Comparison between the Protein Quality of Northern Adapted Cultivars of Common Maize and Quality Protein Maize[†]

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The present study was designed to quantitatively measure and compare the levels and variation of total protein as well as the individual amino acids in three northern adapted (latitude >45° N) cultivars of common maize, namely a typical Dent CO251, a Flint CO255 inbred line, and commercial hybrid maize Pioneer 3953 with the new quality protein maize inbred (QPM-C13), and to assess their nutritive value from their FAO/WHO amino acid scoring pattern. The total protein content was variable among these cultivars ranging from 7.95% in QPM to 8.2% (Pioneer), 10.5% (Dent), and 11.79% (Flint). The QPM maize protein, however, proved to be of higher quality than common maize protein because it contained double the amount of lysine and arginine, higher levels of tryptophan and cysteine, and no change in other amino acids except lower levels of leucine. As a result, the QPM amino acid profile gives a good balance of total essential amino acids, limited only in lysine, and has an amino acid score, adjusted for digestibility, of 67%, compared to 28.5, 31.0, and 33.0% values found for Pioneer, Dent, and Flint, respectively. In common maize the primary essential amino acid deficiencies include lysine, threonine, and tryptophan. These results indicate that breeding maize for high protein quality can be very effective and that a very useful method for evaluating the protein quality of cereals is calculating their protein quality from their amino acid composition.

Keywords: *Maize; common maize; Dent, Flint, quality protein maize; QPM; assessment; protein quality; amino acids; composition*

INTRODUCTION

Maize (*Zea mays* L.) is a major cereal crop for both livestock feed and human nutrition in many countries today. Because of its economic importance, genetic improvements of maize cultivars have played a key role in the development of genotypes that will grow in a wide range of environments, rainfall, and altitudes (CIMMYT, 1985; Hallauer, 1987; National Research Council, 1988). Further breeding studies are presently underway to develop more productive maize hybrids which will germinate, grow, and mature in more northern latitudes (latitude >45° N), which have long daylengths (>16 h) and short growing seasons.

Attempts are also underway to further improve the protein quality of maize cultivars. Maize proteins are limited in certain essential amino acids, particularly lysine (Cromwell et al., 1967, 1968; Villegas et al., 1980; Asche et al., 1985; Bressani et al., 1990). Their protein score, based on the FAO/WHO (1965, 1973) pattern, indicates that lysine and tryptophan are the first and second limiting amino acids, respectively (Bressani, 1966; Eggum and Beams, 1983). The starchy endosperm of maize contains a group of four structurally distinct alcohol-soluble proteins called zeins (Esen, 1987; Wallace et al., 1990; Shewry and Tatham, 1990) which are encoded by specific classes of structural genes that belong to a large gene family clustered in several DNA regions (Pedersen et al., 1980; Hagen and Rubenstein, 1981; Larkins et al., 1984, 1989; Das et al., 1990; Pysh et al., 1993). These proteins, which account for about 50% of the total endosperm protein at maturity in common maize, are characterized by a high content of glutamine, leucine, and proline and are practically devoid of lysine and tryptophan. As a result, the overall lysine and tryptophan contents (Paiva et al., 1991) of normal maize are only 1.81 and 0.35%, respectively. The high quality protein maize (QPM), however, contained higher levels of lysine and tryptophan, with no major change in other amino acids except in leucine (Bressani, 1991).

To genetically improve the quality of maize proteins, therefore, either a reduction in the zein storage protein fraction or an increased proportion of other protein fractions, or a combination of the two, will be required (Glover, 1976; Frey, 1951; Villegas et al., 1980; Soave and Salamini, 1984; Schmidt et al., 1987; Or et al., 1993; Habben et al., 1993). Although several endosperm mutations have been identified that suppress the synthesis of zein proteins and increase other protein fractions affecting the quality of protein in maize (Mertz et al., 1964; Nelson et al., 1965; Ma and Nelson, 1975; Jones et al., 1977a,b; Soave, 1979; Salamini et al., 1983; Larkins et al., 1984; Mertz, 1986; Graham et al., 1990; Or et al., 1993; Aukerman and Schmidt, 1993), only the *opaque-2* mutation has been widely studied (Mertz et al., 1964; Ortega and Bates, 1983; National Research Council, 1988; Hallauer, 1987; Bjarnason and Vasal, 1980, 1992; Geetha et al., 1991; Messmer et al., 1992; Damerval and De Vienne, 1993). *Opaque-2* gene is a mutation in one of the regulatory loci that control storage protein gene transcription in maize. This

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mutant gene affects the quality of protein in maize by reducing the synthesis of zeins (Villegas et al., 1980) in the endosperm, thereby resulting in a significantly increased proportion of other protein fractions that contain higher levels of both lysine and tryptophan (Bressani, 1991).

Since these findings several other mutants have been identified, collectively designated high-lysine genes, all of which control the level of zein accumulation during endosperm development, i.e., *opaque-7* (Misra et al., 1972); *opaque-6* and *floury-3* (Ma and Nelson, 1975); *defective endosperm-B30* and *mucronate* (Salamini et al., 1983). The primary effect or lesion of these high-lysine mutants remains unknown; however, each of these loci reduces zein synthesis to a different degree, ranging from 20 to 80% reduction (Mertz et al., 1964; Nelson et al., 1975; Salamini et al., 1983; Misra et al., 1972; Soave, 1979). *Opaque-2* cultivars, however, have not proven profitable as crops because of the soft, chalky kernel endosperm, lower yields, and increased susceptibility to insects, pathogens, and mechanical damage.

