Relation of Kernel Hardness and Lysine to Alcohol-Soluble Protein Composition in Quality Protein Maize Hybrids†

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High-lysine *opaque-2* (*o2*) maize is floury, but quality protein maize (QPM) *o2* hybrids have harder endosperm. We related kernel density, vitreosity, weight, grinding time, and lysine content to protein compositions in *o2* × QPM crosses. Density distributions of Mo17 *o2* × Pool 29 QPM and B73 *o2* × Population 61 QPM differed from F_2 to F_3 and with direction of cross. Kernels with paternal QPM (one dose) were denser than reciprocal (two QPM doses) crosses. Density correlated with vitreosity in F_2 and F_3 kernels ($r = 0.57$). Grinding time correlated with density ($r = 0.66$) and vitreosity ($r = 0.72$). RP-HPLC showed prolamin amounts to correlate significantly with density, vitreosity, and grinding time. Significant interactions among generation, genotype, and density also occurred. Results varied with inbred and *o2* source. With QPM maternal and *o2* paternal parents, progeny had 16–40% less prolamin and more lysine. RP-HPLC of segregating F_2 and F_3 kernels will help explain genetic control of endosperm development and lysine content.

INTRODUCTION

Mertz et al. (1964) discovered that the *opaque-2* (*o2*) mutant maize endosperm contained twice as much lysine as its normal counterpart. Maize containing the *o2* gene has less A/B-zein and more lysine-containing proteins than does normal maize, making *o2* maize more nutritious. Since *o2* maize kernels are soft and floury in texture, they may break more easily, may be susceptibile to ear rot, and have less desirable dry milling properties. To correct these problems, breeders have sought to retain *o2* genes for high lysine, while increasing endosperm hardness (National Research Council, 1988). The result of these efforts is quality protein maize (QPM).

Recent research and breeding efforts involving QPM lines at Centro Internacional de Mejoramiento de Maize y Trigo (CIMMYT), University of Illinois, Pfister Hybrid Corn Co., Texas A&M University, University of Arizona, and Purdue University have led to new breeding populations with improved hardness and high lysine (Mertz, 1992). Maize endosperm texture and hardness were correlated to protein composition (Ortega and Bates, 1983; Wallace et al., 1990; Paulis et al., 1991; Dombrink-Kurtzman and Bietz, 1993), which can be accurately and quantitatively determined by reversed-phase high-performance liquid chromatography (RP-HPLC).

Early studies of modified *o2* mutants indicated that conversion of a floury to a vitreous endosperm was associated with increased synthesis of a storage protein fraction (Gentinetta et al., 1975; Ortega and Bates, 1983; Wallace et al., 1990; Paulis et al., 1991). The increase in γ-zein [water-soluble alcohol-soluble reduced glutelin (wsASG)] (Paulis and Wall, 1977) was dependent on the dosage of *o2* modifiers in F_1 lines (Lopes and Larkins, 1991). In reciprocal crosses and F_2 lines containing *o2* genes, with or without modifiers, investigators have segregated progeny for degree of vitreosity and increased γ-zein synthesis and have shown these factors to be dose dependent and directly related to each other. To characterize proteins from vitreous and floury endosperm regions, zeins (prolamins) have been quantitatively compared by sodium dodecyl sulfate electrophoresis (SDS–PAGE), enzyme-linked immunosorbent assays (ELISA), and RP-HPLC (Paiva et al., 1991; Dombrink-Kurtzman and Wilson, 1992; Dombrink-Kurtzman and Bietz, 1993). Gupta et al. (1979) also showed that modified *o2* kernels decreased in lysine content as seeds increased in vitreosity and zein content.

To extend these studies, we have now used RP-HPLC to analyze prolamin compositions of reciprocal F_2 and F_3 generations. RP-HPLC results were related to distributions of lysine and protein and to direction of cross (i.e., whether QPM was the maternal or paternal parent). Kernel density distributions were correlated with hardness criteria (kernel weight, vitreosity, and grinding time) to better reveal relationships of proteins to endosperm texture.

