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SUMMARY

Protein and lysine contents of 12,613 common wheats of the USDA World Wheat Collection were determined in order to tentatively identify wheats that may be sources of genes for high protein and high lysine. It will be necessary to grow wheats identified as being high protein or high lysine in an array of environments in order to identify those which are genetically superior.

Percent protein of the samples analyzed ranged from 6.9 to 22.0% with a mean of 12.97%. This wide range of protein indicates that significant genetic differences in protein content probably exist among the common wheats of the World Collection. Wheats with more than 17% protein have been tentatively identified as high protein lines. There are over 500 wheats in the World Collection with protein contents of over 17 percent.

Lysine expressed as a percent of sample is highly correlated with percent protein. The simple correlation coefficient is $r = 0.9014$. It would be possible to improve the lysine content of wheat by selecting for high protein. Lysine percent of sample is more a measure of protein content than protein quality.

Lysine expressed as a percent of protein is a better measure of protein quality. The relationship between lysine percent of protein and protein is negative up to 15% protein. For wheats with more than 15% protein, the effect of protein on lysine content of protein is negligible.

Lysine values (% of protein) were adjusted to the mean protein level to make comparisons of the lysine content of protein among wheats with different protein contents. Comparisons of unadjusted lysine values have little validity. Wheats with the highest adjusted lysine values were tentatively identified as being sources of genes for high lysine. Adjusted lysine values ranged from 2.28 to 3.71 percent of protein with a mean of 3.16%.

Results of Systematic Analyses for Protein and Lysine Composition of Common Wheats (*Triticum aestivum L.*) in the USDA World Collection

K. P. Vogel, V. A. Johnson, and P. J. Mattern[1]

INTERPRETIVE SUMMARY

Protein and lysine contents of 12,613 common wheats of the USDA World Wheat Collection were determined in order to identify wheats that may be sources of genes for high protein and high lysine. Results indicate that significant genetic differences in protein and lysine content probably exist among world collection common wheats. Selection criteria have been developed for further evaluation of World Collection lines high in protein and lysine content.

INTRODUCTION

Wheat has been one of the principal foods of man for centuries. During this long association man has improved the yield and other agronomic characters of the wheat plant by selection among existing genotypes. Modern plant breeding methods have accelerated the rate of improvement of wheat for yield.

Until recently, however, little emphasis was placed on improving the nutritive quality of wheat by breeding. The plant breeder of antiquity could not select for more nutritive types because he did not have the means of determining nutritive value. It has since been determined that the nutritive value of wheat could be improved by increasing its protein content and by improving the quality of the protein by increasing the content of limiting amino acids, particularly lysine.

Although modern plant breeders have had the means of determining protein content of wheat, selection for protein *per se* was not prac-

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ticed until the early 1950's because known genetic differences in protein content of wheat grain were small in comparison with environmental effects (6).

Middleton, Bode, and Bayles (18) in 1954 reported that the soft winter variety "Atlas 66" had significant genetic superiority over commonly grown cultivars in its ability to produce grain with high protein content while maintaining yield at a high level. Subsequent research has demonstrated that the genes controlling the high protein trait of Atlas 66 could be transferred to winter wheats adapted to the Great Plains of the USA with an improvement in the protein content of the derived lines over the parent winter wheat varieties of as much as three percentage points of protein (3, 4, 5, 6, 7, 25). This increase in protein content was accomplished while maintaining a high yield level. Amino acid analysis of these high protein lines has shown that the increase in protein content did not adversely affect the amino acid composition of the wheat protein (7, 12). This work demonstrated that the protein content of wheat grain can be significantly improved through breeding.

The discovery by Mertz, Bates, and Nelson (17) that maize homozygous for the opaque-2 gene has a significantly higher lysine content of protein than commonly grown varieties and hybrids suggests that such a gene or genes may also exist in wheat. The development of amino acid analyzers has made it possible to screen large numbers of samples thus making such a search feasible.

In 1966, the Nebraska Agricultural Experiment Station in cooperation with the Agricultural Research Service, U.S. Department of Agriculture, under a contract with the Agency for International Development, U.S. Department of State, began a systematic analysis of the protein and lysine content of the wheats of the U.S. Department of Agriculture World Collection. Purpose of the research was to determine the variability that exists in the World Wheat Collection for protein and lysine content of wheat grain and to identify lines that may be genetically superior for protein and lysine content.

LITERATURE REVIEW

The World Wheat Collection of the U.S. Department of Agriculture contains more than 12,000 entries of common wheat. Although this collection has not been previously analyzed in its entirety for protein and lysine content, prior studies on variability of protein and lysine content in different wheats have been conducted. Preliminary results of this study have also been reported (4, 7).

Lawrence et al. (8) analyzed 230 wheat varieties selected to represent all recognized market types and classes, 12 different *Triticum* species other than *T. aestivum* and *T. compactum,* 6 varieties of

durum, 13 samples of genera related to wheat, and 15 hybrids of crosses of wheat with *Agropyrum elongatum*. The lysine content expressed as a percent of protein of their winter and spring wheat samples ranged from 2.46 to 3.84 percent with a mean of 3.10 percent. The durum samples ranged from 2.70 to 3.30 percent lysine. The lysine content of samples of the *Triticum* species, genera related to wheat, and the hybrids of wheat and *Agropyrum elongatum* ranged from 2.21 to 3.98 percent. The protein content of these species and hybrids ranged from 9.8 to 21.6 percent with the exception of a *T. dicoccoides* (wild emmer) sample which had a protein content of 29.6 percent. A Mexican variety, CI 5286, was tentatively identified as being high in percent lysine of protein with 3.54 percent. The variety Nanking 393, PI 124340, had a lower content of lysine, with 2.48 percent of protein.

Lawrence et al. (8) observed that in the common wheats, an apparent negative correlation existed between lysine expressed as a percent of protein and percent protein. Statistical analysis showed no correlation of lysine (percent of protein) with percent protein for wheat with more than 13.5 percent protein. However, there was a significant negative correlation between percent lysine and percent protein with those samples high in protein content tending to be low in lysine content (lysine percent of protein).

Villegas et al. (27) studied the variability in the protein and lysine content of 12 varieties of spring wheat, 28 varieties and lines of durum wheat, 64 different *Triticum* species, 125 varieties and species of *Secale,* and 25 varieties or crosses of *Triticale* grown at various locations. The average lysine content of the spring and durum wheats was slightly lower than those reported by Lawrence et al. (8). The protein content of the *Triticum* species, on a 14% moisture basis, ranged from 8.6 to 24.2%. The lysine content (percent of protein) of the *Triticum* species varied from 2.09 to 3.99 percent when protein is calculated as N x 5.7. The *Triticale* and rye samples were found on the average 20-30% higher in lysine content than the spring or durum wheats. A negative correlation between lysine expressed as a percent of protein and percent protein for the spring and durum wheats was also reported.

The inverse relationship between lysine content (percent of protein) and percent protein has been noted by others. Simmonds (21) showed an inverse relationship between lysine content and protein content for six Australian wheats and their flours. Glutamic acid exhibited a reverse trend, i.e., increasing with increase in percent protein. McDermott and Pace (15) reported similar results from their analysis of flours extracted from different wheat varieties.