Extensive field trials have been carried out at the International Maize and Wheat Improvement Center (CIMMYT) in Mexico to identify the most productive maize cultivars, which are high in lysine and tryptophan contents, and to change their soft *opaque-2* endosperm into a conventional hard vitreous type (Jones et al., 1977a,b; Vasal et al., 1980; Ortega and Bates, 1983; Bjarnason and Vasal, 1992). Through backcrossing and several cycles of recurrent selection of maize, CIMMYT's maize breeders have successfully combined the high-lysine potential of the *opaque-2* mutation with genetic endosperm modifiers. Cultivars similar in yield and other important agronomic properties to normal maize, which still maintain high protein quality, have been developed (National Research Council, 1988; Bjarnason and Vasal, 1980, 1992). These new maize genotypes, collectively called Quality Protein Maize (QPM), are becoming of major interest to seed producers, breeders, geneticists, and industry for their large-scale production and for their potential advantages in human nutrition and animal feeding. An accurate assessment of the protein quality and nutritional adequacy of QPM is therefore essential.

The present study was designed to quantitatively measure and compare the levels and variation of total protein as well as the individual amino acids in three northern adapted maize cultivars, a typical Dent CO251 and a Flint CO255 inbred line and a commercial hybrid maize Pioneer 3953, with the new QPM-C13 inbred and to assess their nutritive value.

MATERIALS AND METHODS

Materials. Type DC-5A (lot no. 746) cation-exchange spherical resin, sized to 6.0 ± 0.5 mm, was purchased from Dionex Chemical Co., Sunnyvale, CA. The amino acid standards were obtained as follows: norleucine from Pierce Chemical Co., Rockford, IL; 3-nitrotyrosine from Aldrich Chemical Co., Milwaukee, WI; and standard amino acid calibration mixture from Beckman Instruments, Inc., Palo Alto, CA. Highly purified ninhydrin and hydrindantin (Nin-Sol AF) dissolved in sequenal grade dimethyl sulfoxide was purchased from Pierce Chemical Co., Rockford, IL. Octanoic acid was obtained from Eastman Kodak Co., Phillipsburg, NJ. Hydrochloric acid (Analar), hydrobromic acid (Aristar), formic acid (88.0%), and hydrogen peroxide (30.0%) were purchased from BDH Inc., Poole, England. High-purity sodium hydroxide (50.0% w/w), which was used to prepare all buffers and reagents, was a product of Allied Fisher Scientific, Fair Lawn, NJ. The three highly purified microcolumn citrate buffers (pH 3.283, 0.20 M; pH 4.10, 0.20 M; pH 6.40, 1.0 M) and sample dilution buffer (pH 2.2, 0.20 M) recommended for high-sensitivity single-microcolumn analysis were used as described previously (Zarkadas et al., 1987). All other chemicals and reagents were of the highest purity commercially available and were used without further purification.

Experimental Procedures. *Plant Material and Sample Preparation.* The three northern adapted maize inbred lines selected for this investigation were the Dent inbred CO251, the cold-tolerant Flint CO255, and new quality protein maize (QPM-C13), all developed at the Plant Research Centre, Agriculture Canada, Ottawa. Both Dent inbred CO251 and Flint CO255 are early maturing inbreds with superior combining ability and agronomic traits, and both are well adapted to the more northerly temperate regions of Canada (latitude >45° N), where the low average daily temperature in May and June ranges from 10 to 15 °C and the growing season is short. QPM-C13 is a recent inbred, medium late maturity, in evaluation trials. The choice of a tester adapted high-yielding hybrid to evaluate these new lines was Pioneer 3953, which is a single cross yellow Dent hybrid requiring 2450 corn heat units (FAO 150).

The first cultivar, CO251, is an orange-yellow Dent inbred, which has been used as a male parent in a number of early hybrids in Canada and has shown a broad general combining ability. This inbred was developed from the backcross of (CO109 × CO125) by CO125, with selection for both superior combining ability and agronomic traits. The CO109 genotype had been developed from the cultivar Early Butler, while the CO125 inbred was developed from Pfister 44, a northern U.S. corn belt hybrid introduced into Canada in the 1950s. Assessment of the agronomic performance of CO251 was conducted at short-season locations in Eastern Canada and the breeding nursery site at Agriculture Canada's Central Experimental Farm, Ottawa, in the 1970s.

The second cultivar, a Flint inbred designated CO255, has been used widely in as much as 50% of the silage hybrids in several northern European countries including France, Holland, and Germany. The CO255 cultivar originated from an INRA hybrid, INRA 258, which was a four-way or double-cross hybrid with the following pedigree: (F115 × W33)/(F7 × EP1). The origin of the parental lines of INRA 258 was as follows: F115 is an INRA developed inbred that derived from Iowa 153 and W33, which was developed by the University of Wisconsin from the single cross W9 × WH. W9 originated from cultivar Golden Glow, while WH originated from the Wisconsin germplasm W25. F7 was from a population grown at Lacaune, a high-elevation region of southern France, and EP1 was from Lizargarote, Spain. The CO255 inbred cultivar has shown an exceptionally early low-temperature vigor and superior combining ability with many corn families and has a yellow-orange Flint kernel.

The third inbred cultivar, which is designated quality protein maize, QPM-C13, was developed from the Northern Temperate Zone 1 (NTR-1) QPM gene pool originated from the International Maize and Wheat Improvement Center (CIMMYT). This germplasm line was subsequently inbred to the S5 generation at the Plant Research Centre, Ottawa, and resulted in the new QPM-C13 genotype containing the high-lysine *opaque-2* gene and modifier genes that favor improved kernel characteristics. Agronomic performance and superior combining ability studies on QPM-C13 and other lines were carried out by Spaner et al. (1992) at three different locations in eastern Canada.

Representative samples of seed of the four cultivars were obtained from the Plant Research Centre and Pioneer Hi-Bred International in 1991. The freeze-dried kernels were then pulverized in a standard electrically driven end runner mill (Cyclone Sample Mill, U. D. Corp., Fort Collins, CO), passed through a 0.5-mm mesh seive, lyophilized, and stored at −20 °C in polypropylene bottles until used.