MATERIALS AND METHODS

Maize Genotypes. Two CIMMYT QPM vitreous populations [Pool 29 and Population 61 (Pop. 61 QPM)] were reciprocally crossed with two near-isogenic nonvitreous *o2* maize inbreds to yield four F_1 crosses: (A) Mo17 *o2* × Pool 29 QPM; (B) Pool 29 QPM × Mo17 *o2*; (C) B73 *o2* × Pop. 61 QPM; and (D) Pop. 61 QPM × B73 *o2*. The F_2 and F_3 populations from each cross were produced by hand pollination during the winter of 1989 at Molokai, HI, and during the summer of 1990 at El Paso, IL, respectively. F_2 populations used in these studies were from selfed F_1 plants, and backcrossed (BC) generations were from F_1 plants crossed to a recurrent parent. Seed of each population resulted from a bulk of nine ears.

Density Separation. Nine ears were randomly selected from each cross and were hand-shelled and reduced to a representative sample size by the coning and quartering method (Pierce, 1985) to obtain 110 whole undamaged kernels. Each sample was weighed and floated in sodium nitrate solutions (at 25 °C) of 1.116–1.360 specific gravity (sg). Kernels were first put in the

† Presented at the 76th Annual Meeting, American Association of Cereal Chemists, Seattle, WA, Oct 16, 1991.
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0021-8561/93/1441-2249$04.00/0

1.116 sg solution and stirred for 30 s. Floaters were poured off and drained, after which sinkers were poured out and drained. Kernels were then rinsed with distilled water and towel dried. Floaters and sinkers were air-dried overnight until weights remained constant. Floaters were weighed, while sinkers were refloated using a denser solution. The process was repeated until no kernels sank. Kernels were thus separated into seven fractions (sg 1.116, 1.152, 1.193, 1.235, 1.277, 1.318, and 1.360). Kernels of densities 1.318 and 1.360 were combined since few kernels were in the sg 1.360 fraction. Density distributions (Figure 1) were reported for kernels from nine ears. These densities comprised the sample pools for further analysis.

Visual Inspection of Endosperm Vitreosity. Maize kernels from the same ears used for density separations were visually inspected for endosperm vitreosity. Pale yellow kernels were arbitrarily defined as zero endosperm vitreosity (100% floury endosperm), and completely orange kernels were defined as completely (100%) vitreous.

Grinding Time. Twenty-one kernels selected randomly from density levels 1.116–1.360 of nine ears from each population were ground separately in a Crescent Wig-L-Bug, and grinding times were estimated. Kernels were ground for 5-s intervals and then visually evaluated for particle size. When the kernel was ground to a fine flour and additional grinding did not appear to change the particle size, the totl grinding time was recorded. Total grinding times for different pedigrees varied from 10 to 100 s, with denser kernels having longer grinding times.

Lysine and Protein Analyses. Four kernels were randomly selected from each density pool of each F_2 generation. Nitrogen in ground maize kernels was determined by a semimicro-Kjeldahl method. For amino acid analyses, samples were hydrolyzed with 6 N HCl for 4 h at 145 °C (Gehrke et al., 1987); lysine was quantified by cation-exchange chromatography (Beckman Instruments, Inc., San Roman, CA). These analyses were performed at the Experiment Station Chemical Laboratories, University of Missouri, Columbia, MO.

RP-HPLC of Maize Proteins. A/B-zein and ASG were simultaneously extracted from 0.1 g of ground meal prepared from 21–23 randomly selected kernels of each density class and population. Extraction was for 2 h with 2 mL of 70% ethanol containing 0.5% sodium acetate and 0.2% dithiothreitol (Paulis and Bietz, 1986). Proteins were then fractionated by RP-HPLC on a Vydac C_{18} column (300-Å pore size, 10-μm particle size) using an aqueous acetonitrile (ACN) plus 0.1% trifluoroacetic acid (TFA) gradient (Paulis et al., 1991). Eluting proteins from 5-μL injections were detected at 210 nm (0.1 absorbance unit full scale), where absorbance relates closely to quantity of protein. Data were stored in a computer for subsequent integration and statistical evaluation. Early-eluting peaks (1, 2, and 3) contained water-insoluble alcohol-soluble reduced glutelin (wiASG) (Paulis and Wall, 1977) (peaks 1 and 3) and wsASG (peak 2); later peaks (area 4) contain A/B-zein (Paulis et al., 1986, 1991). This nomenclature relates that used in earlier corn protein RP-HPLC and solubility studies to current α, β, γ, and δ nomenclature (Wilson, 1991).

