A Rapid Turbidimetric Analysis for Zein in Corn and Its Correlation with Lysine Content

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A rapid, simple, and inexpensive method has been developed for estimating lysine in corn of normal and high-lysine types. The procedure is based on an inverse correlation of the zein content with percentage lysine in protein. Zein is determined turbidimetrically after being precipitated from solution in 70% ethanol–0.5% sodium acetate extracts of either meals of whole grain or endosperm. Optimum conditions have been established by which quantitative extraction of zein from corn meals is rapid. The influences of germ, lipid, and age of meal upon the development of turbidity in extracts were determined. By use of a reciprocating shaker to prepare extracts, a single technician can complete analysis of 40 ground and weighed samples in 7 hr. For 37 different whole corn meals, correlation coefficients for absorbance at 590 nm per g of protein of turbid extracts to percentage lysine in proteins were −0.87 and for 17 endosperms −0.89.

The discovery by research scientists at Purdue University that *opaque-2* (o_2) and *floury-2* (fl_2) mutants of corn were higher than ordinary dent corns in the essential amino acids—lysine and tryptophan (Mertz *et al.*, 1964; Nelson *et al.*, 1965)—led to extensive breeding programs to develop high-yielding corn hybrids with quality protein. A major impediment in selecting desirable high-lysine corn genotypes has been the lack of a simple, rapid, inexpensive lysine assay to screen the large numbers of samples required. High-lysine character can be incorporated into kernels with vitreous endosperms resembling normal dent corn and having better test weight and processing properties (International Maize and Wheat Improvement Center, 1970; Nelson, 1966; Paez *et al.*, 1969; Bauman and Aycock, 1970). Consequently, procedures are needed that readily distinguish high-lysine corn from normal corn.

Amounts of various classes of proteins differ markedly in corns also differing in lysine content (Paulis *et al.*, 1969; Jiménez, 1966). These differences in amounts of different proteins, which occur in o_2, fl_2, and normal corn genotypes, affect their lysine contents because the types of proteins vary in amount of lysine. Albumins and globulins are rich in lysine, about 6% by weight, and glutelins contain around 4% lysine, whereas zein is almost devoid of lysine, less than 0.2% (Jiménez, 1966; Mossé *et al.*, 1966). The smaller the amount of zein in the endosperm or total grain protein and the greater the amounts of globulin, albumin, and glutelin, the greater the content of lysine. Salamini and Baldi (1969) observed a correlation coefficient of −0.81 when lysine in several o_2 varieties of corn was related to the amount of the alcohol-soluble fraction (zein) in their meals. Pollmer *et al.* (1971) later showed, with 48 lines from o_2 crosses, that this inverse correlation of lysine and zein in corn could serve as a method of estimating lysine in grain. They found, with defatted whole corn meals, a correlation coefficient of −0.84 between the nitrogen in water-saturated 1-butanol extracts of the meals and amount of lysine in protein. Later they modified their method by correlating the nitrogen in the butanol-extracted insoluble meal residues with lysine in meal protein (Fromberg *et al.*, 1971).

Our practical procedure for estimating lysine in whole corn grain by analysis of zein is based on the demonstration by Craine *et al.* (1957) that a pure sample of zein in ethanolic solution can be determined quantitatively by turbidimetry after zein precipitation by addition of aqueous saline. However, Craine and his coworkers did not determine conditions whereby their method could be applied to grain samples. We established optimum conditions for extracting zein from corn meals and for reproducing turbidimetric analysis of zein in the extracts. We obtained a high negative correlation of turbidity to lysine content of grain. Our procedure offers the advantages of speed, economy, and simplicity over earlier techniques of screening for nutritional quality of proteins in a large number of corn samples.

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MATERIALS AND METHODS

Samples consisted of normal, o_2, and fl_2 genotypes of hybrid seed and field corn selected to provide a wide range of lysine contents. The 43 samples, grown in 1969, 1970, and 1971, came from six sources. They were dried at 100–110°F in forced-air dryers as specified for seed corn to a moisture content of about 14%. The amount of zein extracted from samples dried under more drastic conditions may be reduced. Grain was stored at 4° in air-tight plastic bags.

