Nutritional Evaluation of Hard Endosperm *Opaque-2* Maize (*Zea mays L.*)

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Chemical and biological evaluation of nutritional quality of hard endosperm *opaque-2* maize was undertaken. Amino acid and nitrogen distribution in the endosperm and biological value of kernel protein of various grades of hard endosperm *opaque-2* maize were studied. Albumins, globulins, and glutelins increased while the zein fraction decreased with decrease in kernel vitreosity. Amino acid composition of endosperm proteins indicated a decrease in lysine and tryptophan and an increase in leucine with the increase in kernel vitreosity. Rat feeding experiments showed nutritional superiority of modified *opaque-2* over normal maize. Biological value of modified *opaque-2* was slightly lower compared to *opaque-2*. Utilizable protein values for modified *opaque-2* and *opaque-2* were similar. The fresh weight and protein content of liver were higher in rats fed with modified *opaque-2* and *opaque-2* diet compared with normal maize diet.

The discovery by Mertz et al. (1964) that the maize mutant *opaque-2* (*o2*) has nearly twice as much lysine and tryptophan as normal maize opened up new vistas in improving cereal protein quality. However, the original stocks containing this *o2* mutation are low yielding, and the soft kernels are subject to fungal attack during grain development and to insect attack during storage. Therefore, to overcome these problems efforts are being made to develop hard endosperm *o2* strains (modified *o2*) by genetic manipulation of modifier genes. The nutritional superiority of *o2* (chalky) maize over normal maize is well established. In our earlier nitrogen balance study (Lodha et al., 1976) with albino rats, it was reported that the biological value (b.v.) of protein of Shakti *o2* composite was 90% of milk protein casein while that of normal maize varieties was less than 70% of the casein.

However, information is scanty about the amino acid spectrum, distribution of nitrogen, and biological value of various kernel categories of modified *o2* maize. Therefore, in the present study, the nutritive value of various categories of modified *o2* maize have been compared with that of normal and *o2* maize.

MATERIALS AND METHODS

Sample Preparation. The maize varieties, SO/SN composite (modified *o2* with hard endosperm), Shakti *o2* composite (chalky), and Vijay (normal) were tested. The crop was grown during the rainy season under irrigation at 120, 60, and 40 kg/ha levels respectively of N, P, and K. All varieties were grown under identical agronomy. SO/SN composite was developed at our own station from the hard endosperm *o2* inbred lines derived from three released *o2* composites, namely Shakti, Rattan, and Protina. For separating various categories of hard endosperm *o2* kernels, viz., 100% opaque, 75% opaque–25% vitreous, 50% opaque–50% vitreous, 25% opaque–75% vitreous, and nearly vitreous (normal), the kernels were screened against light. The classification was based on the percent transparency of the kernels. For endosperm studies, kernels were soaked in distilled water at 4 °C for 3 h, then the pericarp was removed, and the endosperm and embryo were separated with a scalpel, and the endosperm was collected. Dried endosperms ground to 100 mesh flour were defatted.

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Protein Fractionation. Protein fractionation was done according to the method of Nagy et al. (1941). At each stage completeness of extraction was checked by measuring the absorbance of the last extract at 280 nm in a spectrophotometer. Nitrogen was determined by micro-Kjeldahl method (AOAC, 1965).

Amino Acid Analysis. An endosperm sample containing 5 mg of protein was hydrolyzed in 2 mL of 6 N HCl at 110 °C for 22 h in evacuated hydrolysis tubes. The hydrolyzed material was freed of acid using a rotary vacuum evaporator and dissolved in 0.1 M sodium citrate buffer (pH 2.0). It was then filtered and amino acid analysis done by an automatic Beckman amino acid analyzer, Model 120 C. Tryptophan was estimated by colorimetric method of Hernandez and Bates (1969).

Nitrogen-Balance Study. Nitrogen balance of each sample was determined in four male Wistar growing rats weighing about 74 g according to the method described by Eggum (1973) in a room maintained at 24 ± 1 °C. Each rat daily received 10 g of dry matter containing 150 mg of N. At the end of the experiment, the rats were sacrificed. Two hours before killing, their food was restricted. Rats were anesthetized by giving chloroform and were quickly cut open, and their livers were removed, weighed, and stored in liquid nitrogen. Nitrogen of the rat liver was estimated by the micro-Kjeldahl method.