RESULTS AND DISCUSSION

Kernel Hardness. F_2 and F_3 kernels from reciprocal crosses derived from CIMMYT *o2* QPM genotypes differed in density distributions (Figure 1). In both F_2 and F_3 generations the density distribution of the Pool 29 QPM × Mo17 *o2* tended toward the middle to lower densities, while the distribution of the reciprocal Mo17 *o2* × Pool 29 QPM tended toward the middle to higher densities. The Pop. 61 QPM × B73 *o2* had its peak of density distribution at the midrange in the F_2 and shifted toward the higher densities in the F_3. The reciprocal B73 *o2* × Pop. 61 QPM was similar to Pop. 61 QPM × B73 *o2* with the exception that 63 of the 200 kernels were at the highest density in the F_2. No conclusions were drawn from these results.

Densities of F_2 and F_3 kernels were correlated with vitreosity ($r = 0.57$) and log grinding time ($r = 0.66$) (Table I). Grinding time and vitreosity were also correlated ($r = 0.72$). These results confirm the suitability of kernel density as a selection criterion for increasing vitreosity during maize breeding. Kernel density, vitreosity, and grinding time are kernel hardness criteria. Determination of vitreosity may reduce the time and cost of measuring hardness: a past test with hybrids showed a correlation of 0.98 between light transmittance (vitreousness) and percentage of floaters (Hall and Anderson, 1991). Equations to predict log grinding time (LGT) [LGT = 2.0430 + 0.35476 × density, ($R^2 = 0.55$); LGT = 2.6456 + 0.02469 × % vitreosity, ($R^2 = 0.57$); and LGT = 2.1269 + 0.20609 × density + 0.01547 × % vitreosity ($R^2 = 0.67$)] were derived from data used to obtain correlations in Table I.

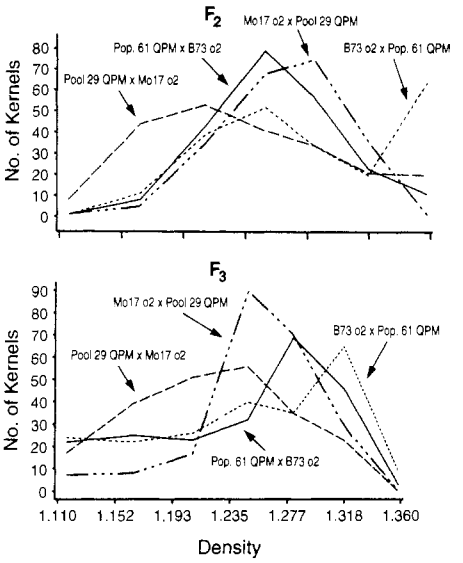

Figure 1. Density distributions (sg, specific gravity) of F_2 and F_3 generations of maize kernels derived from hybrids containing *opaque-2* (*o2*) and QPM genes.

Table I. Correlations of Kernel Hardness Criteria in F_2 and F_3 Maize Kernels Derived from QPM and *o2* Genotypes[a]

	wt	% vitreosity	log grinding time
density	0.26	0.57	0.66
wt		*b*	0.16
% vitreosity			0.72
	(n = 165)		

[a] There were no ($P < 5\%$) significant differences in results for F_2 and F_3 kernels. [b] Correlations between percentage vitreosity and kernel weight ranged from $r = 0.02$ (for F_3 Pop. 61 QPM × B73 *o2*) to $r = 0.86$ (for F_3 B73 *o2* × Pop. 61 QPM).

Variation in Lysine in F_2 QPM × *o2* Crosses. Lysine and protein analyses were performed on kernels of the same density and vitreosity types to determine the amount of variation within each of four populations (Table II). A previous study (Gupta et al., 1979) found that the lysine content of maize with modified *o2* endosperm decreased as amount of zein (A/B-zein or peak area 4) and kernel vitreosity increased. We observed that lysine and protein tended to be negatively and positively correlated, respectively, with vitreosity ($r = -0.33$ and 0.42, respectively) (data not shown). Also, total lysine content of proteins decreased significantly ($r = -0.78$, $P < 0.01$) as total protein increased. The lysine content of Pool 29 QPM × Mo17 *o2*, expressed as percent protein, was greater than that of its reciprocal cross (4.20% vs 3.48%). Similarly, the lysine content of Pop. 61 QPM × B73 *o2* kernels was greater than that of kernels of its reciprocal cross (4.15% vs 3.62%).

