Selection of High-Lysine Corns with Varied Kernel Characteristics and Compositions by a Rapid Turbidimetric Assay for Zein

Jerrold W. Paulis,* Joseph S. Wall, William F. Kwolek,[1] and Gary L. Donaldson

Breeders are discovering and developing new high-lysine corns that have improved kernel characteristics, different starch types, and elevated protein levels. Because of the varied physical characteristics of these grains, an easy method for estimating lysine in corn is required to select corn with better protein quality. Our rapid turbidimetric assay of zein in extracts of meal was tested as a means of estimating lysine on a large number of whole corn and endosperm meals. The meals were from grains varying considerably in hardness and composition. High-oil, high-protein, high-amylose, *sugary-2*, *brittle-2*, and combinations of *sugary-2* and *waxy* with *opaque-2* and other corns were analyzed. A highly significant negative correlation was found between lysine content of the protein and zein content of the meal, a correlation similar to that observed for normal, *opaque-2*, and *floury-2* grains. The method has been used successfully to select high-lysine grains from among vitreous kernels having *opaque-2* backgrounds. These results demonstrate the applicability of our turbidimetric analysis of zein for estimating lysine in corn in breeding programs and for distinguishing high-lysine grain in commerce.

Several new sources of high-lysine corn have been reported since the discovery that mutant *opaque-2* and *floury-2* strains (Mertz *et al.*, 1964; Nelson *et al.*, 1965) are richer in that amino acid than are normal dent types. Baudet *et al.* (1968) and Nordstrom and Meade (1969) found that certain corn genotypes differing from normal corn in the type of starch exceeded the normal grain in lysine content. Combining certain of these mutant genes with those of *opaque-2* modified grain characteristics, such as starch properties and kernel hardness, and elevated lysine content (Baudet *et al.*, 1968; Misra *et al.*, 1972). Analysis of segregated kernels from hybrid *opaque-2* lines revealed that modifier genes exist which cause the endosperm of *opaque-2* kernels to become more vitreous but to retain high-lysine content (Bauman and Aycock, 1970). Because of low yield, fragility of kernel, and susceptibility to disease of the floury types of *opaque-2* corn, current research is directed toward development of high-lysine lines of corn with more vitreous kernels. To facilitate development of these modified types by breeders and to help distinguish them from ordinary corn in commerce, simpler, faster methods for determining lysine in corn are essential.

The automated amino acid analyzer separates amino acids in hydrolysates by chromatography and determines them colorimetrically; it is an accurate means of lysine analysis (Villegas *et al.*, 1968), but the instrument and its maintenance are costly. Although gas chromatography can be used to determine lysine in cereals (Zscheile and Brannaman, 1972), this method also requires sophisticated chemical techniques and instrumentation. Alternate methods to determine lysine in corn include microbiological assay (Snell, 1957) and measurement of CO_2 liberated by the enzyme, lysine decarboxylase (White and Gauger, 1967). Most popular are colorimetric procedures based on reactions of 2-chloro-3,5-dinitropyridine (Tsai *et al.*, 1972) and di- or tribenzenesulfonate (Subramanian *et al.*, 1970; Concon, 1972) with lysine. However, all these methods require considerable technical skill and are time-consuming. A simple technique based on dye-binding employed by Mossberg (1969) and by Kieselwetter *et al.* (1968) reportedly screens grain samples for lysine rapidly.

Northern Regional Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Peoria, Illinois 61604.

[1] Biometrician, North Central Region, Agricultural Research Service, U. S. Department of Agriculture, stationed at the Northern Laboratory.

In our earlier investigations of a simple, fast mass screening to select high-lysine corns, we found that the amount of zein protein in the grains correlated inversely with the amount of lysine in the total protein of the grain or endosperm (Paulis *et al.*, 1974). Zein protein could be rapidly determined turbidimetrically in 70% ethanol–0.5% sodium acetate extracts of freshly ground meals of whole grain or endosperm. To demonstrate that this simple method could approximate lysine in corn and thereby distinguish high-lysine lines, we tested the procedure on a wide variety of corn genotypes. These grains were selected to show the effect that high or low protein, variation in starch type, oil content, grain hardness, and level of lysine had on the accuracy of the method.