Endosperm sections were prepared by soaking 15 g of unbroken corn kernels in distilled water for 0.5 hr at room temperature and then manually removing hull and germ.

Approximately 50 g of each grain sample was ground in a Udy cyclone hammer mill through a 0.024-in. screen. This mill permitted rapid grinding of whole undefatted grain without heating and required only minimal cleaning between samples. For endosperm analysis, 10 g of each sample was ground in a semimicro-Wiley mill with a 40-mesh screen. However, when material is limited, samples as small as 1.0 g may be used if a suitable mill is available.

In some analyses, 5- to 10-g portions of meals were partially defatted by stirring intermittently with 30 ml of pentane–hexane at 4° for 1 hr. The suspensions then were filtered and the solids washed on a Buchner funnel with cold solvent and air-dried overnight.

For reproducible zein analyses, all ground samples must be stored at 4° in sealed containers and analyzed within 3 days after grinding.

Protein and Amino Acid Determinations. Nitrogen contents of meals, extracts, and hydrolysates were determined in duplicate by the micro-Kjeldahl method (Steyermark, 1961). Crude proteins were estimated by multiplying nitrogen content by 6.25 and are given on an as-is basis.

For amino acid analyses, two samples of each defatted whole meal and defatted endosperm meal, equivalent to 2

Table I. Protein Content of Corn Samples

Sample	No. of samples	% protein (as-is basis) Min	Max	Mean
Whole grain				
o_2[a]	12	8.60	11.25	9.90
fl_2	12	9.73	13.35	11.74
Normal	13	7.77	10.93	9.49
All types	37	7.77	13.35	10.37
Endosperm				
o_2	7	6.71	8.03	7.34
fl_2	6	8.06	12.70	11.03
Normal	4	7.12	10.01	8.86
All types	17	6.71	12.70	8.87

[a] o_2 = *opaque-2*; fl_2 = *floury-2*.

Table II. Standard Deviations in Absorbance Determinations of Extracts of Different Meals[a]

Determinations	Absorbance at 590 nm/20 mg of meal: Nondefatted Whole	Nondefatted Endosperm	Defatted Whole	Defatted Endosperm
Duplicates of each extract	0.006	0.006	0.006	0.006
Replicate extractions of each meal	0.030	0.021	0.051	0.029

[a] Based on two turbidimetric determinations carried out on each of two separate extracts of every meal within a 3-day storage period at 4° following grinding.

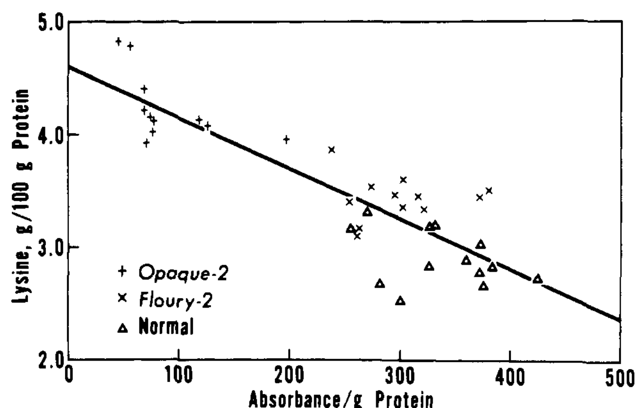

Figure 1. Turbidity of 70% ethanol–0.5% sodium acetate extracts (absorbance at 590 nm) per gram of protein *vs.* per cent lysine of protein of whole grain meals.