Variation in RP-HPLC Peaks of F_2 QPM × *o2* Crosses. RP-HPLC patterns of alcohol-soluble proteins

Table II. Variation in Lysine and Protein in F_2 QPM × *o2* Kernels

	protein, %			mg of lysine in 100 mg of protein			mg of lysine in 100 mg of meal		
pedigree	mean[a]	range	SD[b]	mean	range	SD	mean	range	SD
Mo17 *o2* × Pool 29 QPM	12.5	11.4–13.0	0.69	3.48	3.2–3.7	0.210	0.43	0.40–0.46	0.028
Pool 29 QPM × Mo17 *o2*	9.1	7.8–10.6	1.15	4.20	3.9–4.4	0.216	0.38	0.34–0.45	0.049
difference	3.4**			−0.72*			0.05+		
B73 *o2* Pop. 61 QPM	10.3	8.6–11.7	1.29	3.62	2.7–4.3	0.700	0.36	0.31–0.40	0.039
Pop. 61 QPM × B73 *o2*	9.3	8.9–9.7	0.40	4.15	4.0–4.3	0.129	0.38	0.37–0.41	0.017
difference	0.5+			−1.06			−0.02		

[a] Average of four kernels. Probabilities of 1%, **; <5%, *; and <10%, +. [b] Standard deviation.

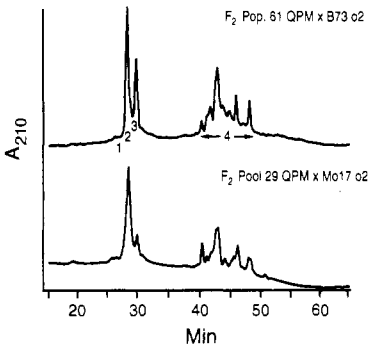

Figure 2. RP-HPLC of alcohol-soluble proteins in F_2 *opaque-2* (*o2*) maize hybrids incorporating quality protein maize (QPM) germplasm.

from combined kernels of two of the four crosses are shown in Figure 2. These QPM crosses are rich in fraction 2 (26 kDa, wsASG) and contain relatively little A/B zein [fraction 4 (22 + 24 kDa)]. Paulis et al. (1991) showed a similar protein distribution for QPM as compared to normal maize genotypes. The increased lysine content of Pool 29 QPM × Mo17 *o2*, expressed as percent protein (Table II), as compared to that of its reciprocal cross, agreed with RP-HPLC data (Table III). These data show 16% less A/B-zein (area 4) and 15% less total area in Pool 29 QPM × Mo17 *o2* than in its reciprocal cross. Since A/B-zeins contain no lysine, Pool 29 QPM × Mo17 *o2* contains more grain protein lysine than its reciprocal cross. The correlation for all populations between total RP-HPLC peak area and lysine in protein was $r = -0.60$. Pop. 61 QPM × B73 *o2* and its reciprocal cross show a similar relationship (Tables II and III). In all hybrids examined that involve the *o2* gene and QPM modifiers, A/B-zeins and total prolamin area were less abundant ($P < 0.01$) when the QPM line was the maternal parent.

RP-HPLC also showed that peak 2 (wsASG or γ-zein) of all crosses correlates positively and significantly with total lysine ($r = 0.74$, $P < 0.01$, $n = 16$) (data not shown), while peak 4 and total area (primarily corresponding to A/B-zeins of peak 4, which lack lysine) are correlated negatively with percent lysine (see Tables II and III). Lysine increases with number of doses of QPM modifier genes in *o2* hybrids, increasing the amount of γ-zein. Thus, total lysine is directly proportional to QPM dose increases in *o2* hybrids. A relationship of dose vs γ-zein concentration was also reported by Lopes and Larkins (1991). Dose effects on A/B-zein inheritance are also apparent by RP-HPLC. When QPM populations are maternal parents, A/B-zeins are 16% or 40% less abundant than when QPM is the paternal parent (Table III). Crosses with QPM as the maternal parent also contain less protein and more lysine (as percent of protein) than their reciprocal crosses (Table II). B73 *o2* × Pop. 61 QPM had the largest peak 4 of any of the four reciprocal crosses. Peak 2 was larger and peaks 3 and 4 were smaller in Mo17 *o2* × Pool 29 QPM and its reciprocal cross than in the B73 *o2* × Pop. 61 QPM crosses.

