account for the traces of radioactivity detected. One possibility could be metabolites of [^{14}C]benzo[*a*]pyrene. However, this would not account for the variable and almost equivalent levels of activity found in many control plant tissues.

A more plausible explanation would be some degradation of the [^{14}C]benzo[*a*]pyrene from the nutrient solutions forming volatile decomposition products that would deposit on the plant cuticles. The waxy cutin would have been sufficiently retentive since tissue dehydration did not result in losses of activity. Since control and test plants were grown in the same laboratory hood area to maintain a similar environment, they would have received similar exposure. This theory was supported by the detection of traces of carbon-14 radioactivity from routine wipe tests made on the hood surfaces at the conclusion of the study.

Decomposition of [^{14}C]benzo[*a*]pyrene was minimal since the nutrient test solutions did not show significant differences in the specific activity (8.83 mCi/g) of the [^{14}C]benzo[*a*]pyrene or in its solution (4 ppb) or total (8 ppb) concentration at the conclusion of the experiments. Significant degradation would have caused at least one of these values to change. The use of opaque plant containers was evidently effective in minimizing any photodecomposition of the benzo[*a*]pyrene.

The higher level of activity found in the cotton plants may therefore be due to greater exposure because of its longer growth period. In contrast, the comparatively low levels in the cantaloupes may be attributed to late appearance of the fruit in its growth cycle. Higher levels in the bean leaves compared with stems and fruit could be explained by their greater surface available for exposure. Assays of these crops, unexposed to [^{14}C]benzo[*a*]pyrene and grown in fields showed no radioactivity above the normal 30 counts/min of background.

The variability of activity found in the samples could be due to a combination of different individual plant growth rates and the extent of which they could have been exposed. Greater variations would be expected with controls because proximity to test plants would influence the extent to which deposition of activity could have occurred.

On the basis of this study, benzo[*a*]pyrene was not found to translocate or concentrate into the three different crops tested under hydroponic growth conditions. In the instance where other parts of the bean plant were examined, the same conclusion could be drawn. The traces of radioactivity found in the plants suggested contamination from volatile degradation products rather than occurrence through a metabolic process.

LITERATURE CITED

Hartwell, J. L., "Survey of Compounds Tested for Carcinogen Activity", 2nd ed, National Institutes of Health, Washington, D.C., 1951.

Howard, J. W., Fazio, T., *J. Agric. Food Chem.* **17**, 527 (1969).

Davis, W. W., Drahl, M. E., Clowes, G. H. A., *J. Am. Chem. Soc.* **64**, 108 (1942).

Davis, W. W., Parke, T. V., Jr., *J. Am. Chem. Soc.* **64**, 101 (1942).

Received for review January 16, 1976. Accepted May 26, 1977.

Protein Supplementation of Navy Beans with Brazil Nuts

Aloisio J. Antunes*[1] and Pericles Markakis

Rat feeding experiments showed that the protein quality of Navy beans, a poor source of sulfur-containing amino acids, can be largely improved by mixing the beans with defatted Brazil nuts, a rich source of methionine. Compared with a PER (protein efficiency ratio) of 2.50 for casein, the PER for beans was 1.53, and for diets containing bean protein and Brazil nut protein at the ratios 80:20, 90:10, and 95:5, the PER's were 2.42, 2.16, and 1.93, respectively, at 10% total protein content in the diet. A comparison of PER with other protein evaluation measures was made for the diets tested here.

Protein malnutrition is a major health problem in the world. A significant portion of the protein in the diet of many people comes from legumes (Aykroyd and Doughty, 1964). A popular legume, especially in Latin America and South Asia, is the dry seeds of the common bean (*Phaseolus vulgaris*). The bean protein, however, is deficient in the sulfur-containing amino acids, methionine and cysteine (FAO, 1970). On the other hand, the protein of Brazil nuts (*Bertholletia excelsa*) is exceptionally rich, among plant proteins, in sulfur-containing amino acids (FAO, 1970). The objective of this research was to study the supplementary effect of Brazil nut protein on Navy bean protein through rat feeding and a microbiological assay.

