Assessment of the Protein Quality of Native White Floury Maize, Designated IAPO-13, by Amino Acid Analysis†

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The present study was designed to quantitatively measure the total protein and amino acid composition of a typical northern adapted (latitude > 45° N) variety of native white floury maize, designated IAPO-13, and to assess its protein quality from its FAO/WHO protein digestibility-corrected amino acid scoring pattern. The native IAPO-13 maize results in the production of maize proteins that closely resemble the amino acid composition of *floury-2* mutant maize. In IAPO-13 maize lysine, tryptophan, histidine, arginine, and aspartic acid are all higher than normal flint CO255 maize, while glutamic acid, leucine, alanine, tyrosine, and phenylalanine are significantly lower. The concentration of methionine is higher than in any other maize tested. As a result, the native IAPO-13 maize amino acid profile gives a good balance of total essential amino acids, limited only in lysine, and has a protein digestibility-corrected amino acid score ranging from 37.0 to 40.0% compared to 27.5, 50.6, and 67.0% values found for flint CO255, *floury-2*, and QPM-C13, respectively. These results indicate that a potentially very useful method for evaluating the protein quality of maize is calculating their protein quality from their amino acid composition.

Keywords: *Maize; native maize; white flour maize; mutant maize; protein quality; composition; amino acids; essential amino acids*

INTRODUCTION

Native white floury maize (*Zea mays* L.) was a staple food of the aboriginal peoples in North America long before European settlers arrived. Hulled maize kernels, used primarily for soup and bread making, were a major source of energy, protein, and other nutrients. Aboriginal people domesticated maize and, by selection over the centuries, were able to develop a wide range of maize types including flint, dent, floury, sweet maize, and popcorn (Knott et al., 1995). In the St. Lawrence River and Great Lakes regions very hardy, early maturing flints and floury maize were developed that would germinate, grow, and mature in the more northern latitudes (>45° N), where there are long daylengths (>16 h) and short growing seasons. These native northern white flints and floury maize varieties are two-eared plants that have 8−10 rows of crescent-shaped white floury kernels per ear (Doebley et al., 1986; Goodman and Brown, 1988). It now appears that some of these early maize selections, although low in yield, contain mutations which express both higher and better quality protein (Cromwell et al., 1968; Asche et al., 1985; Glover and Mertz, 1987).

Although several endosperm gene mutations have been shown to alter the synthesis of seed storage proteins (Mertz et al., 1964; Soave, 1979; Salamini et al., 1983; Bressani, 1992; Mertz, 1986, 1992; Graham et al., 1990; Das et al., 1990; Or et al., 1993; Aukerman and Schmidt, 1993), the two most important mutant genes that have been shown to improve the protein quality of maize are the *opaque-2 (o2)* (Mertz et al., 1964; Mertz, 1986; Ortega and Bates, 1983; National Research Council, 1988; Bjarnason and Vasal, 1992; Schmidt et al., 1987; Geetha et al., 1991; Messmer et al., 1992; Damerval and De Vienne, 1993; Pysh et al., 1993; Habben et al., 1993) and *floury-2 (fl-2)* genes (Nelson et al., 1965). The *opaque-2* gene is a mutation in one of the regulatory loci of chromosome 7 that controls storage protein gene transcription in maize (Kodrzycki et al., 1989; Lending and Larkins, 1989) and is inherited as a simple Mendelian recessive, while *floury-2* gene is semidominant and is located on chromosome 4 (Nelson, 1969; Kodrzycki et al., 1989). Like most cereals, maize seeds contain three major groups of proteins, namely the storage proteins, the enzymes involved in metabolism, and the structural proteins, i.e., ribosomal, chromosomal, and the membrane proteins. The alcohol-soluble prolamin storage proteins called zeins, which can be divided into four structurally distinct types, i.e., α-, β-, γ-, and δ-zeins (Esen, 1987; Wang and Essen, 1986; Larkins et al., 198sen9; Wallace et al., 1990; Shewry anf Tatham, 1990), are present in large quantities, with α-zeins in greatest proportion. Typically, zeins account for approximately 50% of the total endosperm protein, and all are devoid of lysine and tryptophan. Both *opaque-2* and *floury-2* genes in maize reduce the synthesis of prolamins, resulting in a significant increase in lysine and tryptophan (Paiva et al., 1991; Lopes and Larkins, 1991), the two amino acids most deficient in maize endosperm proteins (Bressani, 1991, 1992). Mertz et al. (1964) have shown that *floury-2* and *opaque-2* maize contain 3 and 4 g, respectively, of lysine per 100 g of protein, compared to normal maize which contains 2 g of lysine/100 g of protein. According to Mertz (1992), the *opaque-2* and *floury-2* endosperm also contain higher levels of tryptophan compared to normal. In addition, the *floury-2* mutant gene increases both the total protein content and the methionine concentration (Nelson et al., 1965; Nelson, 1969). *Opaque-2* genotypes, however, have not proven profitable as crops because of their soft, chalky endosperm, lower yields, and increased susceptibility to diseases.