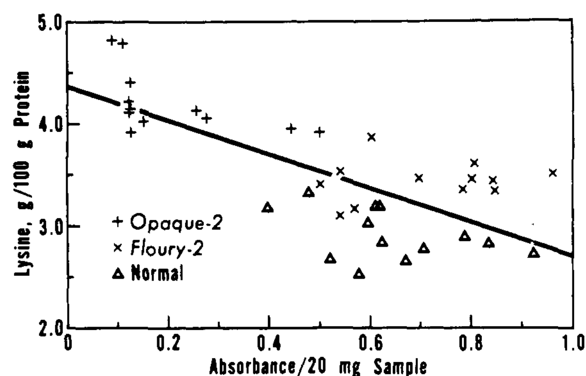

Figure 2. Turbidity of 70% ethanol–0.5% sodium acetate extracts (absorbance at 590 nm) representing 20-mg sample *vs.* per cent lysine in protein of whole grain meals.

mg of nitrogen per sample, were separately hydrolyzed by refluxing in 6 *N* HCl (2 ml per mg sample) for 24 hr. Lysine in the hydrolysate was determined quantitatively on a basic column of a Beckman 120B automatic amino acid analyzer according to Benson and Patterson (1965). ϵ-Aminocaproic acid served as the internal standard (Bates, 1971). Lysine was calculated on the percentage of crude protein as determined from the nitrogen in each hydrolysate sample.

Extraction of Meals. Two procedures for extraction of the corn meal were developed; one had a capacity to handle many samples, the other yielded information quickly on a few samples where rapid identification of high-lysine grains was essential.

The high-capacity extraction procedure, using a reciprocating shaker, was employed in establishing the correlation curves and for determining the effect of variations of the method on the accuracy and precision of analysis. From each sample of well-mixed corn meal, 0.200 ± 0.001 g was weighed into 50-ml capacity polypropylene centrifuge tubes with Teflon-lined screw caps (International Equipment Co.). An all-glass syringe pipet (Repipet-Labindustries) was used to dispense 20 ml of 70% ethanol solution containing 0.5% sodium acetate to each tube. Plastic tubing connections were avoided since some plastics introduced interfering contaminants in the alcohol extract. Sodium acetate was added to the ethanol solution because previous studies showed that it increased the yield of extracted zein (Paulis *et al.,* 1969). The tubes were capped and placed horizontally on a reciprocating shaker parallel to the direction of movement. As many as 40 tubes were wedged into position on a heavy-duty box-type shaker. The contents of the tubes were agitated at the maximum speed of the shaker at room temperature for 1 hr. The tubes were then centrifuged in an International centrifuge (Model HN) for 5 min at 2400 rpm.

For more rapid extraction, the alternative extraction procedure used a Vortex mixer (Scientific Industries Inc.). Samples of ground corn meal (0.1 g) were placed in 16 × 150 mm Pyrex test tubes to which were added 10 ml of 70% ethanol–0.5% sodium acetate. The samples were extracted by agitating the suspension in the tube with a Vortex test tube mixer, four units, for 10 min at medium speed. The test tubes were next centrifuged for 5 min at maximum speed in a table top centrifuge.

Analysis of Zein by Turbidity. The procedure of Craine *et al.* (1957) was followed for determination of zein. Duplicate 2-ml samples of the clear supernatant (equivalent to 20 mg of meal) from each centrifuge tube were removed with a pipet and placed in matched colorimeter tubes (19 × 150 mm, Coleman). Supernatant samples were separated immediately after centrifuging since results may vary if the solution remains in contact with the precipitated meal residue at room temperature. Into each colorimeter tube, 6 ml of 1% solution of sodium chloride in water was vigorously added by blowing the solution from a 10-ml Mohr pipet. After 1 hr, air bubbles were removed from each dispersion by inclining the tubes toward the horizontal and rotating them. The absorbance of the turbid solution was read at 590 nm in a Beckman Model B colorimeter.

Kjeldahl Nitrogen and Lowry Protein in Extracts. In some preliminary experiments, the turbidimetric analysis of zein in extracts was compared to results of analysis of the same extracts for protein by Kjeldahl and Lowry methods. Before Kjeldahl nitrogen analysis, duplicate 5-ml samples of extracts of 70% ethanol–0.5% sodium acetate in 30-ml Kjeldahl flasks were heated on a steam bath to remove the alcohol. For protein determination by the method of Lowry as described by Layne (1957), duplicate 0.2-ml alcoholic extracts were diluted to 1 ml with 0.6 *N* NaOH to dilute the ethanol in the extracts without precipitating zein. A fivefold increase in all reagents enabled us to follow the procedure of Layne (1957) for insoluble proteins.