MATERIALS AND METHODS

The apparatus, reagents, and procedures were based on those previously shown to be most effective in yielding reproducible, accurate results (Paulis *et al.*, 1974). Since the method has now been standardized in our laboratory, details of the instrumentation and techniques are summarized below to permit close duplication of our results by others.

Apparatus. *Mills.* Udy cyclone-hammer mill (Udy Instrument Co.) with 0.024-in. screen. A cyclone-hammer mill permits rapid grinding without apparent heating. Micro-Wiley mill (Arthur Thomas Co.) with 40 U. S. mesh screen.

Balance. A standard single-pan analytical balance accurate to 0.2 mg (Mettler Model B-5 or equivalent).

Shaker. Heavy-duty box-type reciprocating shaker (Precision Scientific Co.) that accommodates 40 centrifuge tubes (50 ml). Shaking times must be varied for different commercial shakers to attain optimum extraction.

Automatic Pipets. All-glass syringe pipets (Repipet-Labindustries, Inc.), adjustable volume 50 and 20 ml, with standard tapered glass joints to fit 2-l. bottles. It is essential that the device to dispense the alcoholic extractant be all glass to prevent extraction of interfering substances from many plastic materials used for tubing or containers.

Centrifuge Tubes. Polypropylene, 50-ml capacity, 104 × 28.6 mm, Oak Ridge type, with Teflon-lined screw caps (International Equipment Co.).

Centrifuge. International Equipment Co. Model HN or equivalent.

Colorimeter. Beckman B spectrophotometer or similar standard laboratory colorimeter.

Colorimeter tubes. Matched 19 × 150 mm (Coleman).

Reagents. *70% Ethanol–0.5% Sodium Acetate.* Add 5 g of anhydrous sodium acetate and 737 ml of 95% ethanol to a 1000-ml flask and make up to volume with distilled water.

1% Sodium Chloride Solution (w/v). Dissolve 1 g of sodium chloride in distilled water to give 100 ml of solution.

Samples. Normal dent, *opaque-2,* and *floury-2* meals used to establish the standard curve were identical with those described previously (Paulis *et al.,* 1974). The grain analyzed represented 24 specialty corns selected to provide a wide range in composition of lysine, oil, protein, and starch. They were grown in 1968, 1969, 1971, and 1972 and came from nine sources. All had been dried in air or forced-air driers as specified for seed corns. Kernels from one *opaque-2* hybrid line were manually separated into three groups differing in degrees of hardness or translucency. Translucency was determined by examining the light transmittancy of kernels placed on a sheet of frosted glass over a fluorescent light source.

Kernels were manually dissected to provide endosperm sections. Lysine analyses were averages of determinations on duplicate acid hydrolysates with a Beckman 121 amino acid analyzer as described earlier by Paulis *et al.* (1974). Protein was calculated as 6.25 times nitrogen determined by semimicro-Kjeldahl.

Zein Determination and Correlation with Lysine. (1) Grind 50 g of each sample of whole corn grain in the Udy mill with 0.024-in. screen or 10 g of each endosperm sample in the Wiley mill with 40 U. S. mesh screen. Where amounts of whole grain are limited, as little as 1.0 g may be ground if a suitable mill is available to yield meal passing a 60 U. S. mesh screen. Meals should be analyzed no later than 3 days at 4° storage after grinding. (2) Weigh 0.200 g ± 0.001 g of each sample of well-mixed corn meal into 50-ml centrifuge tubes. (3) Add 20 ml of 70% ethanol–0.5% sodium acetate into each centrifuge tube. (4) Agitate all the capped centrifuge tubes on a reciprocating shaker for 1 hr at room temperature. Tubes are wedged in shaker horizontally, parallel to direction of movement. (5) Centrifuge tubes for 5 min at about 2400 rpm. (6) Remove with pipet duplicate 2-ml samples of clear supernatant from each centrifuge tube and place each sample in a matched colorimeter tube. Extracts should be analyzed immediately. (7) Add vigorously by blowing from a 10-ml Mohr pipet 6 ml of 1% sodium chloride into each sample of extracts in the colorimeter tubes. (8) After 1 hr, remove air bubbles from each solution by inclining tubes toward the horizontal and rotating them. Read absorbance at 590 nm in colorimeter. (9) Convert absorbance at 590 nm ($A_{590\,nm}$/20-mg sample) to percentage lysine in protein by the prediction equation

$$\text{lysine, g per 100 g of protein} = b - a[(A_{590\ nm}/\text{20-mg sample})/\%\ \text{protein}] \quad (1)$$