Relation of Protein Compositions to Hardness Criteria. An increase in density was associated with an increase in absolute area of all four RP-HPLC peak areas (Table IV). On a relative basis, however, density was correlated positively with areas 1 and 4 and negatively with peak 3. Peak 3 expressed as percent of total area was negatively correlated with density, vitreosity, and grinding time. Peaks 1 and 4, expressed as percentages, were positively correlated with density, vitreosity, and grinding time. Peak 2 percent was positively correlated with grinding time but tended to be negatively correlated ($P < 0.10$) with vitreosity.

Effects of Generation, Pedigree, and Density on Protein Composition. To better relate generation (G) (F_2 vs F_3), pedigree (P) (two sets of reciprocal crosses), and density (D) (levels 1.116–1.360) to protein composition (RP-HPLC peak areas), as well as to kernel grinding time, percent vitreosity, and weight, all data were subjected to analysis of variance. Results are summarized in Table V. Differences associated with pedigree and density were significant ($P < 0.01$) for most individual and total peak areas, as well as for grinding time, percent vitreosity, and kernel weight. Multiple genes appear to be involved; these genes are variable in any pedigree. The relationship of density to RP-HPLC peak areas may vary with population and generation.

The area of peak 1 was less than 2% (Table III) and so was not considered to be of practical significance for this analysis. The combined effect of G, P, and D on peak area 3 was not significant (Table V), so G × D and P × D were examined separately. The G × D interaction for peak 3 was a result of an increase from F_2 to F_3 at densities 1.116 and 1.360, a decrease at densities 1.193 and 1.235, and no change from F_2 to F_3 at densities 1.152 and 1.277. For the P × D interaction, crosses ranked similarly at densities 1.152, 1.193, 1.235, and 1.277 but differed at low (1.116) and high (1.318) densities.

Peak area 4 (A/B-zeins) varied considerably with generation, population, and kernel density. For Pool 29 QPM × Mo17 *o2*, area 4 was larger in the F_3 than in the F_2 genotypes at densities 1.116–1.235. At higher densities (1.277 and above) for Pool 29 QPM × Mo17 *o2*, the area of peak 4 was larger in F_2 than in F_3; in the reciprocal (Mo17 *o2* × Pool 29 QPM), the area of peak 4 was larger in the F_3 than the F_2 generation. In B73 *o2* × Pop. 61 QPM, the F_3 had a larger peak 4 than F_2 only at middle densities (1.235 and 1.277) as compared to its reciprocal cross. These results appear to be unrelated to year or location of growth, since Paulis et al. (1990) reported that environment had minimum effects on alcohol-soluble RP-HPLC protein profiles.

Previous results plus those reported here led us to conclude that the amount of A/B-zeins is genetically

Table III. Mean Peak Areas and Relative Areas of Alcohol-Soluble Proteins for Each F_2 and F_3 Cross[a]

pedigree	gen	n	area 1	area 2	area 3	area 4	area total	% area 1	% area 2	% area 3	% area 4	grinding time, s	% vitreous	kernel wt, g	density
Mo17 *o2* × Pool 29 QPM	F_2	22	7	380	80	557	1025	0.6	38.3	8.3	52.7	32.3	36.4	0.32	1.231
	F_3	20	7	386	101	770	1263	0.5	31.1	8.3	60.1	59.5	33.5	0.28	1.233
Pool 29 QPM × Mo17 *o2*	F_2	21	9	344	51	469	873	0.7	42.1	6.2	51.0	42.4	36.7	0.31	1.221
	F_3	19	7	314	87	554	961	0.7	32.5	9.5	57.7	45.5	33.9	0.26	1.188
B73 *o2* × Pop. 61 QPM	F_2	19	13	313	149	768	1244	1.0	26.2	12.9	60.0	39.7	36.8	0.25	1.237
	F_3	20	29	263	105	868	1265	1.8	24.0	10.5	63.7	52.8	34.2	0.23	1.210
Pop. 61 QPM × B73 *o2*	F_2	22	8	327	145	582	1062	0.7	31.0	14.0	54.3	30.7	22.0	0.28	1.237
	F_3	20	6	231	120	341	699	1.0	31.7	16.9	50.4	26.0	20.0	0.28	1.119

[a] See Table V for ANOVA comparisons of means.