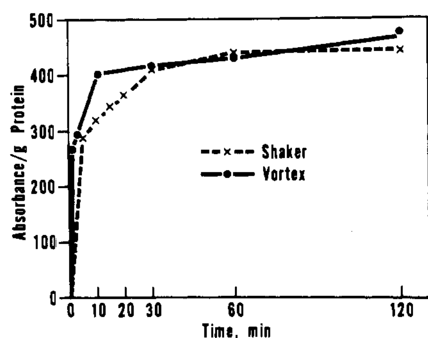

Figure 3. Rates of extracting zein from defatted normal endosperm meal as indicated by absorbance at 590 nm per gram of protein in turbid extracts for Vortex mixer and reciprocal shaker procedures.

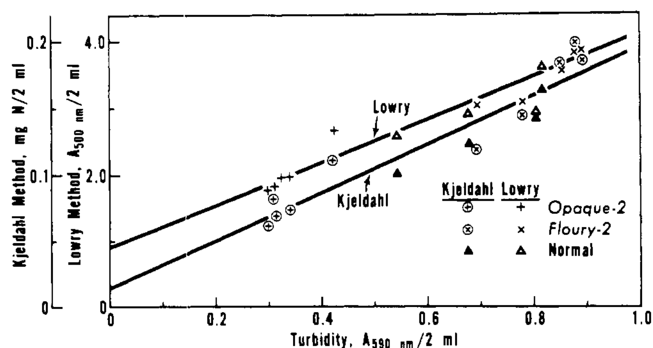

Figure 4. Turbidity measurements of zein (absorbance at 590 nm) *vs.* Kjeldahl nitrogen and Lowry color yield (590 nm) in 2-ml samples of 20 ml of 70% ethanol–0.5% sodium acetate extracts of 0.20 g of endosperm.

Statistical methods for interpreting the data appear in Snedecor and Cochran (1967).

RESULTS

Protein and Lysine Analyses. The quantity of zein in corn depends on total protein in the grain, as well as on the varietal type. The protein content of 37 whole corn samples ranged from 7.77 to 13.35% and for 17 endosperm samples from 6.71 to 12.70% (Table I). The high-lysine mutant grains, especially fl_2, were markedly higher in mean protein content than the normal grain. In contrast, the o_2 endosperm samples were lower in protein than endosperm from normal or fl_2, an indication that larger germs in o_2 might contribute to a higher amount of protein in the whole kernel.

The lysine determinations by ion exchange analysis were based on the average of analyses of two separate hydrolysates of each meal. Precision of the analyses was good since, for a total of 43 whole grain and 26 endosperm samples, the standard deviation per lysine determination was 0.127 g of lysine per 100 g of protein (63 degrees of freedom). The standard deviation did not appear to depend on type of meal (whole or endosperm) or genotype (o_2, fl_2, or normal). The least significant difference in lysine analyses by the amino acid analyzer between two corn samples, based on the mean of two hydrolysates per sample, was 0.254 g of lysine per 100 g of protein.

In the 37 whole meal samples, lysine per 100 g of protein varied from 2.52 to 4.82. Mean values for classes were: o_2, 4.21; fl_2, 3.43; and normal, 2.90 g of lysine per 100 g of protein. Endosperm samples ranged from 1.60 to 3.72 g of lysine per 100 g of protein among the 17 samples. Mean values were: o_2, 3.32; fl_2, 2.50; and normal, 2.00 g of lysine per 100 g of protein in endosperm.

Precision of Turbidimetric Method. If extraction of grain or endosperm meals was conducted within 3 days of grinding, replication of turbidimetric analyses on duplicate extractions was always good. Table II contains the standard deviations for variations in absorbance at 590 nm associated with the mean of two turbidimetric determinations on each of two extractions (replicates) by the reciprocating shaker method. Duplicate turbidimetric determinations on each extract of nondefatted meal indicated no significant differences (Table II). However, the means of two analyses of duplicate extractions of each of these meals showed an appreciable standard deviation. Therefore, the mean of two extractions was used in developing the standard curves and during analysis of unknowns. Analyses of endosperm meals were associated with a higher precision for replicate extractions than those of whole meals. Similar reproducibility of turbidities was observed for extracts of the different meals of the same grain ground at intervals several months apart.