To overcome some of these difficulties, maize breeders

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at the International Maize and Improvement Center (CIMMYT, 1985) in Mexico, through backcrossing and several cycles of recurrent selection, have successfully combined the high lysine potential of the *opaque-2* mutation with endosperm modifiers and have developed new cultivars similar in yield and other agronomic properties to normal maize (Bjarnason and Vasal, 1992; Villegas et al., 1980; Ortega and Bates, 1983; Ortega et al., 1991). These new maize inbreds, collectively called Quality Protein Maize (QPM), yield high levels of lysine and tryptophan without sacrificing crop yields and have a kernel hardness and appearance similar to those of normal maize. A high-quality protein maize inbred (QPM-C13), adapted to more northern regions of Canada, has been reported previously (Zarkadas et al., 1995). However, less is known about maize varieties carrying the *floury-2* gene. An accurate assessment of the protein quality of native white floury maize carrying the *floury-2* mutation is therefore essential.

The aims of the present study were, first, to quantitatively measure the amino acid composition of a typical native northern white floury maize variety from the Indian Agricultural Program of Ontario (IAPO) collection, designated IAPO-13, which originated from the Six Nations reserve of southern Ontario; second, to assess its protein quality from the FAO/WHO/UNU (1985) and FAO/WHO (1991) protein digestibility corrected amino acid scoring pattern; and third, to compare it with the protein and amino acid contents of an established high-lysine mutant maize, *floury-2*, a typical flint CO255 inbred, and the new QPM-13 inbred.

MATERIALS AND METHODS

Materials. Type DC-5A (lot 746) cation-exchange spherical resin, sized to 6.0 ± 0.5 μm, was purchased from Dionex Chemical Co., Sunnyvale, CA. The amino acid standards were obtained as follows: 4-hydroxyproline from Calbiochem-Behring Corp., La Jolla, CA; norleucine from Pierce Chemical Co., Rockford, IL; 3-nitrotyrosine from Aldrich Chemical Co., Milwaukee, WI; and the standard amino acid calibration mixture from Beckman Instruments, Inc., Palo Alto, CA. Octanoic acid was obtained from Eastman Kodak Co., Rochester, NY, and phenol was a product of J. T. Baker Chemical 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. 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 northern adapted native white floury maize selected for this investigation was from the IAPO collection, designated IAPO-13, which originated from the Six Nations reserve of southern Ontario. IAPO-13 is an early maturing inbred with superior combining ability and agronomic traits and is 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.

Representative samples of seed of the native white floury IAPO-13 maize variety were obtained from Dr. R. I. Hamilton of the Eastern Cereal and Oilseed Research Centre and were from experimental trials grown at the Central Experimental Farm for three consecutive years from 1992 to 1994. The whole kernels were freeze-dried and then pulverized in a standard electrically driven end runner mill (Cyclone Sample Mill, U.D. Corp, Fort Collins, CO), passed through a 1.0-mm mesh sieve, 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 mmmHg) 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 hydrolysates were performed on the clear filtrate in duplicate according to methods described previously (Zarkadas et al., 1986, 1988a−c, 1990).

Procedures for Amino Acid Analyses. Amino acid analyses were carried out on a Beckman Spinco System 6300 fully automated high-perfomance amino acid analyzer using single-column expanded protein hydrolysate methodology (Beckman Bulletin A 6300-AN-007, 1987). The automated instrument was equipped with a Beckman Model 406 analog interface module, the system Gold (Beckman Instrument, Inc., Altex Division, San Ramon, CA) chromatographic data reduction system, and an IBM (486-series) compatible personal computer.