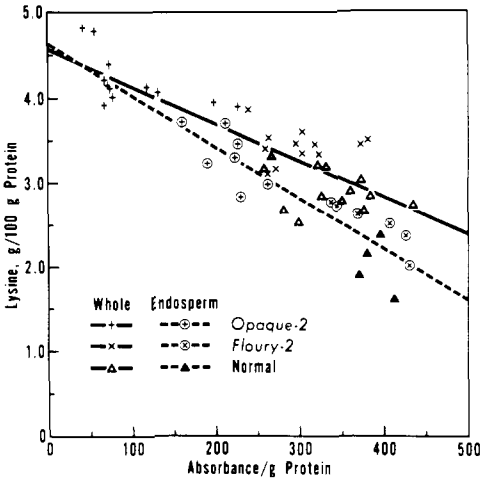

Figure 1. Turbidity of 70% ethanol–0.5% sodium acetate extracts (absorbance at 590 nm) per gram of protein *vs.* per cent lysine of protein of whole grain and endosperm meals of normal, *opaque-2,* and *floury-2* corns. Lines are least-squares plots.

For whole meal, b = 4.60 and a = 22.20. For endosperm meal, b = 4.62 and a = 30.05.

These relationships are based on regression curves computed for 37 whole kernel and 17 endosperm samples (Paulis *et al.,* 1974). Alternatively, percentage lysine in protein may be read from standard plots relating it to zein turbidity measured as absorbance at 590 nm/g of protein (eq 2) for a series of corn grain or endosperm samples. Slopes of standard curves in Figure 1 are equal to a in eq 1 divided by 5000.

$$A_{590}/\text{g of protein} = (A_{590}/\text{20-mg sample})(5000/\%\ \text{protein}) \quad (2)$$

(10) Each laboratory should conduct the turbidimetric determinations on several samples of corn having known, but different, lysine contents to establish that experimental conditions yield the same standard curve. If altered procedures give different results, a new standard curve and equation should be established for the modified procedure.

RESULTS AND DISCUSSION

***Opaque-2, Floury-2,* and Normal Dent Corns.** Data in Table I summarize analyses of grain and endosperm samples from each class of corn used to establish standard regression curves and equations. The good agreement between mean calculated and analyzed lysine contents of the protein in each group of samples further demonstrates that the analyzed value of lysine is inversely correlated to the mean absorbance per gram of protein. However, correlation coefficients between lysine content and absorbance

Table I. Mean Analytical Results and Correlation Coefficients by Class of Corn

Sample type	No. of samples	% protein[a]	Absorb. at 590 nm/20 mg of corn	Calcd g of lysine/100 g of protein	Anal. g of lysine/100 g of protein	Absorb. 590 nm/g of protein[b]	Corr coeff, r
Whole corn							
Opaque-2	12	9.90	0.201	4.16	4.21	99	−0.59[c]
Floury-2	12	11.74	0.709	3.27	3.43	299	−0.04
Normal	13	9.49	0.644	3.11	2.90	336	−0.36
Overall	37				3.50	247	−0.87
Endosperm							
Opaque-2	7	7.34	0.312	3.33	3.32	215	−0.66
Floury-2	6	11.03	0.840	2.30	2.50	386	−0.90[c]
Normal	4	8.29	0.650	2.27	2.00	391	−0.32
Overall	17				2.72	316	−0.89

[a] Variation between classes not significant for per cent protein. [b] Highly significant variation between classes. [c] Significant at the 5% level.

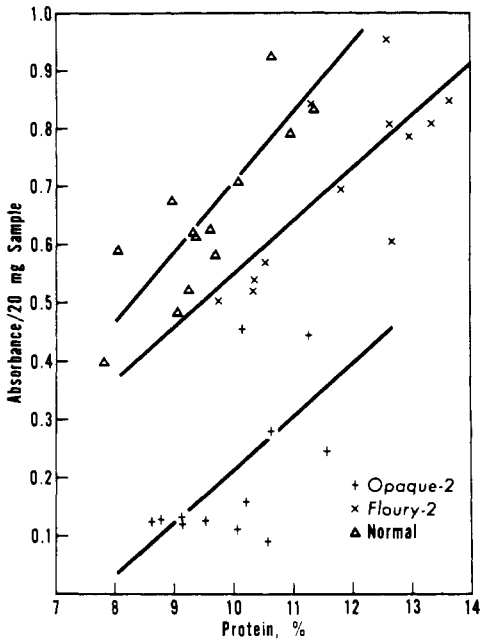

Figure 2. Turbidity of 70% ethanol–0.5% sodium acetate extracts (absorbance at 590 nm) per 20-mg sample *vs.* per cent protein of whole grain of normal, *opaque-2*, and *floury-2* corns. Lines are least-squares plots.

