73-5	BLP (BP) C73-7
GLS (GP KG/HL) 73-29 (Section I)	.99**	.95**	.93**	.97**	.96**	.65**	.63**	.14	.13
GLP (GP KG/HL)		.94**	.94**	.96**	.97**	.64**	.64**	.15	.14
GLS (GP % End)			.99**	.97**	.96**	.66**	.64**	.31**	.29**
GLP (GP % End)				.96**	.97**	.66**	.66**	.31**	.30**
GLS (GP)					.99**	.70**	.68**	.19*	.17*
GLP (GP)						.70**	.70**	.18*	.17*
ELS (EP)							.99**	.01	.01
ELP (EP)								.01	.03
BLS (BP)									.99**

*,** Indicates significance at the .05 and .01 levels of probability respectively.

+ Abbreviations: G = whole grain; E = Endosperm; B = Bran; % End = % endosperm; P = % Protein; LS = Lysine (% of sample); LP = Lysine (% of protein). Factor used to adjust lysine values given in partentheses. Regression model used for adjustment equation listed below adjustment factors.

lysine. The correlation of grain lysine (% of protein) adjusted for percent protein and test weight with grain lysine (% of protein) adjusted for percent protein and percent endosperm was high (r = 0.94). Adjusted endosperm lysine values were not correlated with adjusted bran lysine values.

Means, F ratios, coefficients of variation, and LSD values for grain, endosperm, and bran protein, lysine and adjusted lysine percentages for the check varieties of the World Collection nursery are listed in Table 14. There were highly significant differences among the check varieties for all variables tested. The F ratio for replications was significant at the .01 level of probability only for unadjusted endosperm and bran lysine (% of protein) and adjusted bran lysine (% of protein).

Atlas 66 and Nap Hal were higher in grain and endosperm protein content than the other varieties. Nap Hal had significantly higher bran protein content than the other wheats. Nap Hal also was significantly higher than the other varieties for adjusted grain lysine (% of protein). Nap Hal had the highest adjusted endosperm lysine (% of protein), but the degree of its superiority in comparison with the other varieties was less than for grain adjusted lysine. Atlas 66 had significantly lower adjusted bran lysine (% of protein) than the other varieties. There was no difference among the other varieties for adjusted bran lysine. Centurk had relatively high grain and endosperm adjusted lysine percentages.

Grain, endosperm, and bran protein and lysine percentages of the World Collection wheats with the highest and lowest endosperm protein percentages are listed in Table 15. The wheats that had the highest and lowest grain lysine (% of protein) values adjusted for percent protein

Table 14. Means, coefficients of variation, F ratios, and LSD values for whole grain, endosperm, and bran protein and lysine values for check cultures in the 1973 World Wheat Collection Special Study nursery.+

Variables	Mean of check cultivars r=4						CV %	F ratio for checks	F ratio for reps	LSD.05
	Atlas 66	Triumph 64	Scout 66	Inia 66	Centurk	Nap I I				
GP	19.0	17.4	14.5	16.4	13.9	18.5	5.83	18.36**	2.81	1.46
EP	19.2	17.5	14.4	16.6	13.5	17.8	6.00	19.12**	4.10*	1.49
BP	19.5	18.3	16.8	17.3	17.0	24.0	5.54	27.57**	3.91*	1.57
GLS	.52	.49	.43	.46	.43	.57	5.08	19.35**	2.04	.04
ELS	.44	.41	.36	.38	.35	.45	5.34	15.81**	1.10	.03
BLS	.81	.85	.79	.79	.76	1.11	5.89	26.58**	1.06	.08
GLP	2.76	2.83	2.99	2.83	3.12	3.09	3.00	12.00**	2.57	.13
GLP adj for P & % E	2.86	2.90	2.97	2.88	3.04	3.18	2.27	12.94**	1.46	.10
GLP adj for P	2.87	2.88	2.91	2.83	3.01	3.18	2.36	14.08**	1.74	.10
ELP	2.32	2.36	2.51	2.32	2.64	2.54	3.79	8.22**	5.87**	.14
ELP adj for P	2.44	2.42	2.44	2.34	2.53	2.61	3.15	6.06**	4.28*	.12
BLP	4.20	4.68	4.76	4.59	4.48	4.64	3.12	7.75**	10.69**	.21
BLP adj for P	4.22	4.67	4.71	4.54	4.44	4.76	3.05	8.67**	9.28**	.21
% of Kernel P in E	80.4	81.9	82.5	83.1	79.9	76.2	.72	73.56**	.86	.87
% of Kernel L in E	69.3	69.3	71.2	71.2	69.9	63.7	1.62	24.64**	3.77*	1.69

*,** Indicate significance at the .05 and .01 levels of probability respectively.

+ Abbreviations: G = grain; E = endosperm; B = bran; P = % Protein; LS = lysine (% of sample); LP = lysine (% of protein).

and percent endosperm are listed in Table 16. The wheats that had the highest endosperm lysine (% of protein) values adjusted for percent protein are listed in Table 17. The wheats listed in these tables were not replicated. LSD values from the analysis of variance of the check varieties can be used as estimates of statistical differences among the World Collection wheats for protein and lysine content.

There are significant differences among the World Collection wheats for endosperm protein content. Wheats with high endosperm percentages differ by seven percentage points from those with low endosperm protein percentages. The wheats with high endosperm protein percentages are equal to Atlas 66 in endosperm protein content. Some of the endosperm protein values are slightly higher than whole kernel protein percentages. The differences, however, are within the range of laboratory experimental error.

There are also significant differences among the World Collection wheats for grain lysine (% of protein) adjusted for percent protein and percent endosperm and for endosperm lysine (% of protein) adjusted for percent protein. Seven wheats are in the top 15 of each group. The range between the top 15 and bottom 5 wheats for adjusted grain lysine (% of protein) is 0.6 of a percent. The range between the top 15 and bottom 5 wheats for adjusted endosperm lysine (% of protein) is 0.4 of a percent. Wheats with the highest adjusted grain lysine percentages have adjusted grain lysine values as high or higher than the adjusted grain lysine percentage of Nap Hal. World Collection wheats with the highest adjusted endosperm lysine percentages have higher adjusted endosperm lysine percentages than Nap Hal.

Table 15. Whole kernel, endosperm, and bran protein and lysine values of World Collection Wheats from the 1975 Yuma nursery that had the highest and lowest endosperm protein content of the wheats fractionated.+

CI/PI	Rank	Whole kernel			Endosperm			Bran		
		% protein	lysine % of protein	adj lysine % of protein	% protein	lysine % of protein	adj lysine % of protein	% protein	lysine % of protein	adj lysine % of protein
9053	1	20.9	2.54	2.64	21.0	2.34	2.53	21.7	3.23	3.30
6225	2	19.8	2.86	3.01	20.9	2.42	2.60	20.5	4.63	4.67
192014	3	21.0	2.86	3.08	20.8	2.20	2.38	21.2	4.13	4.19
5022	4	20.1	2.66	2.84	20.8	2.23	2.41	19.8	4.35	4.37
285812	5	20.3	2.79	2.97	20.2	2.39	2.55	23.0	4.32	4.42
8681	6	19.8	2.89	3.02	20.2	2.39	2.55	21.5	4.20	4.26
166788	7	20.0	2.81	2.92	20.0	2.30	2.45	20.6	3.95	3.99
284666	8	20.2	2.83	3.01	19.9	2.46	2.60	21.5	4.20	4.26
191961	9	19.2	2.83	2.98	19.8	2.35	2.49	19.5	4.73	4.74
295350	10	19.6	2.93	3.01	19.7	2.42	2.56	21.2	4.16	4.22
166875	11	20.0	2.62	2.76	19.6	2.31	2.44	24.4	3.89	4.03
167680	12	19.8	2.75	2.88	19.5	2.22	2.35	22.6	4.18	4.27
192290	13	19.5	2.64	2.81	19.5	2.16	2.29	22.4	4.34	4.43
298580	14	19.1	2.60	2.75	19.4	2.14	2.26	20.5	4.28	4.32
254077	15	19.1	3.07	3.23	19.3	2.58	2.70	20.3	4.43	4.46
166292	125	13.4	2.96	2.79	12.3	2.42	2.28	18.1	4.82	4.80
142521	126	12.1	3.23	3.00	12.0	3.08	2.92	13.6	4.09	3.95
135044	127	12.3	3.14	2.92	11.9	2.55	2.39	15.2	4.64	4.54
135076	128	12.5	2.96	2.79	11.8	2.53	2.37	18.6	4.65	4.64
268449	129	12.4	3.27	3.14	11.6	2.77	2.60	16.5	4.72	4.66

+ Whole kernel adjusted lysine adjusted for grain % protein and % endosperm.
Endosperm adjusted lysine adjusted for endosperm % protein.
Bran adjusted lysine adjusted for bran % protein.

