While the data of Table III indicate that Brazil nut protein does supplement bean protein, the quantitative estimate of the supplementation effect varies with the measure used for protein evaluation. Specifically, the amino acid scores drop faster than the protein efficiency ratios or the microbiological values, as the protein quality of the diet diminishes. A possible explanation of these differences is that organisms conserve and utilize the limiting amino acids better, as the diet becomes more severely deficient in these amino acids. This adaptive response is strong for lysine (Hegsted, 1974), and it is apparently operative for S-containing amino acids under the experimental conditions described here. On the other hand, the MEAAI's are rather high estimates of protein quality in comparison to PER's. The authors are aware of the limitations of PER as a protein evaluation measure; yet they use it as a comparison reference only because it is "official" (AOAC, 1975).

No amino acid analysis was carried out on the germinated beans for a possible correlation with the low PER observed in the corresponding diet. Had an increase in PER (similar to that observed in antiscorbutic activity) been obtained as a result of germination, the testing of sprouted beans would have been continued, for whatever practical significance such a product might have.

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LITERATURE CITED

Association of Official Analytical Chemists, "Official Methods of Analysis", Washington, D.C., 1970, 1975.
Aykroyd, W. R., Doughty, J., "Legumes in Human Nutrition", FAO, Rome, 1964.
FAO, "Amino Acid Content of Foods", Rome, 1970.
FAO/WHO, "Energy and Protein Requirements", WHO, Geneva, 1973.
Ford, J. E., *Brit. J. Nutr.* 14, 485 (1966).
Hegsted, D. M., in "Improvement of Protein Nutriture", NAS, Washington, D.C., 1974.
Hirs, C. H. W., Stein, W. H., Moore, S., *J. Biol. Chem.* 211, 941 (1954).
Hoffman, I., Westerby, R. J., Hidiroglou, M., *J. Assoc. Off. Anal. Chem.* 51, 1039 (1968).
Lewis, O. A. M., *Nature (London)* **209**, 1239 (1966).
Mitchell, H. H., in "Symposium on Methods for the Evaluation of Nutritional Adequacy and Status", Spector, H., et al., Ed., NRC, Washington, D.C., 1954.
Spies, J. R., *Anal. Chem.* 31, 1412 (1967).

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Chemical Composition and Nutritional Properties of a Sugary-1/Opaque-2 (su_1/o_2) Variety of Maize (*Zea mays* L.)

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Four maize varieties [Nutrimaiz sugary-1/opaque-2 (su_1/o_2), Piramex sugary-1 (su_1), Maya opaque-2 (o_2), and the Maya XII Normal] were compared with respect to proximate composition, amino acid content, and nutritive value including rate of growth, PER, protein digestibility, nitrogen balance, and apparent biological value of the protein. The su_1/o_2 variety, obtained by combining the su_1 and o_2 genes, presented protein and oil contents similar to those of su_1 but considerably higher than the o_2 varieties. The amino acid profile of the su_1/o_2 was also improved by increasing lysine and tryptophan and decreasing leucine as compared with su_1 and o_2. The new su_1/o_2 variety had a higher apparent biological value and produced a greater growth rate in rats than the o_2 parent, although no significant difference was observed in PER, nitrogen retention, and apparent protein digestibility. Compared with su_1, the double mutant exhibited superior qualities for all parameters studied. The improved nutritional quality is of special interest because of the highly desirable agronomic characteristics of this new synthetic variety.

The fact that the opaque-2 (o_2) mutant in corn had a higher lysine and tryptophan content (Mertz et al., 1964) stimulated a great amount of research aimed at producing varieties of corn with better composition and nutritional characteristics (Mertz et al., 1965; McWhirter, 1971; Misra et al., 1972; Nelson and Chang, 1974; Ma and Nelson, 1975).

Misra et al. (1975a,b) reported in two papers that the endosperm genes which reduce starch synthesis could also decrease the level of zein in the seed, when combined with the o_2 gene, causing a substantial increase in the proportion of lysine and tryptophan. Combinations such as brittle-2/opaque-2 (bt_2/o_2), shrunken-2/opaque-2 (sh_2/o_2), amylose extender/opaque-2 (ae/o_2), and waxy/opaque-2 (wx/o_2), therefore, could be of great interest in increasing the nutritive value of the product. It has been shown by Barbosa (1971) and Tosello (1974) that the combination su_1/o_2 is of particular interest since it codes for high concentrations of water-soluble polysaccharides (WSP) and a high-quality protein in the endosperm.

