# Effect of the Extraction of a Hemagglutinin on the Nutritive Value of *Amaranthus leucocarpus* Seeds

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## ABSTRACT

Hemagglutinins present in *Amaranthus leucocarpus* seed meal were extracted with saline buffer and nutritive value was estimated by PER, NPR as well as Digestible Energy Consumption Evaluations. Protein digestibility was determined both *in vitro* and *in vivo*. Trypsin inhibitor activity was also determined. Saline-extracted Amaranth meal had significantly higher PER and NPR values than whole meal ($p<0.05$). There were no significant differences in digestible energy (Kcal/day/animal) and protein consumption (g/day/animal) between diets ($p<0.05$). *A. leucocarpus* improves in nutritive value after the saline extraction procedures and has a good potential as a complementary food due to its lysine content. In addition purified agglutinin may be a useful biochemical.

## INTRODUCTION

THE NEED for diversification of protein sources is especially imperative in developing countries. Many plants which are being rediscovered are valuable in human nutrition because of the high quality of their protein (Inglett, 1977; Desborough et al., 1981; Hill and Rawate, 1982; Kidwal and Zain, 1969; Mugerwa and Bwabye, 1974; Sugimoto et al. , 1981). One example is *Amaranthus*, a genus of particular interest for the role that it played during the religious ceremonies of the ancient Aztecs in Mexico (Early, 1977; Ruttle, 1976; Branch, 1978; Sánchez Marroquín, 1980). The Indians used the plant in a variety of forms (Early, 1977; Sánchez Marroquín, 1980) and the popped seeds are still used to prepare a confection very popular in some parts of the country.

The seed of *Amaranthus leucocarpus* (syn. *hypochondriacus*) is rich in protein and other elements important in human or animal diets (Sánchez Marroquín, 1980). However, it also contains an extractable protein that binds specifically to N-acetyl-D-galactosamine-containing structures and induces the aggregation of red cells in suspension (Zenteno and Ochoa, 1984). This protein belongs to a group of biological macromolecules called lectins (Goldstein et al., 1980; Lis and Sharon, 1977, Goldstein and Hayes, 1978). Since the presence of lectins in edible seeds has been related with low digestibility and toxic effects (Turner and Liener, 1975; Jaffé and Gaede, 1959), the aim of this study was to determine the nutritional value of a saline extracted meal of *Amaranthus leucocarpus* and compare it with that of the whole ground meal.

## MATERIALS & METHODS

FIVE kg of finely ground seeds (Mill: Arthur H. Thomas Co., PA, USA Mesh #40) were suspended in 10 parts (w/v) 0.9% NaCl solution and stirred 2 hr at 4°C. The pH was then adjusted to 4 with 4M acetic acid and the mixture allowed to stand overnight at 4°C. The sediment obtained by centrifugation (DAMON, IEC Division, JEC-HN-S centrifuge, Needham Hts, MA) at 3000 rpm for 15 min was washed three times with one part (5L) 0.2M acetate buffer pH 4. The supernatants were pooled and concentrated using Amicon filtration (Amicon, Lexington, MA) with a PMIO Diaflo membrane having pores of 10 Kda and/or freeze dried (FTS Systems, Inc. Stone Ridge, NY).

To test the agglutination capacity of the crude extract, a 2% suspension of human erythrocytes in PBS (Phosphate Buffer Saline, 0.02M $KH_2PO_4$, 0.9% NaCl, pH 7.4), was employed following the two fold serial dilution procedure in microtiter plates with an aliquot of the extract corresponding to 25 μg protein. Accordingly, the hemagglutination titer of the saline crude extract was defined as the reciprocal of the maximum dilution factor showing visible hemagglutination capacity.

Amino acid analyses were carried out on an automatic amino acid analyzer Durrum D-500 (Dionex, CA, USA) hydrolyzing 3 mg flour with about 500 μL HCl at 110°C in sealed tubes for 48 hr (Moore and Stein, 1963).

The trypsin-inhibitor capacity of the saline extract was estimated spectrophotometrically according to Kasell (1970), determining the rate of hydrolysis of a synthetic trypsin-substrate, N-benzoyl-DL-arginine-p-nitroanilide (BAPA) (Sigma, St. Louis, MO), at 405 nm (Spectronic 2000, Bausch and Lomb, Orange, CA). One inhibitor unit was defined as 0.1M BAPA not hydrolyzed by 20 μg trypsin in 5 min. The specific inhibition of activity was defined as the number of inhibitor units per mg of protein in the crude extract. Protein concentration for this assay was determined colorimetrically by the method of Lowry et al. (1951).

Whole grain flour and the extracted meal of *Amaranthus* were analyzed for protein (N × 6.25), fat, carbohydrates and ash according to AOAC (1980) procedures. Gross energy was determined using an adiabatic calorimeter (Parr Instruments, Co. Moline, IL).