per gram of protein within each class were significant for only two out of six types. Lysine contents of individual samples of each class of whole grain (Figure 1) indicate that the wider the spread of lysine values the better (more negative) the correlation (r) within the class. Evidently, the method has limitations in distinguishing small differences in lysine within a class but readily distinguishes differences between classes and larger differences within a class.

The considerable differences between whole grain and endosperm in both lysine and absorbance per gram of protein are shown in Table I and Figure 1. These differences arise because endosperm and germ vary in their protein content and quality. Mean protein contents of whole grain and endosperm differ most for *opaque-2*, followed by normal. Probably there is a larger amount of germ tissue in *opaque-2* whole kernels and a lower amount of protein in their endosperms. The average amount of germ in dissected grains used for endosperm analysis was 14.7% for *opaque-2*, 12.0% for *floury-2*, and 10.2% for normal. Bjarnason and Pollmer (1972) also observed a larger proportion of germ and germ protein in *opaque-2* than in normal corns. Since the germ contains practically no zein (Bressani and Mertz, 1958; Concon, 1966) and is rich in lysine-containing ethanol-insoluble protein, the germ protein reduces extract turbidity per gram of protein of whole kernel. The difference in absorbance per gram of protein between *opaque-2* and either *floury-2* or normal is less evident in endosperm than in whole grain. The large absorbance difference between extracts of *opaque-2* whole meals and those of *floury-2* or of normal enhances detection of samples of high-lysine *opaque-2* lines by the zein analysis method.

Protein Content Variability. In the course of analysis of *opaque-2*, *floury-2*, and normal grains, used for development of the standard curve, a wide range of protein contents was observed in each class. This variation was due to differences in genetic background of the lines and conditions of cultivation. As shown in Figure 2, in each class an increase in protein is accompanied by an increase in absorbance at 590 nm of extracts of whole grain. This increase in zein content with higher grain protein is greatest in normal and least in *opaque-2* kernels. The data also revealed that an increase in protein reduced the per cent

Table II. Calculation of Lysine Content of Whole Corn Protein from Zein Turbidity Analysis

Corn[a] type	Av absorb./20 mg of corn	% protein[b]	% lysine, g/100 g of protein	
			Calcd[c]	Anal.[d]
Protein				
High	3.144	26.15	1.93	2.11
Low	0.598	8.00	2.94	3.02
Oil				
9.32%	0.906	12.36	2.98	2.26
8.31%	0.845	11.13	2.92	2.50
6.22%	0.696	10.91	3.18	2.82
Starch				
59% Amylose	0.856	11.54	2.95	3.08
66% Amylose	0.705	11.17	3.20	3.08
77% Amylose	0.650	12.65	3.45	3.17
83% Amylose	0.772	11.68	3.13	3.49
Brittle-2	1.076	15.40	3.05	3.28
Sugary-2	0.811	12.24	3.13	3.07
Waxy-opaque-2 (1)	0.266	10.78	4.05	4.27
Waxy-opaque-2 (2)	0.233	11.24	4.14	4.27
Sugary-2–opaque-2	0.063	10.43	4.47	4.91
Floury				
Floury-1	1.038	11.84	2.66	2.50
Opaque-1	0.915	11.99	2.90	2.61
Vitreous *opaque-2*				
1	0.087	9.56	4.40	4.24
2	0.379	11.78	3.89	3.57
3	0.468	11.59	3.70	3.85
4	0.399	10.14	3.73	3.78
Opaque-2 hybrid segregating selections[e]				
Opaque	0.113	10.05	4.35	4.78
Semitranslucent	0.117	9.23	4.32	4.19
Vitreous	0.371	9.80	3.76	3.58