Preparation of Tissue Hydrolysates. Duplicate samples (50.0 mg) were hydrolyzed in Pyrex (No. 9860) test tubes (18 × 150 mm) under vacuum (below 10 mmHg) with triple-glass-distilled constant-boiling HCl (6.0 M) containing 0.2% (v/v) phenol and 5 μL of octanoic acid at 110 ± 0.5 °C for periods of 24, 48, 72, and 96 h with the precautions described by Zarkadas et al. (1988a,c). Analyses of individual acid hydroly-

sates were performed on the clear filtrate in duplicate according to methods described previously (Zarkadas et al., 1986, 1988a−c, 1993, 1994).

Procedures for Amino Acid Analyses. Amino acid analyses were carried out on a Beckman Spinco Model 121 MB fully automated amino acid analyzer using single-column methodology (Zarkadas et al., 1986, 1987, 1990).

Complete amino acid analyses were carried out on each of the three replicate maize samples (50.0 mg) per cultivar according to the standard procedures described previously (Zarkadas et al., 1986, 1987). Each of the four replicates was divided into two subsamples, i.e., A and B, which were then hydrolyzed in duplicate for 24, 48, 72, and 96 h as described previously (Zarkadas et al., 1988a−c).

Methionine and cyst(e)ine were determined separately (50.0 mg samples) according to the performic acid procedure of Moore (1963). Norleucine was added in the hydrolysate as an internal standard. Recoveries of cyst(e)ine as cysteic acid and methionine as methionine *S,S*-dioxide were calculated in proportion to the yields obtained by the performic acid treatment of standard solutions of these amino acids. The data were then normalized relative to alanine, valine, leucine, and isoleucine present in the sample and represent the average of 24 determinations.

Tryptophan in maize samples (50.0 mg) was also determined separately after alkaline hydrolysis (Hugli and Moore, 1972) on a single column as described previously (Zarkadas et al., 1986), using 3-nitrotyrosine as the internal standard, and the data presented in Table 1 represent the average of 24 determinations.

Protein Determination. Recoveries of amino acids were calculated on the basis of the protein content of individual hydrolysates determined according to the method of Horstmann (1979) as described previously (Zarkadas et al., 1988a−c):

$$\mathrm{WE} = \sum_{i=1}^{18}(a_i b_i) \quad (1)$$

According to this method, a mean residue weight (WE, in micrograms per nanomole) is calculated for the amino acids constituting the proteins in maize; where a is the mole fraction of an amino acid i found in the analyzed aliquot and b is the molecular weight of amino acid residue i (in micrograms). The conversion factor CF, which represents the apparent average residue molecular weight (in micrograms per nanomole) of the proteins in maize but in the absence of tryptophan, methionine, and cyst(e)ine, and the protein concentration of each hydrolysate were then calculated as described previously (Zarkadas et al., 1988a,b, 1994).

The protein content of each sample was calculated by multiplying CF by the nanomoles of total amino acids (χ_i) in each acid hydrolysate as follows:

$$P = CF\sum_{i=1}^{15}\chi_i \quad (2)$$

Predicting Properties of Proteins from Amino Acid Compositions. Previous studies by Khanizadeh et al. (1989) and Zarkadas et al. (1994) have shown that grouping amino acids from tissue compositional data into classes with distinct properties could be partially related to the rather general properties of the proteins in tissue mixtures. One feature of protein structure that is fairly reliable is the tendency of the side chain of charged or very hydrophilic or polar amino acid residues to be external, to interact strongly with water, and to have high solubility in water. At the opposite end of the polarity scale are the apolar or hydrophobic side chains, which tend to have low solubility in water and therefore will be internal (Bigelow, 1967; Nozaki and Tanford, 1971). Barrantes (1973, 1975) has grouped the amino acids into four classes, total charged, hydrophilic, hydrophobic, and apolar, and simply compared the ratio (R) of the frequencies of occurrence (χ) of whatever particular side chains of proteins one wishes to examine, e.g.

$$R = \sum_k \chi_k / \sum_j \chi_j \quad (3)$$

where k can be hydrophilic and j hydrophobic side chains or k polar and j nonpolar as defined by Barrantes (1973). [Basic: histidine + lysine + arginine. Acidic: aspartic acid + glutamic acid + asparagine + glutamine. Total charged: basic + acidic. Hydrophilic: total charged + threonine + serine. Hydrophobic: valine + methionine + isoleucine + leucine + tyrosine + phenylalanine + tryptophan. Apolar: hydrophobic − tyrosine. Ratio 1 (R_1): hydrophilic/hydrophobic. Ratio 2 (R_2): hydrophilic/apolar. Ratio 3 (R_3): total charged/hydrophobic. Ratio 4 (R_4): total charged/apolar.]

Although the particular choice of amino acid residues used to construct these ratios is somewhat arbitrary (Barrantes, 1973, 1975), one particular ratio scale that reliably weighs the tendency of charged or very polar residues to be external is R_3. This ratio is convenient because it spreads different proteins over a wide scale range, from 0.36 to 2.03, and gives a measure with more information about the system.

Statistical Analysis. Data processing of the results was carried out by a FORTRAN computer program developed for this purpose. Analysis of variance, conducted on the amino acid data, for a completely randomized block design (factorial) was carried out by the general linear model procedure (SAS, 1991) and represents the average values from eight subsamples per cultivar.

RESULTS AND DISCUSSION

The overall amino acid composition of three new northern adapted maize cultivars, expressed as grams of amino acid per kilogram of anhydrous fat- and ash-free tissue protein, is summarized in Table 1. Although wide variations in the amino acid composition among the various cultivars were noted, the least variability, in seed tissue amino acid content, was found when the results were expressed on a protein basis. According to Benedict (1987) and Zarkadas et al. (1988a−c), when the data are expressed on a protein basis, they reflect the relative amounts of the amino acids present, since the influence of both fat and moisture is eliminated. In addition, the data on amino acid composition on a protein basis, as presented in Table 1, allow comparisons to be made between the present results and those reported by others, those given in food compositional tables, and the recommended FAO/WHO/UNU (1985) and FAO/WHO (1990) reference amino acid patterns for humans. This method has the added advantage that the percentage recovery of amino acids by weight or on a nitrogen basis can be found by simple summation (Tristram and Smith, 1963; Eastoe, 1967).