Table III. Equations for Estimating Lysine from Zein Content

Meal	No. of samples	Equation[a]	Corr[b] coeff (r)	SE of estimate, % lysine
Whole	37	$L = 4.35 - 1.647A$	−0.73	±0.416
	37	$L = 4.60 - 4.44B10^{-3}$	−0.87	±0.307
Endosperm	17	$L = 3.69 - 1.683A$	−0.67	±0.479
	17	$L = 4.62 - 6.01B10^{-3}$	−0.89	±0.292

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

Correlation of Zein Turbidity and Lysine. Because of fluctuation in protein content, scatter of points was less and correlation was higher when absorbance was based on the protein content (Figure 1) rather than on weight of sample (Figure 2). The estimation of lysine in unknown samples may be read from the curves at the determined value of absorbance per 20-mg sample or per gram of protein.

Our results using this method are summarized in Table III. Equations were derived for estimating lysine (L) from either A (absorbance per 20-mg sample, equivalent to 2 ml of extract) or B (absorbance per gram of protein in the corn, $5000A$/% protein). The equation involving B, which corrects for protein variations in the samples, improved correlation and reduced error. All correlations were significant at the 1% level. Substitution of experimentally determined turbidity values into the equations permitted good estimation of lysine in unknown corn samples, if the determinations were made under the same conditions used for developing the equation.

DISCUSSION

Extraction of Corn Meals. Rate and degree of extraction of zein by the 70% ethanol–0.5% sodium acetate solution were affected markedly by the fineness of grinding of the whole grain or endosperm. Particle size greater than 40 mesh reduced zein extraction. Where possible, grinding to −60 mesh is recommended.

The Vortex mixer method extracted almost all the protein in only 10 min for a quick evaluation of zein content (Figure 3). The reciprocating shaker required 60 min for almost complete extraction. The reciprocating shaker procedure, in addition to permitting a larger number of samples to be run simultaneously, gave better reproducibility. Extraction rates may vary with different commercial shakers and optimum extraction time should be determined for each type of shaker.

Comparison of Turbidity, Lowry, and Kjeldahl Nitrogen for Zein Determination and Lysine Estimation.

Table IV. Correlations of Lysine in Protein with Turbidity, Kjeldahl, and Lowry Methods for Zein in Extracts of Meals

Method	Meal	Corr[a] coeff (r)
Turbidity[b]	Whole grain	−0.90
	Endosperm	−0.91
Micro-Kjeldahl[c]	Whole grain	−0.89
	Endosperm	−0.72
Lowry[c]	Whole grain	−0.92
	Endosperm	−0.77

[a] All correlations significant at the 1% level and based on either turbidimetric absorbance or protein of 70% ethanol–0.5% sodium acetate extracts equivalent to 1 g of protein in nondefatted meal. [b] Based on the turbidimetric data from the same 70% ethanol–0.5% NaOAc extracts of nondefatted meals in Table V (one o_2, five fl_2, and three normal corns used also in micro-Kjeldahl and Lowry analyses). [c] Micro-Kjeldahl and Lowry analyses determined on the same extracts from 14 nondefatted whole meals (five o_2, five fl_2, and four normals) and their 14 nondefatted endosperm meals.

The turbidity procedure for zein analysis gave a linear correlation to protein extracted from the meal by the 70% ethanol–0.5% sodium acetate solution. In Figure 4 are plotted values for zein turbidity *vs.* those for Kjeldahl nitrogen and Lowry reagent color yield. When extrapolated to zero absorbance at 590 nm by turbidity, both Kjeldahl nitrogen and Lowry color yield values intersect the y axis above zero. Nonprotein nitrogen in the meal contributes to Kjeldahl nitrogen while phenolic substances and free tyrosine cause color formation not due to zein protein by the Lowry procedure.