[a] All values are averages of duplicate determinations. Turbidimetric absorbances are averages of two extractions carried out at no later than 3 days after grinding. Only 0.1 g of high protein samples was extracted and turbidimetric analyses were obtained on 1-ml extracts diluted to 2 ml with extractant (indicated absorbance is four times observed). [b] Protein determined on as-is basis and not corrected for moisture. [c] Calculated from prediction (regression) equation. [d] Analytical data from ion-exchange analysis for lysine. [e] Samples were sorted from an *opaque-2* corn based on their transparency over a fluorescent light source.

lysine in normal grain protein slightly but resulted in a smaller decrease in the lysine content of *opaque-2* grain protein. In contrast, we observed that the lysine content of grain protein increased significantly with increased protein in *floury-2*. Despite these effects of protein content, there was no significant correlation between the amount of protein in the standard samples and the accuracy of the estimation of lysine per gram of protein by the turbidimetric procedure. Extraction of zein from grain having the range of protein exhibited by our standard samples does not appear to be influenced by protein content. Calculation of the standard curves and regression equations on the basis of absorbance per gram of protein compensates for the effect of protein variation on lysine estimation.

The zein turbidity determination departs from Beer's law at high levels of zein concentration (Craine *et al.*, 1957). Dilutions of extracts of samples of high-zein content are required. Therefore, corns with extremely high and low protein concentrations were selected for lysine analyses by the zein extraction procedure to determine the accuracy of the method at the limits of protein contents. As shown in Tables II and III calculated and analyzed lysine contents compare favorably for both high- and low-protein whole corn meals and the low-protein en-

Table III. Calculation of Lysine Content of Corn Endosperm Protein from Zein Turbidity Analysis[a]

Corn type	Av absorb./20 mg of endosperm	% protein	% lysine, g/100 g of protein	
			Calcd	Anal.
Protein				
High	4.236	26.56	−0.170	1.34
Low	0.541	7.12	2.34	2.14
Oil				
9.32%	0.922	12.29	2.37	1.35
8.31%	0.929	11.38	2.17	1.54
6.22%	0.825	10.11	2.17	1.72
Starch				
59% Amylose	0.824	11.26	2.42	2.07
66% Amylose	0.744	10.84	2.56	2.04
77% Amylose	0.889	12.70	2.52	2.27
83% Amylose	0.784	11.49	2.57	2.82
Waxy-opaque-2 (1)	0.395	9.72	3.40	3.57
Vitreous-*opaque-2*				
1	0.183	7.37	3.88	3.57
2	0.588	10.68	2.97	3.06

[a] All analyses and calculations made as described in Table II.

dosperm meal. However, agreement is poor for the lysine estimate and analysis of the endosperm of the high-protein grain (Table III). The absorbance at 590 nm of the turbid extracts of the endosperm is so great that the calculation based on it yields a negative lysine value even when the extract sample is smaller. The high-protein endosperm meal contains a slightly higher proportion of protein than whole grain; evidently this endosperm is even richer in protein than the germ fraction. High-protein endosperm contains a high-zein content as observed earlier by Bressani and Mertz (1958). High-protein grain would be readily distinguished by the high turbidity of its alcohol extracts.

High Oil Samples. Our earlier investigation (Paulis *et al.*, 1974) revealed that free fatty acids contribute to the turbidity of extracts during lysine estimation. To determine the effect of large amounts of fat on lysine approximation, three high-oil corns containing varying levels of oil were analyzed for lysine by the zein turbidity procedure and by the amino acid analyzer.

The analyzer data in Table II indicate that the high-oil corn grains contain a normal or less than normal level of lysine in their protein even though the grains all contain larger than normal germs. The 6.2% oil grain contains 12.0% germ, the 8.3% oil grain contains 14.0% germ, and the 9.32% oil grain contains 15.2% germ. The low lysine contents of these grains result because their endosperm proteins contain a lower than usual amount of lysine (Table III). Interestingly, the larger the oil content of the grain, the higher the concentration of protein and the lower the lysine in protein in both whole kernel and endosperm.