Digestible Energy. The digestible energy experiment was carried out on a group of four male Wistar growing rats under similar conditions of nitrogen-balance study. The total experimental period consisted of 4 days preliminary feeding, followed by a 5-day balance period in which feces were pooled. Each rat received 10 g of dry matter daily. Energy content of the diets and feces was determined by using a Gallenkamp Bomb Calorimeter. The percentage digestible energy (D.e.) was calculated as follows:

$$\% \text{ D.e.} = \{[(\text{total energy of diet consumed}) - (\text{energy of feces excreted})]/(\text{total energy of diet consumed})\} \times 100$$

RESULTS

Amino Acid Composition. The complete amino acid spectrum of endosperm protein from kernels with different vitreosity is presented in Table I. Endosperms of 25, 50, 75, and 100% opaque segregating kernels contained respectively 9, 15, 16, and 22% more protein; 37, 86, 114, and 123% more lysine; and 26, 62, 84, and 100% more tryptophan as compared to 100% vitreous maize endosperm.

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Table I. Amino Acid Composition of Endosperm of Various Categories of Modified *Opaque-2* (g/16 g of nitrogen)

amino acid	0% vitreous-100% opaque	25% vitreous-75% opaque	50% vitreous-50% opaque	75% vitreous-25% opaque	0% opaque or 100% vitreous
aspartic acid	9.58	9.13	8.25	6.65	6.06
threonine	3.34	3.73	3.69	3.40	3.34
serine	3.70	4.26	4.26	4.34	4.49
glutamic acid	21.21	20.71	21.90	22.99	24.68
proline	9.90	11.04	11.05	11.39	11.58
glycine	4.34	4.08	3.91	3.23	2.74
alanine	7.21	6.72	7.27	7.94	8.72
valine	5.90	5.71	5.86	5.40	5.12
isoleucine	4.19	3.91	4.11	4.14	4.16
leucine	11.47	11.58	13.00	14.99	17.34
phenylalanine	4.65	4.76	5.17	5.53	5.66
tyrosine	4.03	4.17	4.33	5.00	4.83
lysine	3.74	3.59	3.13	2.30	1.68
histidine	3.64	3.99	3.89	3.63	3.20
ammonia	2.11	2.11	2.12	2.36	2.56
arginine	5.24	5.07	4.85	4.15	3.67
methionine	1.88	1.88	1.96	2.10	2.05
cystine	3.68	3.46	2.73	3.00	2.04
tryptophan	1.00	0.92	0.81	0.63	0.50
% protein	9.44	10.13	10.25	10.97	12.06
chemical score	68.0	65.3	56.9	41.8	30.5

Table II. Nitrogen Distribution in Endosperm of Various Categories of Modified *Opaque-2*

fraction[a]	0% vitreous-100% opaque	25% vitreous-75% opaque	50% vitreous-50% opaque	75% vitreous-25% opaque	0% opaque or 100% vitreous
albumin	12.50	10.72	9.98	7.25	3.35
globulin	6.40	5.69	4.85	4.17	1.68
prolamine (zein)	11.11	13.91	18.28	23.14	42.56
glutelin	58.22	58.67	54.83	48.59	37.53
residue	11.78	11.04	12.06	16.84	14.87
total protein recov.	96.5	94.2	85.9	78.5	86.6

[a] Percent of the total recovered protein.

Table III. Mean True Digestibility, Biological Value, and Other Properties of Proteins of Normal, *Opaque-2*, and Various Categories of Modified *Opaque-2*[a]

			categories of modified opaque-2			*opaque-2*
	casein	normal (Vijay)	25% opaque	50% opaque	75% opaque	(Shakti)
true digestibility	101.58 ± 1.21	92.71 ± 0.27	97.10 ± 0.60	95.49 ± 0.40	94.45 ± 0.85	94.35 ± 0.85
biological value	86.47 ± 0.89	60.60 ± 1.30	72.19 ± 2.11	73.12 ± 1.95	73.27 ± 0.99	78.14 ± 0.80
net protein utilization	87.87 ± 1.74	56.18 ± 1.17	70.14 ± 2.38	69.81 ± 1.82	69.21 ± 1.11	73.74 ± 1.14
utilizable protein	76.66 ± 2.25	5.41 ± 0.14	8.28 ± 0.35	8.20 ± 0.27	8.00 ± 0.16	7.97 ± 0.16
digestible energy, %	94.30 ± 0.45	90.79 ± 0.48		87.60 ± 0.65		88.74 ± 0.76
protein (N × 6.25) in % of dry matter	87.25	9.63	11.81	11.75	11.56	10.81