Table 16. Whole kernel, endosperm, and bran protein and lysine values of World Collection Wheats from the 1973 Yuma nursery that had the highest and lowest grain lysine (% of protein) values adjusted for % protein and % endosperm of the wheats fractionated.+

CI/PI	Rank	Whole kernel			Endosperm			Bran		
		% protein	lysine % of protein	adj lysine % of protein	% protein	lysine % of protein	adj lysine % of protein	% protein	lysine % of protein	adj lysine % of protein
10907	1	17.2	3.27	3.30	16.9	2.37	2.40	18.7	4.20	4.19
176217	2	18.2	3.21	3.29	17.1	2.49	2.52	24.4	4.54	4.68
162008	3	16.8	3.24	3.25	16.6	2.56	2.58	20.6	4.85	4.89
6616	4	16.7	3.25	3.24	16.4	2.73	2.74	19.7	4.73	4.74
245604	5	15.9	3.27	3.23	15.2	2.86	2.83	19.8	4.28	4.30
254077	6	19.1	3.07	3.23	19.3	2.58	2.70	20.3	4.43	4.46
283832	7	16.4	3.17	3.15	16.5	2.65	2.67	18.4	4.41	4.40
225244	8	15.6	3.16	3.14	15.1	2.54	2.50	19.0	4.56	4.56
109368	9	14.6	3.19	3.14	14.0	2.60	2.52	20.3	4.64	4.67
268449	10	12.4	3.27	3.14	11.6	2.77	2.60	16.5	4.72	4.66
166859	11	16.5	3.16	3.14	15.4	2.38	2.35	21.2	4.23	4.29
3342	12	15.9	3.12	3.14	15.5	2.70	2.68	19.3	4.60	4.61
272427	13	17.0	3.09	3.13	16.6	2.62	2.64	18.8	4.31	4.31
13792	14	19.1	2.93	3.12	18.1	2.55	2.63	24.3	4.13	4.27
6192	15	14.0	3.15	3.12	13.3	2.54	2.43	18.4	4.73	4.72
182563	125	16.4	2.62	2.62	16.3	2.29	2.30	18.6	4.49	4.48
151202	126	14.5	2.65	2.60	14.1	2.35	2.27	16.3	4.76	4.69
9393	127	17.5	2.56	2.58	17.4	2.25	2.30	21.0	4.44	4.49
125387	128	15.7	2.59	2.57	15.4	2.41	2.38	18.6	4.34	4.33
9050	129	13.7	2.71	2.53	13.6	2.50	2.40	13.8	3.84	3.71

+ Whole kernel adjusted lysine (% of protein) adjusted for grain % protein and % endosperm. Endosperm adjusted lysine (% of protein) adjusted for endosperm % protein. Bran adjusted lysine (% of protein) adjusted for bran % protein.

Table 17. Whole kernel, endosperm, and bran protein and lysine values of World Collection Wheats from the 1973 Yuma nursery that had the highest and lowest endosperm lysine (% of protein) values adjusted for endosperm % protein of the wheats fractionated.+

CI/PI	Rank	Whole kernel % protein	Whole kernel lysine % of protein	Whole kernel adj lysine % of protein	Endosperm % protein	Endosperm lysine % of protein	Endosperm adj lysine % of protein	Bran % protein	Bran lysine % of protein	Bran adj lysine % of protein
142521	1	12.1	3.23	3.00	12.0	3.08	2.92	13.6	4.09	3.95
245604	2	15.9	3.27	3.23	15.2	2.86	2.83	19.8	4.28	4.30
298587	3	14.6	3.27	3.05	14.5	2.86	2.80	16.4	3.90	3.83
254829	4	15.5	3.16	3.04	14.8	2.82	2.77	18.5	4.45	4.44
6616	5	16.7	3.25	3.24	16.4	2.73	2.74	19.7	4.73	4.75
254077	6	19.1	3.07	3.23	19.3	2.58	2.70	20.3	4.43	4.46
3342	7	15.9	3.12	3.14	15.5	2.70	2.68	19.3	4.60	4.61
174702	8	15.5	3.20	3.12	15.1	2.71	2.67	18.7	4.56	4.55
283832	9	16.4	3.17	3.15	16.5	2.65	2.67	18.4	4.41	4.40
272427	10	17.0	3.09	3.13	16.6	2.62	2.64	18.8	4.31	4.31
192738	11	17.7	3.04	2.86	17.9	2.57	2.64	19.3	3.49	3.50
10003	12	17.7	2.99	3.07	17.5	2.58	2.63	18.2	4.29	4.27
13793	13	19.4	2.81	2.99	19.0	2.52	2.63	23.6	4.18	4.30
13792	14	19.1	2.93	3.12	18.1	2.55	2.63	24.3	4.13	4.27
306513	15	15.2	3.15	3.08	14.4	2.69	2.63	18.1	4.41	4.39
166474	125	15.8	2.70	2.70	15.9	2.23	2.22	19.3	4.35	4.36
6523	126	17.4	2.56	2.66	17.1	2.18	2.22	18.5	4.34	4.33
7514	127	17.4	2.67	2.70	16.4	2.20	2.21	19.6	4.20	4.22
166877	128	13.6	2.93	2.83	12.7	2.34	2.21	16.6	4.66	4.60
245539	129	15.8	2.70	2.71	15.9	2.19	2.18	18.7	4.39	4.38

+ Whole kernel adjusted lysine (% of protein) adjusted for grain % protein and % endosperm. Endosperm adjusted lysine (% of protein) adjusted for endosperm % protein. Bran adjusted lysine (% of protein) adjusted for bran % protein.

Parents and Progenies of High Protein and/or High Lysine Crosses

Grain, endosperm, and bran protein and lysine mean and range values for the parents and progenies of three high protein and/or high lysine crosses are listed in Table 18. The relationships among grain, endosperm, and bran samples for protein and lysine content are the same as for the World Collection wheats. The mean grain, endosperm, and bran protein and lysine values of the parent and progeny rows are higher than those of the World Collection wheats listed in Table 8.

Correlation coefficients for grain, endosperm, and bran protein and lysine for the parent and progeny rows are listed in Table 19. The relationships are similar to those of the World Collection wheats with a few exceptions. Grain lysine (% of sample) and endosperm lysine (% of sample) are negatively correlated to their respective lysine (% of protein) values for the parent and progeny rows. In the parent and progeny rows, the negative correlations of percent endosperm with grain percent protein and grain lysine (% of sample) are larger than for the World Collection wheats. The correlation of percent endosperm with grain lysine (% of protein) is positive for the parent and progeny rows.

The standard partial regression coefficients for whole kernel protein and lysine content for the parent and progeny rows listed in Table 20 are similar to those of the World Collection wheats. Endosperm lysine (% of protein) had a larger effect on grain lysine (% of sample) for the World Collection wheats than it had for the head rows. Although the correlation of percent endosperm with lysine (% of protein) for the parent and progeny rows was positive, its standard partial correlation coefficient is negative.

Table 18. Means, Range values, and standard deviations for whole grain, endosperm, and bran protein and lysine percentages for 176 parent and progeny rows of three crosses from the 1973 Yuma, Ariz. nursery.+

Variable	Mean	Range	Standard deviation
% Protein			
Whole grain	18.0	11.6 - 24.2	3.16
Endosperm	17.4	10.5 - 23.6	3.34
Bran	22.1	15.1 - 26.7	2.10
Lysine (% of sample)			
Whole grain	.55	.38 - .68	.068
Endosperm	.45	.33 - .58	.060
Bran	1.00	.71 - 1.20	.088
Lysine (% of protein)			
Whole grain	3.10	2.63 - 3.66	.235
Endosperm	2.65	2.23 - 3.26	.227
Bran	4.53	3.91 - 5.09	.220

+ Bran includes the germ.

Table 19. Correlation coefficients for whole grain, endosperm, and bran protein and lysine percentages for 176 parent and progeny rows of three crosses from the 1973 Yuma, Ariz. nursery.+

Variable	Whole kernel		Endosperm			Bran			%
	LS	LP	P	LS	LP	P	LS	LP	END
Whole kernel									
% protein	.94**	-.82**	.99**	.94**	-.82**	.71**	.47**	-.54**	-.71**
Lysine (% of sample)		-.58**	.92**	.93**	-.68**	.80**	.63**	-.44**	-.74**
Lysine (% of protein)			-.83**	-.69**	.85**	-.36**	-.08	.58**	.44**
Endosperm									
% protein				.94**	-.84**	.67**	.43**	-.54**	
Lysine (% of sample)					-.60**	.71**	.48**	-.54**	
Lysine (% of protein)						.44**	-.24**	.44**	
Bran									
% protein							.86**	-.40**	
Lysine (% of sample)								.11	

*,** Indicates significance at the .05 and .01 levels respectively.
+ Abbreviations: P = % protein; LS = lysine (% of sample); LP = lysine (% of protein); % End = % endosperm.