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, 1988a−c). The data reported for serine and threonine in Tables 1 and 2 represent the average values of 48 determinations extrapolated to zero time of hydrolysis by linear regression analysis of the results. The values for valine, isoleucine, leucine, and phenylalanine are the average of 36 values obtained from the 72 and 96 h of hydrolysis. All others are reported as the average values of 48 determinations from 24, 72, and 96 h of hydrolysis.

Methionine and cyst(e)ine were determined separately (50.0 mg samples) according to the performic acid procedure of Moore (1963) as described previously (Zarkadas et al., 1988a−c). The data were then normalized relative to alanine, valine, leucine, and isoleucine present in the sample and represent the average of 24 determinations. The yields obtained following performic acid oxidation of these amino acid calibration standards were as follows: cysteic acid, 105.9%; and methionine *S*,*S*-dioxide, 89.0%.

Tryptophan in maize samples (50.0 mg) was also determined separately after alkaline hydrolysis (Hugli and Moore, 1972) on a Beckman Spinco Model 121 MB fully automated amino acid analyzer using single-column methodology as described previously (Zarkadas et al., 1986). 3-Nitrotyrosine was used as the internal standard. The data presented in Tables 1 and 2 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). According to this method, a mean residue weight, WE (in micrograms per nanomole), was calculated for the amino acids constituting the proteins in maize as follows:

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

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).

A 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 protein concentration of each hydrolysate were then calculated as described previously (Zarkadas et al., 1988a,b, 1995).

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

$$P = \mathrm{CF} \sum_{i=1}^{15} X_i \qquad (2)$$

Table 1. Comparison of the Amino Acid (AA) Composition and Protein Contents (Grams of Amino Acids per Kilogram of Total Protein) of a Northern Adapted Cultivar of Native White Floury Maize, Designated IAPO13,[a] with a Typical Flint CO255, QPM-C13, and *Floury-2* Maize