Nutritional work has been conducted using the opaque-2 flour in rats (Mertz et al., 1965), on swine (Vernon, 1968;

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Sikombing et al., 1969; Marroquin et al., 1973), on poultry (Cromwell et al., 1967; Adams and Rogler, 1970), and with humans (Clark et al., 1967). All nutritional studies revealed that the o_2 possess a much higher nutritive value than other varieties of corn due particularly to the higher lysine and tryptophan content of the o_2 proteins. Unfortunately, some undesirable kernel characteristics and low acceptability of the o_2 corn both at the industrial and consumer level have hindered its introduction for animal and human consumption.

Recently, Silva and Teixeira (1975), encouraged by the results of Barbosa (1971), selected a variety of sweet corn with endosperm su_1/o_2 which had 40.4% WSP, 4.37% lysine, and 1.01% tryptophan in the endosperm protein on a dry basis, at 26 days after pollination. The outstanding agronomic qualities of the su_1/o_2 variety described by Silva et al. (1976) prompted us to further study this material of good food potential.

In this paper we present some additional data on the chemical composition and nutritional properties of this variety of corn harvested at the dry stage.

MATERIALS AND METHODS

Corn Varieties. The four varieties used in this study were Piramex sugary-1 (su_1), Maya opaque-2 (o_2), Maya XII Normal, and Nutrimaiz sugary-1/opaque-2 (su_1/o_2). Piramex with su_1 endosperm, as well as the other germplasms, were produced at the Agronomic Institute of Campinas, São Paulo, Brazil. The synthetic su_1/o_2 was derived from a population consisting of 87.5% of Maya o_2 and 12.5% of the variety Pajimaca from Cuba, containing su_1 endosperm. The new double mutant was selected for seven generations until a product was obtained which had none of the undesirable characteristics such as small and conical ears and flint endosperm kernels. The resulting product had only cylindrical ears of deep, soft, yellow endosperm kernels, characteristics of the Tuxpeño type maize.

Chemical Analyses. All of the chemical analyses were performed in duplicate on flours obtained after grinding the dry kernels to pass a 60 mesh screen, unless otherwise stated.

Crude protein was determined by the semi-micro-Kjeldahl procedure as described in the Official Methods of the AOAC (1970).

Nonprotein nitrogen (NPN) was determined by extracting the flour with water and treating the extract with a trichloroacetic acid (TCA) solution until the final concentration of TCA was 0.85 M. Nitrogen was determined in the supernatant (TCA soluble material) by the Kjeldahl procedure after centrifugation.

The lipid material was extracted from the flour for 8 h in a Soxhlet apparatus with petroleum ether. The amount of ether-soluble material was determined gravimetrically after complete evaporation of the solvent.

The content of ash was determined gravimetrically after carbonization of the organic components according to the procedure described in the "Official Methods" of the AOAC (1970).

The amino acid composition was determined on the acid hydrolysate of the defatted flour using the Beckman Model 120C Analyzer and the procedure recommended by the manufacturer. Tryptophan was determined colorimetrically according to procedure W of Spies (1967) using flours ground to pass a 100 mesh screen.

Nutritional Evaluation. Determination of the nutritional properties was performed by utilizing rats of the Wistar strain and procedures based on the "in vivo" and "in vitro" digestibility, growth rate, and nitrogen balance.

Table I. Proximate Composition of Four Varieties of Corn (Dry Basis)

Variety	Total crude protein (% N × 6.25)	Nonprotein nitrogen (% N × 6.25)	Ether-soluble material, %	Ash, %
su_1/o_2	12.5	2.2	9.4	2.3
o_2	8.5	0.9	5.1	2.1
su_1	13.3	1.1	8.5	2.1
Normal	11.9	0.8	4.4	1.7

For the protein efficiency ratio (PER) assay, six rats weighing an average of 54 g were used in each group. The assay was carried out with diets containing approximately 7% corn protein. All the other nutrients were added to the corn flour in the amounts to give the recommended concentration for the growing rat. The test lasted 28 days, the food intake was determined every three days and weights once a week. Water was offered ad libitum throughout the experimental period.

Nitrogen balance tests were done using three rats for each diet. The animals were adapted for 96 h with the experimental diets before starting the study. Following the adaptation period, feces and urine were collected for each animal during 72 h. Protein digestibility, nitrogen retention, and apparent biological value of the protein were calculated from the nitrogen intake and the quantities of urinary and fecal nitrogen. In vitro protein digestibility was performed by the method of Akeson and Stahmann (1964) which consists of successive digestions with pepsin and pancreatin under optimal conditions, followed by precipitation of the protein in 10% TCA and measurement of the nitrogen in the TCA-soluble fraction.