Simmonds (21) fractionated the protein of two different flour samples and analyzed the fractions for amino acid content. The

albumin and globulin proteins were higher in lysine content than the gluten proteins. The gluten proteins, however, were higher in glutamic acid content. Mattern et al. (13) obtained similar results with protein fractions extracted from the flour of the hard red winter wheat variety "Bison."

Pence et al. (20) studied the albumin and globulin content of the flour of 32 different wheats and found that the albumin and globulin content varied from 13 to 22 percent of the total flour protein. The amounts of these soluble proteins (percent of sample) increased directly with increase in the total protein content of the flour, but the relationship was inverted when the amount of globulin and albumin protein was expressed as a percentage of the total protein. Ulmer and Mattern (26) have reported similar results.

Simmonds (21) and McDermott and Pace (15) have postulated that the variability in the lysine content of wheats and flours, as well as the inverse relationship between lysine content expressed as a percent of protein and percent protein, is due to differences in the albumin-globulin:gluten ratio among wheats and flours. They reason that wheats with low protein content have a higher proportion of the albumin and globulin proteins and hence are higher in lysine content expressed as a percent of protein than wheats with a higher protein content.

Variability in the protein and lysine content of wheat determined on a whole kernel basis may also be due to:

1. Variability in the proportion of the major morphological components of the wheat kernel, i.e., bran, germ and endosperm.
2. Variability in the percent protein of each component.
3. Variability in the lysine content of the protein of each component.

A survey of the literature on the composition of the morphological components of the wheat kernel was conducted by MacMasters et al. (10). Their review shows that such variability does exist. In general, the germ and bran are higher in protein content than the endosperm. The proteins of the germ and bran are also higher in lysine content than the endosperm proteins.

Environment also has a definite influence on protein and lysine content of wheat and contributes to variability in lysine and protein content of the grain. McElroy et al. (16) determined the protein and lysine content of the hard red spring wheat variety "Marquis" grown in nine different locations in Alberta, Canada during the same year. Percent nitrogen of the different samples ranged from 1.94 to 4.03. Significant differences were also obtained in the lysine content. An inverse relationship between total nitrogen and lysine nitrogen (percent of total nitrogen) was observed.

Lawrence et al. (8) grew three winter and three spring wheat varieties at three different locations for three years. They reported no influence of environment on percent lysine of protein except as environment affected the percent of protein, i.e., higher lysine content could be attributed to lower protein content.

Stroike and Johnson (24) used stability parameters to study environmental influence on protein and lysine content of wheat in an international array of environments. The varieties studied differed in their sensitivity to change in environment for both protein and lysine content. However, the consistency or repeatability of performance of the cultivars' response to different environments in regard to protein and lysine content was high.

MATERIALS AND METHODS

Materials

Wheat samples analyzed in this study were obtained from the World Wheat Collection maintained by the U.S. Department of Agriculture, Agricultural Research Service. Common wheats totaling 12,613 were analyzed. World Wheat Collection accessions are routinely increased at Mesa, Arizona. Consequently all samples analyzed were grown under irrigation at Mesa, Arizona but not all samples were grown during the same year. Additional accessions to the World Wheat Collection will be analyzed on a routine basis.

Methods

Laboratory Analysis

Whole kernel samples were analyzed. Samples were ground using a Udy Cyclone Sample Mill.[2] Ground samples were brought to uniform moisture levels in a humidity controlled cabinet (11). Samples were then weighed on a dry matter basis for protein and lysine analyses.

Protein values were determined by dye binding using a modification of the shaker method, AACC 46-14 (1). Macro-Kjeldahl procedure AACC method 46-12 (1) was used to determine nitrogen content on all samples with more than 19% protein as determined by the dye binding method or with more than 3.5% lysine of protein. Protein content was calculated as percent N x 5.7.

Ion exchange chromatography is considered a reliable method for determination of amino acids and was used to determine lysine content

[2] Mention of firm or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture or the U.S. Department of State over other firms or similar products not mentioned.

of the samples (19, 23). Samples were analyzed with an automatic amino acid analyzer modified with four short columns. The procedure was programmed so that only the lysine peak was recorded and integrated (14). Samples were acid hydrolyzed prior to lysine analysis (14).

Statistical Analysis

The large number of observations in this study necessitated computer analyses. Means and standard deviations for the 12,613 samples were obtained for percent protein, percent lysine expressed as a percent of sample, and percent lysine expressed as a percent of protein. The frequency distributions of percent protein, percent lysine of sample, and percent lysine of protein were tested for normality by plotting the relative cumulative frequencies on probability graph paper (9). The simple correlation coefficients (r values) were calculated to provide a measure of the degree of association of these factors with each other.

To determine more precisely the relationship of protein content to lysine content of wheat grain, regression analysis of percent lysine on percent protein was done. First, second, and third degree polynomial models were tested. The linear models for these polynomials are (22):

(a) First degree or linear model:

$$Y_i = a + b_1 X_i + e_i$$

(b) Second degree or quadratic model:

$$Y_i = a + b_1 X_i + b_2 X_i^2 + e_i$$

(c) Third degree or cubic model:

$$Y_i = a + b_1 X_i + b_2 X_i^2 + b_3 X_i^3 + e_i$$

The b values are regression coefficients, a the intercept, Y_i the percent lysine of the $_i$th sample, X_i the percent protein of the $_i$th sample, and e_i the residual error.

Six models were tested—three to determine the effect of protein on lysine expressed as a percent of sample and three to determine the effect of protein on lysine expressed as a percent of protein.

The regression equation 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 two criteria met, a maximum coefficient of determination (r^2). In addition to "t" tests, the significance of the regression coefficients also was tested by partitioning the regression sum of squares.

Predicted lysine values for lysine expressed as a percent of sample

and lysine expressed as a percent of protein were calculated using the appropriate regression equation for each. These predictive values were used to plot the regression lines for the relationship between percent protein of sample and lysine content expressed as a percent of sample and as a percent of protein. Deviations of the observed lysine values (% of protein) from the predicted values also were calculated.

To compare the percent lysine of protein of samples differing in protein contents, lysine values (% of protein) were all adjusted to the mean protein level using the equation:

$$Y_i \text{ adj} = Y_i - b_1 (X_i - \overline{X}) - b_2 (X_i^2 - \overline{X^2}) - b_3 (X_i^3 - \overline{X^3}).$$

The b values are the regression coefficients, Y_i the observed lysine content (% of protein), and X_i the percent of protein of the $_i$th sample.

Average error variance for an adjusted value was calculated using the equation (22):

$$s^2\text{adj} = s^2_{y.x} [1 + c_{11} \cdot s^2_x + c_{22} \cdot s^2_{x^2} + c_{33} \cdot s^2_{x^3} + c_{12} \cdot \text{cov } XX^2 + c_{13} \cdot \text{cov } XX^3 + c_{23} \cdot \text{cov } X^2 X^3]$$

The $s^2_{y.x}$ term is the error mean square from regression; the c values are the elements of the inverse matrix of the independent variables used in calculating the regression equation, and the s^2 and cov terms are the variance and covariance terms respectively of the independent variables, i.e., % protein, (% protein)^2, and (% protein)^3.