	native white floury maize cultivars and year of trial[a]									maize cultivars		
	IAPO13, 1992		IAPO13, 1993		IAPO13, 1994		weighted	signif levels among cultivars				
AA	mean ± SEM	CV	mean ± SEM	CV	mean ± SEM	CV	mean	CV	*F*	flint[b]	QPM-C13[b]	*floury -2*[g]
aspartic acid	73.74 ± 0.84	1.98	74.96 ± 1.62	3.76	76.57 ± 0.82	1.87	75.09	2.67	1.50[ns]	55.7	78.9	81
threonine	40.47 ± 0.67	2.91	35.72 ± 1.49	7.25	39.77 ± 1.45	6.34	38.66	5.68	4.09[ns]	28.7	35.8	33
serine	51.83 ± 1.17[d]	3.91	44.37 ± 0.66[e]	2.59	7.33 ± 1.36[e]	4.98	47.84	4.00	11.57**	44.3	43.9	48
glutamic acid	184.33 ± 0.03	0.03	187.81 ± 5.47	5.05	188.76 ± 0.19	0.17	186.96	2.93	0.90[ns]	213	171	191
proline	82.75 ± 0.91[d]	1.92	75.49 ± 2.87[e]	6.59	77.23 ± 0.14[d,e]	0.32	78.49	3.87	4.72*	86.7	91.1	83
glycine	30.73 ± 0.09	0.51	30.63 ± 1.75	9.89	28.12 ± 0.09	0.55	29.82	5.88	2.13[ns]	27.1	43.3	37
alanine	66.52 ± 0.07[e]	0.21	64.59 ± 0.64[f]	1.73	69.29 ± 0.24[d]	0.60	66.81	1.04	34.72***	79.0	56.3	80
cysteine	0.53 ± 0.24[d,e]	1.39	33.67 ± 2.45[d]	12.63	26.57 ± 0.15[e]	1.02	30.25	8.17	6.21*	29.9	53.4	18
valine	47.71 ± 0.07[d]	0.28	44.12 ± 0.71[e]	2.81	46.49 ± 0.20[d]	0.75	46.11	1.62	17.72**	45.9	50.1	52
methionine	43.22 ± 0.34[d]	1.39	40.61 ± 0.62[e]	2.66	38.34 ± 0.22[f]	1.02	40.72	1.84	31.75***	21.3	17.2	32
isoleucine	34.51 ± 0.18	0.90	42.88 ± 4.92	19.88	36.55 ± 0.12	0.57	37.98	12.97	2.35[ns]	38.3	33.8	40
leucine	122.37 ± 0.15[d]	0.22	116.33 ± 4.08[d]	6.08	131.10 ± 0.33[e]	0.44	123.26	3.32	9.85**	141	85.2	133
tyrosine	42.27 ± 0.06	0.24	44.26 ± 1.20	4.72	43.72 ± 0.39	1.55	43.42	2.92	1.98[ns]	47.0	38.1	45
phenylalanine	44.69 ± 0.03[f]	0.11	46.37 ± 0.15[e]	0.54	49.22 ± 0.09[d]	3.47	46.76	1.84	498.87***	53.3	41.8	51
histidine	30.40 ± 0.09[d]	0.54	25.52 ± 0.50[e]	3.43	25.79 ± 0.12[e]	0.83	27.24	1.93	80.74***	25.3	34.4	22
lysine	25.90 ± 0.01	0.03	24.11 ± 0.22	1.59	24.21 ± 0.11	0.81	24.74	1.08	48.82***	17.7	39.8	33
arginine	38.65 ± 1.75[e]	7.86	55.63 ± 7.01[d]	21.83	42.19 ± 0.13[d,e]	0.55	45.49	15.89	4.60*	37.5	73.6	45
tryptophan	9.34 ± 0.23	4.41	12.88 ± 1.96	26.29	8.69 ± 0.11	2.23	10.30	19.14	3.91[ns]	7.07	12.1	8
ammonia	16.89 ± 0.28	2.85	16.87 ± 0.34	1.28	10.41± 0.33	5.55	14.72	30.71	2.05[ns]	10.1	17.6	
WE,[c] g/nmol	0.110776 ± 0.0009	0.14	0.112129 ± 0.0003	0.58	0.111103 ± 0.0006	0.09	0.111336	0.35	9.71**			
CF,[c] g/nmol	0.114424 ± 0.0001	0.12	0.113647 ± 0.0002	0.45	0.113915 ± 0.0002	0.36	0.11399	0.34	3.09[ns]			
total protein, g/kg of dry matter	97.67 ± 0.62[e]	1.10	99.19 ± 1.11[e]	1.93	107.24 ± 0.30[d]	0.49	101.36	1.29	46.47***	117	79.5	136

[a] The new cultivar, IAPO13, was developed by the Indian Agricultural Program of Ontario (IAPO), which is a nonprofit Ontario corporation involved in native crop developmental work. 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, ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant; CV, coefficient of variation. [b] Adapted from Zarkadas et al. (1995). [c] Computed according to the method of Horstmann (1979) and Zarkadas et al. (1988a,b). [d–f] Means along a row with different superscripts are significantly different (Duncan, 1955). [g] Amino acids in the defatted *floury-2* maize endosperm were taken from Nelson et al. (1965).

Table 2. Amino Acid (AA) Composition and Nitrogen Contents (Grams of Amino Acids per 16 g of Nitrogen) of a Northern Adapted Cultivar of Native White Floury Maize, Designated IAPO13[a]