Table 20. Standardized partial regression coefficients for whole kernel protein and lysine content and the coefficients of determination from the regression analysis of 176 parent and progeny rows of three crosses grown at Yuma, Ariz. in 1973.+

Dependent variables	Independent variables	Standardized partial regression coefficients	Coefficient of determination for regression model
whole kernel % protein	endosperm % protein	.9274	.9914
	bran % protein	.0705	
	% endosperm	-.0280	
whole kernel lysine (% of sample)	endosperm % protein	.8768	.9415
	endosperm lysine (% of protein)	.1755	
	bran % protein	.2752	
	bran lysine (% of protein)	.1188	
	% endosperm	-.1088	
whole kernel lysine (% of protein)	endosperm % protein	-.6310	.8453
	endosperm lysine (% of protein)	.4005	
	bran % protein	.2452	
	bran lysine (% of protein)	.2122	
	% endosperm	-.1269	

+ All partial regression coefficients are significant at the .01 level.

Correlation coefficients for grain, endosperm, and bran protein and lysine percentages for the parent and progeny rows of the Nap Hal x Atlas 66 population and for the parent and progeny rows of the Nap Hal x CI 13449 population are listed in Table 21. In general, protein and lysine correlations within each population are similar and they correspond to the protein and lysine correlations of the World Collection wheats and to the entire set of head row wheats. However, the correlations of the Atlas 66 x Nap Hal population differ in magnitude from the correlations of the Nap Hal x CI 13449 population.

Protein and lysine relationships within the Nap Hal x Atlas 66 population differed from those within the Nap Hal x CI 13449 population. Because of these differences, each of the three sets of parent and progeny rows were analyzed separately using regression procedures. The second degree polynomial model provided a slightly better fit than the linear model for the regressions of grain lysine (% of sample) and grain lysine (% of protein) on grain percent protein for the Nap Hal x Atlas 66 population. However, there was no practical difference between the two models. Excluding the Nap Hal x 8156 group, the linear regression models for the regression of grain, endosperm, and bran lysine (% of sample) and lysine (% of protein) values on their respective protein percentages provided the best fit. The linear regression of bran lysine (% of protein) on percent protein was not significant for the Nap Hal x Atlas 66 group.

The Nap Hal x CIMMYT 8156 group consisted of only twelve progeny rows and six parent and check rows. This is a limited sample for regression analysis. The regressions of lysine (% of sample) values on their respective protein percentages were significant but the regressions

Table 21. Correlation coefficients for whole kernel, endosperm, and bran protein and lysine percentages for 94 parent and progeny rows of the Nap Hal x Atlas 66 populations (a) and for 64 parent and progeny rows of the Nap Hal x CI 13449 populations (b) grown at Yuma, Ariz. in 1973.

Variable	whole kernel		endosperm			bran		
	lysine			lysine			lysine	
	% of sample	% of protein	% protein	% of sample	% of protein	% protein	% of sample	% of protein
Whole kernel								
a % protein	.90**	-.53**	.98**	.88**	-.47**	.69**	.46**	-.36**
b	.91**	-.67**	.99**	.94**	-.66**	.86**	.61**	-.64**
a lysine (%		-.11	.86**	.86**	-.25**	.76**	.60**	-.21*
b of sample)		-.31*	.88**	.89**	-.48**	.87**	.76**	-.45*
a lysine (%			-.56**	-.34	.57**	-.09	.13	.40**
b of protein)			-.69**	-.56**	.68**	-.41**	-.04	.66**
Endosperm								
a % protein				.89**	-.48**	.60**	.38**	-.36**
b				.94**	-.69**	.84**	.58**	-.64**
a lysine (%					-.04	.54**	.31**	-.36**
b of sample)					-.41**	.86**	.65**	.58**
a lysine (%						-.31**	-.24*	.09
b of protein)						-.46**	-.21	.52**
Bran								
a % protein							.85**	-.17
b							.83**	-.58**
a lysine (%								.36**
b of sample)								-.03

*, ** Indicates significance at .05 and .01 levels respectively.

for lysine (% of protein) were not. The means of the independent and dependent variables, regression coefficients, and intercept values for the linear regression models for all three sets of parent and progeny rows are listed in Table 22.

The regression coefficients listed in Table 22 were used to adjust grain, endosperm, and bran lysine values of the Nap Hal x Atlas 66 and Nap Hal x CI 13449 samples to the mid-parent protein level for each group. Nap Hal x Atlas 66 bran lysine (% of protein) values were not adjusted. The mid-parent values were used instead of mean values because the progeny rows of each group included segregates for grain, endosperm, and bran protein content. The Nap Hal x CIMMYT 8156 parent and progeny row lysine values were not adjusted for percent protein because the limited size of their sample precluded precise regression analysis. Whole kernel lysine values were not adjusted for percent endosperm.

The correlation coefficients for grain, endosperm, and bran protein and adjusted lysine values for the Nap Hal x Atlas 66 and Nap Hal x CI 13449 populations are listed in Table 23. The coefficients for the correlation of grain and endosperm adjusted lysine (% of sample) values with their respective adjusted lysine (% of protein) values were large ($r = 0.99$ to 0.96). The coefficient for the correlation of Nap Hal x CI 13449 bran adjusted lysine (% of protein) with bran adjusted lysine (% sample) was $r = 0.99$. The coefficient for the correlation of adjusted bran lysine (% of sample) with unadjusted bran lysine (% of protein) for the Nap Hal x Atlas 66 samples was $r = 0.98$.

Correlations between grain and endosperm protein percentages were high ($r = 0.98$ and 0.99) for both the Nap Hal x Atlas 66 and the Nap Hal

Table 22. Means, regression coefficients, 't' values for the regression coefficients, and intercept values for the regression of lysine (% of sample) and lysine (% of protein) on % protein for parent and progeny rows of the crosses Nap Hal x Atlas 66, Nap Hal x CI 13449, and Nap Hal x CIMMYT 8156 +

Population	Dependent variable	Independent variable	Mean Independent variable	Regression Coefficient	't' value	Intercept	Dependent variable mean	Coefficient of determination (r^2)
Nap Hal x Atlas 66 n=94	GLS	GP	20.33	.0226	19.52**	.1325	.59	.8055
	GLP	GP	20.33	-.0341	-6.06**	3.6299	2.94	.2851
	ELS	EP	19.89	.0197	19.02**	.0964	.49	.7972
	ELP	EP	19.89	-.0269	-5.28**	3.030	2.48	.2327
	BLS	BP	22.84	.0406	15.66**	.0846	1.01	.7271
	BLP	BP	22.84	-.0197	-1.74	4.9043	4.45	.0322
Nap Hal x CI 13449 n=64	GLS	GP	15.26	.0236	17.25**	.1417	.50	.8275
	GLP	GP	15.26	-.0628	-7.18**	4.2882	3.33	.4541
	ELS	EP	14.49	.0212	21.44**	.1032	.41	.8812
	ELP	EP	14.49	-.0524	-7.61**	3.6364	2.87	.4828
	BLS	BP	21.66	.0311	11.57**	.3165	.99	.6836
	BLP	BP	21.66	-.0685	-5.64**	6.0917	4.61	.3398
Nap Hal x CIMMYT 8156 n=18	GLS	GP	15.21	.0287	8.73**	.0420	.48	.8265
	GLP	GP	15.21	-.0176	-.82	3.4413	3.17	.0408
	ELS	EP	14.56	.0299	16.29**	-.0381	.40	.9431
	ELP	EP	14.56	.0143	.94	2.5496	2.76	.0525
	BLS	BP	19.99	.0492	15.27**	-.0575	.92	.9358
	BLP	BP	19.99	.0190	1.15	4.2638	4.64	.0769

+ Abbreviations: G = Whole grain; E = Endosperm; B = Bran; P = % Protein; LS = Lysine (% of sample); LP = Lysine (% of protein).

*,** Indicates significance at the .05 and .01 levels of probability respectively.

Table 23. Correlation coefficients for whole kernel, endosperm, and bran protein and adjusted lysine percentages for 94 parent and progeny rows of the Nap Hal x Atlas 66 population (a) and for 64 parent and progeny rows of the Nap Hal x CI 13449 population (b) grown at Yuma, Ariz. in 1973.

Variable	whole kernel		endosperm			bran			unadj.
	adjusted lysine			adjusted lysine			adjusted lysine		lysine
	% of sample	% of protein	% protein	% of sample	% of protein	% protein	% of sample	% of protein	% of protein
whole kernel									
% protein (a)	.00	.00	.98**	.01	.01	.69**	-.25*		-.35**
(b)	.00	.00	.99**	.05	.04	.86**	-.18	-.17	
adj. lysine (a)		.99**	-.04	.42**	.41**	.32**	.32**		.25*
(% of sample) (b)		.98**	-.04	.35**	.35**	.20	.56**	.54**	
adj. lysine (a)			-.04	.42**	.41**	.33**	.32**		.26*
(% of protein) (b)			-.03	.38**	.41**	.24	.55**	.54**	
endosperm									
% protein (a)				.00	.00	.60**	-.26**		-.36**
(b)				.00	.00	.84**	-.20	-.19	
adj. lysine (a)					.98**	.01	-.09		-.08
(% of sample) (b)					.96**	.19	.22	.24	
adj. lysine (a)						-.02	-.11		-.09
(% of protein) (b)						.18	.22	.25*	
bran									
% protein (a)							.00		-.18
(b)							.00	.00	
adj. lysine (a)									.98**
(% of sample) (b)								.99**	

*,** Indicates significance at the .05 and .01 levels respectively.

x CI 13449 populations. The coefficient for the correlation of grain lysine (% of sample) with endosperm lysine (% of sample) was r = 0.86 for the Nap Hal x Atlas 66 samples and r = 0.89 for the Nap Hal x CI 13449 samples (Table 21). The coefficients for the correlation of grain and endosperm adjusted lysine (% of sample) was r = 0.42 for the Nap Hal x Atlas 66 samples and r = 0.35 for the Nap Hal x CI 13449 samples. Adjusting lysine (% of protein) values also decreased the size of the correlation coefficients for the correlation of grain and endosperm lysine (% of protein) for both populations.

