Digestibility and Nutritional Value of Crude Oil from Three Amaranth Species

Luis A. Garcia, Maria Antonieta Alfaro and **Ricardo Bressani***
Division of Agricultural Sciences, Institute of Nutrition of Central America and Panama, P.O. Box 1188, Guatemala, Guatemala, C.A.

Oils from a selection of *Amaranthus caudatus, A. hypochondriacus* and *A. cruentus* were extracted with hexane. The crude oils were analyzed for acid value, saponification and iodine number, and were included in basal casein diets for rat studies at 5 and 10% levels to replace equal amounts of refined cottonseed oil. The oils from *A. cruentus* and *A. hypochondriacus* were similar in the oil properties studied and different from *A. caudatus*. At either 5 or 10% food intake levels, weight gain and PER were not statistically different from values reported for cottonseed oil. True digestibility of amaranth oil was lower than that of cottonseed oil. *A. cruentus* oil gave the lowest digestibility. Oil levels induced statistical differences in food intake and digestibility. Oils from *A. caudatus, A. hypochondriacus* and cottonseed induced similar serum cholesterol levels, while oil from *A. cruentus* gave statistically higher values. Hemoglobin, hematocrit and serum proteins were similar among all groups. Microscopic analysis of the organs of the rats revealed some changes that were also found in cottonseed oil-fed rats. It was concluded that crude amaranth oil has lower digestibility than cottonseed oil, but that it is not responsible for growth-depressing effects when the seed is fed raw as compared to processed materials.

Amaranth grain is considered an excellent food for the future, both for man and animal species, due to its relatively high protein content (13-18%) and quality. Several workers have shown its nutritional value to be similar to that of casein, particularly if it is cooked before being submitted to biological evaluation (1-3). These results suggest the existence of thermolabile antinutritional factors, which inhibit its full nutritive potential, although palatability characteristics and bioavailability of nutrients should not be disregarded.

The chemical composition of amaranth grain has been described elsewhere (3-5), and it shows a lipid content of 5-9%. Some of these analyses demonstrated amaranth oil to contain 4.6% squalene (4). The purpose of this study was to evaluate the nutritional value and possible toxic effects of the oil from amaranth seeds as the factor responsible for the lower quality in raw as compared to thermally processed grain.

MATERIALS AND METHODS

A selection of each of three *Amaranthus* species was used: *A. caudatus, A. cruentus* and *A. hypochondriacus*. The seeds were obtained from the experimental farm of the Institute of Nutrition of Central America and Panama, located in Guatemala. They were ground and analyzed for protein and ether extract contents (6) prior to extraction with hexane during 28-32 hr in a soxhlet apparatus. Part of the hexane extract was stored at room temperature in amber glass bottles before distilling to remove the hexane and proceeding to the chemical analysis of the oil. Analyses carried out included acid value, saponification and iodine numbers by standard AOAC procedures (6). The rest was distilled immediately after extraction and stored under refrigeration at 4 C in amber glass bottles until their biological evaluation.

For biological evaluation, rats of the Wistar strain, 21 days old, from the animal colony of INCAP were used. A total of 64 animals were divided by weight in groups of eight, four males and four females, placed in individual wire-screen cages with raised screen bottoms. They were fed casein diets at a protein level of 9.5% for 28 days; the diets contained the oils at 5% and 10% levels. For comparison purposes, refined cottonseed oil was used as reference at the same levels. All diets contained vitamins (7), 4% mineral mixture (8), 1% cod liver oil, and corn starch to adjust to 100%. The rats were administered feed and water ad libitum and weighed every seven days. Feces were collected during the last seven days to determine the digestibility of the oils. To obtain endogenous fecal fat values, a vegetable oil-free diet was fed to a separate group. The PER value was calculated as weight gain per g of ingested protein. At the end of the assay, four rats from each group (two males and two females) were killed

*To whom correspondence should be addressed.