	native white floury maize and year of trial[b]								signif levels among cultivars	
	IAPO13, 1992		IAPO13, 1993		IAPO13, 1994		weighted mean			
AA	mean ± SEM	CV	mean ± SEM	CV	mean ± SEM	CV	± SEM	CV	CV	*F*
aspartic acid	7.35 ± 0.09[e]	2.17	7.92 ± 0.09[d]	2.13	7.73 ± 0.08[e]	1.89	7.66 ± 0.09[e]	3.76	2.63	10.48**
threonine	4.03 ± 0.07	3.09	4.12 ± 0.14	6.07	3.69 ± 0.27	0.47	3.95 ± 0.11	8.44	14.19	0.61[ns]
serine	5.17 ± 0.12	4.09	4.89 ± 0.13	4.70	4.58 ± 0.20	7.86	4.88 ± 0.11	7.12	10.29	0.06[ns]
glutamic acid	18.37 ± 0.01	0.16	19.54 ± 0.05	0.42	19.35 ± 0.07	0.66	19.08 ± 0.18	2.87	13.45	0.91[ns]
proline	8.25 ± 0.08[d,e]	1.73	7.99 ± 0.02[d]	0.59	7.77 ± 0.05[e]	1.16	8.00 ± 0.07[d]	2.79	7.15	3.61*
glycine	3.06 ± 0.01	0.70	2.91 ± 0.01	0.85	3.15 ± 0.08	4.47	3.04 ± 0.04	4.20	6.57	2.04[ns]
alanine	6.63 ± 0.02	0.39	7.17 ± 0.04	0.91	6.67 ± 0278	7.13	6.82 ± 0.11	5.19	4.14	3.76[ns]
cysteine	3.04 ± 0.03	1.59	2.75 ± 0.02	1.01	3.49 ± 0.36	18.03	3.09 ± 0.15	14.62	7.99	56[ns]
valine	4.75 ± 0.01	0.09	4.81 ± 0.03	1.05	4.55 ± 0.21	8.23	4.71 ± 0.07	4.70	4.14	2.70[ns]
methionine	4.31 ± 0.04	1.59	3.97 ± 0.02	1.02	4.19 ± 0.08	3.61	4.16 ± 0.05	4.14	7.14	2.58ns
isoleucine	3.44 ± 0.01	0.69	3.78 ± 0.02	0.87	4.39 ± 0.37	14.64	3.87 ± 0.17	13.62	2.26	4.91[ns]
leucine	12.19 ± 0.01	0.02	13.57 ± 0.05	0.74	12.03 ± 0.79	11.42	12.59 ± 0.33	7.97	2.46	5.95[ns]
tyrosine	4.21 ± 0.01	0.04	4.538 ± 0.05	1.85	4.56 ± 0.01	0.71	4.43 ± 0.05	3.88	2.99	2.15[ns]
phenylalanine	4.45 ± 0.01	0.30	5.09 ± 0.02	0.63	4.78 ± 0.16	5.95	4.78 ± 0.10	6.54	3.03	3.61[ns]
histidine	3.03 ± 0.01[e]	0.34	2.67 ± 0.02[e]	1.10	2.63 ± 0.03[d,e]	2.09	2.78 ± 0.06[e]	6.94	2.16	4.89*
lysine	2.58 ± 0.01	0.22	2.51 ± 0.02	1.10	2.48 ± 0.05	3.83	2.52 ± 0.02	2.61	3.33	2.26[ns]
arginine	3.85 ± 0.17[d,e]	7.68	4.37 ± 0.02[e]	0.85	4.78 ± 0.44[d]	15.79	4.34 ± 0.19[e]	13.25	3.12	3.80*
tryptophan	0.93 ± 0.02[e]	4.22	0.89 ± 0.01[d]	2.41	1.34 ± 0.24[e]	31.45	1.06 ± 0.10[e]	28.48	4.72	8.65**
ammonia	1.68 ± 0.03	3.94	1.07 ± 0.03	5.42	1.51 ± 0.37	42.75	1.42 ± 0.14	29.76	48.61	0.76[ns]
total AAN[c]										
g of AAN/kg of protein	160.53 ± 0.17	0.18	154.59 ± 0.27	0.30	155.31 ± 4.87	5.43	156.81 ± 1.69	3.23	3.18	2.81[ns]
g of AAN/kg of dry mass	15.68 ± 0.11	1.29	16.57 ± 0.06[f]	0.66	15.39 ± 0.34[e,f]	3.89	15.88 ± 0.21[d,e]	3.93	3.01	6.78[ns]
g of AA/16 g of N	99.67 ± 0.11	0.19	103.49 ± 0.18	0.31	103.22 ±3.23	5.42	102.13 ± 1.12	3.29	3.19	3.04[ns]

[a] The new cultivar, IAPO13, was developed by the Indian Agricultural Program of Ontario (IAPO), which is a nonprofit Ontario corporation involved in native crop developmental work. [b] 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; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant; CV, coefficient of variation. [c] Computed according to the method of Heidelbaugh et al. (1975), Horstmann (1979), and Zarkadas et al. (1988a,b). [d −f]Means along a row with different superscripts are significantly different (Duncan, 1955).