The estimated amount of lysine based on turbidimetric zein analysis, as shown in Table II, is close to the analyzed value for the whole grain with 6.26% oil. However, with increasing oil content in the other two grain samples, calculated values become significantly larger than the analyzed values. If germ oil constituents contribute to the turbidities of the extracts, the higher oil samples should appear to have increased turbidity in extracts and to have less lysine in the protein. The observed variation in lysine analyses appears to be primarily due to factors in the endosperm. The endosperms of the highest oil samples give higher estimated lysine contents than analyzed values (Table III). Possibly the extremely hard horny endosperm of the highest oil strains renders zein extraction from them less effective. Even with lower absorbances at 590 nm of high-oil corn lines, the zein turbidity method is adequate to distinguish these low-lysine, high-oil strains from higher lysine genotypes.

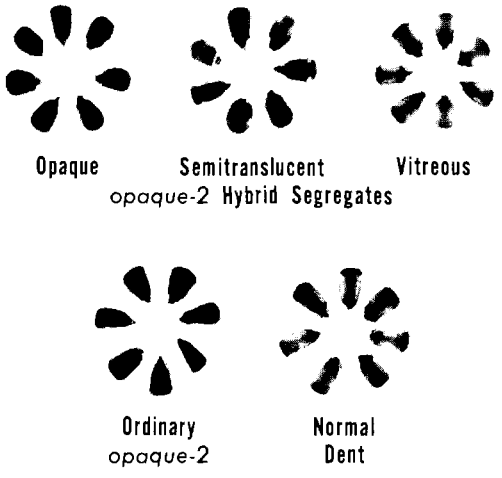

Figure 3. (Top) Separation of kernels of hybrid *opaque-2* cultivar according to translucency—opaque, semitranslucent, and vitreous; (bottom) ordinary *opaque-2* and normal dent grains.

Starch-Modifying Mutations. Several corn mutants which contain modified starches were evaluated for lysine content by the zein extraction method.

Agreement was reasonably good between calculated and analyzed lysine values in a series of whole high-amylose corns (Table II). The series of corns contained 59, 66, 77, and 83% amylose in their starches in contrast to normal dent corn which contains only 27% amylose in its starch. Baudet *et al.* (1968) found that the amylose-extender gene increased lysine content of endosperm protein. The level of lysine in the endosperm of these grains increased slightly as their amylose content was elevated (Table III) as noted earlier by M. J. Wolf (1973). The calculated lysine values, while permitting selection of these corns from ordinary corns, do not distinguish small lysine differences among high-amylose grains.

Nordstrom and Meade (1969) and Misra *et al.* (1972) reported that *sugary-2* and *brittle-2* mutant corns have higher than normal lysine analyses. The meals of *sugary-2* and *brittle-2* whole grains give good agreement between estimated lysine based on zein and the analyzed lysines (Table II).

Combinations of *opaque-2* genes with starch-modifying genes result in high-lysine lines with modified starch. Where the starch-modifying gene gives rise to higher lysine than normal, its combination with the *opaque-2* gene often elevates lysine content over regular *opaque-2* lines. As shown in Table II, two hybrid grains containing both *opaque-2* and waxy starch genes are high in lysine and give good agreement between results of the zein turbidity method of lysine estimation and lysine analysis. A grain representing the combination of *sugary-2–opaque-2* analyzed 4.91% lysine in its protein (Table II), a value higher than the lysine content of any *opaque-2* grain used in the derivation of the estimation equation for lysine in whole meals. A meal extract of this sample gave a low absorbance in the zein turbidity measurement (Table II). The calculated lysine value is almost at the maximum lysine concentration that can be read on the standard curve. The large amount of germ in this grain, 16% by weight of the kernel, may be a factor in its high-lysine content.

Variations in Kernel Hardness. The floury characteristics of regular *opaque-2* and *floury-2* kernels facilitate their grinding to fine powders which may permit more zein extraction. *Opaque-1* and *floury-1* mutants were shown by Nelson *et al.* (1965) to contain only about half as much lysine as the *opaque-2* and *floury-2* genotypes, which they resemble in phenotype by having soft, floury kernels. As shown in Table II, analysis of *opaque-1* and

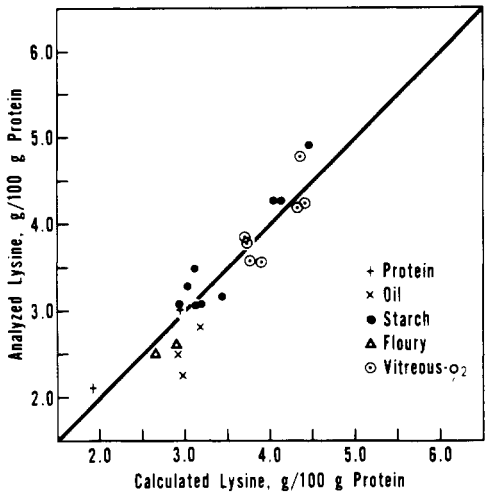

Figure 4. Calculated lysine per 100 g of protein (by zein turbidity) *vs.* analyzed lysine per 100 g of protein for 23 whole corn meals from samples varying in kernel hardness and grain composition. Line at 45° represents points of identical analysis.