The average standard error of a difference between two adjusted values was calculated as follows:

$$s_D = \sqrt{2 \; s^2_{\text{adj.}}}$$

This average standard error of a difference for the adjusted values was used to calculate 95% confidence limits about the mean lysine (% of protein) value. The confidence interval was calculated as $\overline{X} \pm t_{.05} \cdot s_D$.

RESULTS

Means, standard deviations, and range values for percent protein, lysine percent of sample, and lysine expressed as a percent of protein of 12,613 common wheats of the USDA World Collection are shown in Table 1. Frequency distributions of percent protein, lysine percent of sample, and lysine percent of protein are shown in Figures 1, 2, and 3 respectively. The frequency distribution of percent protein and percent lysine of protein approximate normal distributions, but the frequency distribution of lysine expressed as a percent of sample does not.

Simple correlation coefficients (r) are given in Table 2. A signifi-

Table 1. Means, standard deviations, and range for % protein, lysine (% of sample), and % lysine of protein for 12,613 common wheats of the USDA World Wheat Collection.

	Mean	Standard deviation	Range
% Protein[a]	12.97	2.019	6.90 to 22.00
% Lysine (% of sample)[a]	.40	.049	.25 to .66
% Lysine (% of protein)[a]	3.16	.231	2.25 to 4.26

[a] Dry matter basis

Table 2. Simple correlation coefficients (r) for percent protein, lysine percent of sample, and lysine percent of protein for common wheats of the USDA world collection.

	Lysine (% of sample)	Lysine (% of protein)
% Protein	.9014**	-.6779**
% Lysine (% of sample)		-.3046**

** Significant at the .01 level

Table 3. Analysis of variance for the regression of lysine expressed as a percent of sample on percent of protein using the second degree polynomial model.[a]

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F value
Attributable to regression	2	24.7908	12.39542	28446.1522**
Deviation from regression	12,610	5.4948	.00044	
Total (corrected for mean)	12,612	30.2856		

** Significant at the .01 level

[a] The coefficient of determination (r^2) using the second degree polynomial model equals 0.8186.

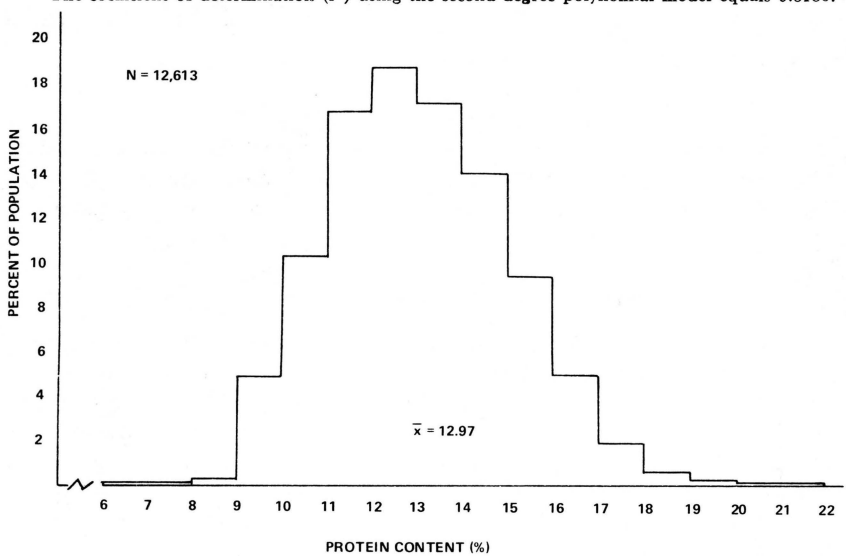

Figure 1. Frequency distribution for grain protein content among 12,613 wheats in the USDA World Collection.

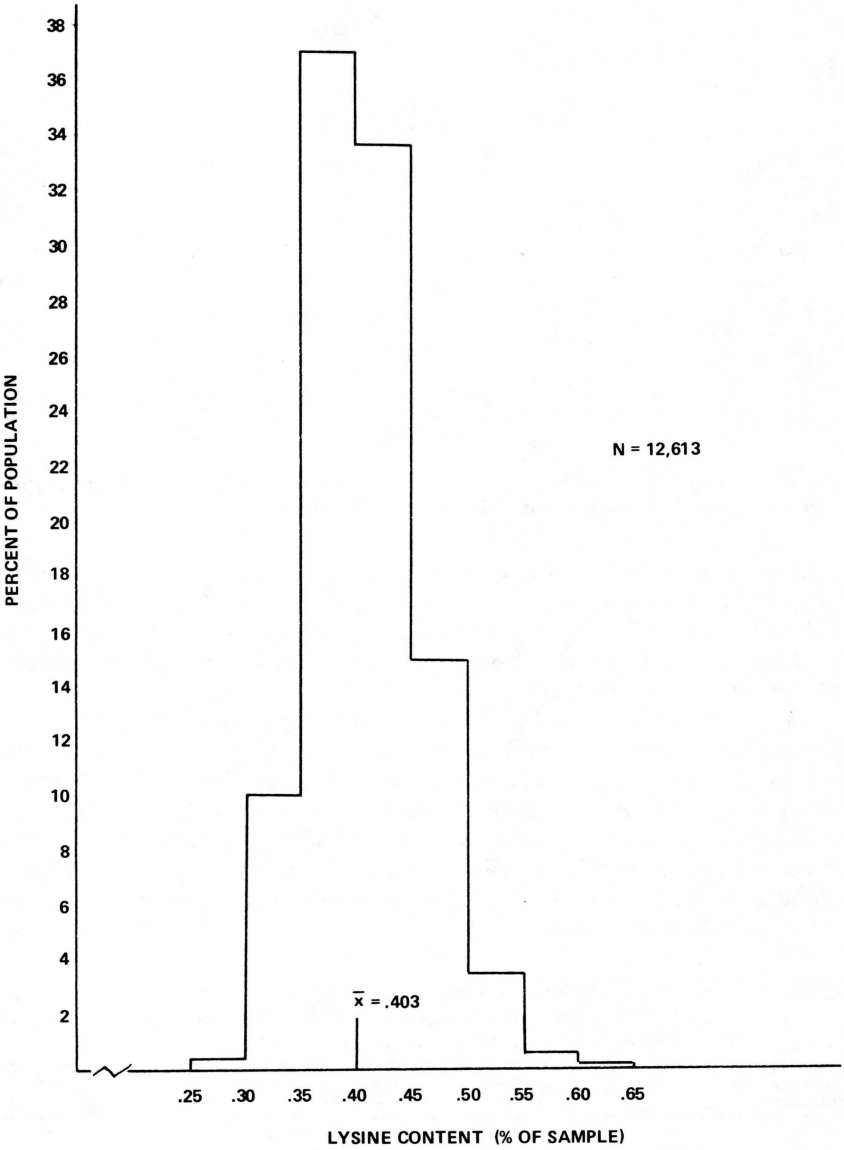

Figure 2. Frequency distribution for lysine expressed as a percent of whole grain sample among 12,613 wheats in the USDA World Collection.

