

CHEMICAL CONSTITUENTS, *in vitro* PROTEIN DIGESTIBILITY, AND PRESENCE OF ANTINUTRITIONAL SUBSTANCES IN AMARANTH GRAINS¹

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SUMMARY

The chemical composition, content of antinutritional factors, and the *in vitro* protein digestibility of grains of the pseudo-cereal *Amaranthus* were analyzed. The plants were grown in Brazil (without fertilizer), Puerto Rico (100 kg N/ha), and California (200 kg N/ha). The seed analysis gave the following values (o/oDM): 14.4 - 16.9 protein (N x 6.25), 4.8 - 6.8 fat, 2.5 - 3.9 ash, and 2.3 - 2.9 crude fiber. The trypsin inhibitors, phenolics and saponine contents were low, and the phyto-hemagglutinin activity, fairly low. The *in vitro* protein digestibility was 61 - 76^o/o. Digestibility was not correlated to the analyzed proximal composition nor to the antinutritional factors. The grain composition indicates a food value equivalent to that of conventional food grains.

INTRODUCTION

A large part of the world's population predominantly consumes vegetable diets, and only industrialized countries consume considerable

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amounts of animal food products. In the developing countries, large segments of the population live on diets with a low protein/calorie content. This risk of being undernourished endangers health; therefore, in 1974 the Commission of International Relations of the National Research Center, selected 23 tropical plant species, to develop more nutritious foods, among them *Amaranthus*. The grain amaranth has a high content of protein as well as lysine. The young plants are also traditional tropical leafy vegetables, and both the green plant and the grains, are much better balanced than the conventional cereals (1). Grain yields from four to six tons/ha have been reported for different *Amaranthus* species, depending on the field population density (2).

In the study herein discussed, seeds of five species were analyzed for their proximal composition and content or presence of antinutritional factors: saponins, polyphenols, phytohemagglutinins, and trypsin inhibitors. The *in vitro* protein digestibility was also studied, as it may be affected by some of those factors (3).

MATERIAL AND METHODS

Plant Material

The following *Amaranthus* species were grown:

A – In Brazil (Belo Horizonte, Minas Gerais):

- *A. anclancalius* – Black-coated seed
- *A. gangeticus* – Black-coated seed
- *A. hypochondriacus* HH5 – “Yellow” coated seed
- *A. cruentus* HH1 – “Yellow” - coated seed

Belo Horizonte represents a dry, tropical climate, at a high altitude (1,000 m above sea level). The plants were not irrigated nor fertilized.

B – In Puerto Rico (Mayaguez):

- *A. anclancalius* – Black-coated seed
- *A. hypochondriacus* HH5 – “Yellow”-coated seed
- *A. mantegazzianus* – “Yellow”-coated seed

Mayaguez represents a hot, humid and rainy subtropical climate at a lowland area. The plants were fertilized with 100 kg N/ha, but not irrigated.

C – In California (Davis):

- *A. anclancalius* – Black-coated seed
- *A. gangeticus* – Black-coated seed
- *A. hypochondriacus* HH5 – “Yellow”-coated seed
- *A. mantegazzianus* – “Yellow”-coated seed

Davis represents a hot temperature climate, at a dry, lowland area. The grains were harvested from irrigated (every two weeks) and fertilized plants (200 kg N/ha).

At all locations, the row distances were 20 ± 5 cm, with plant distances within the row, of about 10 cm. Sowing was always made in the early

summer. Seeds were harvested when they were fully mature, i.e., at the age of 14 weeks in Brazil, 12 in Puerto Rico, and at 16 to 18 weeks in California. *Amaranthus* is a short-day plant, and the flowering and seed setting data, as well as the subsequent maturing time for the seeds, are thus related to the cultivation sites distances from the Equator, and their altitude above sea level.

Analytical Methods

The dried seeds (60°C) were ground to pass a 35-mesh sieve. The following determinations were carried out: moisture, crude protein (N x 6.25), lipids (ether extract) and ash (4), crude fiber (5), *in vitro* protein digestibility (6), trypsin inhibitor activity (7, 8) and phytohemagglutinin (9).

The ethanol-soluble phenolic substances were analyzed as follows: 500 mg samples were extracted three times with ethanol (80%o, 3 x 100 ml, during 3 x 2 hr) in a Soxhlet apparatus. The combined extracts (300 ml) were then analyzed for phenols with Folin-Denis solution (10), at 470 nm, using tannic acid as a reference (Carlsson, unpublished).