Whole kernel, endosperm, and bran protein and lysine values for the parent and progeny rows of the Nap Hal x Atlas 66 population are listed in Table 24. Mean values are listed for the parent and check varieties. The coefficients of variation and LSD values were obtained from the analysis of variance of the parent and check rows. The listed progeny rows are representative of the protein and lysine segregates obtained from the cross. Protein and lysine values for the parent and selected progeny rows of the Nap Hal x CI 13449 and Nap Hal x CIMMYT 8156 crosses are listed in Tables 25 and 26 respectively.

Atlas 66 and Nap Hal have high grain protein content. Atlas 66 has high endosperm protein content while Nap Hal has both high endosperm and high bran protein content. Transgressive segregation was obtained for both high and low grain protein content. The low protein progeny wheats had endosperm protein percentages that were 3 to 4 percentage points lower than the endosperm protein content of either parent. The high protein progeny rows had endosperm protein percentages 2 to 4 percentage points higher than either parent. None of the low protein

Table 24. Whole kernel, endosperm, and bran protein and lysine values, 100 kernel weights, % endosperm of sample, % of kernel protein in endosperm, and % of kernel lysine in endosperm for parents, check, and progeny rows of Nap Hal x Atlas 66. Coefficients of variation and LSD values obtained from the analysis of variance of the parent and check rows.*

Parental/ check lines n=3	Whole Kernel P	Whole Kernel LP	Whole Kernel ALP	Endosperm P	Endosperm LP	Endosperm ALP	Bran P	Bran LP	100 KWT	% END	% of kernel P in END	% of kernel lysine in END
Centurk	15.47	2.98	2.81	15.00	2.52	2.41	19.60	4.48	3.66	87.0	83.7	74.1
Atlas 66	19.43	2.80	2.80	19.33	2.46	2.46	19.80	4.41	3.63	83.1	82.7	72.8
Nap Hal	19.57	3.09	3.09	18.97	2.52	2.51	24.46	4.57	2.66	83.2	79.3	67.7
Coefficient of variation %	2.72	1.52	1.51	2.53	2.32	2.28	2.43	7.16	3.32	0.74	1.20	2.00
LSD.05	.99	.09	.09	.90	----	----	1.03	----	0.22	1.25	1.96	2.86
Progeny row number												
10286	16.3	3.05	2.94	15.7	2.63	2.54	19.4	4.55	2.55	85.1	82.2	72.7
12333	14.4	3.02	2.84	14.1	2.70	2.56	19.2	4.47	3.61	87.4	83.6	75.6
15138	24.2	2.81	2.97	23.6	2.34	2.45	25.8	4.40	2.90	82.5	81.2	69.6
11982	20.0	3.12	3.14	19.1	2.37	2.36	25.4	4.58	2.82	84.8	80.7	68.3
15168	21.0	2.79	2.84	21.3	2.25	2.30	21.9	4.51	3.01	83.1	82.7	70.7
11026	19.6	3.04	3.04	18.7	2.48	2.47	25.2	4.54	3.10	82.3	77.6	65.3
14329	20.7	2.63	2.67	19.9	2.25	2.26	21.8	4.38	2.83	84.9	83.7	72.2
15252	20.0	3.05	3.06	19.6	2.69	2.70	22.7	4.27	3.20	82.6	80.4	72.0
11481	20.5	3.04	3.07	21.0	2.76	2.81	21.8	4.52	3.20	83.5	83.0	74.6
10078	23.3	2.72	2.83	20.9	2.74	2.78	25.5	3.98	2.75	84.0	81.1	74.8
12546	21.6	2.79	2.86	21.3	2.40	2.45	23.1	3.91	2.85	82.7	81.6	73.1
12120	23.6	2.88	3.02	23.2	2.45	2.56	26.7	4.51	3.50	83.0	80.9	69.8

*Adjusted lysine values obtained by adjusting lysine (% of protein) values to mid-parent protein level using linear regression model for this population. Abbreviations: P = % protein; LP = lysine (% of protein); ALP = lysine (% of protein) adjusted for % protein; KWT = kernel weight; End = Endosperm. LSD values listed only when 'F' ratio from the analysis of variance was significant at the .05 level.

segregates had bran protein percentages as high as Nap Hal. All of the high protein segregates had the high bran protein content of Nap Hal. Progeny rows with parental endosperm values had bran protein values that were similar to either the Nap Hal or Atlas 66 parent or were intermediate to them.

Nap Hal had higher grain adjusted lysine (% of protein) than either Atlas 66 or Centurk. Nap Hal also had higher endosperm and bran lysine (% of protein) but the mean differences were not significant. Some of the progeny rows had endosperm adjusted lysine (% of protein) percentages which were lower than the adjusted lysine percentages of either parent. Other rows such as 15252, 11481, and 10078 had endosperm adjusted lysine percentages which were considerably higher than the endosperm adjusted lysine percentage of Nap Hal. Some of the progeny rows had bran lysine (% of protein) values which were lower than those of either parent.

Most of the progeny rows had grain adjusted lysine percentages which were intermediate to the parental values. Some of the progeny rows such as 14329 had grain adjusted lysine values which were lower than the Atlas 66 adjusted lysine percentage. These wheats had lower endosperm adjusted lysine values than either parent. Some of the progeny rows had grain adjusted lysine values which were as high as that of Nap Hal.

Progeny rows 15252 and 11481 had high grain lysine percentages because of the high lysine content of their endosperm proteins. Although progeny row 10078 had high endosperm lysine (% of protein) content, it did not have high grain lysine (% of protein) content because of the

low lysine content of its bran proteins. The progeny rows 11982 and 11026 had grain adjusted lysine (% of protein) values equivalent to that of Nap Hal. Their endosperm adjusted lysines percentages are lower than Nap Hal's adjusted lysine percent but their bran protein content is the same as the bran protein content of Nap Hal.

The head rows of each cross were grown in separate blocks within the head row nursery. The protein percentages for Nap Hal and Centurk rows were lower in the Nap Hal x CI 13449 blocks of the head row nursery than in the Nap Hal x Atlas 66 blocks. Direct comparisons should not be made between the Nap Hal x Atlas 66 and Nap Hal x CI 13449 progeny row protein and lysine percentages because of the mean protein differences between the two areas of the nursery. Comparisons can be made by comparing progeny row protein and lysine percentages to the mean percentages of the Centurk and Nap Hal rows that were grown with each group of head rows. Nap Hal was four percentage points higher than Centurk in grain protein content in both areas of the head row nursery.

The CI 13449 wheat sample used in this study was not grown in the head row nursery. It was grown in an adjacent nursery. When CI 13449 is grown in the same nursery as Nap Hal, it averages 4-5 percentage points lower in protein than Nap Hal.

Although CI 13449 is not known to possess genes for high grain protein content, some of the Nap Hal x CI 13449 head rows had higher grain, endosperm, and bran protein percentages than Nap Hal. Most of the progeny rows had lower grain and endosperm protein percentages than Nap Hal. Approximately half the Nap Hal x CI 13449 head rows had bran protein percentages as high or higher than Nap Hal.

Table 25. Whole kernel, endosperm, and bran protein and lysine values, 100 kernel weights, % endosperm of sample, % of kernel protein in endosperm, and % of kernel lysine in endosperm for parent, check, and progeny rows of Nap Hal x CI 13449. Coefficients of variation and LSD values obtained for the analysis of variance of the parent and check lines.*