X_i is the nanomoles of each amino acid *i* found in the analyzed aliquot. The values reported in Table 1 for the content of total protein in each of the three IAPO-13 trial years maize samples investigated are the averages of 48 determinations.

Statistical Analysis. Data processing of the results was carried out by an EXCEL version 5 for windows spreadsheet computer program developed for this purpose. Analysis of variance, conducted on the amino acid data, for a completely randomized block design (factorial) was done by the general linear model procedure using SAS under the windows operating system, release 6.2 (SAS, 1991), and represents the average values from eight subsamples per genotype.

RESULTS AND DISCUSSION

The native white floury maize (IAPO-13) was chosen for this study as an example of a potentially high nutritional quality indigenous maize genotype. Unlike ordinary maize, which has relatively poor quality protein because of its low lysine and tryptophan contents, the native northern flints and floury maize varieties are considered to be very good sources of protein. Accurate determination of total protein and amino acids of the IAPO-13 maize variety, from three consecutive years (1992−1994) of experimental trials, was carried out at the picomole range by use of the analytical methodology described previously (Zarkadas et al., 1986, 1987, 1988a−c, 1990, 1995). The results, expressed as grams of amino acids per kilogram of protein, are presented in Table 1. Although variations in the amino acid composition of the IAPO-13 maize variety grown during different years were noted, the least variability occurred when the amino acid data were expressed on a protein basis. The mean protein concentration of each sample was determined according to the method of Horstmann (1979) by the summation of the weights of the amino acid residues and represents the mean of 48 determinations. The weighted mean nitrogen contents of IAPO-13 maize, calculated according to the method of Heidelbaugh et al. (1975) by the summation of the amino acid nitrogen contents for each trial year, are summarized in Table 2. These methods have the advantage that the percentage recovery of amino acids by weight or on a nitrogen basis can be found by simple summation.

Significant variations ($P < 0.001$) in the values for protein content among the trial years 1992−1994 were noted. The overall protein content of IAPO-13 maize averaged 10.14% across the trial years, ranging from 9.77% in 1992 to 10.72% in 1994. These data compared favorably with those of Zarkadas et al. (1995) for flint C0255 inbred maize but were considerably lower than those evaluated by Mertz et al. (1964) and Nelson et al. (1965) for *floury-2* maize using the Kjeldahl procedure (i.e., grams of nitrogen × 6.25 = grams of protein) (Table 1). The accuracy of the Kjeldahl method varies depending upon the amount of nonprotein nitrogen present in the sample (Benedict, 1987; Zarkadas et al., 1988a−c); therefore, the commonly used protein conversion factor of 6.25 for assessing the total protein content in cereals is limited. Nelson (1969) stressed that in order to yield more accurate quantitation, especially when breeding maize for high protein content, the Kjeldahl results must be corrected for nonprotein nitrogen present using the conversion factor, 5.70. This factor is based on the amino acid nitrogen content (Heidelbaugh et al., 1975) and has been widely used to convert maize Kjeldahl nitrogen into protein content. However, the best estimate of the protein content in IAPO-13 maize was made by the summation of the weights of the amino acid residues of each sample, as described by Horstmann

Table 3. Essential Amino Acid (EAA) Scores of a New Northern Adapted Cultivar of Native White Floury Maize, Designated IAPO13,[a] a High-Quality Animal Protein, Hen's Whole Egg, and the EAA Requirements of a Preschool 2−5-Year-Old Child

		EAA scores							
		native white floury maize and year of trial[b]				maize cultivars			
EAA	EAA requirements for a preschool child (2−5 years old)	IAPO13, 1992	IAPO13, 1993	IAOP13, 1994	weighted mean	flint[c]	QPM-C13[c]	*floury-2*	egg[a]
		Milligrams of Amino Acid per Gram of Total Protein[d]							
histidine	19	30	26	25	27	25	34	22	22
isoleucine	28	34	36	43	38	38	34	40	54
leucine	66	122	131	116	123	141	85	133	86
lysine	58	26	24	24	25	18	40	33	70
methionine + cyst(e)ine	25	73	75	65	71	50	71	50	57
phenylalanine + tyrosine	63	87	91	93	90	100	80	96	93
threonine	34	40	36	40	39	29	36	33	47
tryptophan	11	9	13	9	10	7	12	8	17
valine	35	48	44	46	46	46	50	52	66
% total protein									
EAA_9[d]	33.9	47	46.6	47	47	45.5	44.2	46.7	51.2
EAA index[e] (%)		79	86	81	82	74.7	82.0		
total EAA,[f] mg/ g of N		2988	3041	3149	3059	3064	2785		3215
		Percent True Protein Digestibility in Man[g]							
		89	89	89	89	89	92	89	97
		Protein Digestibility Corrected Amino Acid Score[g]							
		40	37	37	38	27.5	63	50.6	97