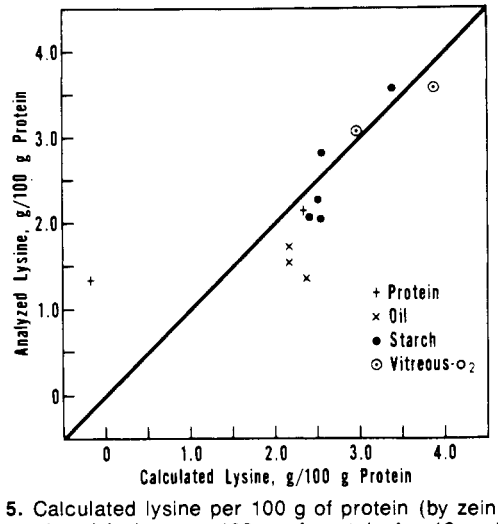

Figure 5. Calculated lysine per 100 g of protein (by zein turbidity) *vs.* analyzed lysine per 100 g of protein for 12 endosperm meals from corns varying in kernel hardness and grain composition. Line at 45° through origin represents points of identical analysis.

floury-1 by the zein turbidity method for lysine gives results close to the analyzed lysine values for these samples. The method can then be used to distinguish these mutants readily from the high-lysine *opaque-2* and *floury-2* grains of similar phenotype.

The analysis of lysine by the zein turbidity method was carried out on several *opaque-2* grains with varying degrees of vitreousness in their endosperm. Calculated lysine of protein in four whole grains and two endosperm samples shows that these vitreous corns are high in lysine (Tables II and III). Estimated lysines are in close agreement with the values obtained by use of the amino acid analyzer.

In one line of hybrid *opaque-2* corn supplied by a commercial corn breeder, an uncommonly large amount of translucent grain (approximately 30–40%) was observed. Kernels from this sample were separated according to hardness into three separate groups as listed in Table II—fully opaque, semitranslucent, and translucent (vitreous). Figure 3 illustrates the appearance of kernels of each group of corn when examined over illumination. Rapid analysis by the zein turbidity method established that the lysine content of each group of these kernels, as listed in Table II, exceeded that of normal dent corn. The protein

Table IV. Regression Equations for Turbidity *vs.* Lysine Analysis for Different Corns

Meal	No. of samples	Equation[a]	Corr coeff[b]	SE of estimation, g of lysine per 100 g of protein
Whole	23	$L = 4.69 - \frac{24.65A_{590}}{\%\ \text{protein}}$	−0.93	0.289
Endosperm	12	$L = 3.66 - \frac{18.80A_{590}}{\%\ \text{protein}}$	−0.76	0.541

[a] L = grams of lysine per 100 g of protein. A_{590} = absorbance at 590 nm per 20-mg sample. [b] All correlation coefficients significant at 1% level.

of the fully translucent sample contained 3.58% lysine/g of protein, a value as high as some vitreous *opaque-2* grain from other sources. The estimated lysine values were consistent with the analyzed values. Lysine content in *opaque-2* kernels is reduced as their degree of vitreousness is increased.

These results indicate that the zein turbidity method can serve as a powerful tool for rapid initial screening of vitreous samples for lysine content. Undoubtedly, lack of such a simple tool in the past caused potentially valuable *opaque-2* germ plasm to be overlooked and considered as probable outcrosses because of their aberrant phenotypes.

