Journal of Agricultural and Food Chemistry

Volume 31, Number 1 January/February 1983

Inherited Characteristics of Composition and Protein Nutritive Value of a New Cultivar of Maize (Nutrimaiz) in Two Stages of Maturity

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A comparative study of four cultivars of maize, i.e., Maya Opaque-2 (Suo_2), Piramex Sweet (suO_2), Nutrimaiz (suo_2), and Maya Normal (SuO_2), was made at 20 days after pollination and at full maturity (dry). Nonprotein nitrogenous substances were higher in the Opaque-2 than in the Sweet and Normal cultivar and still higher in the Nutrimaiz. Lipid content was higher in Opaque-2 and Nutrimaiz, particularly at full maturity. The whole grain flours of Opaque-2 and Nutrimaiz were higher in lysine and tryptophan with a lower leucine/isoleucine ratio in both stages of maturity. Fractionation of the proteins indicated that the Nutrimaiz had the highest albumin, globulin, and glutelin 3 and lowest zein contents. The overall amino acid composition of the isolated protein fractions was generally better for the albumins, globulins, and glutelin 3 than for zein, in all four cultivars. The new cultivar (Nutrimaiz) had a better composition and a higher nutritive value for the rat when compared with those of the other three cultivars.

Since the discovery of Mertz et al. (1964) and Nelson et al. (1965) of the genes opaque-2 and floury-2 which modify the maize endosperm proteins by decreasing the zein and increasing the salt-insoluble and glutelin fractions, many workers have characterized the changes produced by these genes on the Normal corn endosperm proteins (Mertz, 1968; Sodek and Wilson, 1971; Robutti et al., 1974; Jones et al., 1977).

It has also shown that the presence of the gene sugary modifies extensively the sugar content of the endosperm by decreasing the starch and increasing the sugars and water-soluble polysaccharides (Andrew et al., 1944; Black et al., 1966; Creech, 1968).

Misra et al. (1975a) studied several combinations of the gene opaque-2 with genes capable of modifying the starch content of the endosperm, i.e., sugary (su), shrunken (sh), brittle (bt), shrunken-2 (sh_2), and shrunken-4 (sh_4). They reported on an improvement of rotein quality and nutritive value of these mutants.

Da Silva et al. (1978) reported on a double mutant (suo_2) produced at the Agronomic Institute of Campinas, São Paulo, Brazil, by crossing the varieties Maya Opaque-2 (Suo_2) with a Pajimaca (Cuban) Sweet (suO_2) which was named Nutrimaiz by these workers. The overall composition and nutritional properties of the mature kernels of Nutrimaiz were first described by Sgarbieri et al. (1977).

In this paper, the results of a comparative study of composition, nutritive value, amino acid patterns of whole kernel flours, and isolated protein fractions, for the cultivars Nutrimaiz, Maya Opaque-2, Piramex Sweet, Maya Normal in two stages of maturity, i.e., 20 days after pollination and at full maturity, are described.

MATERIALS AND METHODS

Maize Cultivars. The cultivars Maya Normal (SuO_2), Maya Opaque-2 (Suo_2), Piramex Sweet (suO_2), and Nutrimaiz (suo_2) were furnished by Dr. W. J. Da Silva, of the Genetic and Evolution Department, University of campinas (UNICAMP). The Maya Normal was a dent-type maize and the Piramex Sweet was a standard sweet maize. Samples were collected at 20 days after pollination (20 DAP) and at full maturity (60 DAP).

The unripe (milky) samples were frozen and then freeze-dried. Separation of the anatomical parts of the fresh corn was easily done by direct dissection of the freeze-dried fresh kernels. The mature kernels were soaked in ice-cold water and the dissection was done under cold water to minimize enzyme action. The freeze-dried fresh kernels and the mature kernels were ground to pass a 70-mesh screen and stored under refrigeration in sealed

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0021-8561/83/1431-0001$01.50/0

Table I. Proximate Composition of Four Cultivars of Maize in Two Stages of Maturity (Dry Basis)

	Maya Normal		Piramex Sweet		Maya Opaque-2		Nutrimaiz	
determinations, %	fresh	mature	fresh	mature	fresh	mature	fresh	mature
protein, % N x 6.25	13.0	11.9	13.6	14.2	11.2	13.2	14.0	13.3
NPN, % N x 6.25	2.6	0.8	2.3	0.9	3.5	1.5	4.6	1.5
total lipid	3.8	5.0	5.1	8.0	3.7	6.4	4.0	9.3
ash	2.2	0.6	2.1	0.6	2.0	1.8	2.3	1.7
fiber	3.6	1.6	3.4	2.3	2.8	1.6	3.4	3.2
carbohydrate (difference)	74.8	80.1	73.5	74.0	76.8	75.5	71.7	71.0

containers prior to use for the chemical analysis and biological assays.

Chemical Determinations. Moisture, total lipid, and ash contents were determined according to Association of Official Agricultural Chemists (1970) procedures 14004, 7048, and 14006, respectively.