RESULTS AND DISCUSSION

Proximate composition analysis of the four varieties studied is presented in Table I. Higher nonprotein nitrogen (2.2%) and ether-soluble lipids (9.4%) characterized the su_1/o_2 product. The high lipid content of the su_1/o_2, a characteristic inherited from the Piramex su_1, was nearly twice the content of the normal and the o_2 maize. A twofold increase in the oil content, however, was not the mere result of a greater germ size. Germ sizes were (cm^2) 0.39 for the double mutant, 0.33 for o_2, 0.41 for su_1, and 0.32 for the Maya XII Normal variety. The areas correlated well with the total weights of the germs. The high-protein characteristic (13.3%) of the su_1 variety was retained by the double mutant (12.5%), thus contrasting with the low-protein content (8.5%) of the Maya o_2. It is important to emphasize that although the germ size and protein contents in su_1 and su_1/o_2 were essentially the same, the protein quality was much superior in su_1/o_2, showing that the endosperm protein was improved considerably in the double mutant.

Table II is a comparison of the amino acid profiles of the four varieties. In general, the amino acid pattern of the su_1/o_2 maize showed a considerable improvement by the further increase of both lysine and threonine levels while maintaining the low leucine–isoleucine ratio of the o_2 variety.

Concentrations of proline, glycine, and alanine, however, were also found somewhat higher in the su_1/o_2 variety while tryptophan and methionine appeared to be lower than in the o_2 parent. The relatively low levels (0.6–0.9) of methionine in all varieties are probably artifacts due to oxidation losses during hydrolysis.

On the whole, one could view the result of the association between the su_1 and o_2 genes, as a material which approximates the su_1 in the fat and protein content, as well as in the nature of carbohydrate fractions (Silva et al., 1977). Chemical analyses also indicated that the protein

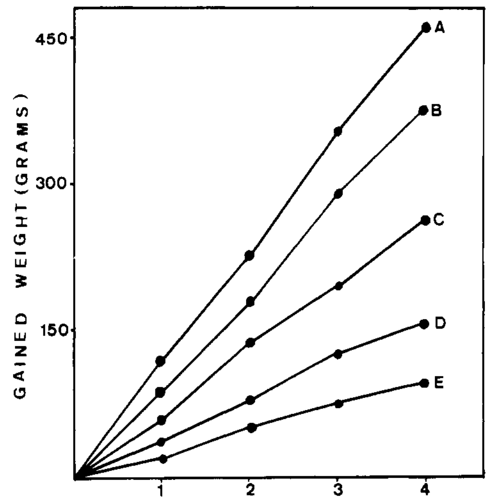

Figure 1. Growth-response curves of rats (six per group) fed diets containing one of the four corn varieties and casein as the sole source of protein: A, casein; B, Nutrimaiz sugary-1/opaque-2; C, Maya opaque-2; D, Piramex sugary-1; E, Maya XII Normal. The values on the y axis represent the total grams gained by the six rats in each group.

Table II. Amino Acid Composition of Four Corn Varieties (g/16 g of Nitrogen)

	Varieties			
Amino acid	su_1/o_2	o_2	su_1	Normal
Lys	5.4	4.6	3.0	2.4
His	3.8	3.3	2.8	2.7
NH_3	1.2	1.2	1.7	2.1
Arg	5.1	3.6	3.3	2.7
Asp	10.5	9.3	7.1	6.7
Thr	4.4	3.5	3.6	3.0
Ser	6.3	4.6	5.8	7.3
Glu	23.3	21.5	27.9	32.9
Pro	11.4	9.6	10.6	11.0
Gly	7.4	5.8	4.7	3.6
Ala	7.9	5.9	8.9	9.6
Half-Cys	1.7	1.5	1.3	1.0
Val	6.2	4.6	5.0	4.8
Met	0.6	0.7	0.9	0.7
Ile	3.1	2.8	3.4	3.5
Leu	9.7	8.7	15.9	18.2
Tyr	2.0	1.5	1.6	1.2
Phe	4.3	3.8	4.8	5.4
Trp[a]	0.8	1.2	0.6	0.4

[a] Tryptophan was determined colorimetrically in a separate experiment.

of su_1/o_2 variety could have an equal or better nutritional quality than that of the o_2 variety, and consequently better than either one of the parents.