An earlier method for expressing amino acid content was based on grams of amino acid per 16 g of total nitrogen. This method was first introduced by Block and Mitchell (1946) for rapid calculation of the amino acid content of diets in nutritional studies. For purposes of comparison, the data from this study have been calculated as recommended by FAO/WHO/UNU (1985) and FAO/WHO (1990) and are presented in Table 2.

Precise protein determinations of each acid hydrolysate were carried out according to the method of Horstmann (1979) as described previously (Zarkadas et al., 1988a,b; Khanizadeh et al., 1992), and the results are summarized in Table 1. This method of calculating the protein mass in seeds or tissues is based upon knowledge of their amino acid composition and yields accurate estimates of the amount of protein present. The mean residue weight equivalent (WE, micrograms per nanomole) and conversion factor (CF, micrograms per nanomole) given in Table 1 were determined using eq 1 and can be used in all subsequent protein quantita-

Table 1. Comparison of the Amino Acid (AA) Composition and Protein Contents (Grams of Amino Acids per Kilogram of Total Protein) of Three New Northern Adapted Maize Cultivars

	maize cultivars[a]								signif levels among cultivars[a]	
	Dent (C0251)		Flint (C0255)		Pioneer (3953)		quality protein maize (QPM-C13; opaque-2)			
AA	mean ± SEM	CV	mean ± SEM	CV	mean ± SEM	CV	mean ± SEM	CV	CV	F
aspartic acid	46.66 ± 4.03^{e}	14.96	55.78 ± 2.16^{e}	6.69	55.05 ± 0.07^{e}	0.22	78.88 ± 5.45^{d}	11.97	11.66	12.08**
threonine	31.11 ± 2.04^{e}	11.36	28.73 ± 1.22^{e}	7.33	29.49 ± 0.19^{e}	1.17	$35.79 \pm 0.54^{c,d}$	2.59	6.23	7.98*
serine	42.89 ± 2.57	10.40	44.28 ± 1.05	4.09	43.34 ± 0.84	3.36	43.89 ± 0.73	2.91	4.27	0.32^{ns}
glutamic acid	207.11 ± 7.46^{d}	6.24	213.20 ± 0.43^{d}	0.35	215.50 ± 0.56^{d}	0.45	171.21 ± 3.27^{e}	3.31	3.17	31.21***
proline	100.12 ± 13.07	22.63	86.74 ± 1.22	2.45	80.16 ± 0.11	0.24	91.13 ± 4.74	9.02	12.11	1.79^{ns}
glycine	29.34 ± 0.59^{e}	3.52	27.15 ± 0.18^{e}	1.17	26.86 ± 0.05^{e}	0.35	43.33 ± 2.74^{d}	10.98	8.12	27.95***
alanine	78.39 ± 2.53^{d}	5.59	79.03 ± 2.88^{d}	6.33	85.69 ± 0.29^{d}	0.59	56.31 ± 4.19^{e}	12.87	6.77	19.13***
cysteine	31.42 ± 1.64^{e}	9.06	29.92 ± 0.69^{e}	4.02	29.11 ± 0.83^{e}	1.41	53.37 ± 4.05^{d}	13.14	9.05	38.46***
valine	49.81 ± 1.66^{d}	5.78	45.93 ± 0.62^{e}	2.35	41.43 ± 0.12^{f}	0.49	50.11 ± 1.59^{d}	5.49	4.02	13.98**
methionine	$19.19 \pm 1.74^{d,e}$	15.73	21.33 ± 0.72^{d}	5.81	16.76 ± 0.15^{f}	1.60	17.24 ± 1.09^{f}	10.97	7.12	7.40**
isoleucine	37.95 ± 0.91^{e}	4.15	$38.28 \pm 0.54^{d,e}$	2.43	40.53 ± 0.12^{d}	0.52	33.82 ± 1.15^{f}	5.91	3.30	15.19**
leucine	140.49 ± 1.39^{d}	1.71	141.84 ± 1.71^{d}	2.08	146.84 ± 0.50^{d}	0.59	85.18 ± 3.53^{e}	7.19	3.18	151.52****
tyrosine	44.95 ± 0.21^{e}	0.80	$47.02 \pm 0.59^{d,e}$	2.18	48.25 ± 0.89^{d}	3.21	38.05 ± 1.30^{f}	5.92	3.37	27.59***
phenylalanine	53.27 ± 1.75^{d}	5.71	53.26 ± 0.88^{d}	2.88	56.31 ± 0.23^{d}	0.69	41.81 ± 1.95^{e}	8.09	4.14	27.42***
histidine	25.29 ± 1.44^{e}	9.90	25.30 ± 0.54^{e}	3.72	20.34 ± 1.48^{f}	12.67	34.40 ± 2.34^{d}	11.79	8.88	18.87***
lysine	19.86 ± 1.75^{e}	15.22	17.71 ± 2.04^{e}	19.93	17.51 ± 0.06^{e}	0.58	39.77 ± 2.91^{d}	12.68	12.82	37.59***
arginine	36.06 ± 1.12^{e}	5.39	37.46 ± 0.83^{e}	3.82	38.57 ± 0.11^{e}	0.53	73.59 ± 1.65^{d}	3.89	2.98	516.44****
tryptophan	6.07 ± 1.06^{e}	30.11	7.07 ± 0.28^{e}	6.94	8.33 ± 0.13^{e}	2.64	12.11 ± 1.49^{d}	21.30	17.89	9.27**
ammonia	15.13 ± 3.83	43.90	10.15 ± 2.16	36.83	15.43 ± 0.54	6.09	17.62 ± 2.63	25.81	27.37	1.88^{ns}
WE,[b] μg/mol	0.109860 ± 0.0006^{e}	0.54	$0.110582 \pm 0.0001^{d,e}$	0.19	$0.110359 \pm 0.0002^{d,e}$	0.044	0.111245 ± 0.0005^{d}	0.81	0.54	2.70^{ns}
CF,[b] μg/nmol	0.110254 ± 0.0002^{e}	0.44	$0.111040 \pm 0.0002^{d,e}$	0.19	$0.110907 \pm 0.00003^{d,e}$	0.051	0.112056 ± 0.0005^{d}	0.80	0.52	4.99*
basic[c]	81.21 ± 1.57^{e}	3.35	80.45 ± 2.68^{e}	5.78	76.42 ± 1.42^{e}	3.22	147.77 ± 4.60^{d}	5.39	4.57	181.09****
acidic[c]	252.77 ± 11.47	7.83	268.97 ± 2.55	1.64	270.56 ± 0.55	0.35	250.09 ± 3.25	2.25	4.33	2.55^{ns}
charged[c]	334.88 ± 12.95^{e}	6.70	349.93 ± 1.24^{e}	0.61	346.98 ± 1.13^{e}	0.56	397.86 ± 4.83^{d}	2.10	3.69	13.25**
hydrophobic[c]	351.73 ± 0.71^{d}	0.35	354.72 ± 3.23^{d}	1.58	358.45 ± 0.76^{d}	0.37	278.32 ± 6.80^{e}	4.23	2.07	92.06****
hydrophilic[c]	408.99 ± 17.56^{e}	7.44	422.44 ± 2.91^{e}	1.19	419.76 ± 1.24^{e}	0.51	477.54 ± 5.48^{d}	1.99	3.87	10.13**
apolar[c]	306.78 ± 0.62^{e}	0.35	307.71 ± 2.96^{d}	1.66	310.21 ± 1.24^{d}	0.69	240.27 ± 5.58^{e}	4.03	2.07	95.48****
R^{c}_{1}	0.863 ± 0.038^{d}	7.72	0.839 ± 0.009^{d}	2.04	0.854 ± 0.004^{d}	0.87	0.583 ± 0.021^{e}	6.16	5.48	29.44***
R^{c}_{2}	1.333 ± 0.056^{e}	7.30	1.373 ± 0.015^{e}	1.96	1.355 ± 0.09^{e}	1.16	1.990 ± 0.069^{d}	6.05	5.99	37.28***
R^{c}_{3}	0.952 ± 0.037^{e}	6.66	0.985 ± 0.011^{e}	2.10	0.965 ± 0.004^{e}	0.88	1.432 ± 0.053^{d}	6.42	6.03	37.82***
R^{c}_{4}	1.099 ± 0.041^{e}	6.57	1.135 ± 0.013^{e}	2.12	1.12 ± 0.006^{e}	0.94	1.658 ± 0.059^{d}	6.19	5.87	41.19***
total protein, g/kg of dry mass	104.85 ± 6.59^{e}	10.88	117.78 ± 4.66^{d}	6.86	82.01 ± 0.60^{f}	1.86	79.50 ± 3.08^{f}	6.73	6.19	28.84***