Nonprotein nitrogen was determined by the micro-Kjeldahl procedure in a water extract from defatted flour, after precipitation of the proteins with Cl_3AcOH (5% w/v), following the method of Becker et al. (1940).

Total crude protein was determined by the micro-Kjeldahl procedure according to the method described in American Association of Cereal Chemists (1976), 46-12. Protein was calculated by multiplying the percent nitrogen by the factor 6.25.

Fractionation of the proteins from the whole kernel flours was performed by the extraction method of Landry and Moureaux (1970) after defatting the samples with acetone and ether at −10 °C, as suggested by Baudet et al. (1966). The salt-soluble proteins (albumins plus globulins) were fractionated by dialysis against distilled water, centrifugation, and lyophilization. The zein and glutelins were all submitted to dialysis against distilled water to eliminate the extracting agents and then freeze-dried.

Amino acid determination was done following hydrolysis in 6 N HCl at 105 °C for 22 h, on a Beckman amino acid analyzer 120C, following the procedure described by Beckman Instruments, Inc. (1973). Tryptophan was determined in the enzymatic hydrolysate (Pronase) by the colorimetric method of Spies (1967).

Biological Evaluation. Preparation of diets and determination of protein efficiency ratio (PER) were done according to AOAC (1970) using male rate of the Wistar strain weighing an average of 50 ± 2 g.

Nitrogen balance was performed on rats of the same strain and initial body weight, following the procedure of Mitchell (1923).

RESULTS AND DISCUSSION

The proximate composition of the four cultivars in the two stages of maturity is shown in Table I. The main differences are the higher concentrations of nonprotein nitrogeneous compounds in Maya Opaque-2 (3.5%) and in Nutrimaiz (4.6%), at 20 days after pollination, and 1.5% for both cultivars at full maturity. The lipid contents in the mature kernels of Piramex Sweet (8.0%) and Nutrimaiz (9.3%) is significantly higher than that of the Normal and Opaque-2 cultivars. It is concluded that the new cultivar (Nutrimaiz) inherited from Opaque-2 the high nonprotein nitrogen content and from the Piramex Sweet the high lipid content.

The high content of free amino acids as a characteristic of Opaque-2 cultivars and of Opaque-2 double mutants was described by Misra et al. (1975c) and by Arruda et al. (1978). Misra et al. (1975c) determined that the introduction of the gene opaque-2 in a cultivar of sweet maize increased of 54% the free amino acid content in the nature double mutant (suo_2). Our results (Table I) show an increase of 60% of NPN in the mature Nutrimaiz (suo_2) and 50% in the fresh kernels (20 DAP) as compared with that of the Piramex Sweet (suO_2). Comparison of NPN of Maya Normal with Nutrimaiz indicates a 56% increase in Nutrimaiz 20 DAP and 53% in the mature kernels. The lipid content (Table I) in the whole kernels increased with maturity in all four cultivars as a result of an increase in the proportion of germ. The comparative weights of 1000 whole kernels (dry) and 1000 dry germs at 20 and 60 DAP and the x-fold increase of germ during maturation are shown in table II, for the four cultivars. The germ increase with maturation was, roughly, 2.5-fold for Maya Normal and Piramex Sweet and 4.0-fold for Maya Opaque-2 and Nutrimaiz. During maturation the proportion of pericarp and endosperm (except for Normal endosperm) and the protein content (dry basis) decreased in these anatomical parts (Table III). As a whole, the mature kernels of Opaque-2 and Nutrimaiz contain more germ and more germ proteins on a dry basis.

Table II. Relative Weight of Whole Kernels and Germs of Four Cultivars of Maize in Two Stages of Maturation

	1000 whole kernels, g		1000 germs, g		germ increase, x-fold
cultivars	20 DAP	60 DAP	20 DAP	60 DAP	
Maya Normal	104.2	185.4	7.0	18.0	2.6
Piramex Sweet	102.5	140.5	8.3	18.0	2.3
Maya Opaque-2	104.3	163.3	5.3	20.7	3.9
Nutrimaiz	101.7	160.8	7.3	30.2	4.1

Table III. Percentage of Pericarp, Germ, and Endosperm from Kernels of Four Cultivars of Maize and Percentage of Protein in Each Part in Two Stages of Maturity (Dry Basis)

		% of kernel		% protein	
cultivars	anatomical parts	fresh	mature	fresh	mature
Maya Normal	pericarp	10.8	6.6	7.1	3.7
	germ	6.7	9.7	13.0	19.5
	endosperm	82.5	83.7	12.8	10.5
Piramex Sweet	pericarp	11.8	11.2	6.0	3.6
	germ	8.1	13.5	12.5	17.7
	endosperm	80.1	75.3	11.8	12.8
Maya Opaque-2	pericarp	11.6	6.1	8.0	3.4
	germ	5.1	12.7	18.3	28.1
	endosperm	83.3	81.2	13.2	9.2
Nutrimaiz	pericarp	13.6	10.3	7.6	4.1
	germ	7.2	18.8	15.9	22.9
	endosperm	79.2	70.9	12.2	9.8