[a] Data from FAO/WHO/UNU (1985) and FAO/WHO (1991). [b] The new native white floury maize cultivar IAPO13 was developed by the Indian Agricultural Program of Ontario (IAPO), which is a nonprofit Ontario corporation involved in native crop developmental work. [c] Data taken from Zarkadas et al. (1995). [d] Calculation of protein ratings was carried out by comparison of the amino acid composition of the native white floury maize (IAPO13) cultivar with that of the reference pattern established by FAO/WHO/UNU (1985) and FAO/WHO (1991) for a preschool child (2−5 years old). [e] Calculated according to the method of Block and Mitchell (1946) and Oser (1951). [f] Computed from reference protein standards (FAO/WHO, 1965). [g] True protein digestibility values were taken from the U.S. Food and Drug Administration (U.S. FDA, 1993) *Federal Register*, Appendix B, pp 2193−2195. [h] Values for the amino acids in the defatted *floury-2* maize endosperm were taken from Nelson et al. (1965).

(1978). The results are summarized in Tables 1 and 2 and show that this method yields accurate estimates of the absolute amount of protein present among the IAPO-13 samples evaluated.

Several studies have shown that the synthesis of zeins is affected by mutations, such as *opaque-2* and *floury-2*, that cause a reduction in the accumulation of zein proteins and result in a significantly increased lysine content (Nelson, 1969; Jones, 1978; Pedersen et al., 1980; Hagen and Rubenstein, 1981; Soave and Salamini, 1984; Ortega et al., 1986, 1991; Kodrzycki et al., 1989; Lending and Larkins, 1989; Wallace et al., 1990; Paiva et al., 1991; Geetha et al., 1991). These mutations affect primarily the synthesis of α-zeins, causing a 50% reduction of both the 19 and 22 kDa α-zein subunits and a 2−3-fold increase of γ-zein (Wallace et al., 1990; Geetha et al., 1991). The *floury-2* mutation causes a reduction of both the 19 and 22 kDa α-zeins in proportional amounts (Jones, 1978). Alexander et al. (1969) and Nelson (1969) have shown that in the breeding of double *opaque-2* and *floury-2* mutant ($o_2/o_2/fl_2/fl_2$) maize, the progeny was considered to be phenotypically near normal but had an increased lysine content.

In the present study there were no differences in aspartic acid, glutamic acid, threonine, glycine, isoleucine, tyrosine, and tryptophan contents of the native white floury maize IAPO-13 variety among the experimental trials from 1992 to 1994 (Tables 1 and 2). Small but significant differences were observed for lysine, serine, proline, alanine, cysteine, valine, leucine, phenylalanine, histidine, and arginine among the three trial years, indicating the high accuracy and precision of the analytical procedures used.

From the amino acid composition of the IAPO-13 maize it is apparent that there are differences in the concentrations of several amino acids as compared to normal flint CO255 maize (Table 1). For example, in IAPO-13 maize the amino acids lysine, tryptophan, histidine, arginine, and aspartic acid are all increased compared to normal flint CO255 maize, while there are substantial decreases in the concentration of glutamic acid, alanine, and leucine and smaller decreases in the concentrations of the aromatic amino acids tyrosine and phenylalanine. In addition to these changes there is a substantially increased concentration of methionine in IAPO-13 maize compared to normal flint CO255 maize. The levels of methionine found in this study are much higher than those reported by Nelson et al. (1965) for *floury-2*. These authors, however, did not indicate whether corrections for recoveries of methionine were made after performic acid oxidation, in which case their values would be approximately 15% higher than the values they reported, bringing them closer to those found in this study. From these data it becomes apparent that the native IAPO white floury maize has increased levels of lysine, tryptophan, and especially methionine and has an amino acid composition resembling that of *floury-2* maize. Further studies are required to establish which of the zeins have been reduced in native white floury IAPO maize selections.