[a] Mean values and standard error of measurements (SEM) for 3 replicates ($N = 3$) and 48 determinations. Significance: F, values from analysis of variance among cultivars; ****, $P < 0.001$; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant; CV, coefficient of variation. [b] Computed according to the method of Horstmann (1979) and Zarkadas et al. (1988a−c). [c] Calculated according to the method Barrantes (1973, 1975) using eq 4. [d−f] Means along a row with different superscripts are significantly different (Duncan, 1955).

tions using eq 2 as described previously by Horstmann (1979) and Zarkadas et al. (1988a, 1994).

The variation noted in the protein content among the three maize cultivars evaluated was statistically highly significant ($P < 0.001$), with the Flint CO255 being consistently higher in total protein than either of the other two maize cultivars. The new QPM-C13 maize cultivar had approximately the same amount of total protein (7.95%) as normal Pioneer 3953 maize (8.2%). These results are in accord with those of Villegas et al. (1990) and Ortega et al. (1991), who reported a range from 7.4 to 8.6% for their hard endosperm *opaque-2* maize cultivars. However, Kniep and Mason (1991) and Bjarnason and Vasal (1992) reported higher values ranging from 8.3 to 9.7% for normal and *opaque-2* maize. The differences in total protein content between the present study and others may be attributed to the quantitative methods used by these authors for determining total nitrogen content in maize. Differences between Kjeldahl nitrogen and nitrogen determined by the summation of the amino acid nitrogen contents among various plant or animal tissues have been reported previously (Zarkadas et al., 1988b; Khanizadeh et al., 1992). These authors indicated that percent differences as a function of method of nitrogen determination ranged from 1.86 to 5.40% in soybean protein products to 30.7 and 36.8% in milk solid nonfat powder and gluten feed, respectively. Similar variability has been reported by Heidelbaugh et al. (1975) for Skylab foods. These results suggest that because the conventional Kjeldahl nitrogen procedure used for the analysis of total nitrogen in cereals greatly overestimates the protein content of maize, quantitative amino acid data should be the preferred method for assessing both the total protein and the protein quality of the new maize genotypes under investigation.

The data presented in Table 1 indicate that total protein in Dent CO215 was 10.5% and in Flint CO255 was 11.8% in contrast to the commercial Dent Pioneer 3953 with 8.2%. This represents an increase of 28% in protein of the Dent CO251 inbred and 43% in the Flint CO255 inbred compared to the normal maize hybrid, Pioneer 3953, corresponding to increases of 2.3 and 3.6 g of protein/100 g of dry mass for the Dent CO251 and Flint CO255 maize cultivars, respectively. The results show that this increase in total protein content was not accompanied by increased protein quality.

The data reported on the total nitrogen and protein contents of the four maize cultivars in Tables 1 and 2 have been calculated according to the method recommended by Heidelbaugh et al. (1975) and Horstmann (1979). The total amino acid nitrogen of these samples ranged from 12.8 to 17.9 g of amino acid nitrogen/kg of dry mass. The endosperm of both Flint CO255 and Dent CO251 cultivars contained a significantly higher ($P < 0.001$) concentration of nitrogen (from 1.65 to 1.8%) compared to those found in the QPM-C13 (1.36%) and Pioneer 3953 maize (1.28%). These data compare favorably with those of Ortega et al. (1991) but were lower than those of Kniep and Mason (1991).