TABLE 1

Protein and Ether Extract Content of Three Amaranth Species[a]

Species	% protein (N × 6.25)	Ether extract %
A. caudatus	12.5	6.7
A. cruentus	15.0	7.9
A. hypochondriacus	14.9	7.1

[a]Results are the average of a triplicate analysis.

TABLE 2

Chemical Analyses of the Oils of Three Species of Amaranth, Cottonseed Oil, Soybean Oil and Corn Oil[a]

Origin of oil	Acid value	Saponification number	Iodine number
A. caudatus	14.6 (0.1)	139 (1)	87.2 (6.6)
A. cruentus	22.3 (0.1)	151 (3)	96 (7)
A. hypochondriacus	23.5 (0.1)	158 (3)	113 (2)
Cottonseed	1-5%[b]	190-198	105-114
Corn	1%[b]	187	109-133
Soybean	approx. 3%[b]	189-195	127-138

[a]Free fatty acids as oleic acid.
[b]Williams, K.T., 1950 (13).
( ), Numbers in parentheses are standard deviations.

TABLE 3

Biological Evaluation of Oils From Amaranth Seeds

Origin and level of oil		% Protein in diet (N × 6.25)	% Ether extract in diet	Average food intake[a,b] (g)	Average weight gain[a,b] (g)	PER[b]	True oil digestibility[b,c]
Cottonseed	5%	9.4	5.32	389.5 (36.6)	95.8 (18.4)	2.6 (0.3)	98.7 (0.4)[a]
A. caudatus	5%	9.5	4.74	387.8 (19.7)	101.4 (13.3)	2.8 (0.2)	94.1 (1.5)[b]
A. cruentus	5%	9.4	5.24	362.9 (49.1)	95.9 (15.8)	2.8 (0.2)	91.7 (0.7)[d]
A. hypochond.	5%	9.8	5.05	349.6 (51.7)	88.4 (17.8)	2.6 (0.2)	93.2 (1.2)[b,c]
Cottonseed	10%	9.8	9.63	353.3 (56.6)	89.9 (15.0)	2.6 (0.4)	98.8 (0.3)[a]
A. caudatus	10%	9.4	9.96	344.8 (31.9)	89.8 (15.0)	2.8 (0.3)	93.8 (0.6)[b]
A. cruentus	10%	9.5	9.50	348.3 (54.0)	92.3 (19.9)	2.8 (0.2)	91.1 (0.7)[d]
A. hypochond.	10%	9.3	9.40	344.8 (14.0)[a]	87.1 (6.4)[a]	2.7 (0.1)[a]	92.0 (0.9)[c,d]

[a]All values are statistically similar (P > 0.05).
[b]Numbers in parentheses are standard deviations.
[c]Statistical difference (P < 0.05) between values with different letters.
Initial average weight: 46.3 g (2.3).
Vegetable oil-free diet, % protein, 9.1; ether extract, 1.17%; average food intake 420.4 g (45.1); average weight gain, 106.5 g (15.0); PER 2.8 (0.1).

TABLE 4

Food Intake and True Digestibility by Species and by Level (5 or 10%) of Oil in Diets

Food intake by species		
Cottonseed oil	371.4 g	All statistically similar
A. caudatus oil	366.3 g	(P > 0.05)
A. cruentus oil	355.0 g	
A. hypochondriacus oil	346.9 g	
Food intake by level of oil in diet		
5%	372.4 g	Statistically different
10%	347.6 g	(P < 0.05)
True digestibility by species		
Cottonseed oil	98.8%	All statistically different
A. caudatus oil	93.9%	(P < 0.05)
A. cruentus oil	91.4%	
A. hypochondriacus oil	92.6%	
True digestibility by level of oil in diet		
5%	94.4%	Statistically different
10%	93.9%	(P < 0.05)

TABLE 5

Cholesterol Content in Serum of Rats Fed Casein Diets with Amaranth or Cottonseed Oil