Table 3 compares the essential amino acid (EAA) patterns (milligrams per gram of dietary nitrogen) of IAPO-13 maize with that of the amino acid pattern for a 2−5-year-old child as the reference pattern. Data for normal (flint CO255), *floury-2*, and QPM-C13 maize and egg are compared. The present data indicate that IAPO-13 maize contains significant amounts of all EAA required for both human and animal nutrition (Block and Mitchell, 1946; Oser, 1951) and is limited only in lysine and, to a lesser extent, by tryptophan and isoleucine. Mean values for total EAA calculated according to FAO/WHO (1965) show that native white floury maize has a lower essential amino acid content (from 2988 to 3149 mg of EAA/g of N) than egg proteins

(3215 mg of EAA/g of N). Similarly, the EAA indices calculated according to the method of Oser (1951) show that IAPO-13 maize provides 82% of the EAA compared to hen's egg protein.

However, as these predictive tests fail to take into account differences in the digestibility and availability of individual amino acids, the Expert Consultation Group of FAO/WHO (1991) adopted an even more accurate method for assessing the protein quality of foods, which is based on the concept of the nine essential amino acids (EAA_9) required for humans, which include histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (FAO/WHO/UNU, 1985). Since cysteine and tyrosine can replace methionine and phenylalanine, respectively, the two sulfur-containing amino acids (methionine, cysteine) and the two aromatic amino acids (phenylalanine, tyrosine) are usually considered together. This protein digestibility-corrected amino acid scoring procedure (U.S. FDA, 1993) uses the reference amino acid pattern for the 2−5-year-old child as the reference pattern (Table 3) in the evaluation of foods, since protein per kilogram requirements of this age group are the greatest, except for infants.

The results presented in Table 3 indicate that IAPO-13 maize proteins are of higher quality than common maize proteins because they contain higher levels of lysine and tryptophan, and also the methionine concentration is considerably higher than in any other maize tested. This mutant may be valuable nutritionally in diets where maize is supplemented with leguminous proteins, which are low in this amino acid. It should be noted, however, that although lysine averages 25 mg/g of IAPO-13 proteins, which is much higher than in other cereals, it is still below the recommended FAO/WHO (1991) reference lysine standard value of 58 mg/g dietary protein for the 2−5-year-old child (Table 3). The IAPO-13 proteins would provide adequate amounts of all of the essential amino acids, limited only in lysine and, to a lesser extent, by tryptophan and have a protein digestibility-corrected amino acid score ranging from 37.0 to 40.0% compared to 27.5, 50.6, and 67.0% values found for flint CO255, *floury-2*, and QPM-C13, respectively.

Early nutritional studies with rats by Benton et al. (1955) have shown that the other limiting amino acid in common maize is isoleucine. These authors have indicated that although common maize is not deficient in isoleucine, the presence of large amounts of leucine in diets of maize has caused both amino acid imbalances in rats and interference of isoleucine absorption. This can increase the niacin requirements in humans fed primarily maize (Harper et al., 1955; Benton et al., 1955; FAO, 1992). The ratio of leucine/isoleucine found in IAPO-13 maize was 3.24 compared to 3.22, 3.70, and 2.50 in *floury-2*, flint CO255, and QPM-13, respectively, suggesting that the IAPO-13 proteins provide an even better EAA balance than is indicated from the calculated amino acid profile.

The data presented in this paper show that the amino acid composition and protein content of native white floury IAPO-13 maize, which is considered as a good energy and protein source for both human and animal nutrition, closely resemble the amino acid composition of *floury-2* mutant maize. Although lysine is the first limiting amino acid in IAPO-13 maize, followed by tryptophan, the overall balance of its essential amino acids is considered superior to other normal maize.

The results of this study indicate that breeding native white floury mutant maize for better protein quality and higher protein content can be very effective and that a potentially useful method for evaluating the protein quality of maize and other cereals would be based on an accurate quantitation of their amino acid composition and protein digestibility-corrected amino acid score.

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