Protein quality was assayed *in vivo* by means of the protein effi-

*Table 1—Basal diet composition*[a]

| Ingredient[b] | % |
|---|---|
| Corn Oil | 8.0 |
| Vitamin Premix[c] | 1.0 |
| Mineral Premix[d] | 5.0 |
| $Cr_2O_3$ | 0.2 |
| Cellulose | 1.0 |
| Water | 5.0 |
| Protein Source to make 10% of diet | |
| Starch and Dextrose to make 100% of diet | |

[a] Corn oil, minerals premix, cellulose and water were adjusted after proximate analysis of ingredients sample was calculated as (1.6% N in sample) × 100, according to AOAC method 43.212, which is applicable to materials with %N above 1.8.

[b] All the ingredients except corn oil were from Bioserv, Inc. (New Jersey).

[c] The vitamin premix supplied the following g/kg of diet: ascorbic acid 0.45, biotin 0.0002, calcium pantothenate 0.03, choline 0.633, folic acid 0.0009, inositol 0.05, menadione 0.02, niacin 0.04, PABA 0.05, pyridoxine 0.01, riboflaviin 0.01, thiamin 0.01, Vitamin A 9,000 IU, Vitamin B-12 0.01mg, Vitamin D 1,000 IU, Vitamin E 25 IU.

[d] Mineral premix supplied the following g/kg of diet: aluminium 0.0005, calcium 11.0865, chlorine 4.7935, copper 0.0175, fluorine 0.0027, iodine 0.0030, iron 0.385, magnesium 0.3812, manganese 0.0055, phosphorus 2.5305, potassium 5.8820, sodium 1,3690, sulphur 0.1162, zinc 0.0637.

*Table 2—Composition of* Amaranthus *seed meal before and after saline extraction*

| | Components (%) | | | |
|---|---|---|---|---|
| Sample | Protein (N×6.25) | Fat | NFE[a] | Ash |
| Whole flour | 14.69 | 8 | 74.04 | 3.27 |
| Extracted flour | 13.25 | 8.5 | 76.58 | 1.67 |

[a] Determined by difference. Only traces of crude fiber were found.

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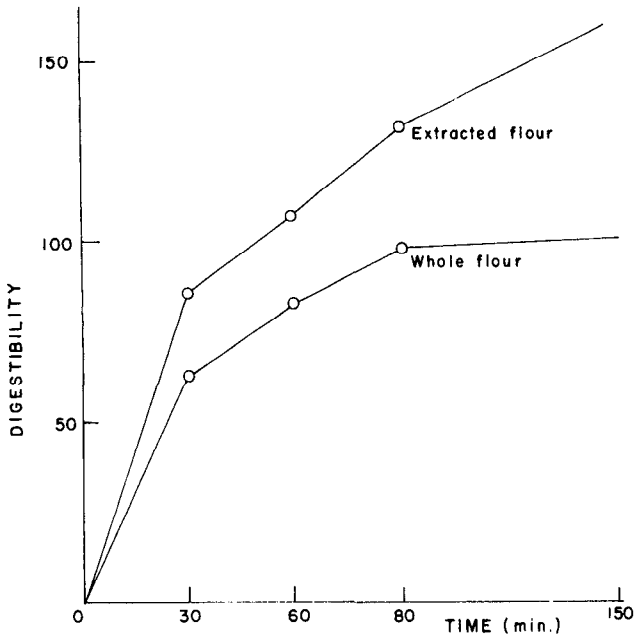


*Fig. 1—In vitro digestibility of extracted and whole seed flour of* Amaranthus leucocarpus *seeds as determined by the liberation of α-amino acid N following trypsin hydrolysis.*

ciency ratio (PER) according to AOAC (1980) procedures. Thirty weanling Sprague Dawley rats were randomly assigned into three groups and each fed either a diet containing saline extracted *Amaranthus* meal, whole meal or reference protein (ANRC Casein) for 28 days. The basal diet is presented in Table 1. All diets contained 10% protein and were of equal caloric densities. The experimental animals were housed individually in stainless steel cages held under controlled environmental conditions, at 22 ± 1°C, 55–65% relative humidity under a 12 hr light-dark cycle. Water and food were provided *ad libitum*.

*Table 3—Essential amino acid composition of whole and extracted* Amaranthus *flour*[a]

| Amino Acid | Whole flour g/16g N | Egg ratio % | Extracted flour g/16g N | Egg ratio % |
|---|---|---|---|---|
| Lysine | 3.65 | 47 | 3.19 | 41 |
| Threonine | 2.69 | 55 | 2.22 | 45 |
| Valine | 4.21 | 59 | 2.97 | 42 |
| Methionine | 2.40 | 75 | 1.83 | 58 |
| Isoleucine | 3.61 | 61 | 2.63 | 45 |
| Leucine | 5.81 | 67 | 4.54 | 52 |
| Phenylalanine | 4.27 | 78 | 3.45 | 63 |
| Tryptophane[b] | - | | - | |
| Chemical score[c] | 47 (Lys) | | 41 (Lys) | |
| Essential amino Acid index[d] | 62 | | 49 | |

[a] Essential amino acids for the human adult.
[b] Was not determined.
[c] Mitchell and Block (1946).
[d] Calculated as the geometric means of the egg ratios of the essential amino acids (Oser,1951).