A comparison of the amino acid profiles of the maize cultivars, as presented in Table 1, shows that three of these four cultivars, i.e., the normal endosperm Dent CO251 and Flint CO255 cultivars and the Pioneer 3953 tester, were very similar in amino acid composition. All three were high in several amino acids, namely glutamic acid (20.7−21.5% of the total amino acids), leucine (14.6%), proline (8.0−10.0%), alanine (7.8−8.5%), phenylalanine (5.3−5.6%), valine (4.1−4.9%), and aspartic acid (4.6−5.6%). Thus, these seven account for 67−69% of the total amino acids. The total basic amino acids, which include lysine, histidine, and arginine, constituted only 7.5−8.1% of the total amino acids. These values are considerably lower than the acidic amino acids, which represent 25% of the total amino acid residues. The rarest amino acid residues in these three maize cultivars were lysine (1.70−1.98%), tryptophan (0.6−0.8%), and methionine, which accounted for a further 1.7−2.1% of the total amino acid residues. These results were close to those reported by Nelson et al. (1965) for normal maize.

In contrast, the QPM-C13 cultivar has an overall amino acid profile that was very different from the other three cultivars investigated in this study. Amino acid content was found to be highly significantly different for each amino acid analyzed, i.e., QPM-C13 versus Dent CO251, Flint CO255, and Pioneer 3953, except for serine and proline (Table 1). Of particular note were the increased levels of lysine and tryptophan in QPM-C13 genotype. Lysine and tryptophan variations were statistically highly significant ($P > 0.001$). QPM-C13 contained 3.98 g of lysine and 1.21 g of tryptophan per 100 g of protein, in accord with the findings of Paiva et al. (1991). These results are also similar to the values presented for lysine by Kniep and Mason (1991), who reported a range from 4.1 to 4.3% for short- and long-season *opaque-2* maize, respectively. By contrast, the lysine values found among the other three hybrids ranged from 1.77 g/100 g of protein in normal Flint CO255 to 1.99 g/100 g in normal yellow Dent CO251 compared to 1.82 g/100 g of protein found in the commercial tester hybrid, Pioneer 3953.

There were other accompanying changes in the proportions of amino acids in QPM-C13, which are in accord with the type of modifications reported in the amino acid profiles of *opaque-2*, *floury-2*, *opaque-6*, *opaque-7*, and *mucronate* high-lysine mutants (Nelson et al., 1965; Misra et al., 1972, 1975a,b; Ma and Nelson, 1975; Salamini et al., 1983; Glover and Mertz, 1987). In addition to lysine and tryptophan, the contents of arginine, histidine, aspartic acid, glycine, and cyst(e)ine increased in QPM-C13 while the levels of glutamic acid, alanine, leucine, tyrosine, and phenylalanine decreased sharply, compared to normal maize varieties. The data presented in Tables 1 and 2 suggest a preferential accumulation of certain amino acids in the kernels of these four cultivars. These data also indicate a highly significant increase ($P > 0.0001$) in basic and total charge ($P < 0.001$) amino acids in the R_3 ratio (Barrantes, 1973, 1975) and a decrease in hydrophobicity of the storage proteins in QPM-C13, compared to Pioneer 3953, and the normal Dent CO251 and Flint CO255 cultivars. These results suggest that the observed decrease in hydrophobicity reflects the structural changes that occur in the subunit composition of the QPM-C13 storage proteins as a result of differential gene expression that determine protein transformation patterns (Goldberg, 1986) in the seeds of this new cultivars.

QPM maize, which combined the *opaque-2* genes with genetic modifiers, yields high levels of lysine and tryptophan and has a kernel hardness and appearance similar to normal maize. According to Ortega and Bates (1983) and Ortega et al. (1986, 1991), the *opaque-2* gene present in QPM hybrids partially inhibits zein synthesis. This is then accompanied by proportional increases in other protein fractions, i.e., albumins, globulins, glutelins, and free amino acids (Misra et al., 1975b), so that the endosperm protein in QPM maize has about double the lysine and tryptophan content of normal endosperm maize.

Table 2. Amino Acid Composition and Nitrogen Content of Three New High-Protein Maize Cultivars (Grams of Amino Acid per 16 g of Nitrogen)