Saponins were analyzed by a modified method (11, 12). Samples weighing 30 mg were mixed with 20 ml of a suspension of washed erythrocytes. (In the present study human blood, type A, Rh⁺, was used; 20 ml of washed erythrocytes were suspended in 980 ml of 0.85%o NaCl). Hemolytic activity was noted between 30 minutes and up to 12 hours, and the degrees of hemolysis were attributed as follows:

No hemolysis within 12 hours	— 0	} low activity
100%o hemolysis within 12 hours	— 1	
20 — 40%o hemolysis within 12 hours	— 2	
50 — 90%o hemolysis within 12 hours	— 3	} high activity
100%o hemolysis within 12 hours	— 4	
100%o hemolysis within 30 minutes	— 5	

All analytical data were based on duplicates and average values are given. Standard deviations were less than $\pm 5\%$.

RESULTS AND DISCUSSION

The proximal composition of the amaranth grains is given in Table 1. As the data reveal, there was little variation in their composition, in spite of differences in fertilizer levels, growth conditions and climates.

Values for crude protein in grain amaranth have been reported between 11 and 19%o of dry matter (2, 13-29). Our values varied from 14.4 to 16.9%o, being within the normal range. The lowest crude protein content was detected in the non-fertilized Brazilian samples. Also, the seeds from plants which received the highest fertilizer level, seemed to have the highest crude protein level, a finding which confirmed the results obtained by Carlsson (13).

The fat (ether extract) contents varied from 4.8 to 6.6%o and were similar to values cited in the literature (2, 13, 16, 19-22, 24-29).

TABLE 1

PROXIMAL COMPOSITION OF *Amaranthus* SPECIES SEEDS
(Values are expressed per 100 g, dry-weight basis)

Sample ^a	Crude protein ^b	Ether extract	Ash	Crude fiber
AhBy	14.4	6.4	2.7	2.6
AhPRy	15.0	6.2	2.9	2.6
AhCy	14.4	6.2	3.5	2.6
AaBb	14.4	5.1	2.5	2.9
AaPRb	16.2	5.3	3.0	2.6
AaCb	16.2	5.4	3.5	2.6
AgBb	14.4	6.6	3.5	2.6
AgCb	15.6	6.5	3.8	2.3
AmPRy	15.0	5.8	3.2	2.5
AmCy	16.9	5.8	3.9	2.8
AcBy	14.4	4.8	2.8	2.4

a Ah (*A. hypochondriacus* HH5), Aa (*A. anclancalius*), Ag (*A. gangeticus*), Am (*A. mantegazzianus*), Ac (*A. cruentus* HH1); B (Brazil), PR (Puerto Rico), C (California); y (yellow), b (black).

b N x 6.25.

The ash contents (2.5 - 3.0^o/o were found to be in accordance with literature values: 2.3 - 5.8^o/o (13, 15, 20, 22, 24-29). Higher nitrogen fertilizer levels resulted in a higher ash content (Table 1).

Crude fiber (2.3 - 2.9^o/o) was lower than values reported in literature: 2.9 - 8.8^o/o (13, 15, 20, 22, 24-28). This difference could be due to the use of different analytical methods, but also to species differences.

The proximal composition of some cereals grown in Brazil, such as rice, corn, sorghum and wheat (30), were compared to that of amaranth. The latter grains had higher protein content than rice, corn and sorghum, while it proved to be similar to wheat. The fat, ash and crude fiber contents of amaranth seeds were similar to those of corn, sorghum and wheat and higher than values found in rice.

The antinutritional factors were measured as the activity values of trypsin inhibitors, phytohemagglutinins and saponins, and as the polyphenols content (Table 2). The *in vitro* protein digestibility is also given in the same Table.

The trypsin inhibitors ranged from about 900 to 2,300 TIU/g for seeds with black coating (*A. anclancalius*, *A. gangeticus*) and from 3,000 to 5,454 TIU/g for seeds with yellow coating. The highest value was detected in *A. hypochondriacus* HH5. For *A. hypochondriacus* S. Wats, a value of 8,000 TIU/g was recorded (20). Raw soybeans have values comprised between 15,000 and 110,000 TIU/g (31). Therefore, based on these results, the several amaranth grain varieties studied have a much lower trypsin inhibitors activity than soybean.