For whole grain, negative correlation coefficients were similar between lysine in the meal protein and the protein extracted by 70% ethanol–0.5% sodium acetate solution as determined by the three methods (Table IV). This similarity of correlation coefficients was unexpected in view of differences in interfering substances that increase zein analysis by the three different methods. However, when endosperms were analyzed for lysine in protein and these values correlated with analysis of proteins in the extracts of those endosperm meals, correlation was highest (r = 0.91) for the turbidity method.

Meal Storage Time *vs.* Extract Turbidity. When ground whole corn samples were re-analyzed by the turbidimetric method for zein after several weeks of storage in plastic bags at 4°, the addition of salt solution to the 70% ethanol–0.5% sodium acetate extracts resulted in greater turbidity than during the earlier analysis. Turbidities of extracts prepared from 0.20 g of meal 60 days after grinding averaged 0.149 absorbance unit higher than when analyzed 1 day after grinding. The three classes of corn differed significantly in mean rate of increase in turbidity; o_2 gained the most in turbidity (Figure 5). Endosperm meals stored under the same conditions before turbidimetric analysis for zein exhibited a much smaller, but significant, increase in extract turbidity with time. However, when both whole grain and endosperm meals were analyzed turbidimetrically for zein after 3 days of storage at 4° after grinding, the results did not differ significantly from those obtained on the same samples 1 day after grinding.

Extracts were prepared from ground meals of whole grain and endosperm of the three classes of corn at various intervals after grinding and analyzed for nitrogen to establish if increase in zein extractability was responsible for the increase in extract turbidity with time. No change in extractable nitrogen with time after grinding was observed; therefore, zein extraction did not increase with time. Fatty acids, which are soluble in alcoholic solutions (Ponte *et al.*, 1967) and increase in corn meals during storage (Gardner and Inglett, 1971), might have caused the increased development of turbidity with time.

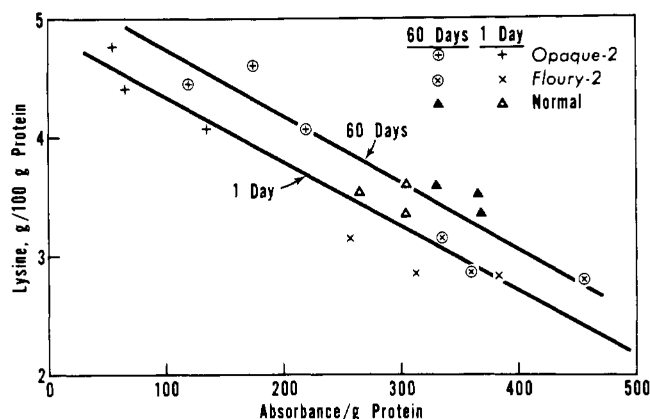

Figure 5. Changes in turbidity of extracts after storage of whole grain meal for 60 days. Turbidity measured as absorbance at 590 nm per gram of protein. Meals from same grain are identified by identical lysine contents.

Defatting Samples *vs.* Zein Turbidity. To investigate the possible involvement of fat in the changes in turbidity in extracts of corn meal after storage, the meals were partially defatted with pentane–hexane to remove most neutral lipids and fatty acids. Protein contents of defatted whole grain meals were 1.8–9.5% (5.26% mean) greater than those of the initial meals and defatted endosperm protein ranged from 1.01 to 6.00% (3.01% mean) greater than those of undefatted endosperm meals on an as-is basis.

Defatting whole grain meals resulted in lower absorbances of 70% ethanol–0.5% sodium acetate extracts than those of corresponding nondefatted meals (Table V). Only small differences in turbidity were observed between extracts of defatted and nondefatted endosperms. Turbidity differences between nondefatted and defatted meals were slightly greater on sample total protein basis than on a sample weight basis, as expressed in Table V because samples contain slightly more protein after defatting. The standard deviation between analyses of two separate extracts of each meal was higher for defatted than for nondefatted meals (Table II).

The correlation of absorbance at 590 nm per gram of protein *vs.* lysine per gram of protein (r) was better (more negative) for both whole grain and endosperm meals before defatting (Table VI). The standard error (s) of lysine determination by zein turbidity was greater with defatted meals than nondefatted meals. Evidently fat in meals not only increases turbidity of zein in the extracts, but also improves the correlation of turbidity with lysine.