Parental/ check lines n=2	Whole kernel P	Whole kernel LP	Whole kernel ALP	Endosperm P	Endosperm LP	Endosperm ALP	Bran P	Bran LP	Bran ALP	100 KWT	% END	% kernel P in END	% kernel lysine in END
Centurk	12.75	3.16	2.96	12.00	2.86	2.72	16.25	4.89	4.56	3.48	87.9	84.5	76.0
Nap Hal	16.15	3.20	3.22	15.15	2.86	2.87	21.90	4.76	4.84	2.72	85.5	80.3	71.0
CI 13449	15.55	3.14	3.12	14.55	2.83	2.81	19.65	4.44	4.36	2.21	83.1	78.4	69.6
CV %	6.63	3.35	1.76	6.31	3.81	2.44	5.97	2.11	0.48	7.42	.35	.25	1.07
LSD	2.31*	----	.17**	2.08*	----	----	3.66*	0.31**	0.07**	.38**	.96**	.65**	2.46**
Progeny row number													
15958	14.5	3.30	3.21	13.7	2.87	2.81	21.4	4.27	4.31	2.98	84.6	77.9	70.2
16551	11.8	3.65	3.40	10.8	3.11	2.89	19.2	4.62	4.51	2.24	85.5	76.8	68.8
16240	19.9	3.14	3.39	19.7	2.68	2.93	25.5	4.28	4.60	2.62	84.7	81.0	72.5
16900	19.4	3.35	3.57	18.5	2.73	2.92	25.4	4.56	4.87	2.92	83.1	78.1	68.1
16593	11.6	3.66	3.39	10.5	3.26	3.03	19.3	5.00	4.89	2.31	85.7	76.5	68.0
18640	16.8	3.41	3.46	15.6	2.98	3.01	24.9	4.69	4.97	2.51	83.5	76.0	66.7
16691	16.1	3.30	3.32	15.3	2.59	2.61	22.4	4.54	4.65	3.15	85.5	80.1	69.5
16686	14.2	3.49	3.39	13.6	2.96	2.89	20.9	5.03	5.03	3.42	85.8	79.7	69.7
16681	14.9	3.48	3.42	14.4	2.95	2.93	21.4	5.09	5.13	3.49	85.9	80.4	70.3
15958	14.5	3.30	3.21	13.7	2.87	2.81	21.4	4.27	4.31	2.98	84.6	77.9	70.2
15986	13.7	3.25	3.11	13.3	2.84	2.75	20.8	4.47	4.47	3.10	85.8	79.5	70.9
16946	16.5	3.24	3.28	15.6	2.63	2.67	22.6	4.59	4.71	3.10	84.3	78.7	68.0

*,** Indicates significance at the .10 and .05 levels respectively.

\+ Adjusted lysine values obtained by adjusting lysine (% of protein) values to mid-parent protein level using linear regression model for this population.

LSD values listed only when 'F' ratio from the analysis of variance was significant at the level indicated. Abbreviations: P = % protein; LP = lysine (% of protein); ALP = lysine (% of protein) adjusted for % protein; KWT = kernel weight; END = Endosperm.

Nap Hal and CI 13449 had higher grain adjusted lysine percentages than Centurk. Endosperm adjusted lysine percentages of Nap Hal and CI 13449 also were higher than the endosperm lysine percentage of Centurk but the mean differences were not statistically significant. Nap Hal had higher adjusted bran lysine than either Centurk or CI 13449.

There was evidence of transgressive segregation for whole grain, endosperm, and bran adjusted lysine (% of protein). Forty-two rows had grain adjusted lysine percentages larger than the grain adjusted lysine percentage of Nap Hal and two had grain adjusted lysine percentages smaller than the grain adjusted lysine percentage of CI 13449. Twenty-five of the progeny rows had endosperm adjusted lysine values larger than the endosperm adjusted lysine value of Nap Hal. Twenty of the progeny rows had endosperm adjusted lysine percentages smaller than the endosperm adjusted lysine percentage of CI 13449. Nap Hal and some of the head rows had bran lysine (% of sample) values as large as 1.0%.

Nap Hal had higher grain, endosperm, and bran protein percentages than Centurk or CIMMYT 8156. The grain, endosperm, and bran protein percentages of the Nap Hal x CIMMYT 8156 progeny rows were within the parental protein range. Some of the head rows had grain and endosperm protein percentages almost as large as those of Nap Hal. Most of the progeny bran protein percentages were at the mid-parent level.

The lysine values of Nap Hal x CIMMYT 8156 samples were not adjusted for percent protein. Nap Hal has unadjusted grain and endosperm lysine (% of protein) values that are as high or higher than those of Centurk and CIMMYT 8156 even though Nap Hal is considerably higher in protein content than the other wheats. Progeny rows such as 19010,

Table 26. Whole kernel, endosperm, and bran protein and lysine values, 100 kernel weights, % endosperm of sample, % of kernel protein in endosperm and % of kernel lysine in endosperm for parent, check, and progeny rows of Nap Hal x CIMMYT 8156. Coefficients of variation and LSD values obtained from the analysis of variance of parent and check rows.

Parental/ check lines n=2	Whole kernel		Endosperm		Bran		100 kernel weight (g)	% endosperm	% kernel protein in endosperm	% kernel lysine in endosperm
	P	LP	P	LP	P	LP				
Centurk	13.20	3.20	12.70	2.81	16.95	4.66	3.65	87.6	81.1	75.9
Nap Hal	17.05	3.17	16.30	2.83	22.40	4.70	2.49	85.1	1.6	71.4
CIMMYT 8156	12.80	3.06	12.65	2.74	16.20	4.42	3.92	87.4	4.4	76.7
Coefficient of variation (%)	1.63	2.49	2.71	3.73	2.22	2.74	2.77	.60	1.07	1.52
LSD.05+	.75	----	1.20	----	1.31	----	.30	1.66	2.82	3.60
Progeny row number										
19165	13.7	3.36	12.6	2.66	19.4	4.81	3.42	85.5	79.3	67.7
19170	14.5	3.25	13.7	2.55	20.2	4.84	3.79	86.4	81.2	69.6
18932	15.2	3.12	14.5	2.78	21.0	4.80	3.36	85.3	80.1	69.9
19010	15.0	3.53	14.5	2.85	20.5	4.93	3.18	86.7	82.2	72.6
19704	16.2	3.15	15.3	2.80	21.8	4.54	2.51	84.4	79.1	69.6
19754	16.6	3.17	15.8	2.82	22.6	4.60	2.58	83.8	78.3	68.6

+ LSD values listed only when 'F' ratio from the analysis of variance was significant at the .05 level.
Abbreviations: P = % protein; LP = lysine (% of protein).

19704, and 19756 are 2 to 3 percentage points higher in protein content than CIMMYT 8156. Even though they are higher in protein content, their lysine (% of protein) values are higher than those of CIMMYT 8156.

Nap Hal is a wheat of poor agronomic type while CIMMYT 8156 is a high yielding semi-dwarf wheat. The Nap Hal x CIMMYT 8156 head rows examined in this study were of good agronomic type.

DISCUSSION

Mill Bran and Flour Protein and Lysine Content

The 70% extraction flours of the four wheats milled with the Kansas State University pilot mill were lower in protein and lysine content than the whole grain or bran samples. Mill bran samples were higher in both protein and lysine content than their whole grain samples. These results agree with those reported in the literature (Mill Feed Manual, 1967; Baumgarten et al., 1946; Waggle et al., 1967).

Mill bran proteins contain more of the albumin and globulin proteins and less of the gliaden proteins than the endosperm (Teller and Teller, 1932). Albumins and globulins contain more lysine and arginine and less glutamic acid than gliadins (Simmonds, 1962; Mattern et al., 1968). In addition to having a higher lysine content, bran proteins have also been reported to contain more arginine and less glutamic acid than endosperm proteins (Hepburn et al., 1957; 1960; Pomeranz et al., 1966). Wheat bran proteins are higher in lysine and arginine content than endosperm proteins probably because they contain more of the albumin and globulin proteins and less of the gliaden proteins than the endosperm.

Wheat mill bran, excluding the adherent starchy endosperm, has several morphological layers and of these layers, only the aleurone layer is high in protein content (Ferrand and Hinton, 1974; Stevens et al., 1963). Analysis of aleurone cell contents by Stevens et al. (1963) has shown that the aleurone proteins are high in lysine content. The pericarp and testa have not been analyzed for lysine content. The aleurone layer is stained intensively by the Sakaguchi reaction which is specific for arginine (Fulcher, et al., 1972). The other bran layers

are not stained by the Sakaguchi reaction. This is indirect evidence that the aleurone cells are the storage sites of the lysine-and arginine-rich proteins in wheat bran.

Bran Flour Protein and Lysine Content

Bran flour samples are higher in protein content than 70% extraction flour samples. The high protein content of the outer layers of the starchy endosperm, particularly the subaleurone cells, has been reported previously (Ferrand and Hinton, 1974; McDermott and Pace, 1960; Normand et al., 1965, Pomeranz and Shellenberger, 1961). The high protein of the bran flour samples provides further evidence that there is a strong protein gradient within a wheat kernel, even for wheats that are very high in protein content.

The bran flour proteins are higher in lysine content than the 70% extraction flour proteins. This indicates that the endosperm proteins from the outer endosperm cells, particularly the subaleurone cells, are higher in lysine content than the endosperm proteins from the interior of the endosperm. However, the results of McDermott and Pace (1960) and Kent and Evers (1969) indicate that the proteins of the outer endosperm cells are lower in lysine content than the proteins of the inner endosperm.

McDermott and Pace obtained samples from the exterior and interior areas of wheat endosperm using a micro-drilling procedure. Lysine (% of protein) values were lower for the outer samples than for the inner samples. McDermott and Pace used the micro-drilling procedure to avoid contamination of endosperm samples with aleurone cells or cell contents. It is possible that the outer endosperm samples of McDermott and Pace did not include the subaleurone cells.

