Effect of Germination on the Chemical Composition and Nutritive Value of Amaranth Grain

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ABSTRACT

Changes in chemical composition and in nutritive value during germination of amaranth grain were studied. One variety each of *Amaranthus hypochondriacus, A. cruentus,* and *A. caudatus* was germinated for 0, 24, 48, and 72 hr. The sprouts were dried with air at 40°C for 18 hr and ground for proximate chemical analyses, total and reducing sugars, damaged starch, thiamin, and riboflavin. Only *A. cruentus* was analyzed for raffinose, stacchyose, ascorbic acid, phytic acid, niacin, and biotin. *A. caudatus* and *A. cruentus* were further subjected to protein fractionation at all stages of germination. These samples were also assayed raw and cooked for protein quality. No changes on a dry weight basis were observed in protein, crude fiber, and ash content, whereas lipid and phytic acid content decreased with respect to germination time. Reducing sugars, total sugars, and damaged starch increased with respect to germination time, whereas raffinose and stacchyose were not detected after 48 and 24 hr, respectively. All vitamins increased with respect to germination time, particularly riboflavin and ascorbic acid. There was an increase in albumins and a decrease in globulins with little change in glutelins. The alcohol-soluble proteins increased slightly. Germination did not change protein quality of raw grain, but cooking of germinated grain did.

The practice of sprouting seeds has been used to improve their nutritional value (Chen et al 1975, Wang and Fields 1978). Germination of the grain has important effects on the chemical composition, nutritive value, and acceptability characteristics of products for human consumption.

During seed germination, a breakdown of seed reserves, carbohydrates, and in some cases protein (Vanderstoep 1981) takes place. Germination causes an increase in several vitamins (Chen et al 1975). Stacchyose and raffinose, which are generally assumed to be responsible for flatulence, decrease during this process (Jaya and Venkataraman 1981).

After germination there is a decrease in the caloric content of the seed. Hence, the nutrient-energy ratio of some vitamins is higher than in the original seed.

Amaranth grain is a good source of high-quality protein (Senft 1979). It is thus important to know amaranth protein content and its nutrient composition during germination. This study was carried out to evaluate the chemical and nutritional changes that occur during germination of amaranth grain.

MATERIALS AND METHODS

Three amaranth species were used: *Amaranthus hypochondriacus,* variety 4EU; *A. cruentus,* variety 7EU; and *A. caudatus* variety 8 Peru. All varieties were grown at INCAP's experimental station in Guatemala in 1984.

For germination, the seeds were washed and soaked in a disinfecting solution (ethyl alcohol 70% and $CaCl_2$ 3%) for 5 min. Then they were washed thoroughly and soaked in distilled water (seed-to-water ratio of 1:5, w/v) for 5 hr at room temperature. The seeds were placed over a sterile sponge covered with a sterile paper towel to keep moisture constant, and germinated at 32°C.

Germination was carried out during 0, 24, 48, and 72 hr. After germination, the sprouts were dried in an air oven at 40°C for 18 hr. Then they were ground to pass 40 mesh, and the following analyses were performed in all samples: moisture, crude protein, lipid, crude fiber, and ash content by AOAC methods (1984). Thiamin and riboflavin were determined by the AOAC fluorometric method (1984), total soluble sugars by the phenol-sulfuric method (Southgate 1976), reducing sugars by the method described by Southgate (1976), and damaged starch by the method suggested by Farrand (1964). *A. cruentus* and *A. caudatus* were also analyzed for ascorbic acid content using 2,6-dichlorophenol-indophenol (Osborne and Boogt 1978), for niacin and biotin by the microbiological method of the AOAC (1984), for raffinose and stacchyose using the method described by Tanaka et al (1975), and for phytic acid by the method suggested by Wheeler and Ferrel (1971). Protein fractions were extracted stepwise following the method of Bressani and Mertz (1957). Five grams of each sample was mixed with 50 ml of extractant under magnetic stirring. Fractions 1 (H_2O) and 2 (5% KCl) were obtained from two 60-min periods of stirring, whereas fraction 3 (70% aqueous ethanol) was obtained from two 60- and one 30-min period and fraction 4 (0.2% NaOH) from one 60-min period. After extraction, the mixtures were centrifuged for 15 min at 3,000 rpm and nitrogen was determined in each of the combined supernatants and in the residue using the micro-Kjeldahl method.