The amino acid composition of the whole grain flours of all four cultivars, in the two stages of maturity, is shown in Table IV. The amino acid patterns of these cultivars at full maturity have already been reported by Sgarbieri et al. (1977), and determinations were repeated in the present investigation for comparative purposes. The lysine contents decreased with maturation for the cultivars Maya Normal and Priamex Sweet, whereas it remained essentially constant at a higher level in the Maya Opaque-2 and in Nutrimaiz. The contents of tryptophan were also higher in the Maya Opaque-2 and in Nutrimaiz, and the leu-

Table IV. Amino Acid Composition of Flours from Four Cultivars of Maize in Two Stages of Maturity (g/100 g Recovered)

amino acid	Maya Normal		Piramex Sweet		Maya Opaque-2		Nutrimaiz	
	fresh	mature	fresh	mature	fresh	mature	fresh	mature
lysine	3.3	1.7	3.8	2.6	4.4	4.4	4.5	4.8
histidine	2.4	2.5	2.0	2.5	2.2	3.3	3.1	3.2
arginine	3.1	3.0	3.8	3.6	4.8	6.7	4.2	7.4
aspartic acid	7.8	6.1	8.9	6.4	10.2	9.0	10.9	9.5
threonine	3.3	2.6	3.5	3.6	3.4	3.3	3.4	4.8
serine	5.5	5.5	5.3	4.5	4.8	4.7	5.0	5.4
glutamic acid	21.6	24.5	23.5	24.5	24.2	21.4	21.7	16.0
proline	7.4	9.6	8.5	9.0	6.1	9.1	6.4	8.6
glycine	3.3	3.0	3.5	4.1	4.5	5.7	4.1	6.0
alanine	8.6	7.9	9.6	8.1	8.7	6.8	10.2	7.0
half-cystine	0.9	1.2	0.9	1.1	0.7	1.4	1.3	1.5
valine	4.7	4.4	5.0	4.5	4.7	3.8	4.7	5.4
methionine	1.2	1.5	1.3	1.4	1.6	1.5	1.6	1.4
isoleucine	3.0	3.1	3.5	3.4	3.2	3.0	3.2	2.7
leucine	13.0	14.9	10.2	13.6	9.1	8.4	8.6	8.8
tyrosine	2.7	3.3	2.7	2.4	2.9	2.8	2.6	3.1
phenylalanine	3.3	4.4	3.4	4.3	3.7	3.4	3.8	3.6
tryptophan[a]	0.5	0.4	0.6	0.4	0.8	1.1	0.9	0.8
total recovered, g/16 g of N	122.4	114.1	112.9	110.2	109.6	105.3	110.3	110.2

[a] Determined by the method of Spies (1967).

cine/isoleucine ratios were much lower in these two cultivars than in the Maya Normal and Piramex Sweet at both stages of maturity.

Comparisons of the results of Table IV with those of other investigators are very difficult since most of the published results are on endosperm and not on whole maize proteins and often determined in different stages of maturity. Partial comparison can be made with Bressani and Mertz (1958), Baudet et al. (1966), and Mertz (1968) for the mature Normal and Opaque-2 after conversion to the same unit (g/100 g recovered). In general a good agreement was found except for glutamic (24.5% for Maya Normal) which ranged from 13.7% to 18.2% (Bressani and Mertz, 1958), 20, 1% (Baudet et al., 1966), and 19.7% (Mertz, 1968). For Maya Opaque-2 21.4% compares with 16.8% (Mertz, 1968).

Figure 1 is a graphical representation of the relative proportions of the various protein fractions of the four cultivars of maize in two stages of maturity, i.e., fresh (20 DAP) and mature (60 DAP). Total recovery of nitrogen on extraction ranged from 90 to 94% in the fresh and 92 to 95% in mature kernels. In Maya Normal and Piramex Sweet, zein was the predominant fraction in the mature stage whereas in Maya Opaque-2 and Nutrimaiz the predominant protein fractions were the salt-soluble proteins (albumins plus globulins) and glutein 3 in both stages of maturity. In Maya Normal and Piramex Sweet an increase in glutelin 3 was also observed with maturation although the main changes were a marked decrease in albumins plus globulins and a corresponding increase in zein. It is important that albumins and globulins are, by far, the most abundant proteins in the fresh kernels of all four cultivars, i.e., 56–70% of the total protein.

A great reduction of albumins plus globulins with a large increase in zein during maturation of the Normal corn endosperm was described by Bressani and Conde (1961), Murphy and Dalby (1971), and Landry and Moureaux (1976). An increase in the ratio of salt-soluble protein (albumins plus globulins) to zein had been observed by Murphy and Dalby (1971) and Misra et al. (1975b), on introduction of the gene opaque-2 in the Normal corn endosperm. Landry and Moureaux (1976) found that the glutelin 3 also had increased considerably in an opaque-2 mutant corn. Some investigators (Misra et al., 1975a,b; Arruda et al., 1978), among others, made the important observations that the combination of the gene opaque-2