Origin and level of oil		Cholesterol content (mg/dl)
Cottonseed	5%	156 (2)[d]
A. caudatus	5%	157 (5)[c,d]
A. cruentus	5%	176 (10)[a,b]
A. hypochondriacus	5%	171 (7)[b,c]
Cottonseed	10%	182 (7)[a,b]
A. caudatus	10%	189 (16)[a]
A. cruentus	10%	185 (6)[a,b]
A. hypochondriacus	10%	176 (1)[a,b]

Vegetable oil-free diet: 158 (1)[c,d].
( ), Numbers in parentheses are standard deviations values with different letters.
[a,b,c,d]Statistical difference (P < 0.05) between values with different letters.

to determine organ weight and for macro and microscopic analyses. Hemoglobin (9) and hematocrit (10) were determined in blood, and cholesterol (8), total proteins, albumins and globulins (11) were determined in serum.

The statistical analyses included one- and two-way analysis of variance, followed by a media comparison in which Tukey's student range was used at a level of 5% probability (12).

RESULTS AND DISCUSSION

Table 1 presents the results for protein and ether extract contents in the flours used in this experiment. Ether extracts were 6.7, 7.9 and 7.1% in *A. caudatus, A. cruentus* and *A. hypochondriacus,* respectively.

Table 2 shows the results of the chemical analysis of the oils extracted from the three species, as well as values reported in the literature for cottonseed oil, soybean oil and corn oil. The acid value indicates a high content of free fatty acids in amaranth oil, while the low saponification number, compared to other vegetable oils, shows a high content of unsaponifiable fat, which could be due to the presence of such substances as squalene or wax. Iodine numbers of the oils of *A. cruentus* and *A.*

TABLE 6

Hemoglobin, Hematocrit and Serum Protein Contents in Rats Fed Casein Diets With Amaranth or Cottonseed Oil

Origin and level of oil		Sex	Hb[a]	Ht[a]	Total prot[a]	Albumins[a]	Globulins[a]		
Cottonseed	5%	M	15.5	42.0	6.40	3.01	2.07	0.93	0.43
		F	15.9	44.5	5.35	2.72	1.37	0.82	0.43
Vegetable oil-free diet		M	16.0	43.0	6.40	2.82	2.11	0.91	0.54
		F	15.1	45.5	5.60	2.69	1.74	0.72	0.54
A. caudatus	5%	M	15.4	46.5	5.30	2.36	1.83	0.71	0.38
		F	14.6	46.0	6.20	2.90	1.88	0.98	0.42
A. cruentus	5%	M	14.7	44.0	6.35	2.40	2.23	1.06	0.60
		F	15.9	45.0	5.10	2.16	1.59	0.86	0.48
A. hypochondriacus	5%	M	15.5	44.0	5.85	2.41	1.94	0.99	0.51
		F	14.1	46.5	6.05	2.56	1.94	1.07	0.47
Cottonseed	10%	M	15.2	43.0	5.55	2.33	1.82	0.93	0.46
		F	16.0	43.0	6.35	2.64	2.04	1.15	0.51
A. caudatus	10%	M	16.1	43.0	5.90	2.37	2.04	1.06	0.43
		F	15.0	45.5	5.70	2.32	1.74	1.01	0.62
A. cruentus	10%	M	14.8	46.5	6.00	2.45	2.02	0.97	0.55
		F	14.9	45.5	6.90	2.93	2.18	1.25	0.54
A. hypochondriacus	10%	M	13.9	45.0	6.50	2.69	1.91	1.06	0.82
		F	14.8	45.0	6.15	2.51	2.15	1.03	0.50

[a]Values in g/dl.