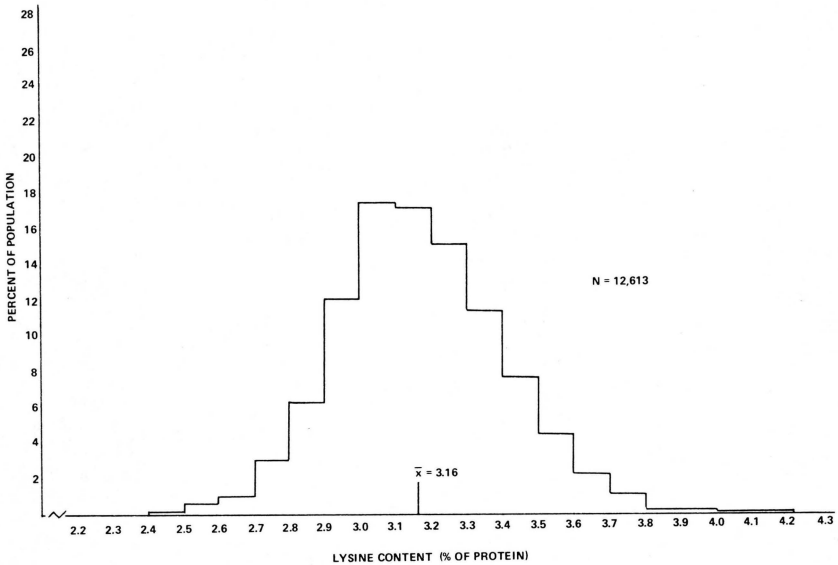

Figure 3. Frequency distribution for lysine expressed as a percent of grain protein among 12,613 wheats in the USDA World Collection.

cant negative relationship between percent protein and lysine percent of protein exists in the common wheats of the World Collection. Likewise, a highly significant positive correlation exists between percent lysine of sample and percent protein as shown by the magnitude of the correlation coefficient.

The second degree polynomial or quadratic model gave the best fit for the regression of lysine percent of sample on percent protein. The analysis of variance for this regression is given in Table 3. Regression coefficients, standard errors of the regression coefficients, and the computed "t" values are in Table 4. The coefficient of determination (r^2) value for the regression of lysine percent of sample on percent protein using this model was .8186 which indicates that about 81% of the total variation in lysine expressed as a percent of sample can be

Table 4. Regression coefficients, standard errors of the regression coefficients and computed "t" values for the regression of lysine percent of sample on percent of protein for the second degree polynomial model.[a]

Independent variable	Regression coefficient	S.E. of regression coefficient	Computed "t" value
% Protein	$b_1 = .00348$	.000899	3.8767**
(% Protein)^2	$b_2 = .00069$	.000034	20.5549**

** Significant at the .01 level

[a] Intercept = a = 0.23814

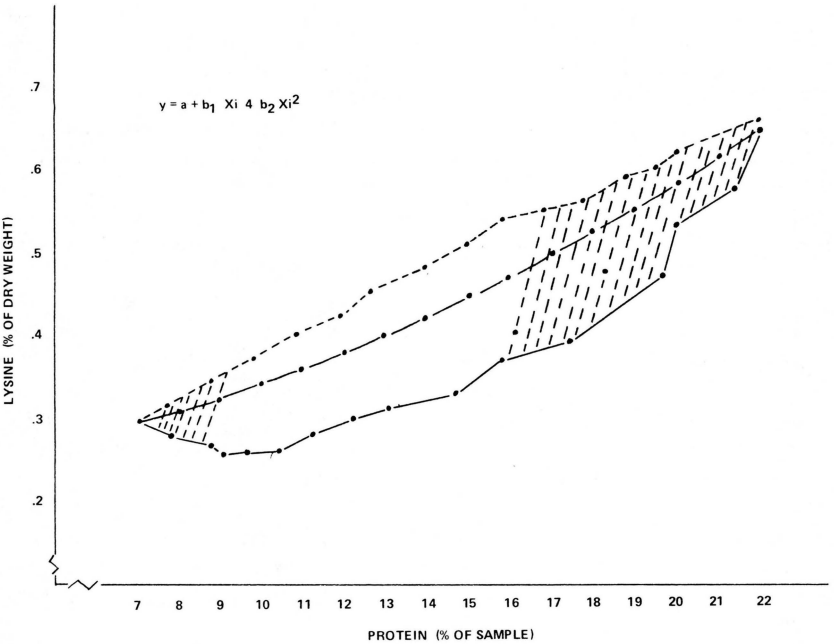

Figure 4. Curvilinear regression (second degree polynomial model) of lysine percent of dry weight on protein and the range of dispersion of lysine values about the regression line computed from the analysis of 12,613 common wheats from the USDA World Collection.

attributed to variation in protein content of the wheats analyzed. The linear regression of lysine percent of sample on percent protein was also significant but this regression resulted in a slightly smaller coefficient of determination than the quadratic model. The slightly curvilinear relationship between lysine percent of sample and percent protein is shown in Figure 4.

The third degree polynomial model provided the best fit for the regression of lysine percent of protein on percent protein. The analysis of variance for this regression is given in Table 5. Regression coeffi-

Table 5. Analysis of variance for the regression of lysine expressed as a percent of protein on percent protein using the third degree polynomial model.[a]

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F value
Attributable to regression	3	354.50676	118.16892	4610.90**
Deviations from regression	12,609	323.14521	.02563	
Total (corrected for mean)	12,612	677.65196		

** Significant at the .01 level

[a] The coefficient of determination (r^2) using the third degree polynomial model = 0.5231

Table 6. Regression coefficients, standard errors of the regression coefficients and computed "t" values for the regression of lysine percent of protein on percent protein for the third degree polynomial model.[a]

Independent variable	Mean	Regression coefficient	Standard error of reg. coef.	Computed "t" value
% Protein	12.97	$b_1 = -.61999$	.041884	-14.803**
(% Protein)[2]	172.40	$b_2 = .03277$	.003105	9.750**
(% Protein)[3]	2344.93	$b_3 = .00048$	.000076	-6.399**

** Significant at the .01 level

[a] Intercept = a = 7.12171

Correction: b2=0.03028; b3=-0.00048

cients, standard error of the regression coefficients and the computed "t" values are given in Table 6. The coefficient of determination (r^2) value for the regression of lysine percent of protein on percent protein using this model was .5231. This indicates that approximately 52% of the total variation of lysine percent of protein can be attributed to variation in protein content of the wheats analyzed. The strongly negative curvilinear relationship between lysine percent of protein and percent protein is shown in Figure 5. The relationship between lysine percent of protein and percent protein is clearly negative for wheats with less than 15% protein but for wheats with more than 15% pro-