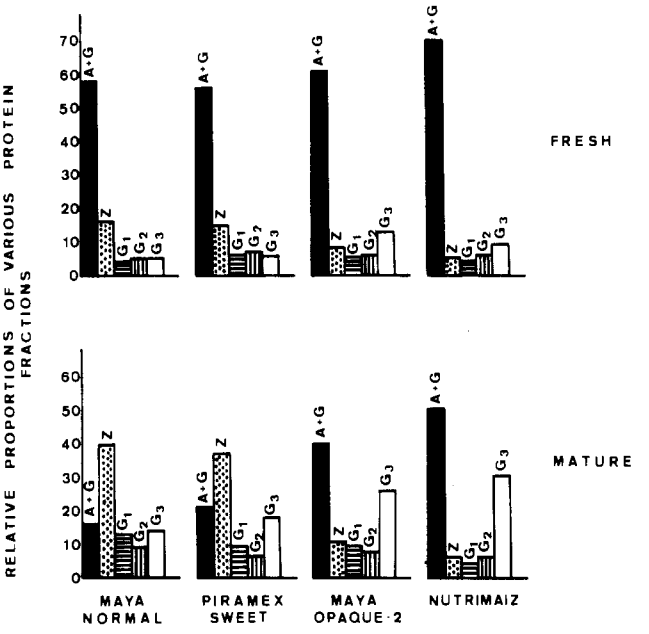

Figure 1

(o_2) with the sugary (su) and with other carbohydrate-modifying genes (Sh, Sh_2, bt, bt_2, and fl_2) increased even more the ratio of salt-soluble proteins and glutelin 3 (combined) to zein, with an even stronger suppression of zein biosynthesis.

Mossé et al. (1966) utilized whole grain and the classic method of Osborne to fractionate the Normal and Opaque-2 corn proteins. They found for the salt-soluble proteins 21% and 39%, respectively, for the Normal and Opaque-2 which can be compared with 16% and 40% (Figure 1), zein 44% and 18% with 40 and 11% (Figure 1), and glutelins 35% and 43% with 36% and 43% (sum of glutelins 1, 2, and 3 of Figure 1).

Landry and Moureaux (1970) found the following in mature Normal whole grains: albumins plus globulins, 19% (Figure 1, 16%); zein, 38% (Figure 1, 40%); glutelin 1, 11.5% (Figure 1, 13%); glutelin 2, 10% (Figure 1, 9%); glutelin 3, 10% (Figure 1, 14%). When comparable, our results seem to be in good agreement with published data.

The amino acid patterns of six isolated protein fractions from all four cultivars studied, in the fresh and full mature stages, are shown in the Tables V and VI. The overall

Table V. Amino acid composition of the isolated protein fractions of four maize cultivars harvested 20 days after pollination (g/100g recovered).

Amino	Albumin				Globulin				Zein				Glutelin 1				Glutelin 2				Glutelin 3			
Acid	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Lys	7.0	7.2	5.2	5.2	6.6	6.0	7.5	6.6	0.1	0.7	0.2	0.2	0.1	0.8	0.5	0.1	2.0	1.8	1.7	2.5	4.6	4.0	4.4	4.5
His	2.3	2.0	1.5	1.6	2.0	2.3	2.2	2.3	0.9	1.0	1.0	0.5	1.2	1.0	2.8	2.3	8.6	7.4	6.9	10.2	2.6	3.2	2.6	2.7
Arg	5.6	8.2	5.7	5.7	5.0	5.1	6.4	5.8	1.1	1.3	1.0	1.2	1.6	1.5	2.5	1.9	3.5	4.0	4.6	5.4	4.0	3.9	5.2	4.7
Asp	11.4	10.4	12.4	11.4	10.7	10.5	11.0	10.7	5.3	4.9	4.6	4.9	4.6	5.8	5.1	5.2	1.6	1.6	1.3	2.3	9.2	9.1	10.1	8.6
Thr	5.4	4.8	4.9	4.9	6.8	3.9	4.1	4.2	2.5	2.4	2.5	2.2	3.2	2.8	2.4	2.6	4.5	5.0	5.8	7.3	3.7	3.3	5.0	4.3
Ser	6.3	4.4	5.2	5.2	4.7	3.9	4.9	4.7	5.3	4.9	5.3	5.1	6.2	5.7	5.1	6.1	2.9	2.9	5.7	6.2	5.1	5.6	5.4	5.4
Glu	10.6	16.8	17.1	15.6	15.7	16.7	16.5	16.7	27.7	28.1	28.3	30.4	26.0	24.2	27.6	22.0	26.3	27.1	26.9	32.9	2.3	20.4	18.5	20.0
Pro	5.6	5.5	5.1	5.7	3.9	4.7	4.1	4.3	9.1	9.0	9.5	8.0	12.3	10.4	10.3	12.0	18.4	17.6	16.7	18.2	7.9	8.4	6.8	7.5
Gly	6.0	5.9	5.7	5.7	4.9	5.0	5.1	5.2	1.3	1.6	1.6	1.2	3.2	1.9	2.5	3.0	4.6	3.8	3.9	4.5	4.5	4.3	5.3	5.3
Ala	7.7	7.8	7.6	8.0	7.0	6.8	6.7	6.9	9.1	9.1	9.2	10.7	13.0	10.6	10.8	11.2	4.0	3.6	2.4	4.0	6.9	6.4	7.8	7.2
1/2 Cys	1.5	1.7	1.9	1.9	1.0	1.0	1.1	0.9	t	t	t	t	t	t	t	1.4	0.8	1.0	1.3	1.0	t	t	t	t
Val	7.4	3.1	5.5	6.8	6.5	8.0	5.8	5.7	3.1	2.5	3.4	3.0	4.1	3.6	2.8	3.3	6.3	7.1	6.9	6.8	6.1	6.7	6.6	5.6
Met	2.1	1.5	1.9	2.4	2.1	2.1	2.2	2.2	0.7	0.7	1.3	0.5	2.6	2.5	1.8	2.4	1.4	1.3	1.5	0.9	1.6	1.8	1.0	1.7
Ile	4.5	4.4	3.6	4.3	4.1	4.7	3.9	4.1	3.5	3.4	3.1	3.4	3.1	3.8	2.6	3.0	1.9	1.7	1.4	2.6	3.5	3.5	3.5	3.4
Leu	9.2	9.0	9.0	2.8	10.1	10.7	9.9	10.7	20.0	19.6	18.0	17.6	17.5	14.9	15.1	14.9	9.6	9.1	8.9	10.8	10.9	11.5	9.9	10.5
Tyr	2.6	2.4	3.0	2.8	3.4	3.1	3.5	3.5	4.5	4.4	4.8	4.2	3.4	5.0	3.8	4.2	1.9	1.8	1.7	2.1	2.8	3.0	1.9	3.5
Phe	3.9	4.4	3.7	4.3	4.3	4.7	4.2	4.6	5.5	6.0	5.7	6.5	3.0	5.3	4.3	4.0	1.7	2.5	2.0	2.3	4.3	4.2	4.6	3.7
Trp	0.7	0.6	0.9	0.8	1.2	0.7	0.7	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.3	0.3	0.8	0.8	1.0	1.2
TOTAL REC (g/16gN)	116.8	113.3	113.0	105.7	112.6	114.4	122.6	109.3	126.2	127.2	127.7	116.9	113.2	113.7	116.8	114.2	100.1	98.7	101.2	88.1	98.0	98.7	102.7	98.5