Figure 1 shows the growth rate curves for rats receiving diets which contained one of the four varieties as the sole source of protein. It became evident that the capability of the su_1/o_2 corn to promote growth in young rats was much greater than that of any of the other three varieties. The superiority of the new variety could also be appreciated by examining the data of Table III. Both growth rate and apparent biological value of the proteins were higher for the su_1/o_2 than for the o_2 while PER, nitrogen retention, and apparent digestibility did not seem to be significantly different. The su_1/o_2 variety was considerably superior to the su_1 for all parameters studied. The superior ability of the new su_1/o_2 variety to promote growth confirms the excellent nutritional properties of this double mutant.

It was interesting to note that, although the PER's of o_2 and su_1/o_2 were similar, the double mutant had the property of inducing higher food intakes, therefore further stimulating growth in the rats. The daily average food intake per rat was 12.5, 9.0, 7.7, and 7.0 g for the su_1/o_2, o_2, su_1, and normal, respectively. The greater food consumption seems to be the direct consequence of a better amino acid profile of the protein in the double mutant.

The su_1/o_2 variety was also tested at the milky stage with respect to the organoleptic properties of texture and flavor. The panel ranked the new variety first in relation to the other three varieties studied at the same developmental stage. In addition to these favorable nutritional properties, the new variety has desirable agronomic characteristics such as high yield at the green corn stages and high leaf disease resistance (Silva et al., 1977). The same authors have determined that the agronomical yield of the new Nutrimaiz variety at the dry stage is 70% of that of the Maya XII normal; this is slightly higher in yield than the sweet Piramex su_1. Considering the above advantages, the new synthetic variety seems to offer the possibility of successful commercial cultivation and utilization in foods and feeds. Its use as human food in the green (unripe) stage appears to be quite promising since the su_1 is a variety largely employed at this stage of ripening for canning and for other uses.

In a recent study, Rosa (1976) reported on the nutritional properties of double mutants involving combinations of o_2 with waxy (wx) and sugary-2 (su_2). Rosa's results showed no significant advantage in using the double mutants over the homozygous opaque-2 (o_2) type for the nutrition of the rat and the swine. Judging from our results and those of Barbosa (1971) and Tosello (1974), combinations of the o_2 and the su_1 genes seem to be superior to other double mutants so far studied that include the o_2 gene.

Biochemical, nutritional, and technological (i.e., food processing and food formulation for human consumption) studies at three stages of ripening are under way for the Nutrimaiz (su_1/o_2) and results will be reported in a future paper.

Table III. Nutritional Characterization of the Proteins of Four Corn Varieties

	Determination				
Protein source	PER[a]	Nitrogen retention, g	Apparent digestibility, %	Apparent biological value, %	Digestibility "in vitro", %
su_1/o_2	2.6	2.3	79.2	69.9	99.0
o_2	2.5	2.3	78.7	67.4	59.9
su_1	1.6	1.3	75.8	62.5	64.7
Normal	1.2	1.2	73.5	51.9	64.2
Casein	2.9	2.4	91.3	81.2	99.1

[a] PER was determined in a 28-day feeding experiment. All the other values in this table were calculated from the 72-h nitrogen balance experiment.