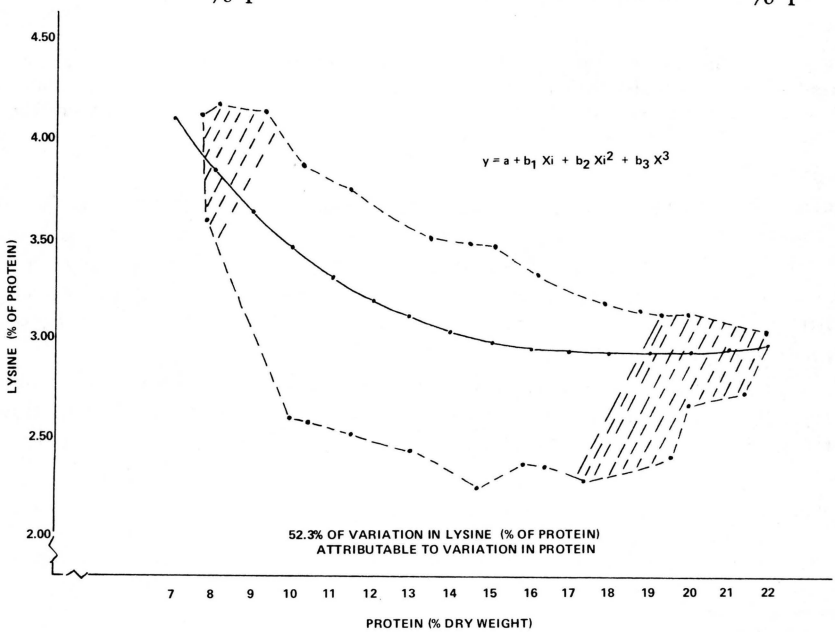

Figure 5. Curvilinear regression (third degree polynomial model) of lysine percent of protein on protein and the range of dispersion of lysine values about the regression line computed from the analysis of 12,613 common wheats from the USDA World Collection.

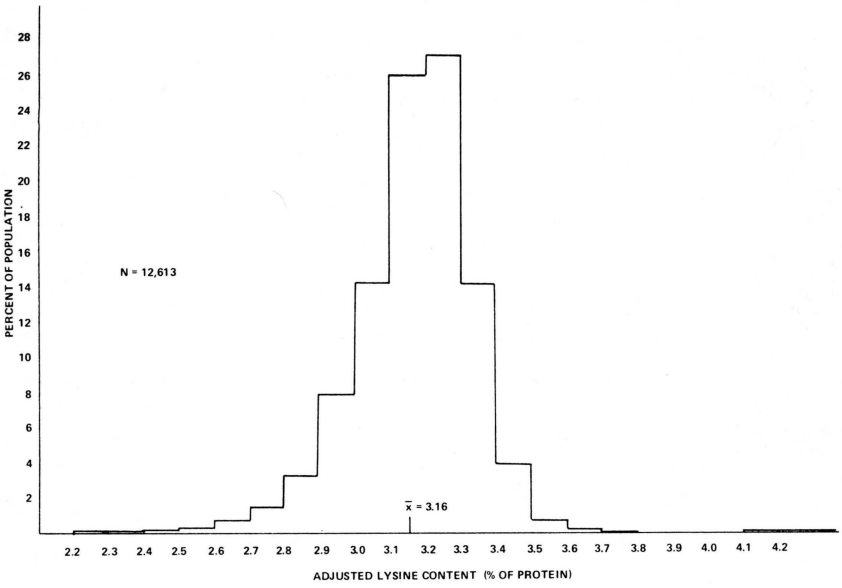

Figure 6. Frequency distribution of lysine adjusted to 12.97 percent protein among 12,613 wheats in the USDA World Collection.

tein there is little apparent effect of protein percent on percent lysine of protein.

Since 52% of the variation in lysine percent of protein is attributable to variation in protein, lysine values (% of protein) were adjusted to the mean protein level, thus removing as much as possible that portion of the variability due to variation in percent protein. The frequency distribution of the adjusted lysine (% of protein) values is shown in Figure 6. The mean for the adjusted lysine values is the same as the unadjusted mean. Adjusting the lysine (% of protein) values to the mean protein level permits valid comparison of lysine values to be made between wheats of differing protein contents.

The average error variance of an adjusted lysine value, s^2_{adj}, for the wheats of the World Collection is 0.0256 and the standard error of an adjusted value, $\sqrt{s^2_{adj}}$, is 0.1601%. The standard error of a difference between two adjusted values, s_D, is 0.2264%. The 95% confidence interval for the adjusted lysine values about the mean lysine value (% of protein) is $3.16 \pm .44\%$. Adjusted lysine values equal to or larger than 3.60% can be considered as being significantly different than the mean for lysine expressed as a percent of protein.

The 12,613 common wheats analyzed were ranked in descending order for percent protein, lysine percent of sample, lysine percent of protein, and adjusted lysine percent of protein. The 50 highest and 10 lowest entries of the World Collection for these criteria are listed

in Tables 7, 8, 9, and 10 respectively. Deviations of the measured lysine (% of protein) values from the values predicted by the third degree polynomial prediction equation are also given in these tables. Adjusted lysine values and lysine deviations are merely two different but equivalent ways of expressing the same values.

Table 7. Protein and lysine values for common wheats among 12,613 analyzed from the U.S.D.A. world collection exhibiting the highest and lowest protein values.

Rank	C.I. or P.I. No.	% Protein[a]	% Lysine[a]	Lysine/ protein %	Adjusted lysine/ protein[b]	Deviation lysine/ protein[c]
1	185700P	22.0	.66	3.04	3.22	.06
2	225252P	21.4	.58	2.74	2.92	–.24
3	6654	21.1	.59	2.83	3.02	–.15
4	185343P	21.0	.60	2.86	3.05	–.11
5	272423P	21.0	.63	3.01	3.20	.04
6	298577P	20.9	.59	2.86	3.05	–.11
7	202800P	20.9	.62	3.00	3.19	.03
8	204008P	20.7	.61	2.98	3.17	.01
9	225248P	20.7	.60	2.90	3.09	–.07
10	174680P	20.6	.58	2.81	3.00	–.16
11	191796P	20.3	.58	2.87	3.07	–.10
12	185349P	20.2	.59	2.94	3.14	–.02
13	174701P	20.0	.55	2.79	2.99	–.17
14	174684P	20.0	.62	3.14	3.34	.18
15	3275	20.0	.53	2.68	2.88	–0.28
16	178005P	19.9	.56	2.84	3.04	–0.12
17	168794P	19.8	.56	2.84	3.04	–0.12
18	166726P	19.8	.60	3.03	3.23	.07
19	184220P	19.8	.57	2.92	3.12	–.04
20	286000P	19.8	.60	3.05	3.25	.09
21	225244P	19.7	.57	2.93	3.13	–.03
22	3384	19.7	.47	2.42	2.62	–.54
23	185388P	19.6	.59	3.01	3.21	.05
24	185233P	19.6	.56	2.89	3.09	–.07
25	272422P	19.6	.53	2.73	2.93	–.23
26	192812P	19.5	.60	3.08	3.29	.12
27	6225	19.5	.58	2.99	3.20	.03
28	174702P	19.5	.59	3.04	3.25	.08
29	192750P	19.4	.58	2.99	3.20	.03
30	13793	19.4	.58	2.99	3.20	.03
31	184523P	19.3	.60	3.14	3.35	.18
32	298584P	19.3	.54	2.82	3.03	–.14
33	298583P	19.2	.56	2.92	3.13	–.04
34	298587P	19.2	.58	3.05	3.26	.09
35	272426P	19.2	.56	2.94	3.15	–.02

Table 7 (cont.)