1 = Maya Normal (SuO_2) 2 = Piramex Sweet (suO_2) 3 = Maya Opaque-2 (Suo_2) 4 = Nutrimaiz (suo_2) t = trace amount

Table VI. Amino acid composition of isolated protein fractions of four maize cultivars harvested 60 days after pollination (g/100g recovered)

Amino	Albumin				Globulin				Zein				Glutelin 1				Glutelin 2				Glutelin 3			
Acid	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Lys	5.5	4.3	6.0	6.7	5.2	6.0	5.2	5.6	0.1	0.1	0.8	0.5	0.1	0.1	0.1	0.2	1.7	1.2	1.2	1.1	4.2	5.7	4.2	5.8
His	2.8	3.0	2.6	2.3	3.4	3.3	3.3	3.5	0.9	0.9	0.8	1.0	2.0	2.0	1.1	1.7	7.3	8.2	6.9	6.7	3.5	4.0	4.2	3.8
Arg	6.6	7.9	8.1	7.5	9.9	10.3	12.9	11.4	1.3	1.3	2.4	2.1	2.2	1.6	1.7	2.2	4.1	4.1	4.1	4.5	5.9	7.9	7.3	8.6
Asp	9.4	9.7	10.5	9.8	6.6	7.0	7.2	7.7	5.3	5.6	5.1	4.9	4.1	3.5	3.9	3.9	3.1	2.5	2.9	3.0	7.7	4.3	8.2	8.7