For net protein ratio (NPR) assays, seeds were germinated and cooked after germination. From raw and cooked germinated samples, flour was made to provide the diets with a 10% crude protein level. Composition of the diets included 4% mineral salts (Hegsted et al 1941), 1% cod liver oil, and 5% cotton seed oil. The diets were adjusted to 100% with cornstarch. For every 100 g of diet, 5 ml of vitamin solution was added (Manna and Hauge 1953). Diets were analyzed for their protein content (AOAC 1984).

The rats were of the Wistar strain from INCAP's animal colony. Diets were fed ad libitum to groups of 21-day-old rats distributed by weight among all diet groups so that initial weight did not differ more than ±5 g. Rats were placed in individual all-wire screen cages, with water available at all times, assigning eight animals per dietary treatment. NPR experiments were conducted for 14 days. Casein was used as the control (Bender and Doell 1957).

RESULTS AND DISCUSSION

After 24 hr at 32°C, from 30 to 40% of the seeds had germinated, and germination increased to 75-85% after 48 hr and did not change after 72 hr. Moisture, crude protein (N × 6.25), ether extract, crude fiber, and ash content of the samples are presented in Table I, in a wet and dry weight basis. The results are the average of two determinations. During germination there was a marked increase in moisture content. Protein, ether extract, crude fiber, and ash expressed on a dry and wet weight basis did not change significantly in any of the amaranth species used. The results on the proximate analysis of *A. hypochondriacus* agree with the findings of Paredes-López and Mora-Escobedo (1989).

Thiamin, riboflavin, ascorbic acid, niacin, biotin, and folic acid contents of amaranth during its germination are presented in Table II on a wet and dry weight basis. Even though thiamin content did not increase significantly in *A. caudatus,* it did in *A. cruentus* and *A. hypochondriacus.* Riboflavin and ascorbic acid increased significantly during germination. These results are similar to the

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findings of other investigators (Vanderstoep 1981, Chen and Thacker 1978). There was no significant difference in niacin content between species and among germination times. The biotin content was significantly higher in *A. caudatus* than in *A. cruentus*. During germination these values increased significantly. The folic acid content was significantly higher in *A. caudatus*. These results probably reflect the metabolic activity of the grain during germination.

Table III shows reducing sugars, total soluble sugars, raffinose, and stacchyose contents. During germination, there was a decrease in storage carbohydrates and an increase in total soluble and reducing sugars due to the energy needs of the growing plant. The raffinose and stacchyose contents decreased quickly during the first 24 hr of germination and almost disappeared after 48 hr of the process. These results agree well with the results of investigations on other seeds (Jaya and Venkataraman 1981, Kon et al 1973).

Phytic acid content of amaranth at different germination times is shown in Table IV. Because germination is mainly a catabolic process that supplies important nutrients to the growing plant

TABLE I
Proximate Analysis of Amaranth Seeds During Germination[a]

Sample/ Germination Time (hr)	Moisture	Protein (%)	Ether Extract (%)	Crude Fiber (%)	Ash (%)
A. caudatus					
0	7.5	13.8 (14.9)	6.4 (6.9)	2.3 (2.5)	2.8 (3.0)
24	56.7	6.7 (15.5)	2.4 (5.6)	1.0 (2.4)	1.4 (3.2)
48	76.3	3.9 (16.3)	1.0 (4.1)	0.5 (2.3)	0.7 (3.1)
72	85.4	2.2 (15.1)	0.5 (3.7)	0.4 (2.4)	0.5 (3.2)
A. cruentus					
0	10.6	14.6 (16.3)	6.4 (7.1)	2.2 (2.5)	3.0 (3.4)
24	66.3	5.3 (15.8)	1.8 (5.5)	0.8 (2.4)	1.2 (3.5)
48	77.5	3.8 (17.1)	1.0 (4.6)	0.6 (2.6)	0.7 (3.2)
72	85.2	2.5 (16.9)	0.6 (4.0)	0.3 (2.5)	0.5 (3.4)
A. hypochondriacus					
0	11.4	14.3 (16.1)	6.3 (7.1)	2.0 (2.3)	3.2 (3.6)
24	61.4	6.1 (15.0)	2.5 (6.4)	1.0 (2.9)	1.3 (3.3)
48	81.7	3.0 (16.0)	1.0 (5.3)	0.5 (2.6)	0.6 (3.2)
72	88.3	2.0 (16.8)	0.6 (5.1)	0.3 (2.4)	0.4 (3.4)

[a] Values are expressed on a wet weight basis followed by dry weight basis values in parentheses.