Kent and Evers (1969) obtained samples of subaleurone cell particles by air classification followed by differential sedimentation. The protein content of these subaleurone cell particles was higher than the protein content of the inner endosperm. Lysine (% of protein) values of the subaleurone endosperm particles were lower than the lysine (% of protein) values of the inner endosperm. The proteins of the subaleurone cell particles analyzed by Kent and Evers (1969) are undoubtedly low in lysine content. However, this does not necessarily mean that all the proteins in the subaleurone cells are low in lysine content.

Fulcher et al. (1972) reported that inclusions in the subaleurone cells stained intensely for arginine while portions of the subaleurone cells were largely unstained. The wheat proteins that contain the highest concentrations of arginine also contain the highest concentrations of lysine. It is likely that inclusions in the subaleurone cells that stained intensely for arginine also contain high concentrations of lysine. The subaleurone cell particles analyzed by Kent and Evers (1969) could have been from the areas of the subaleurone cells that were not stained by the Sakaguchi reaction. Subaleurone endosperm particles separated and photographed by Kent (1966b) resemble the unstained areas of the subaleurone cells shown in the photomicrographs of Fulcher et al. (1972).

Horn milled samples of the same wheat at two extraction rates, 71.5 and 84.9%. Protein, lysine (% of sample) and calculated lysine (% of protein) values were larger for the 84.9% extraction flour than for the 71.5% extraction. These results support the results of this study. The higher lysine content of 84.9% extraction flour could have been due to its having more bran contamination than the 71.5% extraction flour.

Bran flour samples in this study did not contain visible bran contamination. The bran flour samples resembled defatted flour in appearance and consistency. The aleurone layer of the washed bran samples was largely intact and a few small pieces of endosperm could be observed adhering to it. Washed bran pieces were smaller than pieces of mill bran. Aleurone cells along the torn edges could have been torn free or open by the washing process. Aleurone cells or aleurone cell particles could have contaminated the bran flour by passing through the 237 micron mesh of the silk screen used to separate the washed bran and bran flour.

The high protein content of bran flour samples is due to the high protein content of the outer starchy endosperm cells. The high lysine (% of protein) of the bran flour is due to either the contamination of the bran flour by aleurone cells or aleurone cell contents or to a high lysine (% of protein) content of the starchy endosperm that is not separated from the bran by milling. The latter explanation is believed to be correct. Contamination of subaleurone endosperm by an equal amount of aleurone cells would be required to raise the lysine (% of protein) content from the 1.59% reported by Kent and Evers (1969) for subaleurone endosperm to the lysine (% of protein) values obtained in this study for the bran flour samples.

The high protein and lysine content of the bran flour samples was not due to contamination by the germ. The germ was separated from the bran for the wheats milled on the KSU pilot mill.

Comparison of Grain and Flour Samples

Nap Hal had the highest grain protein and lysine (% of protein) percentages of the four wheat varieties milled on the Kansas State University pilot mill. The protein content of the 70% extraction flour of Atlas 66 was higher than that of Nap Hal. Flour lysine (% of protein) content of Nap Hal was not significantly higher than that of Centurk or Atlas 66.

These results illustrate the need for comparisons of whole grain, endosperm, and bran protein and lysine percentages to determine the within kernel site of protein and lysine variability among wheats. Because of the high protein and lysine content of the bran flour, it is necessary to obtain complete and uniformly separated samples of endosperm for protein and lysine analysis. Variation in milling yield could easily affect the protein and lysine content of endosperm samples. The use of samples milled at a uniform extraction rate could slightly distort endosperm protein and lysine relationships among wheats. Seventy percent extraction flour from a wheat with 78% endosperm will contain more of the high protein outer endosperm than 70% extraction flour from a wheat with 85% endosperm.

Modified Milling Procedure

Over 300 20g samples of wheat were separated into endosperm and non-endosperm components using the modified milling procedure. It would not have been possible to hand dissect this many samples in any reasonable period of time. Other mechanical and chemical methods have been used to separate and isolate kernel components for chemical analysis (Kent and Evers, 1969; Stevens et al., 1963; Shetlar et al., 1947). In

comparison with the other methods, the modified milling procedure is relatively simple.

The bran samples obtained by milling on the Quadraplex mill contained both germ and bran. Except for the bran samples from the wheats milled on the Kansas State University pilot mill, the terms "bran" or "washed bran" refer to the non-starchy endosperm kernel components. On large mills such as the KSU pilot mill, the germ is sifted from the bran.

The amount of endosperm removed from the bran by the washing process decreased significantly as milling yield increased. This high negative correlation between milling yield and bran flour weight provides evidence that the bran and endosperm of wheats differing in milling quality were uniformly separated by the bran washing process. Figures 1 and 2 provide visual evidence for the uniformity of separation of bran and endosperm. Although there was more endosperm adhering to the mill bran of Atlas 66 than to the bran of Scout 66, there is no apparent difference between the washed bran samples of the two wheats for endosperm contamination. The washed bran samples are almost free of endosperm.

Wheats of both nurseries differed in milling quality. This is indicated by large ranges in milling percentage and bran flour weights. The amount of bran flour obtained from the bran of some of the soft wheats was large. If the mill flour samples had been used for protein and lysine analysis, much of the variation among wheats for endosperm lysine content would have been due to variation in milling yield.

The range for percent endosperm of sample is considerably smaller than the range in milling yield. The percent endosperm range values for the head row nursery correspond to percent endosperm values that have

been reported by Hinton (1953, 1959), MacMasters et al. (1971), and Farrand and Hinton (1974) for hand-dissected samples. The percent endosperm values are realistic considering the variability for test weight and kernel weights that exists among the wheats analyzed. The head row nursery endosperm percentages are inflated 2 to 3 percentage points. Comparisons of the check varieties which were in both nurseries show that the percent endosperm relationships among the wheats of a nursery is similar in both nurseries. Centurk had high and Nap Hal had low endosperm percentages in both nurseries.

The parent and check varieties of both nurseries differed significantly for milling yield, flour weight, bran flour weight, washed bran weight, and percent endosperm. Although field replication had some effect on these variables for the World Collection wheats, most of the variation was due to differences among the wheats analyzed. The coefficients of variation for milling yield, flour weight, bran flour weight, washed bran weight, percent endosperm, and sample recovery percent were low, indicating that laboratory experimental error was low for the bran and endosperm separation procedures. Sample recovery percentage was not affected by variety or field replication. Considering the number of steps in the modified milling procedure, the sample recovery percentages are satisfactory.

Grain protein and lysine percentages which were calculated using endosperm and bran weights and protein and lysine percentages were almost identical to measured grain protein and lysine percentages. These results show that very little if any protein was lost in the washing process by solubilization of the bran and bran flour in the 80:20

ethanol:acetone washing solution. These results are consistent with Osborne's (1907) results for the solubility of wheat proteins. It was evident that some lipids were dissolved in the washing solution because the bran flour had the consistency of defatted flour. The loss of these lipids had no apparent effect on the protein and lysine content of the endosperm or bran samples.

These results and the results discussed previously demonstrate that the modified milling procedure can be used to obtain reasonably complete and uniform separation of the endosperm and non-endosperm components of the wheat kernel without any appreciable loss of either component or component proteins.

Endosperm Percent of Sample

The range in percent endosperm of sample was large for both the head row nursery and the World Collection nursery. Part of the variation for percent endosperm can be attributed to within-nursery environmental variation since replications had a slight effect on percent endosperm in the World Collection nursery. Most of the variation was probably genetic in origin because there were highly significant differences among the parent and check varieties in both nurseries.

Soft wheats such as Atlas 66, Nap Hal, and CI 13449 had lower endosperm percentages than the hard wheats Centurk and Scout 66. The endosperm of soft wheats is less dense than the endosperm of hard wheats. Consequently, soft wheats have lower endosperm percentages than hard wheats when percent endosperm is calculated on a weight basis. Part of the variation among the wheats for percent endosperm could be due to some wheats having thicker bran and aleurone layers than other wheats (Bates,

1943; Larkin et al., 1951). Seed size could also be a factor. Large kernels have a higher proportion of endosperm than small kernels (Zelany, 1971). The washed bran samples contain both the germ and bran. Variation in endosperm percent could also be due to some wheats having larger germs than other wheats.

The wheats analyzed represent a wide range of kernel types. Test weight provided a better estimate of milling yield and percent endosperm than kernel weight. These results are consistent with those of Section I where it was suggested that test weight had more of an effect on lysine content than kernel weight because it provided a better estimate of percent endosperm. Although test weight provides a better estimate of percent endosperm than kernel weight, it does not provide a good estimate. The correlation between test weight and percent endosperm is low.

Milling yield is highly correlated to percent endosperm. This is to be expected since the maximum yield of white flour from a sample of wheat is determined by the percent endosperm. The modified milling procedure may be useful to millers for estimating milling yield.

Endosperm Proportion of Kernel Protein and Lysine

On the average, 80% of the protein in a wheat kernel is located in the endosperm. This is higher than values reported by Ferrand and Hinton (1974) and Morris et al. (1946). The wheats dissected by these authors, however, were lower in endosperm protein content than the wheats grown at Yuma. MacMasters et al. (1971) had estimated that only 50% of the lysine was endosperm lysine. Their estimation is low because they apparently based it on flour lysine percentages rather than starchy endosperm lysine

percentages. The large range values for percent of protein and lysine that is in the endosperm indicates that the distribution of protein and lysine within the wheat kernel varies significantly among wheats.