Rank	C.I. or P.I. No.	% Protein[a]	% Lysine[a]	Lysine/ protein %	Adjusted lysine/ protein[b]	Deviation lysine/ protein[c]
36	272427P	19.2	.56	2.95	3.16	–.01
37	3300	19.2	.50	2.61	2.82	–.34
38	272420P	19.1	.57	3.00	3.21	.05
39	191160P	19.1	.58	3.07	3.28	.12
40	184176P	19.0	.56	2.97	3.18	.02
41	298580P	19.0	.56	2.96	3.17	.01
42	176223P	19.0	.57	3.00	3.21	.05
43	178008P	18.9	.56	2.97	3.18	.02
44	166841P	18.9	.57	3.03	3.24	.08
45	286004P	18.9	.58	3.09	3.30	.14
46	285965P	18.9	.59	3.13	3.34	.18
47	203985P	18.9	.55	2.93	3.14	–.02
48	243746P	18.9	.54	2.87	3.08	–.08
49	12371	18.9	.56	2.97	3.18	.02
50	184148P	18.8	.55	2.95	3.16	–.00
.	.					
.	.					
.	.					
12604	121815P	8.1	.32	4.04	3.37	.21
12605	127079P	8.1	.30	3.76	3.09	–.07
12606	125352P	8.0	.28	3.59	2.90	–.26
12607	222670P	8.0	.32	4.03	3.34	.18
12608	127097P	7.8	.28	3.63	2.90	–.27
12609	119317P	7.7	.29	3.85	3.09	–.07
12610	117421P	7.7	.31	4.12	3.36	.20
12611	166759P	7.6	.29	3.94	3.16	–.01
12612	135073P	7.5	.30	4.09	3.28	.12
12613	181329P	6.9	.29	4.26	3.30	.13

[a] Dry weight basis

[b] Adjusted to 12.97% protein using curvilinear equation

[c] Deviation of measured lysine (% of protein) from lysine (% of protein) predicted from curvilinear regression equation.

Table 8. Protein and lysine values for common wheats in the U.S.D.A. World Collection with the highest and lowest lysine (% of sample) values.

Rank	C.I. or P.I. No.	% Protein[a]	% Lysine[a]	Lysine/ protein %	Adjusted lysine/ protein[b]	Deviation lysine/ protein[c]
1	185700P	22.0	.66	3.04	3.22	.06
2	272423P	21.0	.63	3.01	3.20	.04
3	202800P	20.9	.62	3.00	3.19	.03
4	174684P	20.0	.62	3.14	3.34	.18
5	204008P	20.7	.61	2.98	3.17	.01
6	225248P	20.7	.60	2.90	3.09	−.07
7	185343P	21.0	.60	2.86	3.05	−.11
8	192812P	19.5	.60	3.08	3.29	.12
9	184523P	19.3	.60	3.14	3.35	.18
10	166726P	19.8	.60	3.03	3.23	.07
11	286000P	19.8	.60	3.05	3.25	.09
12	285965P	18.9	.59	3.13	3.34	.18
13	298577P	20.9	.59	2.86	3.05	−.11
14	254077P	18.8	.59	3.16	3.37	.21
15	174702P	19.5	.59	3.04	3.25	.08
16	185349P	20.2	.59	2.94	3.14	−.02
17	185388P	19.6	.59	3.01	3.21	.05
18	6654	21.1	.59	2.83	3.02	−.15
19	6225	19.5	.58	2.99	3.20	.03
20	191160P	19.1	.58	3.07	3.28	.12
21	191796P	20.3	.58	2.87	3.07	−.10
22	192750P	19.4	.58	2.99	3.20	.03
23	225252P	21.4	.58	2.74	2.92	−.24
24	174680P	20.6	.58	2.81	3.00	−.16
25	298587P	19.2	.58	3.05	3.26	.09
26	286004P	18.9	.58	3.09	3.30	.14
27	285812P	18.8	.58	3.09	3.30	.14
28	285914P	18.6	.58	3.16	3.37	.21
29	13793	19.4	.58	2.99	3.20	.03
30	13790	18.8	.57	3.04	3.25	.09
31	13792	18.6	.57	3.06	3.27	.11
32	272420P	19.1	.57	3.00	3.21	.05
33	176223P	19.0	.57	3.00	3.21	.05
34	176221P	18.7	.57	3.06	3.27	.11
35	166841P	18.9	.57	3.03	3.24	.08
36	184220P	19.8	.57	2.92	3.12	−.04
37	225244P	19.7	.57	2.93	3.13	−.03
38	192738P	18.6	.57	3.09	3.30	.14
39	191273P	17.9	.57	3.19	3.40	.24
40	185233P	19.6	.56	2.89	3.09	−.07
41	192746P	18.4	.56	3.05	3.26	.10
42	196903P	18.4	.56	3.09	3.30	.14
43	191782P	18.8	.56	3.01	3.22	.06
44	243727P	17.8	.56	3.18	3.39	.23
45	184176P	19.0	.56	2.97	3.18	.02

Table 8 (cont.)

Rank	C.I. or P.I. No.	% Protein[a]	% Lysine[a]	Lysine/ protein %	Adjusted lysine/ protein[b]	Deviation lysine/ protein[c]
46	168794P	19.8	.56	2.84	3.04	−.12
47	178008P	18.9	.56	2.97	3.18	.02
48	178005P	19.9	.56	2.84	3.04	−.12
49	286008P	18.5	.56	3.04	3.25	.09
50	285880P	18.0	.56	3.15	3.36	.20
.	.					
.	.					
.	.					
12604	10005	11.2	.28	2.58	2.45	−.72
12605	164501P	8.5	.27	3.29	2.71	−.45
12606	127091P	8.8	.27	3.14	2.62	−.54
12607	166878P	8.5	.27	3.26	2.68	−.48
12608	166475P	9.8	.26	2.67	2.34	−.83
12609	10013	10.4	.26	2.59	2.35	−.81
12610	9047	9.0	.26	2.99	2.51	−.65
12611	9393	9.7	.26	2.78	2.43	−.74
12612	9050	9.9	.25	2.60	2.28	−.88
12613	135050P	9.4	.25	2.76	2.36	−.81

[a] Dry weight basis

[b] Adjusted to 12.97% protein using curvilinear equation

[c] Deviation of measured lysine (% of protein) from lysine (% of protein) predicted from curvilinear regression equation.

Table 9. Protein and lysine values for common wheats in the USDA World Collection with the highest and lowest lysine per unit protein values.

Rank	C.I. or P.I. No.	% Protein[a]	% Lysine[a]	Lysine/ protein %	Adjusted lysine/ protein[b]	Deviation lysine/ protein[c]
1	181329P	6.9	.29	4.26	3.30	.13
2	184250P	8.1	.33	4.17	3.50	.34
3	13449	9.2	.38	4.15	3.71	.55
4	117421P	7.7	.31	4.12	3.36	.20
5	135073P	7.5	.30	4.09	3.28	.12
6	166757P	8.5	.34	4.05	3.47	.31
7	121815P	8.1	.32	4.04	3.37	.21
8	222670P	8.0	.32	4.03	3.34	.18
9	166951P	8.3	.33	3.99	3.37	.21
10	166946P	8.6	.34	3.97	3.41	.25
11	173438P	9.0	.35	3.97	3.49	.33
12	268449P	9.0	.35	3.97	3.49	.33
13	225221P	8.3	.33	3.97	3.35	.19
14	112344P	9.1	.36	3.95	3.49	.33
15	234860P	9.7	.38	3.94	3.59	.42
16	166759P	7.6	.29	3.94	3.16	−0.01
17	191043P	9.5	.37	3.93	3.54	.38
18	167455P	8.4	.32	3.92	3.32	.16
19	166859P	8.3	.32	3.91	3.29	.13
20	166901P	9.2	.35	3.90	3.46	.30

Table 9 (cont.)