TABLE II
Thiamin, Riboflavin, Ascorbic Acid, Niacin, Biotin, and Folic Acid Contents (mg/100 g) During Germination of Amaranth Seed[a]

Sample/ Germination Time (hr)	Thiamin	Riboflavin	Ascorbic Acid	Niacin	Biotin	Folic Acid
Wet weight basis						
A. caudatus						
0	0.093 ± 0.02	0.18 ± 0.03	6.5 ± 0.3	0.93 ± 0.24	47.5 ± 1.5	38.9 ± 1.2
24	0.102 ± 0.008	0.11 ± 0.02	4.19 ± 0.04	0.52 ± 0.12	21.7 ± 0.6	20.0 ± 0.7
48	0.033 ± 0.002	0.102 ± 0.002	2.86 ± 0.04	0.29 ± 0.06	12.7 ± 0.4	11.3 ± 0.3
72	0.022 ± 0.004	0.096 ± 0.003	2.75 ± 0.02	2.75 ± 0.02	8.3 ± 0.2	7.2 ± 0.2
A. cruentus						
0	0.072 ± 0.02	0.19 ± 0.03	4.13 ± 0.12	0.89 ± 0.25	38.0 ± 1.3	39.2 ± 1.3
24	0.030 ± 0.003	0.12 ± 0.02	1.44 ± 0.11	0.41 ± 0.09	13.9 ± 0.6	14.3 ± 0.4
48	0.025 ± 0.006	0.101 ± 0.002	2.01 ± 0.12	0.28 ± 0.06	10.8 ± 0.3	9.7 ± 0.4
72	0.018 ± 0.003	0.089 ± 0.003	1.80 ± 0.07	0.29 ± 0.04	6.9 ± 0.1	6.5 ± 0.2
A. hypochondriacus						
0	0.21 ± 0.02	0.24 ± 0.02	...	...	...	...
24	0.104 ± 0.004	0.158 ± 0.004	...	...	...	...
48	0.058 ± 0.007	0.097 ± 0.007	...	...	...	...
72	0.054 ± 0.006	0.084 ± 0.006	...	...	...	...
Dry weight basis						
A. caudatus						
0	0.10 ± 0.02	0.19 ± 0.03	7.05 ± 0.30	1.00 ± 0.26	51.3 ± 1.6	42.1 ± 1.3
24	0.11 ± 0.02	0.25 ± 0.05	9.68 ± 0.09	1.21 ± 0.29	50.2 ± 1.5	64.3 ± 1.7
48	0.14 ± 0.01	0.43 ± 0.01	12.05 ± 0.15	1.21 ± 0.27	53.4 ± 1.7	47.6 ± 1.2
72	0.15 ± 0.03	0.66 ± 0.02	18.83 ± 0.16	1.09 ± 0.28	56.6 ± 1.3	49.4 ± 1.5
A. cruentus						
0	0.08 ± 0.02	0.21 ± 0.03	4.62 ± 0.14	1.00 ± 0.28	42.5 ± 1.5	43.8 ± 1.5
24	0.09 ± 0.01	0.36 ± 0.05	5.28 ± 0.33	1.23 ± 0.27	41.3 ± 1.7	42.5 ± 1.2
48	0.11 ± 0.03	0.45 ± 0.01	8.92 ± 0.55	1.25 ± 0.26	48.2 ± 1.2	43.1 ± 1.8
72	0.12 ± 0.02	0.60 ± 0.02	12.16 ± 0.51	1.35 ± 0.29	46.4 ± 1.0	44.0 ± 1.3
A. hypochondriacus						
0	0.24 ± 0.02	0.27 ± 0.02	...	...	...	...
24	0.27 ± 0.01	0.41 ± 0.01	...	...	...	...
48	0.32 ± 0.04	0.53 ± 0.04	...	...	...	...
72	0.46 ± 0.05	0.72 ± 0.05	...	...	...	...

[a] Values are mean ± standard deviation.