MATERIALS AND METHODS

Navy beans, cv Sanilac, grown in Michigan, were obtained from the Michigan Foundation Seed Association. Brazil nut kernels were shipped by air from the Brazilian Institute of Food Technology. These nuts were defatted with hexane. A fire-proof Waring blender was used to grind the kernels with hexane. After blending, the slurry was filtered under reduced pressure and the residue was blended with fresh hexane and filtered five more times. The residue, henceforth referred to as Brazil nut defatted flour (BNDF), was air dried in a ventilated hood for 24 h and stored in a refrigerator. For the feeding studies, the beans were ground in a feed grinder, autoclaved as a thin layer (about 5 mm) in stainless steel pans at 121 °C for 10 min and dried in a ventilated hood. For amino acid

Michigan State University, Department of Food Science and Human Nutrition, East Lansing, Michigan 48824.

[1]Present address: Instituto de Technologia de Alimentos, Campinas, Brazil.

Table I. Composition of Navy Bean Flour and Brazil Nut Defatted Flour (BNDF)

	Bean flour	BNDF
Moisture, %	11.5	8.7
Fat, %	1.1	1.0
Protein, %N × 6.25	24.0	54.5
Ash, %	3.7	7.3
Crude fiber, %	3.7	5.9
Carbohydrate, %[a]	56.0	22.6
Selenium, ppm		40[b]

[a] By difference; it does not include crude fiber carbohydrate. [b] Two shipments of Brazil nuts were received from Brazil and analyzed; the first contained 63 ppm Se and the second 17 ppm Se in BNDF. The other constituents differed less than 5% among the shipments.

Table II. Amino Acid Composition of Navy Bean Flour and Brazil Nut Defatted Flour (BNDF), in mg/g of Total N

	Bean flour	BNDF
Alanine	254	217
Arginine	390	910
Aspartic acid	799	516
Cysteine	37	133
Glutamic acid	994	1200
Glycine	232	250
Histidine	174	153
Isoleucine	310	229
Leucine	546	452
Lysine	445	206
Methionine	47	386
Phenylalanine	401	284
Proline	284	290
Serine	411	284
Threonine	333	175
Tryptophan	96	99
Tyrosine	203	174
Valine	383	347

analysis, both the beans and the BNDF were passed through a Wiley mill, using the 60-mesh screen. The Beckman Model 120 C amino acid analyzer was used for the amino acid determination in 22- and 72-h hydrolysates. The concentrations of histidine, serine and threonine were obtained from the 22- and 72-h values by extropolation to zero time (Hirs et al., 1954). Cystine and methionine were determined after prior oxidation with performic acid (Lewis, 1966). Tryptophan was measured colorimetrically after hydrolysis of the protein with pronase (Spies, 1967).

The AOAC (1970) procedure was followed in determining protein efficiency ratios (PER). Ten weanling Sprague-Dawley rats were assigned to each diet. Six diets were used, all containing 10% protein (N% × 6.25): a standard casein diet; a diet in which all protein originated from beans; a diet in which all protein originated from BNDF; a diet in which 80% of the protein was provided by beans and 20% by BNDF; a diet in which the ratio of protein from beans and BNDF was 90:10; and a diet in which the same ratio was 95:5. In one experiment, beans were germinated at 26 °C for 60 h (the sprout length was 5 to 10 mm by that time), ground in a meat grinder, autoclaved at 121 °C for 10 min, dried in air, and included in a rat diet as the sole source of protein for PER determination.

For the microbiological evaluation of bean protein quality, use was made of *Streptococcus zymogenes* NCDO 592, obtained from the National Collection of Dairy Organisms, Reading, U.K. Ford (1966) used this microorganism to measure the relative nutritive value (RNV) of food proteins and found a close correlation between microbiological and biological estimates of protein quality. His method was employed in this study. It was found advisable for this test to grind the beans and BNDF down to a 100-mesh particle size, using a water-cooled grinder (Chemical Rubber Co.). Selenium was measured fluorometrically as described by Hoffman et al. (1968). Moisture, fat, ash, crude fiber, and Kjeldahl nitrogen were measured according to AOAC (1970). The carbohydrate content, other than that present in the fiber, was calculated by difference.

