PHYSICAL PROPERTIES AND BIOLOGICAL EVALUATION OF HIGH-LYSINE MAIZE

P. VAN TWISK, National Food Research Institute, P.O. Box 395, Pretoria, South Africa, 0001; G. V. QUICKE, Department of Biochemistry, University of Natal, P.O. Box 375, Pietermaritzburg, South Africa, 3200; and H. O. GEVERS, Department of Agricultural Technical Services, Natal Region, Private Bag X9059, Pietermaritzburg, South Africa, 3200

ABSTRACT

Cereal Chemistry 53(5): 692-698

A locally bred, open-pollinated, white, *opaque*-2 maize variety was found to differ from normal hybrids in dry-milling properties, starch particle size, relative density, water absorption index, and water solubility index. Starch yield on wet milling, however, was comparable to normal white hybrids and superior to local yellow hybrids. The *opaque*-2 maize was nutritionally superior to normal maize.

The principal uses of maize in South Africa, as far as processing for human nutrition is concerned, are in the wet- and dry-milling industries. The introduction of new maize cultivars which differ in certain respects from the maize grain normally used for industrial processing may require a reassessment of the existing milling processes, or may even be regarded with disfavor, a diversion in physical properties probably proving more objectionable than differences in chemical composition. A study in Colombia (1), for example, resulted in the conclusion that the obstacles to a rapid expansion of commercial production and consumption of *opaque*-2 maize are primarily associated with its softness and appearance. For this reason, a detailed physical and chemical evaluation was done on MPF 9, a relatively soft-kernelled, locally bred, open-pollinated, white, *opaque*-2 variety of the Natal Potchefstroom Pearl type which, in its present or slightly modified form, has earlier been evaluated nutritionally using both rats and chicks (2,3).

MATERIALS AND METHODS

Dry Milling

Using normal, white dent maize as standard, the test material was dry milled by means of a Miag Multomat experimental mill according to a procedure to produce 'special sifted granulated' maize meal, one of the more popular grades of maize meal milled in South Africa. Sifting tests of the ground products were carried out on a Miag laboratory plansifter.

Wet Milling

The laboratory method as described by Pelshenke and Lindemann (4) was applied and the properties of MPF 9 were compared with those of different South African white and yellow maize cultivars.

Soluble nitrogen content was determined by shaking a known amount of the material in a certain volume of water with the aid of a mechanical shaker, followed by filtering and determining the nitrogen content of the filtrate.

Determination of the main components of the kernel was carried out on hand-dissected kernels after steeping in SO_2 solution, and drying and weighing the fractions.

Particle size of starch fractions was measured by means of the Coulter Counter technique.

Enzymatic hydrolysis of the starch in maize was done according to the procedure described by Van Twisk and Tegge (5) and Van Twisk (6).

Number of kernels per liter was measured by determining the amount of kernels needed to fill a 1-liter measuring cylinder, while for 100-kernel weight 100 randomly selected unbroken kernels were counted out in duplicate.

The value for 'per cent floaters,' which gives an indication of kernel density, was calculated according to the method as described by Wichser (7).

Water absorption index (WAI) was measured by stirring 10 g material with water in a centrifuge tube, centrifuging, decanting the supernatant, and determining the amount of water absorbed; water solubility index (WSI) was found by determining the solids content of the supernatant.

Viscosity determinations were done in triplicate in a Brabender Viscograph® on 10% (dry basis) suspensions of ground material.

Protein (N × 6.25) was determined by the Kjeldahl procedure and lysine was assayed by means of an automatic amino acid analyzer (Beckman 120B) after hydrolysis *in vacuo* with 6*N* HCl.

Protein quality of the whole-kernel meals was evaluated as net protein utilization (NPU) by the procedure of Bender and Doell (8) as recommended for chicks by de Muelenaere *et al.* (9) and as net protein ratio (NPR) according to Bender and Doell (10) using the rat as experimental animal.

RESULTS AND DISCUSSION

Dry Milling

The yield of meal and the fat and fiber content of the meal obtained during milling are given in Table I. The results show that the yield from normal maize was 2.2% higher than that for the high-lysine variety and, at the same time, the fat content of the normal maize meal was also higher. This is contrary to the findings in Colombia, where it was established that it was more difficult to separate germ and endosperm in high-lysine maize than in normal hybrids, leading to meal with increased fat content. The data of Wichser (7) also indicate that both the meal and grits of *opaque*-2 maize have a higher fat content than normal maize; however, Wichser found that the *opaque*-2 maize gave a higher yield of milled products than normal maize, while our results indicated the opposite. These results may not be critical, as cultivars and hybrids of different genetic backgrounds were compared.