[a] LSD for TD at 1%, 1.51; at 5%, 1.14. LSD for BV at 1%, 3.39; at 5%, 2.25.

The endosperm protein of 75, 50, and 25% opaque and nearly normal maize segregating kernels contained respectively 0.96, 13.3, 30.7, and 51.2% more leucine compared to 100% opaque endosperm. Histidine, arginine, aspartic acid, valine, glycine, and cystine decreased with increase in kernel vitreosity while there was not much change in the amino acids such as threonine, isoleucine, and methionine. However, the content of the rest of the amino acids increased with the increase in kernel vitreosity. The chemical score, based on lysine as the first limiting amino acid, decreased with the increase in kernel vitreosity. The chemical score value for 75% opaque was twice that of segregating normal and comparable to 100% opaque maize.

Protein Fractions. The results presented in Table II reveal that, as kernel vitreosity decreases or kernel opacity increases, the endosperm fractions (on percent basis) such as albumins, globulins, and glutelins increase, although the extent of increase varies from fraction to fraction. Total extraction of the proteins in the endosperm of more vitreous kernels declined considerably compared to the more opaque ones. Zein fraction decreased with the increase in kernel opacity, but not as a direct relationship. An initial increase in opacity by 25% reduced zein content to half that of 100% vitreous maize. Further increase in opacity (or decrease in vitreosity) decreased zein content but to a much lesser extent. The *o2* maize contained only 26% of the zein of the vitreous normal endosperm. Similar trends were also observed when the protein fractions were expressed on per endosperm basis (Figure 1). Zein content per endosperm showed marked decrease (4.3 mg) in 25% opaque–75% vitreous, whereas further increase in opacity to 100% opaque resulted in an additional decrease of only 2.14 mg of zein. On the other hand, the absolute increase for albumins, globumins, and glutelins was respectively 1.15, 0.59, and 1.22 mg/endosperm in 100% opaque compared to the levels in 100% vitreous maize.

Nitrogen-Balance Study in Rats. The results of nitrogen balance study carried out with albino rats utilizing three kernel categories of SO/SN composite, viz., 25, 50, and 75% opaque, Vijay normal, and Shakti *o2*, are presented in Table III. The true digestibility (t.d.) of all the maize varieties/types under test was more than 92%. The true digestibility of all modified *o2* kernels was higher

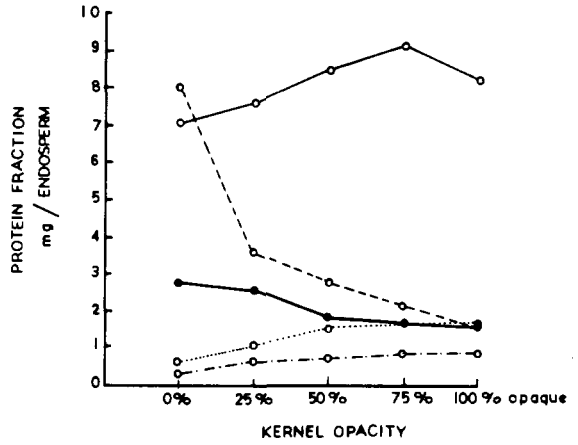

Figure 1. Protein fractions (milligram/endosperm) in various categories of modified *opaque-2*: (··O··) albumin; (·–O–·) globulin; (- -O- -) zein; (—O—) glutelin; (-●-) residue.