AA	maize cultivars[a]								signif levels among cultivars[a]	
	Dent (C0251)		Flint (C0255)		Pioneer (3953)		quality protein maize (QPM-C13; opaque-2)			
	mean ± SEM	CV	mean ± SEM	CV	mean ± SEM	CV	mean ± SEM	CV	CV	F
aspartic acid	4.726 ± 0.339^{c}	12.44	5.844 ± 0.293^{c}	8.69	5.617 ± 0.018^{c}	0.57	7.323 ± 0.579^{b}	13.69	11.61	7.48**
threonine	3.153 ± 0.161	8.86	3.004 ± 0.097	5.58	3.003 ± 0.013	0.77	3.318 ± 0.071	3.73	5.64	2.17^{ns}
serine	4.349 ± 0.191^{b,c}	7.62	4.634 ± 0.089^{b}	3.21	4.421 ± 0.073^{b}	2.86	4.067 ± 0.014^{c}	0.62	3.34	7.72**
glutamic acid	21.014 ± 0.421^{c}	3.47	22.219 ± 0.308^{b}	2.89	21.986 ± 0.133^{b,c}	1.05	15.873 ± 0.368^{d}	4.02	2.67	91.85****
proline	10.217 ± 1.527	25.89	9.082 ± 0.230	4.39	8.178 ~ 0.029	0.63	8.456 ± 0.521	10.66	13.99	2.46^{ns}
glycine	2.982 ± 0.112^{c}	6.51	2.842 ± 0.15^{c}	0.93	2.739 ± 0.009^{c}	0.61	4.015 ± 0.245^{b}	10.56	8.22	15.56**
alanine	7.981 ± 0.382^{b}	8.31	8.279 ± 0.391^{b}	8.17	8.743 ± 0.059^{b}	1.18	5.221 ± 0.400	13.28	7.61	22.87***
cysteine	3.198 ± 0.22^{c}	12.96	3.134 ± 0.107^{c}	5.91	2.966 ± 0.037^{c}	2.16	4.947 ± 0.384	13.45	8.72	26.87***
valine	5.05 ± 0.088^{b}	3.04	4.806 ± 0.038^{c}	1.37	4.227 ± 0.026^{d}	1.08	4.642 ± 0.0843^{c}	3.14	2.49	26.50***
methionine	1.955 ± 0.214^{b,c}	12.26	2.234 ± 0.099^{b}	7.72	1.710 ± 0.021^{c,d}	2.18	1.601 ± 0.120^{d}	12.99	8.94	8.45**
isoleucine	3.853 ± 0.053^{c}	2.38	4.006 ± 0.015^{b,c}	0.67	4.134 ± 0.026^{b}	1.11	3.135 ± 0.116^{d}	6.40	3.25	39.60***
leucine	14.278 ± 0406^{b}	4.93	14.844 ± 0.051^{b}	0.59	14.981 ± 0.102^{b}	1.18	7.897 ± 0.347^{c}	7.62	4.07	125.16****
tyrosine	4.567 ± 0.105^{b}	3.98	4.922 ± 0.084^{b}	2.97	4.922 ± 0.079^{b}	2.97	3.526 ± 0.107^{c}	5.24	4.22	36.59***
phenylalanine	5.405 ± 0.084^{b}	2.68	5.573 ± 0.036^{b,c}	1.10	5.745 ± 0.043^{b,c}	1.10	3.873 ± 0.153^{d}	6.90	3.03	91.42****
histidine	2.576 ± 0.196^{b}	13.17	2.649 ± 0.078^{c}	5.11	2.074 ± 0.149^{c,d}	12.44	3.193 ± 0.245^{d}	13.30	9.92	9.28**
lysine	2.012 ± 0.147^{c}	12.69	1.849 ± 0.189^{c}	17.71	1.786 ± 0.005^{c}	0.50	3.682 ± 0.234^{b}	11.01	11.26	35.60***
arginine	3.659 ± 0.059^{d}	2.78	3.919 ± 0.135^{c}	3.44	3.935 ± 0.011^{c}	0.50	6.818 ± 0.053^{b}	1.35	1.61	1240.52****
tryptophan	0.621 ± 0.119^{c}	33.44	0.741 ± 0.033^{c}	7.77	0.850 ± 0.015^{b,c}	3.18	1.127 ± 0.152^{b}	23.41	18.67	5.76*
ammonia	1.473 ± 0.327	38.44	1.057 ± 0.211	34.66			1.626 ± 0.216	22.99	23.32	1.80^{ns}
total AAN[e]										
g of AAN/kg of protein	157.60 ± 2.91^{c}	3.19	152.888 ± 1.86^{c}	2.11	156.835 ± 0.53^{c}	0.54	127.644 ± 2.57^{b}	2.01	2.01	22.98**
g of AAN/kg of dry mass	16.50 ± 1.31^{b}	13.77	17.949 ± 0.934^{b}	9.01	12.799 ± 1.38^{c}	1.86	13.643 ± 0.745^{c}	8.10	8.10	11.46**
g of AA/16 g of N	101.592 ± 1.88^{b}	3.22	104.682 ± 2.185^{b}	2.08	102.019 ± 0.34^{b}	0.59	92.717 ± 1.361^{c}	1.97	1.97	20.80***

[a] Mean values and standard error of measurements (SEM) for 3 replicates ($N = 3$) and 48 determinations. Significance, F values: ****, $P < 0.0001$; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant; CV, coefficient of variation. [b–d] Means along a row with different superscripts are significantly different (Duncan, 1955). [e] Total amino acid nitrogen (N) was determined according to the methods of Heidelbaugh et al. (1975), Horstmann (1979), and Zarkadas et al. (1988a–c, 1993, 1994).

Table 3. Comparison of the Essential Amino Acid (EAA) Composition of Four Northern Adapted Maize Cultivars with the Suggested EAA Pattern of Requirements for Humans

EAA	EAA[a] requirements preschool child (2–5 years)	maize cultivars: Dent (C0251)	Flint (C0255)	Pioneer 3953	quality protein maize (QPM-C13 Opaque-2)	CIMMYT[b] maize: QPM Population-63	La Posta (normal)	soybean[c] AC Proteus CV	animal products[d]: egg	cow's milk
	Milligrams of Amino Acid per Gram of Total Protein									
histidine	19	25	25	20	34	38	31	23	22	27
isoleucine	28	38	38	40	34	29	36	48	54	47
leucine	66	140	141	146	85	84	132	74	86	95
lysine	58	20	18	17	40	39	26	58	70	78
methionine + cyst(e)ine	25	50	50	46	71	29	28	30	57	33
phenylalanine + tyrosine	63	98	100	104	80	73	81	85	93	102
threonine	34	31	29	29	36	42	36	39	47	44
tryptophan	11	6	7	8	12	9	6	11	17	14
valine	35	50	46	41	50	53	52	50	66	64
% of total protein										
EAA_{10}[e] including Arg		49.4	49.2	48.9	51.6			44.9		
EAA_9[a] minus Arg	33.9	45.8	45.5	45.0	44.2	39.6	42.8	41.8	51.2	50.4
EAA_8[a] minus Arg + His	32.0	43.3	42.9	43.0	40.8	35.8	39.7	39.5	49.0	47.7
total EAA,[f] mg/g of nitrogen		2985	3064	3016	2785					
EAA index[g]		72.8	74.7	72.5	82.0					
	Percent Protein Digestibility in Man									
		98.1[h]	98.1[h]	98.1[h]	96.6[h]	96.6[h]	98.1[h]	86[a]	95	97
	Percent Amino Acid Score[a]									
		34	31	29	69	67	55	100	100	100
	Protein Digestibility Corrected Amino Acid Score[a]									
		33.4	30.5	28.5	67	65	53	93	95	97