A smaller change in turbidity of extracts after storage of samples was observed after defatting both whole grain and endosperm samples, thus implicating changes in soluble lipids as responsible factors. The change in extract turbidity that was noted in stored defatted meals was not associated with changes in extractable nitrogen in the extracts and may therefore be due to unextracted polar lipids.

Effect of Sodium Acetate in Solvent. The presence of 0.5% sodium acetate in the 70% ethanol extractant might either influence the salting out of zein or enhance the extraction of lipids and thereby affect the accuracy and reproducibility of turbidimetric analysis. Turbidimetric analyses on extracts prepared with and without sodium acetate in the solvent are compared in Table V. All meals, except defatted whole meals and nondefatted o_2 whole meals, yielded higher turbidimetric absorbances from acetate-containing extracts. Variations between determinations were the same for both 70% ethanol or 70% ethanol–0.5% sodium acetate extracts of nondefatted or defatted meals (Table II).

Table V. Effects of Defatting Meals and of Including Acetate in Solvent on Turbidity of Extracts

Meals[a]	Acetate[b]	Mean absorbance at 590 nm per 20-mg sample		
		o_2	fl_2	Normal
Whole grain				
Nondefatted	+	0.170	0.734	0.716
Nondefatted	−	0.192	0.721	0.630
Defatted	+	0.046	0.438	0.343
Defatted	−	0.095	0.590	0.554
LSD[c]		0.067	0.059	0.091
Endosperm				
Nondefatted	+	0.314	0.839	0.676
Nondefatted	−	0.270	0.752	0.601
Defatted	+	0.274	0.874	0.731
Defatted	−	0.219	0.747	0.619
LSD[c]		0.010	0.015	0.025

[a] Based on turbidimetric analysis of 70% ethanol (−) and 70% ethanol–0.5% NaOAc (+) extracts of 13 whole corn meals [four o_2, six fl_2, and three normals] and their 13 endosperm meals. [b] With (+) or without (−) 0.5% sodium acetate in 70% ethanol. [c] LSD = least significant difference at the 5% level.

The correlations between extract absorbance per gram of protein and percentage lysine of proteins were improved slightly by addition of 0.5% sodium acetate to the 70% ethanol extractant (Table VI). The improvement of correlation was most marked for nondefatted samples.

CONCLUSIONS

The analysis of zein in corn meals by measuring the turbidity of extracts is complicated by the presence of other components in the grain. Lipids, especially in whole grain, increase the turbidity of 70% ethanol–sodium acetate extracts to which saline solution is added. This lipid effect is more pronounced in stored ground samples, possibly due to free fatty acids formed by lipase activity. Sodium acetate in the extraction solvent, prompt analysis of ground grain, and use of nondefatted grain or endosperm improved reliability of the turbidity method.

On the basis of experimental data, two modifications of the turbidimetric method of analysis of zein may be used to estimate protein quality and lysine content of corn. A rapid estimation can be made by quickly extracting zein from a few samples of ground corn in a Vortex mixer. A subsequent turbidimetric analysis of zein on a meal weight basis can distinguish normal grain from high-lysine types with adequate reliability. This procedure would be useful in commercial channels whenever it is necessary to recognize high-lysine corn shipments and to detect adulteration. In breeding studies where large numbers of samples are produced, greater convenience and accuracy would require use of the reciprocating shaker procedure and correction of turbidity values for protein content of the sample. A single technician could conduct as many as 40 turbidimetric analyses on ground weighed samples in a single day. Nitrogen analyses would probably be available since protein content is a major concern of breeders.

The advantages and limitations of the zein turbidity method using extracts prepared with the shaker are demonstrated in another paper (Paulis *et al.,* 1974). The second paper describes the application of this technique to the evaluation of protein quality in a large number of corn samples of diverse genetic background.