TABLE 7

Weight of Organs of Rats Fed Casein Diets With Amaranth or Cottonseed Oil[a]

Origin and level of oil		Sex	Liver	Kidneys	Heart	Lungs	Spleen	Pancreas	Testicles
Cottonseed	5%	M	6.88	1.28	0.71	1.19	0.29	0.36	2.24
		F	6.94	1.32	0.65	1.29	0.37	0.36	—
Vegetable oil-free diet		M	7.34	1.28	0.81	1.27	0.44	0.51	2.19
		F	5.68	1.14	0.71	1.25	0.27	0.31	—
A. caudatus	5%	M	7.25	1.20	0.71	1.20	0.38	0.27	1.79
		F	5.48	1.15	0.66	1.18	0.33	0.29	—
A. cruentus	5%	M	7.91	1.23	0.57	1.21	0.34	0.43	2.43
		F	6.49	1.33	0.64	1.15	0.35	0.32	—
A. hypochondriacus	5%	M	6.46	1.13	0.64	1.01	0.30	0.31	2.11
		F	6.46	1.09	0.55	1.07	0.33	0.31	—
Cottonseed	10%	M	6.35	1.19	0.59	1.12	0.29	0.33	1.85
		F	5.58	1.08	0.57	1.07	0.34	0.27	—
A. caudatus	10%	M	6.55	1.19	0.52	1.14	0.32	0.31	2.13
		F	6.63	1.11	0.61	1.14	0.32	0.29	—
A. cruentus	10%	M	6.37	1.12	0.59	1.02	0.26	0.28	1.79
		F	8.06	1.27	0.69	1.12	0.36	0.29	—
A. hypochondriacus	10%	M	6.72	1.13	0.61	1.07	0.29	0.36	2.24
		F	6.07	1.18	0.62	1.01	0.34	0.25	—

[a]Values given in g.

TABLE 8

Microscopic Changes in Organs of Rats Fed Casein Diets With Amaranth or Cottonseed Oil

Origin and oil level		Observations
Cottonseed	5%	Tissues of liver, muscle and spleen were normal; kidneys showed a slight glomerular congestion.
A. caudatus	5%	Turbid tumefaction and hydropic degeneration of some cells in the liver; turbid tumefaction in kidneys; slight interstitial edema in muscle.
A. cruentus	5%	Slight turbid tumefaction in cells of the liver and kidneys; hydropic degeneration in cells of the kidneys; presence of small hyaline cylinders. Interstitial edema in the heart.
A. hypochondriacus	5%	Severe turbid tumefaction and hydropic degeneration in hepatic tissue. Slight turbid tumefaction in kidneys.
Cottonseed	10%	Slight turbid tumefaction in liver and kidneys; muscle, heart and spleen were normal.
A. caudatus	10%	Slight turbid tumefaction in liver. Hydropic degeneration of tubular cells of kidneys. Interstitial edema in muscle tissue. Spleen was normal.
A. cruentus	10%	Slight turbid tumefaction in liver; kidneys had cells with slight hydropic degeneration; muscle, heart and spleen were normal.
A. hypochondriacus	10%	Turbid tumefaction and slight hydropic degeneration in hepatic tissue. Renal tissue presented hydropic degeneration of tubular cells and slight edema in the renal pelvis. Muscle showed slight perivascular edema.
Vegetable oil-free diet		Slight edema in Kienan spaces of the liver. Muscle showed congested blood vessels; kidneys, heart and spleen were normal.

hypochondriacus are comparable to those of cottonseed and corn oils. The oil from *A. caudatus* seems to have a higher content of saturated fatty acids. It is noteworthy to mention that amaranth oils showed sedimentation of white matter after storage at 4 C for 4–5 days. This precipitated matter appeared like an amorphous solid and became liquid again when the temperature increased. This amorphous solid could very well be wax.

The biological evaluation shown in Table 3 indicated amaranth oil to be as good as cottonseed oil as judged by food intake, weight gain and PER values, which were all statistically similar ($P > 0.05$). Still, animals fed with diets containing cottonseed oil consumed more diet at the two oil levels than did the animals fed amaranth oil. True digestibility was higher for cottonseed oil than for amaranth oil.