TABLE III
Reducing Sugars, Total Soluble Sugars, Stacchyose, and Raffinose Contents in Amaranth During Germination[a]

Sample/ Germination Time (hr)	Reducing Sugars (%)	Total Soluble Sugars (%)	Raffinose (%)	Stacchyose (%)
Wet weight basis				
A. caudatus				
0	nd[b]	3.7 ± 0.04	0.86 ± 0.06	0.20 ± 0.08
24	nd	2.6 ± 0.3	0.12 ± 0.03	nd
48	0.52 ± 0.02	6.2 ± 0.7	nd	nd
72	1.08 ± 0.06	8.0 ± 0.6	nd	nd
A. cruentus				
0	nd	3.6 ± 0.4	0.67 ± 0.07	0.26 ± 0.05
24	nd	3.2 ± 0.3	0.08 ± 0.03	nd
48	0.74 ± 0.02	6.3 ± 0.5	nd	nd
72	1.22 ± 0.06	8.7 ± 0.7	nd	nd
A. hypochondriacus				
0	nd	3.5 ± 0.4	...	...
24	0.23 ± 0.04	2.4 ± 0.2	...	...
48	0.62 ± 0.04	10.6 ± 0.5	...	...
72	0.83 ± 0.07	7.7 ± 0.6	...	...
Dry weight basis				
A. caudatus				
0	0.0 ± 0.0	4.0 ± 0.4	0.93 ± 0.07	0.22 ± 0.09
24	0.0 ± 0.0	6.1 ± 0.8	0.28 ± 0.08	nd
48	2.2 ± 0.1	26 ± 3	nd	nd
72	7.4 ± 0.4	55 ± 4	nd	nd
A. cruentus				
0	0.0 ± 0.0	4.0 ± 0.5	0.75 ± 0.08	0.29 ± 0.06
24	0.0 ± 0.0	9.5 ± 0.8	0.25 ± 0.09	nd
48	3.3 ± 0.1	28 ± 2	nd	nd
72	8.3 ± 0.4	59 ± 5	nd	nd
A. hypochondriacus				
0	0.0 ± 0.0	4.0 ± 0.5	...	...
24	0.6 ± 0.1	6.1 ± 0.6	...	...
48	3.4 ± 0.2	58 ± 3	...	...
72	7.1 ± 0.6	66 ± 5	...	...

[a] Values are mean ± standard deviation.
[b] nd = Not detectable.

through hydrolysis of reserve nutrients, reduction in phytic acid was expected. Phytic acid is a source of phosphorus and cations during germination. Generally, phytase activity increases during sprouting (Reddy et al 1982). Since phytic acid may be one of the factors responsible for reducing mineral bioavailability, its reduction during germination may enhance the nutritional quality with respect to mineral bioavailability of amaranth. Table IV also shows damaged starch in the samples. The relativly high values for damaged starch of 20–25% in ungerminated samples may have resulted from sample milling. Damaged starch increased significantly during germination, probably due to enzymatic activities during this process.

Table V shows protein distribution of the germinated samples in terms of albumins, globulins, prolamins, and glutelins expressed as percentages of extracted protein on a wet and dry weight basis. The variability between species was not considerable. In the non-germinated samples, the most abundant proteins in *A. caudatus* were the glutelins, followed by albumins, globulins, and prolamins, whereas in *A. cruentus* the most abundant proteins were glutelins, followed by globulins, albumins, and prolamins. These results agree with those reported by García and Bressani (1987), who used a more complex fractionation technique. During germination, albumin content increased and globulin content decreased. Since total albumins plus globulins remained constant, it may be assumed that globulin breakdown products became part of the nitrogen determined under albumins, explaining the increase in this fraction. The prolamin content in amaranth during germination slightly increased.

Table VI presents the NPR results for the diets made with amaranth flour, averaged from eight determinations. Statistical analysis showed significant differences in weight gain, food intake, and NPR between the two amaranth species. There were also significant differences in weight gain, food intake, and NPR values between raw and cooked samples—the cooked, ungerminated amaranth being the best. From these results, it can be inferred that antinutritional factors in amaranth, if present, are thermolabile.