Table VI. Correlation of Lysine in Meal Protein and Absorbance per Gram of Protein for Various Meal and Extract Conditions

Meal	Acetate[a] in solvent	r^b	s^c
Whole grain			
Nondefatted	+	−0.87	0.307
Nondefatted	−	−0.83	0.337
Defatted	+	−0.84	0.331
Defatted	−	−0.83	0.339
Endosperm			
Nondefatted	+	−0.89	0.291
Nondefatted	−	−0.83	0.358
Defatted	+	−0.86	0.326
Defatted	−	−0.83	0.361

[a] Meals extracted with either 70% ethanol–0.5% sodium acetate (+ acetate) or 70% ethanol (− acetate). [b] Correlation coefficients for all conditions were based on the same 36 whole grain meals and 17 endosperm meals. All are significant at 1% level. [c] Standard deviation in per cent lysine in protein based on deviations from the least-squares line.

ACKNOWLEDGMENTS

The following people supplied corn samples: D. E. Alexander, University of Illinois; O. E. Nelson, University of Wisconsin; D. Storck, P-A-G; A. V. Paez, Pioneer Hi-Bred Corn; L. F. Bauman, Purdue University; and C. Lable, Funk Bros. Seed Co. We thank J. F. Cavins and G. L. Donaldson for lysine analysis by ion-exchange chromatography and Miss I. M. Cull for dissection of whole grain for the endosperm samples.

LITERATURE CITED

Bates, L. S., *Anal. Biochem.* **41,** 158 (1971).
Bauman, L. F., Aycock, H., *Proc. Annu. Corn-Sorghum Res. Conf., 25th,* 139 (1970).
Benson, J. V., Jr., Patterson, J. A., *Anal. Chem.* **37,** 1108 (1965).
Craine, E. M., Jones, C. A., Boundy, J. A., *Cereal Chem.* **34,** 456 (1957).
Fromberg, H. K., Christ, W., Pollmer, W. G., *Crop Sci.* **11,** 567 (1971).
Gardner, H. W., Inglett, G. E., *J. Food Sci.* **36,** 645 (1971).
International Maize and Wheat Improvement Center, Mexico, *CIMMYT News* **5**(5–8), 3 (1970).
Jiménez, J. R., "Proceedings of the High Lysine Corn Conference," Mertz, E. T., Nelson, O. E., Ed., Corn Industries Research Foundation, Washington, D. C., 1966, p 74.
Layne, E., *Methods Enzymol.* **3,** 448 (1957).
Mertz, E. T., Bates, L. S., Nelson, O. E., *Science* **145,** 279 (1964).
Mossé, J., Baudet, J., Landry, J., Moureaux, Thérèse, *Ann. Physiol. Veg.* **8,** 331 (1966).
Nelson, O. E., Proceedings of the High Lysine Corn Conference, Mertz, E. T., Nelson, O. E., Ed., Corn Industries Research Foundation, Washington, D. C., 1966, p 156.
Nelson, O. E., Mertz, E. T., Bates, L. S., *Science* **150,** 1469 (1965).
Paez, A. V., Helm, J. L., Zuber, M. S., *Crop Sci.* **9,** 251 (1969).
Paulis, J. W., James, C., Wall, J. S., *J. Agr. Food Chem.* **17,** 1301 (1969).
Paulis, J. W., Wall, J. S., Kwolek, W. F., *J. Agr. Food Chem.* **22,** 318 (1974).
Pollmer, W. G., Fromberg, H. K., Christ, W., *Z. Pflanzenzucht.* **65**(1), 47 (1971).
Ponte, J. G., Jr., De Stefanis, V. A., Cotton, R. H., *Cereal Chem.* **44,** 427 (1967).
Salamini, F., Baldi, G., *Genet. Agr.* **23,** 111 (1969).
Snedecor, G. W., Cochran, W. G., "Statistical Methods," Iowa State University Press, Ames, Iowa, 1967.
Steyermark, A., "Quantitative Organic Microanalysis," 2nd ed, Academic Press, New York, N. Y., 1961, p 188.

Received for review July 19, 1973. Accepted December 3, 1973. Presented in part at the 88th Annual Convention, American Seed Trade Association, St. Louis, Mo., June 28–29, 1971. Naming firms or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.