According to the results obtained after sieving (Table II), high-lysine maize produced a much finer meal than normal maize. This was to be expected, as it is known that high-lysine maize has a relatively soft, floury endosperm.

TABLE I
Yield and Fat and Fiber Content of Meal Dry Milled from Normal and High-Lysine Maize

Variety	Yield %	Fat %	Fiber %
Normal maize	80.1	2.7	1.1
High-lysine maize	77.9	2.1	1.0

Wet Milling

From Table III, it is evident that the starch yield and recovery from MPF 9 compared favorably with the values obtained for the white varieties, and were better than the yield and recovery for the yellow varieties. Protein impurities in the starch recovered from MPF 9 amounted to 0.38%, which can be considered normal. The yield of maize gluten, however, was only 8.7%, and this could be attributed to the high soluble nitrogen content of MPF 9; it was found that MPF 9 contained 0.33% soluble nitrogen, which is more than double the amount for normal maize. This is also reflected in the relatively high solids content of the steepwater of MPF 9. Another factor which has to be taken into consideration is that, according to Gevers (11), the endosperm of *opaque*-2 kernels usually has a lower protein content than that of their normal counterparts.

From the viewpoint of starch yield, the results obtained on MPF 9 are very satisfactory, as both Watson (12) and Dimler (13) quoted higher starch yields for normal hybrids than for *opaque*-2. The starch content of the high-lysine samples used by these authors was, however, much lower than that of the normal hybrids, with the result that the recovery figures (percentage recovered from the chemically determinable starch) for normal and high-lysine maize were comparable.

The yield (dry basis) of endosperm, germ and pericarp after steeping and dissecting by hand of MPF 9 and normal maize is given in Table IV.

According to these figures, normal maize has a larger endosperm-to-germ ratio. This is in general agreement with the findings of Gevers (11), who reported that the normal kernel almost invariably has a higher endosperm:whole grain ratio (E:W ratio) than *opaque*-2 kernels in comparable segregating populations, but there are big differences in the magnitude of this ratio in different populations.

Starch Particle Size

Starch particle size of MPF 9 was compared with the average particle size of a number of white and yellow maize hybrids. In Table V, the figures in the first column represent the equivalent spherical diameter, while the figures in the following three columns are the percentage of particles larger than the corresponding diameter given in the first column.

According to these data, starch from high-lysine maize, on the average, had a larger diameter than starch from normal white and yellow maize, a result which could be expected in view of the more open structure and the lack of horny material of high-lysine maize endosperm.

TABLE II
Particle-Size Distribution of Dry-Milled Maize

	Particle Size, μ					
Variety	**+532**	**+450**	**+305**	**+224**	**+180**	**−180**
Normal maize	6.3	20.5	28.6	15.8	10.4	18.4
High-lysine maize	4.9	10.8	28.2	15.9	17.3	22.9

Direct Enzymatic Hydrolysis

Ground high-lysine maize presented no problem when subjected to hydrolysis by means of a bacterial amylase followed by a fungal amyloglucosidase. It was found, however, that the nitrogen content of the hydrolysate was twice as high for high-lysine maize as for the hydrolysate obtained from normal maize. This can be attributed to the high soluble nitrogen content of high-lysine maize. It was

TABLE III
Dry Solids Recovery during Wet Milling

Variety	Starch Content of Kernels %	Starch Recovery	Dry Solids Recovered per 100g Kernels: Starch g	Steepwater Solids g	Fiber g	Gluten g
MPF 9	73.8	64.1	86.8	5.7	15.1	8.7
SA 4[a]	74.4	58.0	77.9	5.2	19.9	14.1
SA 100[a]	75.6	58.9	77.9	4.0	19.8	12.9
Asgrow[a]	71.7	53.1	74.0	4.9	22.6	13.5
PP × K64R	73.6	63.5	86.3	4.6	18.0	9.8
SA 5	71.5	63.8	89.3	4.9	16.7	11.0
DS 19	74.4	60.6	86.1	4.4	21.6	9.6
NPP × K64R	76.2	64.0	84.0	4.5	17.7	9.6
PPP × K64R	73.0	62.1	85.1	4.5	16.1	12.9

[a] Yellow hybrids.