TABLE IV
Phytic Acid and Damaged Starch Content of Amaranth During Germination[a]

Sample/ Germination Time (hr)	Phytic Acid (%)	Damaged Starch (%)
Wet weight basis		
A. caudatus		
0	0.41 ± 0.03	18.0 ± 0.3
24	0.134 ± 0.009	11.8 ± 0.2
48	0.047 ± 0.0007	7.4 ± 0.1
72	0.020 ± 0.001	6.06 ± 0.06
A. cruentus		
0	0.29 ± 0.02	23.1 ± 0.5
24	0.081 ± 0.003	9.9 ± 0.2
48	0.009 ± 0.002	7.8 ± 0.2
72	nd[b]	6.2 ± 0.1
Dry weight basis		
A. caudatus		
0	0.44 ± 0.03	19.5 ± 0.3
24	0.31 ± 0.02	27.3 ± 0.5
48	0.20 ± 0.03	31.2 ± 0.6
72	0.14 ± 0.01	41.5 ± 0.4
A. cruentus		
0	0.32 ± 0.02	25.8 ± 0.6
24	0.24 ± 0.01	29.5 ± 0.6
48	0.04 ± 0.01	34.6 ± 0.8
72	nd	41.8 ± 0.7

[a] Values are mean ± standard deviation.
[b] nd = Not detectable.

TABLE V
Protein Fraction Distribution in Grain Amaranth During its Germination, Expressed as Percentage of Extracted Protein[a]

Germination Time (hr)	Albumins (%)	Globulins (%)	Prolamins (%)	Glutelins (%)	Residue (%)
A. cruentus					
0	13.9 (19.9)	15.9 (22.8)	3.0 (4.3)	20.3 (29.2)	23.1 (23.8)
24	5.9 (24.5)	4.2 (17.6)	1.4 (5.9)	6.2 (26.0)	8.7 (25.9)
48	5.5 (33.2)	1.8 (11.0)	0.4 (2.4)	4.5 (27.4)	5.8 (25.9)
72	3.2 (35.1)	0.8 (7.3)	0.7 (6.2)	3.3 (26.8)	3.6 (24.5)
A. caudatus					
0	16.8 (24.3)	14.8 (21.4)	2.6 (3.8)	18.6 (25.9)	21.7 (23.5)
24	8.7 (26.6)	6.8 (20.8)	1.3 (3.8)	7.8 (24.0)	10.7 (24.6)
48	6.1 (33.6)	2.7 (15.9)	0.8 (4.2)	4.7 (26.3)	5.0 (21.1)
72	4.0 (36.8)	0.5 (4.8)	0.5 (4.6)	3.3 (29.6)	3.5 (24.0)

[a] Values are expressed on a wet weight basis followed by dry weight basis value in parentheses.

TABLE VI
Net Protein Ratio of Raw and Cooked Germinated Samples

Sample/ Germination Time (hr)	Net Protein Ratio[a]
A. caudatus	
Raw	
0	2.2 ± 0.3
24	2.3 ± 0.4
48	2.1 ± 0.4
72	2.4 ± 0.4
Cooked	
0	3.4 ± 0.4
24	1.7 ± 0.4
48	2.0 ± 0.4
72	1.4 ± 0.3
A. cruentus	
Raw	
0	2.0 ± 0.3
24	2.3 ± 0.4
48	2.1 ± 0.4
72	2.7 ± 0.4
Cooked	
0	3.6 ± 0.4
24	1.5 ± 0.4
48	1.7 ± 0.4
72	1.4 ± 0.4

[a] Mean ± standard deviation.

There were no significant differences in weight gain, food intake, or NPR values for germination time in raw amaranth. Germination did not increase amaranth protein value. However, there was a significant difference in these same parameters in the germinated samples that were cooked. The statistical test (Tukey's) shows that the cooked, ungerminated sample was better than the others. Its NPR value was similar to that of casein. However, the cooked, germinated samples showed a decrease in NPR values. Even though this decrease was not statistically significant, it can be explained by the increase in number of free amino acids during germination, which makes them more available for thermal damage due to amino acid destruction or for formation of new linkages that rats are unable to hydrolize during digestion.

CONCLUSION

The germination process changes the chemical composition of amaranth grain. All vitamin contents increased during the process, particularly riboflavin and ascorbic acid. Total and reducing sugars and damaged starch values increased, whereas stacchyose and raffinose decreased. Germination did not increase the protein value of the grain or its biological utilization. Cooking germinated seeds decreased their nutritive value.

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