Wheat Germ

Wheat germ is the least variable of the non-endosperm kernel components for protein and amino acid composition (Mill Feed Manual, 1967). Wheat germ is slightly higher in protein and lysine content than the bran (Mill Feed Manual, 1967). Washed bran samples from the World Collection and head row nurseries contain both the germ and bran. The terms "washed bran" and "bran" are used for convenience of expression. Variation in non-endosperm protein or lysine percentages among wheats could be due to variation in germ content. The hand dissection results of Hinton (1953, 1959) and Farrand and Hinton (1974) indicate that the germ comprises up to one-third of the washed bran samples.

The uniform composition of the germ and the relative size of its contribution to the total non-endosperm sample weight suggest that most of the variability among wheats for non-endosperm protein and lysine composition is due to variation in protein and lysine composition of the bran layers. The variability in protein and lysine content of the washed bran samples for the wheats milled at Kansas State University supports this contention. These bran samples did not contain any germ. For the purposes of this study, it is not essential to know the reasons for the variation of non-endosperm protein and lysine content but only the effect this variation has on grain protein and lysine content.

Correlations of Grain, Endosperm, and Bran Protein and Lysine Percentages

The coefficients for the correlation of whole grain and endosperm protein percentages were very high for both the World Collection wheats and the wheats of the head row nursery. This high correlation can be attributed to the high percentage of the total protein of wheat kernels that is endosperm protein and to the fairly high correlation of endosperm and bran protein percentages. Because of the high correlation of grain and endosperm protein, wheat breeders can reliably use grain protein percentages as a selection criterion for endosperm protein content.

The coefficients for the correlation of bran percent protein with endosperm and grain percent protein were lower than those for the correlation of grain protein with endosperm protein. This indicates that wheats which have high endosperm protein percentages do not necessarily have high bran protein content.

Grain, endosperm, and bran lysine (% of sample) were positively correlated with their respective protein percentages for the wheats of both nurseries. Total lysine content of the grain, endosperm, and bran samples increased as protein content increased. Grain and endosperm lysine (% of protein) were negatively correlated to their respective protein percentages. The explanation for this relationship is discussed in Section I.

Bran lysine (% of protein) was negatively correlated to bran percent protein for the wheats of both nurseries. The correlation of bran lysine (% of protein) was negative, but not significant for the Nap Hal x Atlas 66 parent and progeny rows. The effect of bran protein content on bran lysine (% of protein) was less than the effect of endosperm

protein content on endosperm lysine (% of protein). Evidence for this is the smaller correlation coefficients and the smaller coefficients of determination from the regression analyses.

Bran proteins contain proportionately less gluten proteins than endosperm proteins (Teller and Teller, 1932). High protein brans probably do not differ greatly from low protein brans in the size of their albumin--globulin gluten ratios. Consequently, bran percent protein does not have as large an effect on bran lysine (% of protein) as endosperm protein percent has on endosperm lysine (% of protein).

Kernel Factors Determining Grain Protein and Lysine Content

Grain protein and lysine (% of sample) were negatively correlated with percent endosperm for the wheats of both nurseries. This relationship is due to the bran being higher in protein and lysine content than the endosperm. The correlations of grain lysine (% of protein) with percent endosperm were either not significant or positive. The regression coefficient for percent endosperm from the multiple regression of grain lysine (% of protein) on grain percent protein and percent endosperm were negative. This indicates that grain lysine (% of protein) decreases as percent endosperm increases. This relationship is evident only when the effect of grain percent protein is taken into account.

Standard partial regression coefficients give an indication of the relative effect of the independent variables on the dependent variables. Standard partial regression coefficients were similar for the wheats of both nurseries. Grain protein content is largely determined by endosperm percent protein although bran percent protein does influence grain protein

content. Percent endosperm has a slight effect on grain protein content.

Endosperm and bran percent protein and lysine (% of protein) and percent endosperm all influence grain lysine content. Of these factors, endosperm percent protein is the most important. Endosperm lysine (% of protein) had more effect on grain lysine (% of sample) than bran percent protein for the World Collection wheats. Bran percent protein had the greater influence on grain lysine (% of sample) for the wheats of the head row nursery because of the high bran protein content of Nap Hal and some of the Nap Hal progeny rows. Bran lysine (% of protein) and percent endosperm are of lesser importance than the other factors in determining grain lysine content.

Correlations of Adjusted Lysine Percentages

The coefficients for the correlation of grain lysine percentages with endosperm lysine percentages were smaller than the coefficients for the correlation of grain protein percentages with endosperm protein percentages. Adjusting grain and endosperm lysine percentages to their respective mean or midparent protein values decreased the correlation of grain and endosperm lysine content.

Grain and endosperm protein percentages are highly correlated. Because of the large effect protein has on lysine content, the coefficients for the correlation of grain and endosperm lysine values were also high. Removal of the lysine variability attributable to percent protein decreased the correlation of grain and endosperm lysine values because the grain samples of some wheats contained more of the lysine rich bran proteins than other wheats.

The adjustment of grain lysine values for percent protein and percent endosperm or test weight resulted in an additional decrease in the correlation of grain and endosperm lysine values. This additional decrease is due to the effect of percent endosperm on grain protein and lysine content.

After adjustment for percent protein, the correlation of grain and endosperm lysine content was still reasonably high for the World Collection wheats (r = 0.70). The correlation of adjusted grain and endosperm lysine values was considerably lower for the head row wheats (r = 0.41). This lower correlation was probably the result of segregation for the high bran protein content of Nap Hal.

Prior to adjustment, grain, endosperm, and bran lysine (% of sample) values were either not correlated or were negatively correlated with their respective lysine (% of protein) values. After adjustment the coefficients for the correlation of lysine (% of sample) and lysine (% of protein) were r = 0.96 to 0.99 indicating that after adjustment for percent protein both lysine values provide the same relative measure of protein quality for grain, endosperm and bran samples. The same general conclusion applies to grain lysine values adjusted for percent protein and percent endosperm.

Grain lysine values adjusted for percent protein and percent endosperm and grain lysine values adjusted for percent protein and test weight provide almost the same relative measure of the protein quality of whole grain samples. The high correlation between these two sets of adjusted lysine values is due primarily to the effect of adjusting for percent protein.

Atlas 66 and Nap Hal Protein and Lysine Content

Atlas 66 was included as a check in the World Collection nursery because of its known genetic superiority for high grain protein content. Nap Hal was included as a check because of its high grain protein and lysine content. The results of grain endosperm, and bran protein analyses of the check entries in the World Collection nursery indicate that Atlas 66 is high in grain protein content because of its high endosperm protein content. Nap Hal is high in grain protein content because of its high endosperm protein content and its high bran protein content.

Nap Hal bran is 5 to 7 percentage points higher in protein content than the brans of the other check varieties. Bran proteins contain almost twice as much lysine as the endosperm proteins. Nap Hal has significantly higher adjusted grain lysine content than the other check varieties partly because of its high bran content. With the exception of Centurk, Nap Hal also has significantly higher endosperm adjusted lysine (% of protein) than the other check varieties. The high adjusted lysine content of Nap Hal grain thus is due to both its high bran protein content and to the high lysine content of its endosperm protein.

Cobb (1905) reported that wheats differ significantly in aleurone cell wall thickness and for the amount of space occupied by the aleurone cell contents. Larkin (1951) reported significant differences among wheats for aleurone cell thickness. Nap Hal is probably high in bran protein content because its aleurone layer is thick and the volume of the aleurone cells is large. Nap Hal may also be high in bran protein content simply because its aleurone cells contain more protein than the aleurone cells of other wheats.

World Collection Wheats

World Collection wheats differ significantly for endosperm protein content. The differences found in this study are mostly genetic because within-nursery environmental variation was small. Some of the differences in endosperm protein content, however, may be due to differences among the wheats for genotype x environment interaction effects. The wheats with high endosperm protein percentages are equal to Atlas 66 in endosperm protein content. The World Collection wheats are diverse in origin. It is probable that some of the World Collection wheats with high endosperm content contain genes different from those of Atlas 66 for endosperm protein content.

Whole grain lysine (% of protein) values were adjusted for percent protein and percent endosperm. Even though percent endosperm was included in the adjustment equation, some of the wheats with high grain adjusted lysine values had high grain lysine percentages because of high bran protein and lysine percentages. Seven of the wheats with high grain adjusted lysine percentages have endosperm adjusted lysine percentages as high as that of Nap Hal. The wheats with low adjusted grain lysine percentages all had low endosperm adjusted lysine percentages. These results indicate that grain adjusted lysine values are usable for selecting wheats that are likely to have high endosperm lysine content from those that have low endosperm lysine content.