Thr	4.7	5.4	5.8	5.3	3.4	3.9	3.9	3.4	2.4	2.6	2.2	3.0	3.1	2.8	2.6	2.6	4.1	4.2	3.8	4.4	4.0	4.2	4.0	4.3		
Ser	5.6	5.5	6.2	5.5	4.9	6.0	5.7	5.8	5.0	5.9	5.6	5.3	5.7	5.0	4.9	4.4	4.1	4.0	5.4	4.3	4.9	4.9	4.7	5.1		
Glu	15.0	15.7	16.8	16.1	17.2	17.6	18.5	18.0	26.8	24.4	20.8	25.6	23.7	23.8	27.0	25.8	25.4	22.5	25.1	24.6	19.1	16.2	17.8	15.3		
Pro	6.9	6.8	7.1	6.7	6.8	4.5	4.4	4.5	8.6	10.0	10.9	6.6	11.8	16.7	17.0	11.6	15.9	16.5	18.4	19.6	7.7	7.3	6.3	5.9		
Gly	9.0	8.9	9.1	8.3	5.4	5.7	5.3	5.5	0.8	1.2	2.3	2.9	2.8	3.1	2.3	4.7	5.2	5.0	5.0	5.3	4.9	5.2	5.7	5.4		
Ala	10.3	9.2	9.1	8.3	6.6	6.1	5.8	5.6	13.0	12.0	2.7	10.3	10.9	9.3	8.9	8.9	4.1	4.4	4.2	4.5	7.0	6.6	6.5	6.7		
1/2 Cys	2.8	2.8	2.5	2.8	2.0	1.3	1.4	1.6	t	t	t	0.5	t	t	t	0.4	1.3	0.9	t	t	t	t	t	t		
Val	4.8	5.3	5.4	4.8	5.9	5.7	5.0	6.0	3.2	3.5	2.5	3.1	3.6	2.9	2.9	3.7	5.8	5.8	6.1	5.7	5.9	5.7	5.8	5.9		
Met	1.4	1.3	1.5	1.2	1.0	1.7	1.3	1.3	0.9	1.0	0.6	1.0	4.1	2.9	1.7	2.2	1.2	1.2	0.7	0.8	1.9	1.8	1.6	1.9		
Ile	3.3	2.8	2.9	2.8	4.0	3.4	3.6	3.9	3.2	3.6	4.0	4.0	2.7	3.1	2.5	2.3	2.4	2.6	2.0	2.2	3.5	3.5	3.3	3.4		
Leu	6.0	5.0	5.6	5.5	8.0	7.4	6.8	6.5	19.7	15.8	19.6	19.5	12.7	13.9	13.0	14.2	8.4	9.5	9.0	9.4	10.5	9.8	9.9	9.7		
Tyr	2.1	2.5	2.9	2.7	3.5	3.6	3.4	3.9	4.3	4.9	3.5	3.7	4.7	4.6	4.4	4.3	2.6	4.1	2.5	3.0	3.7	3.4	4.2	3.6		
Phe	2.2	2.7	3.1	2.5	4.9	5.3	4.8	4.8	6.5	7.0	5.8	5.7	5.6	4.7	5.8	6.7	2.7	3.0	2.0	2.2	4.4	4.1	5.2	4.7		
Trp	0.7	1.0	0.9	0.8	0.9	1.0	1.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.2	0.4	0.4	1.2	1.2	0.9	1.1		
TOTAL (g/16gN)	106.7	108.1	98.4	105.1	101.2	99.7	102.4	103.6	126.5	129.3	97.8	99.4	115.9	97.6	116.8	119.9	96.2	111.1	94.3	87.8	103.1	96.5	95.7	105.0		

1 = Maya Normal (SuO_2) 2 = Piramex Sweet (suO_2) 3 = Maya Opaque-2 (Suo_2) 4 = Nutrimaiz (suo_2) t = trace amount

characteristics of the amino acid composition of these protein fractions are the extremely low contents of lysine and tryptophan in the zein and gluetlin 1 fractions, which also show very high leucine/isoleucine ratios; albumins, globulins, and glutelin 3 had much higher lysine and tryptophan contents and a lower leucine/isoleucine ratio. These parameters had intermediate values in gluetlin 2.

Certain amino acids predominated in particular protein fractions: i.e., glutamic acid, proline, alanine, and leucine in zein and glutelin 1, glutamic acid histidine, and proline in glutelin 2, glutamic acid, aspartic acid, and leucine in glutelin 3, and lysine, aspartic acid, glutamic acid, alanine, and leucine in the albumins and globulins.

The high content of glutamic acid, particularly in zein and glutelins, must be derived, in part, from glutamine during acid hydrolysis. The very low concentration of half-cystine and methionine found in several fractions may reflect destruction of these amino acids during extraction and hydrolysis. Paulis and Wall (1971) and Misra et al. (1976) reported much higher half-cystine and methionine values for the zein and glutelin fractions from Normal corn endosperms when the proteins were reduced with 2-mercaptoethanol and alkylated prior to hydrolysis. According to Paulis and Wall (1971) the reported values for methionine and half-cystine converted to g/100 g recovered were respectively zein, 1.3 and 1.4, glutelin 1, 6.6 and 4.2, glutelin 2, 0.8 and 4.4, glutelin 3, 3.2 and 2.2% (w/w) of the protein. Except for these two amino acids our results are in general agreement with those of Paulis and Wall (1971) and Misra et al. (1976).

In general, the amino acid patterns were characteristic of each protein fraction and more or less independent of cultivar and stage of maturity. Modifications in total amino acid composition are then the result of genetic changes causing alternations in the proportion of the various protein fractions. Similar observations were reported by Sodek and Wilson (1971) based on the amino acid composition of the albumins and globulins from Normal and Opaque-2 cultivars.

Direct comparison of data from Tables V and VI with published results is different because of differences in the nature and stage of maturity of the samples studied. Most published results refer to endosperm analysis while we worked with whole grain. Mertz (1968) studied the amino acid composition of Normal and Opaque-2 endosperm and whole grains. In the Normal cultivar, lysine, arginine, aspartic acid, and glycine were higher and methionine was lower for the whole kernels as compared with the endosperm. In Opaque-2 the major differences were found in the lysine and arginine contents which were higher for the whole kernels as compared with those for endosperms. The amino acids which are higher in the whole kernels are the same as those which predominate in the salt-soluble proteins found, primarily, in the germs. The albumin and globulin fractions present, as a whole, more satisfactory amino acid patterns as compared with the other maize proteins, with a higher content of basic and sulfur-containing amino acids and tryptophan and lower contents of proline, leucine, and glutamic acid (Paulis and Wall, 1969; Sodek and Wilson, 1971; Mossé et al., 1966; Misra et al., 1976).