Lysine Estimates for All Corn Types. The corn types listed in Table II represent a broad spectrum of grains differing in protein, oil, starch, and kernel hardness. To determine how well the zein turbidity procedure for estimating lysine in these corns worked, calculated lysines were plotted against the analyzed values in Figure 4 for whole grain and in Figure 5 for endosperm. The points for whole corn (Figure 4) are closely grouped about the 45° line, a condition that indicates agreement of analyzed and estimated values. The estimated values for these 23 different whole corn meals showed a standard deviation of only 0.288 g of lysine/100 g of protein from the analyzed values. For the 12 endosperm samples, the standard deviation is higher, 0.620. The major source of error in this group of analyses is the single high-protein endosperm sample whose extract turbidity exceeded values on the standard curve. Exclusion of this sample in the estimation reduces the standard deviation to 0.460, a value that still reflects the major differences between estimated and analyzed values of lysine in high-oil corn endosperms. However, the values of lysine generally indicate that zein turbidity measurements discriminate between high-lysine and low-lysine grains over a wide range of characteristics.

Calculation of linear regression equations for relating lysine in grain to zein turbidity of extracts can be based on almost any series of grains having reasonably diverse lysine and protein contents regardless of their other physical characteristics. In Table IV are summarized calculated regression equations, correlation coefficients, and standard errors based on analysis of the diverse samples given in Table II. For the 23 whole corn samples, the regression equation closely approximates the standard equation given under Materials and Methods. The correlation coefficient is high and standard error low. Evidently the inverse relationship between zein and lysine content of protein in corn generally does not depend on corn type. For endosperm, the equation in Table IV relating absorbance to lysine exhibits lower slopes and intercepts than the endosperm standard curve. The anomalous behavior of the high-protein and high-oil endosperm samples contributed to these differences, as well as to the poorer correlation coefficient and greater standard error.

The turbidimetric analysis of zein should find wide applicability in screening corn in breeding programs for high-lysine lines of improved kernel structure. It should

also be useful in commerce to distinguish high-lysine lines of corn, which may exhibit diverse physical and compositional differences from low-lysine types. Modifications are being tested to determine whether the accuracy of this method can be improved to permit analysis of small differences in lysine within a class of corn and whether the standard curve can be extended to include samples containing higher protein and higher lysine levels.

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The Semiautomated Determination of Niacin and Niacinamide in Food Products

David C. Egberg,* Richard H. Potter, and Guy R. Honold

A general continuous flow scheme has been developed which employs an in-phase reference flowcell to eliminate blank color interference. The flow scheme was used for automating a colorimetric determination of niacin in a wide variety of food products. A simplified sample preparation was developed which yielded recoveries ranging from 94 to 100%. The semiautomated procedure was shown to compare favorably with a specific microbiological assay for 63 different products (0.9937 correlation coefficient). The average relative standard deviation of the automated method was 1.5% for the 63 products, with niacin levels ranging from 0.83 to 54.7 mg/100 g. The main advantage of this procedure is the large number of analyses which can be rapidly effected. Fifty samples per hour can be analyzed after sample preparation.

The development of more rapid and accurate methods for determining the nutritional quality of food products is becoming increasingly more important. The determination of vitamins such as niacin is a prime consideration. Because of the inherent difficulties with chemical methods, microbiological assays are often used for this vitamin (Association of Vitamin Chemists, 1947; Strohecker and Henning, 1965). Although these assays are specific and sensitive, they are tedious, time consuming, and lack desirable reproducibility.

Studies have been conducted comparing manual chemical assays with microbiological methods (Melnick, 1942; Steele, 1945). While some workers have found good agreement, other workers have observed wide discrepancies. Gorin and Schütz (1970) observed good correlation between a microbiological method and a spectrophotometric method after thin-layer chromatography cleanup.

Colorimetric methods for the determination of nicotinic acid are based on the König reaction (König, 1904) of pyridine derivatives with cyanogen bromide and an aromatic amine to form polymethine dyes. It has been reported that the colorimetric method suffers from poor reproducibility due to color instability and pH sensitivity and that the method is tedious and subject to color interference when analyzing natural products (Association of Vitamin Chemists, 1947; Strohecker and Henning, 1965).

In view of the inherent reproducibility and ease of operation of continuous flow systems, it was felt that these problems could be eliminated. An automated niacin method applicable to natural products has been reported (Technicon Instrument Company, 1972); however, the sample preparation is time consuming, a reference flowcell to eliminate interference is not used, and the accuracy of the method has not been established. In the

General Mills, Inc., James Ford Bell Technical Center, Minneapolis, Minnesota 55427.