It is important to note that food intake and true digestibility values were statistically different at the 5 and 10% levels, independent of the origin of the oil. For the diets at the 5% level, the average food intake was 372.4 g/rat and true digestibility 94.4%, while for those at the 10% level, values were 347.6 g/rat and 93.9%, respectively (Table 4). This, of course, is to be expected because at 10% oil, energy in the diet was higher, which resulted in lower intake. Even though oil intake increased 1.8 times at the 10% oil level over the intake at 5% oil level, the reduced weight gain on amaranth oil was of the same magnitude as that for cottonseed oil, which may be indicative of its lack of toxicity. However, digestibility at the higher intake level was reduced somewhat. It is difficult, however, to accept this reduction as being responsible for the lower quality of raw grain.

Cholesterol analysis of the serum of rats showed that oils from *A. caudatus* and *A. hypochondriacus* are similar to cottonseed oil. The oil from *A. cruentus* at the 5% level, however, had a cholesterol content similar to those at the 10% level for the other oils (Table 5).

Hemoglobin, hematocrit and serum protein contents are shown in Table 6. They were normal for all animals.

As was to be expected, the weights of the organs were similar in all the animals (Table 7).

The macroscopic analysis of the organs of the rats did not show any damage. However, the microscopic analysis revealed several tissue changes (Table 8). Damage to liver and kidneys was characterized by turbid tumefaction and sometimes hydropic degeneration. Edema was commonly found in different sites. An important finding to point out is that animals that consumed the diet with 10% cottonseed oil showed a slight turbid tumefaction in both kidneys and liver, which indicates that this damage was not due to any toxic constituent of amaranth. Also, kidney congestion probably was caused by the violent death of the rats.

The results of the present research indicate that, except for the lower digestibility of amaranth oil than of cottonseed oil, it is not responsible for the lower weight gain and lower protein quality of raw amaranth grain as compared to the processed material. However, it would be of interest to learn the reasons for the lower digestibility of amaranth oils.

ACKNOWLEDGMENTS

This study was carried out with the financial assistance of the National Academy of Sciences, Washington, D.C. (Grant-in-aid INC-NUT-381/PN/85-85/CA).

REFERENCES

1. Betschart, A., D. Wood Irving, A.D. Shepherd and R.M. Saunders, *J. Food Sci. 46*:1181 (1985).
2. Bressani, R., in Boletín No. 3, *El Amaranto y su Potencial*, edited by Archivos Latinoamericanos de Nutrición, Guatemala (1983).

3. Sánchez-Marroquín, A., S. Maya and J.L. Pérez, in *Proc. Second Amaranth Conference* (1980).
4. Becker, R.O., E.L. Wheeler, K. Lorenz, A.E. Stafford, O.K. Grosjean, A.A. Betschart and R.M. Saunders, *J. Food Sci. 46:*1175 (1981).
5. Carlsson, R., *Proc. Second Amaranth Conference* (1980).
6. *Official Methods of Analysis of the Association of Official Analytical Chemists,* 12th edn., AOAC, Washington, DC (1975).
7. Manna, L., and S.M. Hauge, *J. Biol. Chem 202:*9196 (1953).
8. Hegsted, D.M., R.C. Mills, C.A. Elvehjem and E.B. Hart, *J. Biol. Chem. 138:*459 (1941).
9. Eilers, R.J., *Am. J. Clin. Pathol. 47:*212 (1967).
10. McInroy, R.A., *J. Clin. Pathol. 7:*32 (1954).
11. *Instruction Manual, Beckman, Model R. Paper Electrophoresis Cell* (1965).
12. Snedecor, G.W., and W.G. Cochran, *Statistical Methods,* 7th edn., The Iowa State University Press, 1980, pp. 215–54.
13. Williams, D.T., in *Oils, Fats and Fatty Foods,* 3rd edn., The Balkiston Company, 1950.

[Received March 31, 1986]