LITERATURE CITED

Adams, K. L., Rogler, J. C., *Poult. Sci.* **49**, 1114 (1970).
Akeson, W. R., Stahmann, M., *J. Nutr.* **83**, 257 (1964).
Association of Official Agricultural Chemists, "Official Methods of Analysis", 11th ed, Washington, D.C., 1970.
Barbosa, H. M., Ph.D. Thesis, Purdue University, Lafayette, Ind., 1971.
Clark, H. I., Allen, P. E., Meyers, S. M., Tucket, S. E., Yamamura, Y., *Am. J. Clin. Nutr.* **20**, 825 (1967).
Cromwell, G. L., Rogler, J. C., Featherstar, W. R., Pickett, R. A., *Poult. Sci.* **46**, 705 (1967).
Ma, Y., Nelson, O. E., *Cereal Chem.* **52**, 412 (1975).
Marroquin, C. R., Cromwell, G. L., Hays, V. W., *J. Anim. Sci.* **36**, 253 (1973).
McWhirter, K. S., *Maize Genet. Coop. Newslett.* **45**, 184 (1971).
Mertz, E. T., Bates, L. S., Nelson, O. E., *Science* **145**, 279 (1964).
Mertz, E. T., Vernon, O. A., Bates, L. S., Nelson, O. E., *Science* **148**, 1741 (1965).
Misra, P. S., Jambunathan, R., Mertz, E. T., Glover, D. V., Barbosa, H. M., McWhirter, K. S., *Science* **76**, 1435 (1972).
Misra, P. S., Mertz, E. T., Glover, D. V., *Cereal Chem.* **52**, 161 (1975a).
Misra, P. S., Mertz, E. T., Glover, D. V., *Cereal Chem.* **52**, 844 (1975b).
Nelson, O. E., Chang, M. T., *Crop Sci.* **14**, 374 (1974).
Rosa, J. C., M.S. Thesis, Purdue University, Lafayette, Ind., 1976.
Sikombing, D. T. H., Cromwell, G. L., Hays, V. W., *J. Anim. Sci.* **29**, 921 (1969).
Silva, W. J., da, Teixeira, J. P. F., *Ciênc. Cult. (Sao Paulo), Suppl.* **27**, 613 (1975).
Silva, W. J., da, Teixeira, J. P. F., Lovato, M. B., Arruda, P., *Ciênc. Cult. (Sao Paulo)*, in press (1977).
Spies, J. R., *J. Anal. Chem.* **39**, 1412 (1967).
Tosello, G. A., Ph.D. Thesis, Purdue University, Lafayette, Ind., 1974.
Vernon, O. A., *Diss. Abstr. B* **28**, 421 (1968).

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Relationship between Tannin Levels and in Vitro Protein Digestibility in Finger Millet (*Eleusine coracana* Gaertn.)

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Determination of the total phenol and tannin levels of finger millet varieties indicated wide variations in phenolic contents. White-grain varieties had lower phenolic content than the brown-grain varieties. In vitro protein digestibility values of low tannin samples were higher than those of the high tannin samples. Dehulling had the effect of removing most of the phenolics from finger millet grain with concomitant increase in in vitro protein digestibility. Addition of tannic acid to low tannin or dehulled finger millet samples decreased the in vitro protein digestibility. Tannins were found to be associated mostly with the glutelin fraction of finger millet protein.

Utilization of protein in animal and human diet is adversely affected by phenolic constituents and tannins (polyphenols) since they have the ability to bind with and precipitate proteins. Growth retardation has been observed in chicks and rats fed on diets containing high tannin sorghum (Chang and Fuller, 1964; Fuller et al., 1966; Jambunathan and Mertz, 1973). Tannins have been shown to be growth depressing and toxic to chicks (Vohra et al., 1966). Availability of amino acids in high tannin sorghums is reported to be much lower than in low tannin sorghums (Rostango, 1972). Extraction of tannins from high tannin sorghums results in increased weight gain and feed efficiency of chicks and rats (Armstrong et al., 1974; Featherstone and Rogler, 1975). Addition of phenolics to diets decrease the nutritive value of proteins (Horigome ad Kandatsu, 1968). Further, tannins of sorghum cause a reduction of in vivo and in vitro dry matter disappearance and protein digestibility (Stallcup and Davis, 1962; McGinty, 1968; Maxson et al., 1973; Schaeffert et al., 1974; Featherstone and Rogler, 1975).

The occurrence of tannins in cereals is rare and has so far been reported only in strains of barley and sorghum. Tiemann and Blumenberg (1959) reported that among some cereals tested tannin–like substances were present only in barley while these were absent from wheat, rye, oats, and maize. They reported, however, that all these cereals contained chlorogenic acid.

Finger millet (*Eleusine coracana* Gaertn.) is an important food crop of India and Africa. The protein quality of finger millet varieties has been the subject of earlier studies from this laboratory (Virupaksha et al., 1975). This report deals with the tannin and phenolic contents and their relation to the in vitro protein digestibility (IVPD) in finger millet.

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MATERIALS AND METHODS

Sample. Thirty-two samples of finger millet consisting of 19 Indian, 10 African, and 3 Indian × African cross-bred varieties were procured from the germ plasm collection of the Millet Research Centre, University of Agricultural Sciences, Hebbal, Bangalore, India. Samples had a range of seed color from white to very dark brown. Samples designated by the prefix HP or HX represent crosses between white-grain and brown-grain varieties.

Samples were ground to a fine powder with a mortar and pestle. Dehulling of the finger millet varieties was performed by soaking the grain for 1 min in concentrated H_2SO_4 and pouring tap water on the acid. This treatment loosened the pericarp which was rubbed off the grain by hand and removed by decantation with large volumes of water. The dehulled grain was air-dried at room tem-