RESULTS AND DISCUSSION

The composition of the Navy bean flour and BNDF used in this work is shown by Table I. The amino acid composition of these two flours is shown by Table II.

The total content in S-containing amino acids of the Brazil nut flour is over six times greater than that of the bean flour, while the lysine content of bean flour is more than double that of BNDF. Since the S-containing amino acids are limiting the protein value of beans and lysine is the limiting amino acid in Brazil nuts the prospects of mutual supplementation for these two proteins appeared good.

Before testing mixtures of beans and Brazil nuts for protein supplementation, BNDF was incorporated at the 10% protein level in a AOAC rat diet. The animals lost weight during the first 5 days and died at the end of 2 weeks. A control group fed a casein-based diet showed normal growth. The deaths were attributed to the high selenium content of the BNDF, 63 ppm. The diet made with this BNDF contained 11.5 ppm Se, and it is known that Se concentrations exceeding 7 to 8 ppm are toxic to rats. It is of interest that when the toxic diet was modified to contain 10% casein (by replacing starch) in addition to the 10% BNDF protein, the toxicity was removed and the rats grew at a rate similar to that of a 10% casein diet.

In subsequent experiments the BNDF which contained 17 ppm Se was used. The diet with the highest proportion of BNDF contained 0.6 ppm Se originating in this ingredient. The results of the rat feeding studies are summarized in Table III. In the same table the following are also shown: protein evaluation results obtained microbiologically (SZRNV); amino acid scores (AAS) based on the FAO/WHO 1973 reference pattern, plus the same scores as percent of that for casein; essential amino acid indices (MEAAI) derived according to Mitchell (1954), and the same indices as percent of that for casein.

Table III. Protein Efficiency Ratio (PER), *S. zymogenes* Relative Nutritive Value (SZRNV), Amino Acid Score (AAS), and Modified Essential Amino Acid Index (MEAAI) of Beans and Mixtures of Beans with Brazil Nut Defatted Flour (BNDF)[a]

	PER[b]	% PER	SZRNV	AAS	% AAS	MEAAI	% MEAAI
Casein	2.50 ± 0.06[c]	100	100	69	100	91	100
Beans + BNDF, 80:20	2.42 ± 0.07[c]	97	82	64	92	86	95
Beans + BNDF, 90:10	2.16 ± 0.03	84	76	47	68	84	92
Beans + BNDF, 95:5	1.92 ± 0.03	77	75	39	56	83	91
Beans	1.57 ± 0.03	63	69	31	45	82	90
Germinated beans	0.89 ± 0.07	36					

[a] The mixture ratios refer to protein. The beans were autoclaved. [b] $\bar{X}$ ± SEM. Adjusted to PER = 2.50. [c] These two means did not differ significantly at 0.05 P. All others, differed among themselves and the first two means at levels lower than 0.05 P.

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.

ACKNOWLEDGMENT

The donation of materials by the Michigan Foundation Seed Association and the Brazilian Instituto de Technologia de Alimentos has been greatly appreciated. Thanks are also due to Ursula Koch for assistance in the amino acid analysis.

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).

Received for review March 7, 1977. Accepted May 9, 1977. Michigan Agricultural Experiment Station Journal Article No. 8001.

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

Valdemiro C. Sgarbieri,* William J. da Silva,[1] Pedro L. Antunes, and Jaime Amaya-F.

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).

Departamento de Planejamento Alimentar e Nutricão, Faculdade de Engenharia de Alimentos, UNICAMP, Caixa Postal 1170, 13100 Campinas, Sao Paulo, Brasil.

[1]Instituto de Biologia, Departamento de Genética, UNICAMP, Caixa Postal 1170, 13100 Campinas, Sao Paulo, Brasil.

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;