Rank	C.I. or P.I. No.	% Protein[a]	% Lysine[a]	Lysine/ protein %	Adjusted lysine/ protein[b]	Deviation lysine/ protein[c]
21	222671P	9.3	.36	3.90	3.48	.31
22	184194P	9.4	.36	3.90	3.50	.33
23	225243P	8.3	.32	3.90	3.28	.12
24	222674P	8.7	.33	3.89	3.35	.19
25	157600P	8.4	.32	3.89	3.29	.13
26	137740P	8.4	.32	3.88	3.28	.12
27	167697P	9.0	.34	3.87	3.39	.23
28	166624P	9.3	.36	3.87	3.45	.28
29	166921P	8.3	.32	3.87	3.25	.09
30	166916P	9.4	.36	3.87	3.47	.30
31	121814P	8.7	.33	3.87	3.33	.17
32	225223P	8.5	.32	3.87	3.29	.13
33	94540P	8.8	.34	3.87	3.35	.19
34	13447	10.3	.39	3.87	3.61	.45
35	220358P	9.1	.35	3.86	3.40	.24
36	220350P	9.0	.34	3.86	3.38	.22
37	225232P	8.5	.32	3.85	3.27	.11
38	119317P	7.7	.29	3.85	3.09	−.07
39	135070P	8.4	.32	3.85	3.25	.09
40	135061P	8.4	.32	3.85	3.25	.09
41	137737P	9.2	.35	3.84	3.40	.24
42	166674P	8.4	.32	3.84	3.24	.08
43	181325P	9.4	.36	3.84	3.44	.27
44	211099P	9.0	.34	3.83	3.35	.19
45	222683P	8.5	.32	3.83	3.25	.09
46	167508P	8.5	.32	3.83	3.25	.09
47	167681P	9.0	.34	3.83	3.35	.19
48	225233P	8.1	.31	3.83	3.16	.00
49	117018P	9.0	.34	3.83	3.35	.19
50	94442P	9.6	.36	3.82	3.45	.29
.	.					
.	.					
.	.					
12604	9402	13.9	.34	2.46	2.57	−.59
12605	6477	16.0	.39	2.44	2.63	−.53
12606	13322	13.1	.31	2.44	2.50	−.67
12607	12144	13.6	.33	2.43	2.52	−.64
12608	9458	16.5	.40	2.43	2.63	−.53
12609	3384	19.7	.47	2.42	2.62	−.54
12610	10379	15.8	.37	2.38	2.57	−.60
12611	9432	16.4	.39	2.37	2.57	−.59
12612	94597P	17.4	.39	2.28	2.49	−0.67
12613	117714P	14.7	.33	2.25	2.40	−0.76

[a] Dry weight basis

[b] Adjusted to 12.97% protein using curvilinear equation

[c] Deviation of measured lysine (% of protein) from lysine (% of protein) predicted from curvilinear regression equation.

Table 10. Protein and lysine values for common wheats in the USDA World Collection with the highest and lowest adjusted lysine values.

Rank	C.I. or P.I. No.	% Protein[a]	% Lysine[a]	Lysine/ protein %	Adjusted lysine/ protein[b]	Deviation lysine/ protein[c]
1	13449	9.2	.38	4.15	3.71	.55
2	9364	11.4	.43	3.76	3.66	.49
3	271075P	15.1	.52	3.47	3.64	.47
4	10517	14.5	.50	3.49	3.63	.47
5	94460P	12.4	.45	3.63	3.63	.46
6	117628P	15.8	.54	3.43	3.62	.45
7	9450P	12.6	.45	3.60	3.61	.45
8	13447	10.3	.39	3.87	3.61	.45
9	12189	14.9	.51	3.45	3.61	.44
10	13058	14.2	.49	3.48	3.61	.44
11	2092	15.1	.52	3.44	3.61	.44
12	4191	13.5	.47	3.52	3.60	.44
13	117500P	13.1	.46	3.54	3.60	.43
14	94500P	14.4	.49	3.46	3.60	.43
15	8877	12.8	.45	3.57	3.60	.44
16	4731	13.4	.47	3.51	3.59	.42
17	3663	13.9	.48	3.48	3.59	.43
18	3392	15.1	.51	3.42	3.59	.42
19	9441	12.6	.45	3.58	3.59	.43
20	234860P	9.7	.38	3.94	3.59	.42
21	254053P	13.1	.46	3.52	3.58	.41
22	4556	13.6	.47	3.49	3.58	.41
23	5005	14.1	.48	3.46	3.58	.41
24	12052	12.9	.45	3.54	3.58	.42
25	86209P	13.0	.45	3.53	3.58	.41
26	178649P	11.0	.41	3.74	3.58	.42
27	168650P	12.3	.44	3.59	3.58	.41
28	9352	11.6	.42	3.65	3.57	.41
29	9341	13.4	.46	3.49	3.57	.40
30	185224P	13.2	.46	3.51	3.57	.41
31	191150P	14.2	.48	3.44	3.57	.40
32	191300P	13.3	.46	3.50	3.57	.41
33	181455P	13.7	.47	3.46	3.56	.39
34	192528P	15.9	.53	3.37	3.56	.40
35	9317	11.5	.42	3.66	3.56	.40
36	2473	14.3	.49	3.43	3.56	.40
37	13249	15.9	.53	3.37	3.56	.40
38	173248P	11.4	.41	3.67	3.56	.40
39	117760P	14.6	.49	3.41	3.56	.39
40	254068P	13.1	.45	3.50	3.56	.39
41	171023P	13.6	.47	3.46	3.55	.39
42	13269	14.8	.50	3.40	3.55	.39
43	1915	14.4	.49	3.41	3.55	.38
44	4570	13.3	.46	3.48	3.55	.39
45	4066	14.7	.50	3.40	3.55	.39

Table 10 (cont.)

Rank	C.I. or P.I. No.	% Protein[a]	% Lysine[a]	Lysine/ protein %	Adjusted lysine/ protein[b]	Deviation lysine/ protein[c]
46	9361	10.9	.40	3.72	3.55	.38
47	180612P	13.5	.46	3.47	3.55	.39
48	191292P	13.0	.45	3.50	3.55	.38
49	81793P	11.9	.42	3.60	3.55	.38
50	12186	14.3	.49	3.42	3.55	.39
.	.					
.	.					
.	.					
12604	10005	11.2	.28	2.58	2.45	–.72
12605	9230	12.1	.30	2.48	2.45	–.72
12606	166758P	10.7	.28	2.65	2.45	–.71
12607	9393	9.7	.26	2.78	2.43	–.74
12608	165549P	11.5	.28	2.52	2.42	–.74
12609	117714P	14.7	.33	2.25	2.40	–.76
12610	135050P	9.4	.25	2.76	2.36	–.81
12611	10013	10.4	.26	2.59	2.35	–.81
12612	166475P	9.8	.26	2.67	2.34	–.83
12613	9050	9.9	.25	2.60	2.28	–.88

[a] Dry weight basis

[b] Adjusted to 12.97% protein using curvilinear equation

[c] Deviation of measured lysine (% of protein) from lysine (% of protein) predicted from curvilinear regression equation.