Table IV. Correlations between Peak Areas and Kernel Densities, Percent Vitreosity, and Log Grinding Time (Combined Data for All F_2 and F_3 Crosses)[a]

	area 1	area 2	area 3	area 4	area total	% area 1	% area 2	% area 3	% area 4
density	0.29**	0.34**	0.16*	0.42**	0.48**	0.16*	−0.11	−0.16*	0.17*
% vitreosity	0.33**	0.24**	−0.01	0.38**	0.41**	0.26*	−0.15+	−0.26*	0.26**
log grinding time	0.42**	0.33**	−0.04	0.59**	0.61**	0.28*	0.25*	−0.38**	0.41**

[a] $^{**}P < 1\%$; $^{*}P < 5\%$; $^{+}P < 10\%$.

Table V. Analysis of Variance Results: Effects of Generation, Pedigree, and Density on RP-HPLC Peak Areas and Kernel Characteristics [Combined Data for All Crosses (n = 165)]

source of variation	df	peak area 1	peak area 2	peak area 3	peak area 4	peak area total	grinding time, s	% vitreosity	kernel wt
generation (G)[a]	1	ns[b]	0.06	ns	ns	ns	<0.01	ns	0.02
pedigree (P)[c]	3	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
density (D)	5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
G × P	3	<0.01	ns	<0.01	<0.01	<0.01	<0.01	ns	0.03
G × D	5	<0.01	ns	<0.01	<0.01	0.04	ns	ns	ns
P × D	15	<0.01	0.07	<0.01	<0.01	<0.01	<0.03	ns	ns
G × P × D	15	<0.01	0.03	ns	<0.01	<0.01	<0.01	ns	ns
R^2[d]		0.78	0.60	0.76	0.77	0.74	0.79	0.43	0.36

[a] G, generation (F_2 vs F_3). [b] Not significant. [c] P, pedigree (Mo17 *o2* × Pool 29 QPM; Pool 29 QPM × Mo17 *o2*; B73 *o2* × Pop. 61 QPM; and Pop. 61 QPM × B73 *o2*). [d] R^2, coefficient of determination.

determined but the genetics are not simple and will not respond to selection unless careful consideration is made as to pedigree, generation, and individual kernel density. For B73 *o2* × Pop. 61 QPM, F_3 kernels had longer grinding times than F_2 kernels at densities 1.193 and 1.235. In F_2 and F_3 generations of the reciprocal cross, grinding times of kernels of all densities were similar. For Mo17 *o2* × Pool 29 QPM and its reciprocal cross, F_3 kernels tended to have longer grinding times than F_2 kernels. Overall, at similar densities, F_3 lines had longer grinding times than did F_2 lines, with the exception of Pop. 61 QPM × B73 *o2*. It is unclear whether continued selection would increase grinding time.

Conclusions. F_2 and F_3 maize populations derived from crosses of CIMMYT QPM with *o2* inbreds differ, from kernel to kernel, in endosperm hardness (density, vitreosity, and grinding time). These differences relate significantly ($P < 0.01$) to prolamin compositions, as measured by RP-HPLC. Differences between generations varied with pedigree, and the relationship of density to protein composition varied both with generation and with pedigree. Percent vitreosity and kernel weight were related to density and pedigree. Protein compositions and grinding time were influenced by generation, pedigree, and density.

Thus, selection for protein distribution may or may not influence density, depending on which parents are used. This indicates that more than one gene is involved in hardness. When a QPM line is the maternal parent in *o2*-containing hybrids, A/B-zeins decrease, while lysine is elevated. These effects are greater than in reciprocal crosses. Thus, early-generation screening of segregating F_2 and F_3 populations by methods described should be helpful in identifying promising maize segregants that combine desired hardness and nutritional characteristics.

ACKNOWLEDGMENT

We thank Elizabeth Schmalzried for excellent technical assistance.