The World Collection wheats differ significantly for endosperm adjusted lysine content. Within-nursery environment variation did have some effect on the endosperm adjusted lysine percentages of the check varieties and, therefore, also had some effect on the endosperm lysine

content of the World Collection wheats. However, much of the variability among wheats for endosperm adjusted lysine content is probably genetic in origin. Some of the World Collection wheats with the highest endosperm adjusted lysine percentages are as high as Atlas 66 in endosperm protein content and have higher endosperm adjusted lysine percentages than Nap Hal. The wheats PI 254077 and CI 13793 should be retested along with the other top 15 wheats for potential use in breeding programs as high lysine lines.

Selection Criteria for Lysine

The best selection criterion to use for selection of wheats with high endosperm lysine content is endosperm lysine adjusted for percent protein. Selection of wheats for grain lysine content will result in some wheats being selected because of high bran protein and lysine content. Because of the work involved in separating bran and endosperm, initial selection could be made on whole grain samples.

Although the correlation of endosperm adjusted lysine with grain lysine adjusted for percent protein is slightly higher than its correlation with grain lysine adjusted for percent protein and test weight, the latter adjusted lysine value is the better selection criterion to use for whole grain samples. The use of grain lysine values adjusted only for percent protein could result in wheats with smaller than average endosperm percentages being selected. The quantity as well as the quality of endosperm is important. Even when endosperm adjusted lysine percentages are being used for selection of high lysine wheats, some attention should be given to percent endosperm or seed size. Adjusting whole grain samples for percent protein and percent endosperm is of little practical

value because separation of bran and endosperm is required to obtain endosperm percentages.

Nap Hal x Atlas 66 Parent and Progeny Rows

The results of endosperm protein analysis of Nap Hal x Atlas 66 parent and progeny rows indicates that Nap Hal and Atlas 66 have different genes for endosperm protein content. The large range in endosperm protein content of the progeny rows suggests the segregation of several major genes for endosperm protein content. The head rows 15138 and 12120 are eight percentage points higher in endosperm protein content than the conventional variety Centurk. This difference in endosperm protein content is largely due to genetic differences among these wheats.

Nap Hal in addition to having genes for high endosperm protein content also has genes for high bran protein content. There appear to be at least two genes governing bran protein content because the progeny rows either have parental bran protein percentages or values intermediate to them. All the progeny rows with endosperm protein percentages of 23% also had bran protein percentages as high or higher than that of Nap Hal. This suggests that the Nap Hal genes for high endosperm protein content may be linked to the genes for high bran protein content.

The Nap Hal x Atlas 66 parent rows differed significantly for grain lysine (% of protein) but did not differ for endosperm lysine (% of protein). However, Nap Hal was significantly higher than Atlas 66 for adjusted endosperm lysine (% of protein) in the World Collection nursery. The range in grain adjusted lysine (% of protein) of the progeny rows suggests segregation for grain lysine (% of protein). Part of the

variation among the progeny rows for grain lysine (% of protein) is due to segregation for bran protein and lysine content.

The endosperm adjusted lysine percentages of the progeny rows indicate transgressive segregation for lysine (% of protein) that is independent of segregation for percent protein. Some of the progeny rows such as 1148 and 10078 have protein percentages as high or slightly higher than either parent and considerably higher lysine (% of protein). Although Atlas 66 does not have high endosperm lysine (% of protein), its adjusted lysine percentage was considerably higher than those of the World Collection wheats with low endosperm adjusted lysine. This suggests that the increase in endosperm lysine (% of protein) content of the progeny rows over that of the Atlas 66 may be due to additive gene effects. These results indicate that Nap Hal in addition to having genes for high endosperm protein content also has genes for higher than average endosperm lysine (% of protein).

Nap Hal x CI 13449 Parent and Progeny Rows

Interpretation of the results of the grain, endosperm and bran analyses of the Nap Hal x CI 13449 progeny rows is difficult because the CI 13449 sample used for protein and lysine analysis was not grown in the head row nursery. However, there is substantial evidence of transgressive segregation for grain lysine (% of protein). Part of the variation for grain lysine content is due to variation among the progeny rows for bran percent protein. Some of the head rows inherited the high bran protein and lysine content of Nap Hal.

There is also evidence for transgressive segregation of endosperm

adjusted lysine (% of protein). Some of the progeny rows have adjusted endosperm lysine values 0.2 of a percent higher than those of either parent. Both the parent varieties have higher endosperm adjusted lysine values than the check variety Centurk. Centurk had higher endosperm adjusted lysine content than the other commercial varieties which were analyzed in this study. These results indicate that Nap Hal and CI 13449 possess different genes for high endosperm lysine (% of protein). CI 13449 is a high lysine wheat because of the high lysine content of its endosperm proteins.

It is difficult to explain the high protein content of the Nap Hal x CI 13449 head rows 16240 and 16900 since they are 3 percentage points higher in protein content than Nap Hal. CI 13449 does not have any known genes for high grain protein content. The high protein contents of these rows may be due to complementary gene interactions or to micro-environmental effects within the head row nursery.

Nap Hal x CIMMYT 8156 Parent and Progeny Rows

The protein and lysine analyses of the Nap Hal x CIMMYT 8156 progeny rows indicate that some of the wheats inherited the high protein and lysine genes of Nap Hal and the desirable agronomic characteristics of CIMMYT 8156. Most of these head rows were of good agronomic type. The endosperm and bran protein values of these head rows suggest that Nap Hal has several genes for both endosperm and bran protein content. Progeny rows with parental and mid-parent endosperm protein percentages were obtained. Progeny row bran protein percentages were either at the mid-parent level or at the level of Nap Hal.

Implication for Wheat Breeders

The progeny rows were not replicated. Some of the differences among the head rows of a particular cross may be due to within-nursery environmental variation. The results of the whole grain analyses for the Nap Hal x Atlas 66 and Nap Hal x CI 13449 head rows are similar to those reported by Johnson et al. (1973) for the F_2 progeny bulks from which these head rows were selected. This similarity in results and the magnitude of the differences among the progeny rows strongly suggests that the differences among the parent and progeny rows of a particular cross for protein and lysine content are mostly genetic. Additional testing of the parent and progeny wheats in replicated nurseries is required before more definitive statements can be made.

Nap Hal x Atlas 66 progeny wheats with high endosperm protein and lysine content should be extremely useful for improving the nutritional quality of commercial wheats by breeding. These wheats have the high protein genes of both parents and the high lysine genes of Nap Hal. Some of the Nap Hal x CI 13449 progeny wheats should also be useful to wheat breeders because they have the high lysine genes of both parents and the Nap Hal genes for protein content.

Some of the World Collection wheats may have genes different from those of Nap Hal and Atlas 66 for protein content and genes different from those of Nap Hal and CI 13449 for lysine (% of protein). If so, then additional progress could be made in improving the nutritive value of wheat by their use in breeding programs.

In addition to protein quantity and quality, the nutritive value of wheat also depends upon other factors such as protein and starch

digestibility. As high protein and high lysine lines are developed, human nutrition studies will be needed to determine if the net nutritive value of wheat grain has been improved.

SUMMARY

Most societies eat only the ground endosperm or flour of wheat. Most of the breeding and genetic research involving the protein and lysine content of wheat has been done using whole grain samples. Comparisons of the protein and lysine content of whole grain and endosperm samples were needed to determine the validity of this practice.

Endosperm samples obtained by milling are not satisfactory for making protein and lysine comparisons among wheats. The outer part of the endosperm is higher in protein and lysine (% of sample) than the interior of the kernel. Wheats of different hardness mill differently. Some protein and lysine differences among wheats could be due to milling differences. The method tested and used in this study to obtain endosperm samples is a modified milling procedure. After milling the endosperm adhering to the bran is removed in a washing procedure, dried, and added to the mill flour to reconstitute the starchy endosperm.

Endosperm and bran samples of wheats differing in whole grain protein and lysine content were analyzed. The wheats studied were from the USDA World Wheat Collection and the parents and progenies of three crosses of high protein and/or high lysine lines. Over 300 samples were analyzed.

The correlation for grain protein and endosperm protein was r = .95** showing that whole grain protein percentages accurately reflect endosperm protein content. The correlations of adjusted whole grain and adjusted endosperm lysine percentages were lower. The combined effect of percent bran, bran percent protein, and bran lysine (% of protein) on whole grain lysine content was as great as the effect of endosperm

lysine (% of protein) content. Wheats with high grain lysine content did not always have high endosperm lysine content. Significant differences existed among the wheats tested for endosperm protein and lysine content.

Analyses of the whole grain, endosperm, and bran protein and lysine content of the parents and progenies of high protein and/or high lysine crosses provided useful genetic information. On the basis of whole kernel analyses, the variety Atlas 66 previously had been identified as a high protein line, Nap Hal, PI176217, as a high protein/high lysine line, and CII3449 as a high lysine line. Endosperm and bran (including the germ) analyses show that the high protein of Nap Hal resides in both endosperm and bran. Atlas 66 shows the high protein effect only in its endoseperm. Transgressive segregates with endosperm protein values as high as 23% demonstrate that the genes for endosperm protein of Nap Hal are different from those of Atlas 66. The parents averaged 19% protein. The high grain lysine content of Nap Hal is largely due to the high protein content of its bran. The high lysine of CII3449 is largely due to the high lysine content of its endosperm protein.

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