DISCUSSION

The influence of environment on protein and lysine content of wheat has been noted (8, 16, 24). The protein and lysine values obtained in this study are the results of measurements made on phenotypes. The phenotypic expression of a trait such as protein content of the grain may be considered as a linear function of the genotype and the environment in which the genotype was grown. The following linear model applies to the wheats analyzed in this study (2):

$$P_{ijk} = \mu + g_i + l_j + y_k + (gl)_{ij} + (gy)_{ik} + (yl)_{jk} + (gly)_{ijk} + e_{ijk}.$$

P_{ijk} is the measurement of a trait of the ith genotype at the jth site in the kth year; μ is the general mean of all genotypes; g_i, l_j, and y_k are the average effects of the ith genotype, lth location, and the kth year, respectively; $(gl)_{ij}$, $(gy)_{ik}$, $(yl)_{jk}$, and $(gly)_{ijk}$ are the interaction effects, and e_{ijk} is the microenvironmental effect at a site plus the random error of measurement.

Since the wheats analyzed were all grown at Mesa, Arizona and since the year to year variation at Mesa under irrigated conditions is believed to be small, the amount of variation due to location and year effects is probably small. The genotype and environmental inter-

actions, however, are probably large. The magnitude of these interaction effects can be illustrated by comparing the results obtained by Lawerence et al. (8) for wheats grown at Pullman, Washington with the results of this study. Lawerence et al. reported that the entry CI No. 5286 had a lysine content expressed as a percent of protein of 3.54% and the entry PI No. 124340 had 2.48% lysine. In this study CI No. 5286 had 2.98% lysine of protein and PI No. 124340 had 3.26%. The adjusted lysine values for both these entries in this study are less than the mean adjusted lysine value.

With only one measurement made on each entry, the genotypic effect is completely confounded with the environmental and the genotype x environment interaction effects. Hence it is not possible from the results of this study to state that a particular entry in the World Collection is genetically superior to any other entry.

It is, however, possible to tentatively identify wheats likely to be genetically superior in their ability to produce grain of high protein and lysine content. It will be necessary to grow these wheats in an array of environments in order to determine which are genetically superior in regard to protein and lysine content of the grain. The results of Stroike and Johnson (24) have shown that wheats known to possess genes for high protein content consistently have protein contents higher than the mean of ordinary wheats over an array of environments. This superiority in an array of environments for which the wheats are adapted must be used as the criterion for the selection of high protein and lysine lines from the World Collection for breeding purposes.

The wide range of values for protein content suggests that significant genetic variability for protein content does exist in the common wheats of the World Collection. Comparison of the variability for protein content of the World Collection common wheats with the protein content of other *Triticum* species and genera related to wheat (8, 27) would indicate that the World Collection contains as much genetic variability for protein content as the non-hexaploid sources. The common wheats of the World Collection with more than 17% protein differ by two standard deviations from the mean percent of protein. There are 500 such wheat entries. These wheats represent the best potential source of genes for high protein.

Part of the variability for protein content in the World Wheat Collection is non-genetic in origin. The yielding ability of the wheats in the World Collection undoubtedly differs in the Mesa, Arizona environment. Lower yielding wheats have a general tendency to be higher in protein content than higher yielding wheats. Many wheats of the World Collection have weak straw and lodge easily, especially when heavily fertilized and grown under irrigation. Lodging can result in

poorly filled, shriveled grain. Shriveled grain is usually higher in protein content than plump, well-filled grain. It is possible that some wheats did not fill properly because of high temperatures that can occur in Mesa, Arizona. Hence, some of the wheats found to be high in protein content may not have genes for high protein but are high in protein because of environmental and genotype x environment interaction effects.

Lysine content measured as a percent of sample is highly correlated with percent protein. This is to be expected since lysine is a constituent of protein. Those samples which were highest in percent protein were also the highest in lysine content expressed as percent of sample. Improvement in the lysine content of the grain can be accomplished by selecting for higher protein content. The wheats with the highest lysine percent of sample do not necessarily have the highest lysine percent of protein. In fact, the opposite is true.

Wheats low in protein content of the grain tend to be high in lysine percent of protein. This is shown by the regression of lysine (% of protein) on percent protein. This negative relationship can be explained by a general decrease in the proportion of globulin and albumin protein as the protein content increases (13, 15, 20, 21, 26). If this explanation is correct, the albumin-globulin:gluten ratio should be relatively stable for wheats with more than 15% protein, i.e., not affected by the percent of protein.

The range of variability that exists for protein and lysine among the common wheats of the World Collection is equal to that reported (8, 27) for the *Triticum* species other than the common wheats and for the genera related to wheat.

Forty-eight percent of the variability of lysine (% of protein) is not explained by the regression of lysine on protein. This portion of the variability of lysine percent of protein is due to genotypic, environmental, and genotype x environment interaction effects. At least a portion of this variability is probably due to genetic differences among the wheats tested. The influence of protein content on lysine content of protein can be largely removed by adjusting all lysine values to the mean protein level. Because of the influence of protein, comparisons of unadjusted lysine values of samples of different protein content have little validity.

Wheats that have the largest positive deviation of their unadjusted lysine values from the lysine values predicted by the regression equation are the most likely sources of genes for high lysine. These wheats have the highest adjusted lysine values. Adjusted lysine values can be used to compare the lysine content of wheats across the entire protein range exhibited by the World Collection common wheats (6 to 22%). In comparison with lysine expressed as a percent of sample, lysine percent of protein measures protein quality rather than protein

quantity. Adjusted lysine values provide the best criteria for selection for protein quality in regards to lysine content.

Wheats with adjusted lysine values larger than 3.60% should be considered a good potential source of genes for high lysine. These wheats and others with high adjusted lysine values should be tested in an array of environments. 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.

Identification of wheats genetically superior in protein content and in lysine content of protein will make it possible to combine genes for high protein with genes for high lysine. This should result in a significant improvement in the nutritive value of wheat grain. The analysis of the common wheats of the World Collection was the first step of this identification procedure.

Since the germ and the bran of wheat are higher in both protein content and lysine content of protein than the endosperm (10), selection for protein and lysine on a whole kernel basis could result in a decrease in the proportion of endosperm of the wheat kernel. For those societies that use primarily the endosperm in flour production, it will be necessary to select on the protein and lysine content of the endosperm.

In addition to protein quantity and quality, the nutritive value of wheat is dependent upon other factors such as starch digestibility. Likewise, protein quality is dependent upon the balance of the other essential amino acids as well as upon lysine content. Consequently biological assays, i.e., feeding trials, should be conducted with high protein and high lysine cultivars as they are developed to determine if any positive improvement in the nutritive value of the wheat grain has been achieved.

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