LITERATURE CITED

Dombrink-Kurtzman, M. A.; Bietz, J. A. Zein composition in hard and soft endosperm of maize. *Cereal Chem.* **1993**, *70*, 105.

Dombrink-Kurtzman, M. A.; Wilson, C. M. Characterization of proteins in hard and soft endosperm of quality protein maize. In *Quality Protein Maize*; Mertz, E. T., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1992; pp 155–173.

Gehrke, C. W.; Rexroad, P. R.; Schisla, R. M.; Absheer, J. S.; Zumwalt, R. W. Quantitative analysis of cystine, methionine, lysine and nine other amino acids by a single oxidation—4 hour hydrolysis method. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 171.

Gentinetta, E.; Maggiore, T.; Salamini, F.; Lorenzoni, C.; Pioli, F.; Soave, C. Protein studies in 46 opaque-2 strains with modified endosperm texture. *Maydica* **1975**, *20*, 145.

Gupta, H. O.; Lodha, H. O.; Rastogi, D. K.; Singh, J.; Mehta, S. L. Nutritional evaluation of hard endosperm *opaque-2* maize (*Zea mays* L.) *J. Agric. Food Chem.* **1979**, *27*, 390.

Hall, G. E.; Anderson, D. E. Light transmittance through corn to determine its hardness. In *Uniformity by 2,000*; Hill, L. D., Ed.; Scherer Communications: Urbana, IL, 1991; pp 139–141.

Lopes, M. A.; Larkins, B. A. Gamma zein content is related to endosperm modification in quality protein maize. *Crop Sci.* **1991**, *31*, 1655.

Mertz, E. T., Ed. *Quality Protein Maize*; American Association of Cereal Chemists: St. Paul, MN, 1992.

Mertz, E. T.; Bates, L. S.; Nelson, O. E. Mutant gene that changes protein composition and increases lysine content of maize endosperm. *Science* **1964**, *145*, 279.

National Research Council. *Quality-Protein Maize*; National Academy Press: Washington, DC, 1988.

Ortega, E. I.; Bates, L. S. Biochemical and agronomic studies of two modified hard-endosperm *opaque-2* maize (*Zea mays* L.) populations. *Cereal Chem.* **1983**, *60*, 107.

Paiva, E.; Kriz, A. L.; Perxoto, M. J. V. Y. D.; Wallace, J. C.; Larkins, B. A. Quantitation and distribution of γ-zein in the endosperm of maize kernels. *Cereal Chem.* **1991**, *68*, 276.

Paulis, J. W.; Bietz, J. A. Separation of alcohol-soluble maize proteins by reversed-phase high-performance liquid chromatography. *J. Cereal Sci.* **1986**, *4*, 205.

Paulis, J. W.; Wall, J. S. Fractionation and characterization of alcohol-soluble reduced corn endosperm glutelin proteins. *Cereal Chem.* **1977**, *54*, 1223.

Paulis, J. W.; Bietz, J. A.; Bogyo, T. P.; Darrah, L. L.; Zuber, M. S. Expression of alcohol-soluble proteins in maize single and double mutants. *Theor. Appl. Genet.* **1990**, *79*, 314.

Paulis, J. W.; Bietz, J. A.; Lambert, R. J.; Villegas, E. Identification of modified high-lysine maize genotypes by reversed-phase high-performance liquid chromatography. *Cereal Chem.* **1991**, *68*, 361.

Pierce, J. G. Sampling. In *Feed Manufacturing Technology III*; McEllhiney, R. R., Ed.; American Feed Industry Association: Arlington, VA, 1985; pp 336–343.

Wallace, J. C.; Lopes, M. A.; Paiva, E.; Larkins, B. A. New methods for extraction and quantitation of zeins reveal a high content of γ-zein in modified *opaque-2* maize. *Plant Physiol.* **1990**, *92*, 191.

Wilson, C. M. Multiple zeins from maize endosperms characterized by reversed-phase high performance liquid chromatography. *Plant Physiol.* **1991**, *95*, 777.

Received for review May 28, 1993. Accepted October 4, 1993.[⊗] The mention of firm names or trade products does not imply that they are endorsed or recommended by the USDA over other firms or similar products not mentioned.

[⊗] Abstract published in *Advance ACS Abstracts,* November 15, 1993.