Table IV. Average Fresh Liver Weight and Protein Content of Liver in Rats Fed on Normal, Modified *Opaque-2*, and *Opaque-2* Maize Diets

diet source	fresh liver weight, g	protein in liver	
		%	mg/liver
normal (Vijay)	5.07	15.16	768.6
modified *opaque-2* (50% opaque)	5.67	16.65	944.1
opaque-2 (Shakti)	6.40	17.28	1105.9

compared to either normal or *opaque-2* and appears to be related to the protein content of the kernels. Higher t.d. values were associated with higher protein content in kernels. Biological value (b.v.) was about 20% higher for different categories of modified *o2* compared to normal maize. The net protein utilization (n.p.u.) was also substantially higher (23%) for different categories of modified *o2* compared to normal. The utilizable protein (u.p.) was found to be highest for 25% opaque. Utilizable protein was respectively 47 and 52% higher for Shakti (*o2*) and modified *o2* as compared to normal maize. Although the b.v. of Shakti *o2* was slightly better (6.6–8.2%) than that of various categories of modified *o2*, the u.p. values of *o2* and three categories of modified *o2* were not significantly different from one another. This is mainly due to the increase in the amount of endosperm protein with the increase in kernel vitreosity in the modified *o2* maize. Since the u.p. takes into account both the protein quality and quantity, the higher u.p. values of modified *o2* compared to normal maize indicate nutritional superiority of modified *o2* over normal maize. The digestible energy of modified *o2* (50% opaque) diet (87.6%) was comparable to that of *o2* (88.74%), whereas it was slightly higher for normal diet (90.79%).

Effect of Maize Protein Quality on Liver Weight and Protein Content. Albino rats were fed *o2*, 50% opaque (modified *o2*), and normal maize, and at the end of the experimental period, liver weight and protein in liver were determined. The data are presented in Table IV. The liver weight was maximum (6.4 g) in rats fed on *o2* maize diet and least (5.07 g) in rats fed with normal maize diet. The rats fed modified *o2* had intermediate fresh liver weight (5.67 g). Thus modified *o2* maize supported better liver growth compared to normal maize. Similar trend was also observed for protein percent in liver. The protein content per liver, however, showed major differences. It was 43% higher for *o2* diet and 22.8% higher for modified *o2* diet compared to normal diet.

DISCUSSION

Amino acid analysis indicated a decrease in endosperm protein quality with the increase in kernel vitreosity. Protein fractionation studies indicate that a relationship exists between zein protein synthesis and kernel vitreousness. However, this was not a direct relationship since the decrease in zein was considerable with initial decrease in kernel vitreosity. The results in the present study also suggest that the quality improvement of the grain character of *o2* is possible with retention of better protein quality since 75% vitreous (25% opaque) kernels have substantially higher nutritional quality (Table III) and half the zein of 100% vitreous kernels (Table II and Figure 1).

The nutritional quality of different categories of hard endosperm *o2* maize was found to be almost similar. The utilizable protein (u.p.) of different categories of modified *o2*, viz., 25, 50, and 75% opaque, was similar to that of Shakti *o2*, although b.v. was slightly inferior. This change was mainly due to higher protein content in modified *o2* maize compared to Shakti *o2*. In a 28-day rat feeding experiment the protein efficiency ratio (p.e.r.) values of hard endosperm *o2* and soft endosperm *o2* were also found comparable (2.83 and 2.90) by Mertz et al. (1975). A strong positive correlation between fresh liver weight and b.v. (γ = 0.956) and between protein content per liver and b.v. (γ = 0.976) also suggests that with the improvement in protein quality, liver weight as well as protein content of liver increases. Similar correlation was also observed between diet protein quality and rat growth by Chinchalkar and Mehta (1978).

Although the chemical analysis showed greater difference in endosperm protein quality of various categories of modified *o2*, rat feeding experiments showed more or less similar nutritional quality of the grain protein based on u.p. values. The u.p. values, indicated substantially superior nutritional quality of modified *o2* compared to normal maize. In addition to the decrease in zein content, possibly larger germ may also have contributed to nutritional improvement of modified *o2* compared to normal maize. Modified *o2* has better 100 kernel weight and acceptability compared to chalky *o2*. Similarity in nutritional value of modified *o2* and chalky *o2* based on u.p., therefore, shows that improvement in grain character of *opaque-2* is possible without affecting nutritional quality of the grain.

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