TABLE IV
Ratio of Endosperm, Germ, and Pericarp in Normal and High-Lysine Maize

Fraction	Percentage on Dry Basis: Normal	MPF 9
Endosperm	85.0	81.0
Germ	7.6	9.9
Pericarp	7.4	9.0

TABLE V
Particle-Size Distribution of Starch Isolated from Normal White, Yellow, and High-Lysine Maize (MPF 9)

Particle Diameter μ	Percentage Particles Larger than Stated Diameter: White	Yellow	High-lysine
49	0.62	0.63	0.16
39	1.4	0.70	0.24
31	6.2	1.1	1.6
24	8.5	5.3	16.2
19	34.7	25.5	55.2
15	71.5	66.2	91.8
12	90.4	92.1	92.7
10	93.9	94.3	95.6
8	97.2	97.5	98.4
6	99.4	99.4	99.3
5	99.8	99.9	99.6
4	100.0	100.0	100.0

found that, by washing the ground raw material with water prior to hydrolysis, the nitrogen content of the hydrolysate could be decreased by about 50%, and it is therefore recommended that this procedure be followed should high-lysine maize be used as raw material for direct enzymatic starch hydrolysis.

As stated earlier, it is of interest that MPF 9 was shown to have higher soluble nitrogen content than normal maize. This appears to support the findings of other workers cited by Mertz *et al.* (14), who reported a higher level of water-soluble free amino acids in *opaque*-2 than in normal maize. On this basis, Mertz *et al.* (14) have devised a simple analytical procedure by which the normal kernel and the *opaque*-2 variants may be identified either in the laboratory or in the field by differences in their free amino acid content. This method is the more promising to the maize breeder, as it appears that it can also readily distinguish between normal maize and hard-kernelled *opaque*-2 variants.

Physical Properties

High-lysine maize, MPF 9, showed an average of 1644 kernels/l. against the average of 1910 for the control sample of white maize; this figure indicated relatively large kernels for MPF 9. The mass of 100 kernels of high-lysine maize was 41.6 g as compared to 40.7 g of the control. The small difference recorded between 100 kernel mass for MPF 9 and normal kernels clearly shows that, despite a much larger kernel in the case of MPF 9, its mass is relatively low. Gevers (11) reported, however, that true *opaque*-2 types may have larger kernel volume than their normal counterpart, and he reported relative densities for high-lysine samples ranging between 1090 and 1200 kg/m^3 as against 1240 to 1260 kg/m^3 for normal hybrids. This is also in fairly close agreement with the results reported by Wichser (7).

The values for 'per cent floaters' of MPF 9 and two white and two yellow hybrids are tabulated in Table VI and the lower density of MPF 9 is clearly illustrated by the results.

All these data on physical properties point toward a low-density kernel, probably with loosely packed starch grains and relatively little horny endosperm; this could possibly prove a distinct disadvantage to the dry millers.

Brabender viscosities done on high-lysine and normal maize were almost identical, and it is doubtful whether the small differences have any practical value. In contrast, WSI and WAI of maize meal cooked from high-lysine and normal maize differed appreciably, as can be seen in Table VII. The higher WSI

TABLE VI
'Per Cent Floaters' in Solutions of Different Specific Gravity (S.G.)

Variety	'Per Cent Floaters' S.G. 1,112	S.G. 1,145	S.G. 1,220	S.G. 1,258
MPF 9	19	29	61	97
SA 100 (yellow)	0	3	6	91
SA 200 (yellow)	1	10	17	90
PP × K64R	5	16	35	100
SSPP × K64R	5	5	25	99

of high-lysine maize is probably associated with its high soluble nitrogen content.

Biological Evaluation

Details of the diets and the experimental procedure used in the determination of net protein utilization by chicks and the net protein ratios for rats have been previously described (3). The results of these trials, which included whole kernel meals prepared from three genetically different *opaque*-2 (high-lysine) and three normal white maize types, are presented in Table VIII. Also included in the table is the NPU value of a commercial yellow maize meal. The superior quality of the high-lysine meals is clearly evident in both sets of data, but the differences between high-lysine and normal meals are less marked when the chick is used as test animal. This was due to the better utilization of the protein of the normal maize meals by the chick than by the rat. It must be stressed that the beneficial effect of the introduction of the *opaque*-2 gene into normal maize is not limited to a doubling of the lysine content. As reviewed elsewhere (2,16), tryptophan levels are also doubled, while the proportion of leucine is lowered, resulting in a much improved amino acid balance. The lowered leucine content also promotes better utilization of the vitamin nicotinic acid, which is notoriously unavailable in normal maize.