The amino acid patterns found for the albumins and globulins in this investigation (Tables V and VI) are in good agreement with the results of Paulis and Wall (1969), Sodek and Wilson (1971), Robutti et al. (1974), and Misra et al. (1976).

The amino acid compositions of albumin and globulin fractions were quite similar and did not change appreciably

Table VII. Protein Biological Evaluation in the Integral Flour of Four Cultivars of Maize in Two Stages of Maturity

source of protein (flours)	PER[a]		N retention,[b] g		apparent digestibility,[b] %		apparent biological value,[b] %	
	fresh	mature	fresh	mature	fresh	mature	fresh	mature
Maya Normal	1.9 ± 0.29	1.2 ± 0.21	1.6	1.1	78.6	73.0	69.6	50.8
Piramex Sweet	2.0 ± 0.22	1.4 ± 0.20	1.7	1.2	79.0	75.6	70.0	55.0
Maya Opaque-2	2.6 ± 0.20	2.5 ± 0.24	1.9	1.8	80.0	79.0	74.0	70.7
Nutrimaiz	2.75 ± 0.22	2.6 ± 0.20	2.0	1.9	80.6	79.5	74.4	73.8
casein	2.9 ± 0.25		2.1		88.4		81.0	

[a] PER: determined in experiments of 28 days, using weanling rats (five per group) of the Wistar strain on diets with 9% protein. [b] Other parameters calculated from data of the 72-h nitrogen balance experiment using three rats per diet.

with kernel maturation. There was a small increase in glycine and a decrease in valine, leucine, and isoleucine with maturation in the albumin fraction. In the globulin fraction lysine, aspartic acid, threonine, alanine, valine, methionine, and leucine decreased slightly and argine increased with maturation.

Zein and glutelin 1 were also similar in amino acid composition. Both zein and glutelin 1 were very low in lysine and in tryptophan and high in glutamic acid, proline, alanine, and leucine in all four cultivars and in the two stages of maturity. Zein was also low in histidine, arginine, and glycine as compared with the other protein fractions. When comparisons are made between zein and glutelin 1, zein has lower contents of histidine, arginine, and proline and a higher content of leucine.

The results reported in this paper for zein and for glutelin 1 are in general good agreement with those of Baudet et al. (1966), Landry and Moureaux (1970), Paulis and Wall (1971), Sodek and Wilson (1971), Robutti et al. (1974), and Dimler (1966), except that we did not find higher contents of lysine and tryptophan in glutelin 1 than in zein as reported by some of the above-cited authors.

The amino acid patterns of glutelin 3 were more similar to those of the albumins and globulins than to those of glutelins 1 and 2. Glutelin 3 had higher contents of lysine, arginine, aspartic acid, and tryptophan and lower contents of glutamic acid and proline than the glutelins 1 and 2. Glutelin 2 had the highest histidine and the lowest aspartic acid contents of all six protein fractions. The contents of lysine and tryptophan of glutelin 2 were intermediate between those of the glutelins 1 and 3. There was an increase of arginine in glutelin 3 with maturation.

The amino acid contents of thee glutelins, except for cystine (Tables V and VI) were similar and followed the same general trend of those reported by various authors (Paulis and Wall, 1971; Robutti et al., 1974; Misra et al., 1976).

The relative deficiencies of the essential amino acids in the various protein fractions were evaluated based on the FAO/WHO provisional amino acid scoring pattern (FAO/WHO, 1973). On the basis of this reference pattern the albumin, the globulin, and the glutelin 3 fractions are, by far, the most complete proteins. Disregarding the sulfur-containing amino acids (methionine plus cystine) which suffer degradation during extraction and hydrolysis of the proteins, all the other essential amino acids, in the albumin, globulin, and glutelin 3 fractions, represented from 71% to above 100% of the FAO/WHO reference pattern, which is considered adequate for humans of all ages.

From the nutritional point of view the most deficient proteins from maize kernels are contained in the zein and glutelin 1 fractions. The most limiting essential amino acids in these proteins are tryptophan and lysine. Tryptophan is practically nonexistent and lysine ranged from about 2 to 15% of the reference pattern in four cultivars and two stages of maturity of the kernels.

In glutelin 2 tryptophan and lysine contents represented from 20 to 40% of the reference pattern in all four cultivars and in both stages of maturity. Isoleucine was alsos low (generally below 50% of the reference) in glutelin 2, in both stages of maturity.