TABLE 2
 TRYPSIN INHIBITORS, POLYPHENOLS, PHYTOHEMAGGLUTININ AND
 SAPONINS IN AMARANTH SEEDS AND THEIR *in vitro* PROTEIN
 DIGESTIBILITY

Samples ^a	Trypsin inhibitors (TIU/g) ^b	Polyphenols (g tannic acid/100 g DM)	Phytohemagglutinin (extract dilution) ^c	Saponins (degree of hemolysis) ^d	<i>In vitro</i> protein digestibility ^e
AhBy	5,150	0.35	2	2	76
AhPRy	5,006	0.42	3	2	70
AhCy	5,454	0.56	2	2	70
AaBb	2,286	0.35	2	2	64
AaPRb	1,119	0.29	2	2	61
AaCb	1,175	0.20	3	1	64
AgBb	2,104	0.23	2	2	62
AgCb	938	0.41	2	2	62
AmPRy	4,784	0.33	2	1	67
AmCy	3,886	0.54	2	2	68
AcBy	2,994	0.27	2	2	70

a For abbreviations, see Table 1.

b TIU/g: trypsin inhibitors units per gram of sample.

c Numbers indicate the highest extract dilution (sample: 0.85^o/o NaCl = 1:10) that gave visible agglutination with blood type A, Rh⁺.

d 1 and 2 degrees of hemolysis represent low hemolytic activity.

e Values were corrected for casein (100^o/o digestible).

The polyphenols content, given as tannic acid equivalents of the dry matter (Table 2), ranged from about 0.2 to 0.4 for the black-coated seeds, and from 0.3 to 0.6 for the yellow-coated seeds. For different *Amaranthus* species, ranges of 0.02 to 0.25^o/o (13) and 0.05 to 0.46^o/o (25) have been recorded. Low-tannin content cultivars of sorghum have reported values of 0.40 to 0.46^o/o, while the high-tannin cultivars ranged from 3.44 to 3.60^o/o (32). Thus, amaranth grains seem to contain low tannin values.

The phytohemagglutinin activity can be considered low (Table 2). *A. hypochondriacus* HH5 presented activity to the third dilution. An activity to the fifth dilution level had been observed for the same species but of a different variety (20). For comparison purposes, an activity to the sixth level of dilution of bean extracts had been reported (33).

The hemolytic activity of amaranth seeds was also low (Table 2). Nevertheless, whether a low polyphenol content is harmless or not, remains to be investigated.

The *in vitro* protein digestibility varied from 61 to 64^o/o for the black-coated seeds, and from 67 to 76^o/o for the yellow-coated seeds (Table 2). Similar results had been notified for several *Amaranthus* species (13), as well as values of 65 and 53^o/o for *A. hypochondriacus* and *A. cruentus*,

respectively (27). The difference observed between the *in vitro* protein digestibility of black ($62 \pm 1^{\circ}/o$; $\bar{x} \pm SD$) and yellow seeds ($70 \pm 3^{\circ}/o$) confirmed the assumption that the protein of "white/naked" seeds was more digestible than the one of black-seeded *Amaranthus* (13). On the other hand, it is worthwhile to note that Carlsson (13) observed that *A. cruentus*, which has thick yellow seed cover, gave lower daily weight gains in a rat bioassay than *A. hypochondriacus*, with a thin yellow seed cover.

Variation in the *in vitro* protein digestibility showed no simple correlation for any of the analyzed chemical factors supposed to be anti-nutritional, or for the proximal composition. A negative correlation was found, however (13), between *Amaranthus*, *Atriplex* and *Chenopodium* grains and daily weight gains of rats ($r = -0.89$, $n = 17$).

The present results indicate that *Amaranthus* sp. seeds are equivalent in food value to food grains now of conventional use.

RESUMEN

COMPOSICION QUIMICA Y PRESENCIA DE SUBSTANCIAS ANTINUTRICIONALES EN SEMILLAS DE AMARANTO

Se determinó la composición química, el contenido de factores antinutricionales y la digestibilidad *in vitro* de semillas del pseudo-cereal *Amaranthus*. Las plantas fueron cultivadas en el Brasil (sin fertilización), Puerto Rico (100 kg N/ha) y California (200 kg N/ha). El análisis químico proximal reveló los valores siguientes ($^{\circ}/o$ MS): 14.4 - 16.9 proteína (N x 6.25), 4.8 - 6.8 grasa, 2.5 - 3.9 ceniza y 2.3 - 2.9 fibra cruda. Los tenores de inhibidores de tripsina, polifenoles y saponinas fueron bajos. La actividad fitohemaglutinante, relativamente baja. La digestibilidad *in vitro* de la proteína varió de 61 a 76 $^{\circ}/o$. La digestibilidad no se correlaciona con los constituyentes de la composición proximal, ni con los factores antinutricionales. La composición de las semillas indica ser un alimento de calidad equivalente a la de los granos de uso convencional.

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