While the nutritional superiority of high-lysine over normal maize has been clearly demonstrated in all experiments (16) in which maize meal provides the only source of dietary protein, the nutritional advantage of high-lysine maize is

TABLE VII
Water Solubility Index (WSI) and Water Absorption Index (WAI) of Cooked Maize Meal

Sample	WSI g/g Dry Solids	WAI g/g Dry Solids
High-lysine maize	0.112	3.0
Control	0.097	3.4

TABLE VIII
Net Protein Utilization (NPU) Values for Chicks and Net Protein Ratio (NPR) Values for Rats of Three *Opaque*-2 (O) and Three Normal (N) Whole-Kernel White Maize Meals and a Commercial Yellow Maize Meal

Meals Used as Protein Source	Chicks NPU	Rats	
		NPR	Estimated NPU[a]
MPF-6 (O)	74.6	4.38	76.7
MPF-7 (O)	70.6	4.25	74.7
MPF-9 (O)	68.1	4.18	73.2
MPF-5 (N)	59.2	2.89	50.6
MPF-8 (N)	63.8	2.67	46.6
MPF-10 (N)	61.7	n.d.[b]	n.d.
Commercial yellow meal	58.8	n.d.	n.d.

[a] NPU = NPR × 17.5 (Morrison (15)).
[b] n.d. = not determined.

overshadowed when it is incorporated into high-protein diets containing lysine-rich supplementary protein feeds such as soybean meal. Under these circumstances, the economic advantage of high-lysine maize must be assessed in terms of the saving with respect to more expensive supplementary protein feeds (16).

CONCLUSION

Despite the obvious nutritional advantages of high-lysine maize, it is conceivable that certain physical properties of types such as MPF 9 may cause problems in both dry and wet milling. Although it will be possible to minimize these problematic features of high-lysine maize by selection and breeding, this is likely to require a considerable time. In view of the great nutritional potential of high-lysine maize, ways and means of coping with a somewhat different product should be found, and a study of milling techniques to overcome these problems is warranted.

Literature Cited

1. CENTRO INTERNACIONAL DE AGRICULTURA TROPICAL. The feasibility of introducing opaque-2 maize for human consumption in Colombia. Tech. Bull. 1 (1971).
2. QUICKE, G. V., and GEVERS, H. O. Higher lysine levels and improved protein quality of opaque-2 maize. S. Afr. Med. J. 46: 1579 (1972).
3. DU PREEZ, J. J., GEVERS, H. O., QUICKE, G. V., and GOUS, R. M. Utilisation of protein from opaque-2 maize by chicken and rat. S. Afr. J. Anim. Sci. 4: 97 (1974).
4. PELSHENKE, P. F., and LINDEMANN, E. Untersuchungen über die Eignung verschiedenen Maissorten für die Stärkefabrikation. Staerke 6(8): 177 (1954).
5. VAN TWISK, P., and TEGGE, G. Enzymatischer Abbau von Stärke in trocken entkeimten Mais. Staerke 20(10): 319 (1968).
6. VAN TWISK, P. Neue Erfahrungen bei der Herstellung von Stärkeverzuckerungsprodukten aus Maisgrits. Staerke 22(7): 228 (1970).
7. WICHSER, W. R. Comparison of the dry milling properties of opaque-2 and normal dent corn, p. 104. Proc. High-Lysine Corn Conf., Corn Industries Res. Found.:Washington, D.C. (1966).
8. BENDER, A. A., and DOELL, B. H. Note on the determination of net protein utilization by carcass analysis. Brit. J. Nutr. 11: 138 (1957).
9. DE MUELENAERE, H. J. H., QUICKE, G. V., and WESSELS, J. P. H. The applicability to chicks of the carcass analysis method for the determination of net protein utilization. S. Afr. J. Agr. Sci. 10: 113 (1960).
10. BENDER, A. E., and DOELL, B. H. Biological evaluation of protein: a new aspect. Brit. J. Nutr. 11: 140 (1957).
11. GEVERS, H. O. Breeding for improved protein quality in maize. Trans. Roy. Soc. S. Afr. 40(2): 81 (1972).
12. WATSON, S. A. Comparison of the wet milling properties of opaque-2 and normal dent corn, p. 117. Proc. High-Lysine Corn Conf., Corn Industries Res. Found.:Washington, D.C. (1966).
13. DIMLER, R. J. Report on kernel structure and wet milling of high-lysine corn, p. 121. Proc. High-Lysine Corn Conf., Corn Industries Res. Found.: Washington, D.C. (1966).
14. MERTZ, E. T., MISRA, P. S., and JAMBUNATHAN, R. Communication to the Editor: Rapid ninhydrin color test for screening high-lysine mutants of maize, sorghum, barley, and other cereals. Cereal Chem. 51: 304 (1974).
15. MORRISON, A. B. Proteins and their reactions p. 361. In: Symposium on foods, ed. by H. W. Schultz, and A. F. Anglemier. Avi Pub. Co.: Westport, Conn. (1964).
16. QUICKE, G. V. Better quality maize protein. S. Afr. J. Anim. Sci. 4: 265 (1974).

[Received August 25, 1975. Accepted November 13, 1975]