Under the same experimental conditions, NPR and estimated NPU were determined according to Bender and Doell (1957). Apparent protein digestibility and digestible energy were determined according to Valencia et al. (1979) and Edwards and Gillis (1959) respectively, utilizing a $Cr_2O_3$ marker, fed throughout the experiment and collecting fecal samples during the last 10 experimental days.

The experimental arrangement was a randomized design. Common variance was tested previous to the selection of parametric or non-parametric procedures, namely t test and Man-Whitney (Zar, 1974) respectively.

*In vitro* digestibility was determined according to Marquez and Lajolo (1981) with trypsin, measuring the α-amino nitrogen produced (Kabat, 1961). A sample of *A. leucocarpus* seed meal containing 5 mg protein/mL in 0.05M phosphate buffer pH 7.0 was incubated with an enzyme solution (1 mg/mL) in $10^{-3}$M HCl at 37°C. The enzyme:substrate ratio was maintained at 1:40 and at different incubation intervals aliquots of 5 ml were taken and mixed with trichloroacetic acid (TCA) to a final TCA concentration of 5%. After standing period of 1 hr, the precipitated undigested material was separated by centrifugation at 3000x *g*, and the liberated amino acids present in the supernatant were assayed colorimetrically with ninhydrin. Leucine was used as a standard. Digestibility was expressed as mg of amino acid liberated per gram of protein.

*In vitro* digestibility with trypsin of both whole and the extracted flours was tested to estimate growth depressing effects due to the presence of only trypsin inhibitors (Turner and Liener, 1975).

## RESULTS & DISCUSSION

THE TRYPSIN inhibitor activity of *A. leucocarpus* crude extract was 3.3 specific units. However, unextracted trypsin inhibitors may be responsible for differences in α-amino nitrogen liberation provoked by the trypsin digestion of the meals, as suggested by Fig. 1.

As shown in Tables 2 and 3 there were few differences in chemical composition between the whole flour and the saline-extracted flour.

The essential amino acid composition of the whole and extracted *Amaranthus* flour showed a decrease in all amino acids after the extraction procedure which is reflected in an Essential Amino Acid Index change from 62 to 49 in whole and extracted four, respectively. Lysine was the limiting amino acid in both cases (Table 3). These methods are not very good indicators of protein quality and did not agree with the biological evaluation.

No significant differences ($p<0.05$) were found in apparent digestible energy or protein consumption between the saline extracted and the whole Amaranth meal (Table 4), thus the biological methods utilized reflected only the effect on protein quality. The analysis of the Amaranth meals showed significant differences ($p<0.05$) between the whole and extracted meals by means of the PER, NPR and estimated NPU, where an increase in protein quality after the saline extraction was observed (Table 4).

The results suggest that the presence of agglutinating activity in *Amaranthus leucocarpus* might have a possible negative effect in protein quality ($p<0.05$), that could possibly be due to the binding of agglutinins to receptor sites on the surface of the intestinal epithelial cells, interfering with the absorption of nutrients across the intestinal wall. This type of mechanism has been suggested by Jaffé and co-workers who reported that a decrease in *in vivo* digestibility in rats was observed when

*Table 4—Biological evaluation of the whole and saline extracted* Amaranthus leucocarpus *flours*

| Source of diet | Apparent digestible energy consumption[a] (Kcal/day/animal) | PER[a] | PER[a] corrected | NPR[a] | NPU[a] estimated (%) | Apparent protein digestibility (%) | Apparent digestible[a] protein consumption (g/day/animal) |
|---|---|---|---|---|---|---|---|
| Casein[c] (ANRC) | 46.37 | 3.43 | 2.50 | 5.22 | 84.83 | 90.81 | 1.27 |
| Extracted flour | 43.96[a] | 2.95[a] | 2.15[a] | 4.57[a] | 74.14[a] | 79.29 | 1.10[a] |
| Whole flour | 39.91[a] | 2.68[b] | 1.95[b] | 4.06[b] | 66.23[b] | 77.79 | 1.00[a] |

[a] Means with different superscript are significantly different ($p<0.05$).
[c] Bioserv. (New Jersey).

agglutinins from *P. vulgaris* were added to diets containing casein (Jaffé et al., 1955; Jaffé and Camejo, 1961).

## CONCLUSIONS

It appears that the presence of hemagglutinating activity in *Amaranthus leucocarpus* seeds affected protein quality. Measured agglutinating activity was considerable whereas trypsin inhibitor activity in the same saline extract was extremely low. Additionally, no relationship was found between the protein quality evaluated by the biological methods and the amino acid composition; the saline-extracted flour showed higher values in PER and NPR and had a generally lower Essential Amino Acid Index and Chemical Score. Finally, the extraction procedure can yield a potentially useful biochemical and a residual flour with higher nutritive value.

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