[a] Data from FAO/WHO/UNV (1985) and FAO/WHO (1990). [b] Data taken from Bjarnason and Vasal (1992). [c] Data taken from Zarkadas et al. (1994). [d] Data taken from Bodwell (1987). [e] Calculated according to the methods of Lee et al. (1978) and Pellet and Young (1984). EAA_{10}: threonine, valine, histidine, isoleucine, leucine, phenylalanine, lysine, tryptophan, histidine, and arginine. PER_{10} values were calculated from eq 5 [$PER = 0.06320(EAA_{10}) - 0.1534$]. [f] Computed from reference protein standards (FAO/WHO, 1965). [g] Calculated according to the methods of Block and Mitchell (1946) and Oser (1961). [h] Data taken from National Research Council (1988).

The results in Tables 1 and 2 showed that the amounts of cysteine and histidine present in QPM-C13 were 70–80% more than the amount found in normal maize varieties, i.e., Pioneer 3953. Wallace et al. (1990) presented data showing that QPM contained 2–4 times more γ-zein than normal maize varieties or *opaque-2* and *floury-2* genotypes. Paiva et al. (1991) and Lopes and Larkins (1991) have indicated that both soft and hard regions of QPM endosperm are enriched in γ-zein and that both α- and β-zeins are significantly reduced in QPM cultivars. Sequence studies have shown that γ-zein contained 7% cysteine and 7% histidine (Shewry and Tatham, 1990), which might explain the results in the present study. Thus, on the basis of the finding that the γ-zein genes isolated from normal maize do not code for any lysine or tryptophan (Prat et al., 1985; Wang and Essen, 1986), the high lysine and tryptophan content found in QPM-C13 cannot be explained by the increase in γ-zein. According to Paiva et al. (1991), the only function of γ-zein might be in disulfide bond formations and interactions that influence kernel hardness in the QPM genotypes. Since zeins contain no lysine or tryptophan (Nelson et al., 1965; Shewry and Tatham, 1990), the increase in lysine and tryptophan as percentage of total protein in QPM maize reflects a higher proportion of other protein fractions, i.e., albumins, globulins, glutelins, etc., which have markedly higher lysine contents.

Table 3 compares the essential amino acid (EAA) compositions of QPM-C13 and two typical hard endosperm Dent CO251 and Flint CO255 inbreds with a commercial hybrid tester, Pioneer 3953. Comparison of the EAA patterns (milligrams per gram of dietary nitrogen) indicates that these maize cultivars contain significant amounts of EAA required for both human and animal nutrition (Block and Mitchell, 1946; Oser, 1951; FAO/WHO, 1965), with lysine, tryptophan, and threonine as the major limiting amino acids.

However, as these predictive tests fail to take into account differences in the digestibility and availability of individual amino acids, the FAO/WHO/UNU Expert Consultation Group (FAO/WHO/UNU, 1985; FAO/WHO, 1990) and the Expert Work Group (FSIS, 1984) recommended that an amino acid score, based on the amino acid composition and corrected for true digestibility of protein or bioavailability of amino acids, should be the preferred method for assessing protein quality of plant and animal proteins. They also recommended that the use of the reference amino acid pattern for the 2–5-year-old child be used in the evaluation of foods for all persons except infants. This amino acid scoring method is based on the nine essential amino acids (EAA_9) required for humans: histidine, isoleucine, leucine, lysine, methionine and cyst(e)ine, phenylalanine and tyrosine, threonine, tryptophan, and valine.

The results presented in Table 3 indicate that the QPM-C13 maize protein proved to be of higher quality than common maize proteins because it contained double the amount of lysine and arginine, higher levels of tryptophan, cysteine, and threonine, and no change in other amino acids except lower levels of leucine and isoleucine, which are known to influence the efficiency of protein utilization. It should be noted that although lysine averaged 39.78 mg/g of QPM-C13 proteins, which is considerably higher than in other cereals, it is still below the recommended FAO/WHO (1990) reference lysine standard value of 58 mg/g of dietary protein for the 2–5-year-old child (Table 3). As a result, the QPM-C13 amino acid profile gives a good balance of total essential amino acids, limited only in lysine, and has an amino acid score, adjusted for digestibility, of 67%, compared to 28.5, 31.0, and 33.0% values found for Pioneer 3953, Dent CO251, and Flint CO255, respectively. The large difference in the adjusted amino acid scores between common maize cultivars and QPM-C13 is attributed to their low lysine and tryptophan values (Table 3), which ranged from 17 to 20 and from 6 to 8 mg of amino acid/g of total protein, respectively. Thus, in common maize the primary essential amino acid deficiencies include lysine, tryptophan, and threonine. However, from early nutritional studies with rats, Benton et al. (1955) have shown that the other limiting amino acid in common maize after lysine and tryptophan is isoleucine. These authors have indicated that although common maize is not deficient in either isoleucine or threonine, the presence of large amounts of leucine in diets of zein or maize has caused amino acid imbalances in rats and interferred with their absorption and utilization of isoleucine (Harper et al., 1955; Benton et al., 1956). It has also been reported that high consumption of leucine along with the protein in maize increases niacin requirements and that this amino acid could be partly responsible for the development of pellagra in humans fed primarily maize (FAO, 1992). In the present studies the ratio of leucine/isoleucine found in QPM-C13 was only 2.5 compared to 3.62, 3.70, and 3.70 found in Pioneer 3953, Dent CO251, and Flint CO255, respectively, suggesting that the QPM-C13 proteins provide an even better essential amino acid balance than is indicated from the calculated amino acid profile.

These results indicate that breeding maize for high protein quality can be very effective and that a very useful method for evaluating the protein quality of cereals is calculating their protein quality from their amino acid composition.

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