The results of the biological assays performed on the rat with the whole grain flours of all four cultivars, in the two stages of maturity, showed a marked superiority of Maya Opaque-2 and Nutrimaiz over the Maya Normal and Piramex Sweet (Table VII). The results of Table VII, relative to the mature stage, have already been reported (Sgarbieri et al., 1977) and were repeated here for comparison purposes. The PER values in the fresh stage, of 1.9 and 2.0 for Maya Normal and Piramex Sweet, dropped to 1.2 and 1.4, respectively, at full maturity, whereas these values were 2.5–2.6 and 2.6–2.8 for Maya Opaque-2 and Nutrimaiz, respectively, at both stages of maturity. Apparent digestibility and protein biological values also decreased considerably in Maya Normal and Piramex Sweet, during maturation. The digestibility dropped from 78.6% to 73.0% and the biological value from 69.6% to 50.8% for the Maya Normal. For the Piramex Sweet these values changed from 79.0% to 75.6% and from 70.0% to 55.0%. For Maya Opaque-2 and Nutrimaiz the apparent digestibility (~80%) was the same at both stages of maturity. The protein biological value dropped from 74% to 70.7% in Maya Opaque-2 with maturation and remained constant (~74%) in Nutrimaiz.

The following values were found for casein: PER, 2.9; apparent digestibility, 88.4%; apparent biological value, 81%.

Although the PERs and biological values for Maya Opaque-2 and Nutrimaiz were similar, the rat growth rate was considerably higher in the diets containing Nutrimaiz.

Registry No. Lys, 56-87-1; Trp, 73-22-3; Leu, 61-90-5; Ile, 73-32-5.

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Received for review June 25, 1980. Revised manuscript received October 1, 1981. Accepted October 20, 1982.

Monosaccharide Composition of Alcohol- and Detergent-Insoluble Residues in Maturing Reed Canarygrass Leaves

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Total cell wall sugars and hemicellulosic sugars of reed canarygrass leaves (*Phalaris arundinaceae* L.) increased with increasing plant maturity. The predominant hemicellulosic polymers appeared to be xylans. Neutral detergent residues contained less noncellulosic sugars than the 80% ethanol insoluble residues. Neutral detergent may have solubilized acidic hemicellulosic polysaccharides in addition to pectic polysaccharides. The acid detergent residues contained considerable amounts of arabinose, xylose, and uronic acids. The high xylose/arabinose ratio present in the acid detergent fiber residues may reflect the presence of linear xylans associated with cellulose in a manner sufficient to render the xylans resistant to dilute acid hydrolysis. As a result, the detergent methods underestimated total hemicellulose in the leaves of maturing reed canarygrass. Hydrolysis of alcohol-insoluble residues with 72% sulfuric acid followed by dilution to 2 N is proposed as a means for estimating noncellulosic polysaccharides.

Most research concerned with the evaluation of forage plants for nutritional purposes has relied on gravimetric methods for the determination of plant cell wall components. The gravimetric detergent methods have been well accepted because of their usefulness for prediction of various parameters, such as dry matter intake, with reasonable accuracy (Van Soest et al., 1978). The detergent methods have been recommended for use in establishing hay standards (Rohweder et al., 1977) and have been used recently for the selection of high-yielding reed canarygrass clones that have low amounts of cell wall constituents (Marum and Hovin, 1979).

The neutral and acid detergent procedures and the crude fiber procedures are similar approaches since, in each case, the plant components are defined in terms of laboratory operations rather than constituent chemicals. Examination of the carbohydrate types present in residues of neutral detergent fiber (NDF) and acid detergent fiber (ADF) has shown that neither method completely partitions cellulose and noncellulosic polysaccharides (Bailey and Ulyatt, 1970; Collings and Yokoyama, 1979; Theander and Aman, 1980; Morrison, 1980). Morrison (1980) reported the presence of hemicellulosic sugars in the ADF residues prepared from a wide variety of plant species. Theander and Aman (1980) reported the presence of hemicellulosic sugars and crude protein in the ADF residues of several forage species including alkali-treated straw. The NDF residues prepared from the same forages also contained substantial amounts of crude protein in addition to hemicellulose, uronic acids, and Klason lignin. In view of the presence of extraneous substances in the detergent-insoluble residues, the general validity of these methods for the accurate assessment of cell wall constituents is subject to question.

Theander and Aman (1980) defined hemicellulose in terms of monosaccharides other than glucose that were quantitated after acid hydrolysis of plant cell wall preparations. Xylans substituted with arabinose, galactose, and uronic acid are known to be characteristic of the hemicellulosic polysaccharides in grasses (Wilkie, 1979) and have been proposed as the principal hemicellulosic polymers in the cell walls of monocotyledonous plants (Anderson and Stone, 1978). The quantitation of grass hemicellulose and other cell wall polymers in terms of sugar constituents would thus appear to be a more accurate approach to the evaluation of plant composition and the nutritional potential of these forage plants than is provided by the existing empirical methods.

This manuscript reports the results obtained from a detailed study of the acid detergent, neutral detergent, and aqueous ethanol insoluble cell wall polysaccharides in maturing reed canarygrass leaves. This study was performed for the purpose of evaluating methodology designed

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0021-8561/83/1431-0007$01.50/0