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Digestibility and Protein Quality of Raw and Heat-Processed Defatted and Nondefatted Flours Prepared with Three Amaranth Species

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The study was carried out to determine whether removing hexane extractables from three amaranth species improved protein quality of the grain as compared to heat processing. It also examined whether or not feeding the grain induced pathological damage to rats organs. Hexane-extracted samples were cooked at atmospheric pressure or drum dried. These preparations were fed in 10% protein diets to weanling rats for standard PER digestibility studies. Macroscopic examination of the organs was done on rats fed *Amaranthus cruentus*. Atmospheric cooking of the whole and of the defatted materials gave higher food intake, weight gain, and PER than non-heat-treated samples. Drum drying of the fat-free flours always resulted in lower quality than atmospheric cooking. Organ weight and appearance for *A. cruentus* fed rats were normal. Results showed growth-reducing factors of amaranth grain to be still present in the raw fat-free flour.

Amaranth grain is considered an excellent source of nutrients for man. Its composition has been fully described by various authors (Becker et al., 1981; Carlsson, 1980; Sánchez-Marroquín et al., 1980). These studies report protein and lipid contents of 12–17% and 6–9%, respectively, with its protein being rich in lysine and sulfur amino acids (Carlsson, 1980; Sánchez-Marroquín et al., 1980) and its fat containing high amounts of squalene (Becker et al., 1981), an intermediate in the biosynthesis of cholesterol.

The nutritional value of amaranth seeds has been reported as comparable to that of casein (Sánchez-Marroquín et al., 1980). Moreover, other biological evaluations (Betschart et al., 1985; Bressani, 1983) have pointed out that wet heat-processed amaranth seeds give a higher PER value than nonprocessed seeds, suggesting the existence of unknown thermolabile factors or of chemical structures not readily available to the animal. Even though it has been reported that amaranth grain contains tannins, trypsin inhibitors, and hemagglutinins (Imeri, 1985), the amounts are too small to explain the improvement in protein quality obtained with controlled heat processing. There is need, therefore, to study the significance of other organic components in the grain. In a previous study (García et al., 1986) the nutritive value of the oil from three species was evaluated. The results indicated that oil induced normal animal performance although its true digestibility was lower than that of cottonseed oil.

The purpose of this study was to evaluate the effect of wet-heat treatment on the protein value of defatted and nondefatted amaranth seed flours and on the possible damage caused to organs of rats fed these flours.

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MATERIAL AND METHODS

One selection from each of three species of amaranth was evaluated: *Amaranthus caudatus*, *Amaranthus cruentus*, and *Amaranthus hypochondriacus*. The seeds were obtained from INCAP's experimental farm in Guatemala and were milled to prepare the flours.

Protein (AOAC, 1975) and ether extract content (AOAC, 1975) were determined in the individual flours prior to defatting. The results have been reported previously (García et al., 1986). Portions from each flour were extracted in a Soxhlet apparatus with hexane during 28–32 h. Percentage oil extractions varied from 75 to 85% of the original content in each species (García et al., 1986).

Defatted and nondefatted flours of each of the three cultivars were divided into three portions. The first had no further processing, while the second was cooked for 10 min at atmospheric pressure with constant manual stirring in three parts of boiling water, dehydrated in an air oven at 60 °C, and ground. The third portion was mixed with three parts of cold water and drum dried. The drums were heated with vapor pressure at 70 psi, which gave a temperature of approximately 134 °C. The drums were set to rotate at 3 rpm for a residence time of 10–12 s. The product was ground into a flour.

The biological assay was done using 21-day-old rats of the Wistar strain, from INCAP's animal colony. A total of 144 animals was divided by weight in groups of eight, four males and four females, and placed in individual all-wire screen cages with raised screen bottoms. They were fed diets containing the amaranth flours at a protein level of 10% for 28 days. These diets were supplemented with a vitamin solution (Manna and Hauge, 1953), mineral mixture (Hegsted et al., 1941), 5% cottonseed oil, 1% cod

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Table I. Effect of Fat Removal and of Processing of Three Amaranth Species on Animal Performance[a]

species	food intake, g		weight gain, g		PER		digestibility, %	
	N-D	D	N-D	D	N-D	D	N-D	D
			Raw Samples					
A. caudatus	314[a,b]	313[a,b]	68[b]	65[b,c]	2.0[a,b]	2.0[a,b]	78.8[a]	82.3[a]
A. cruentus	257[b]	339[a]	41[c]	82[a,b]	1.6[b]	2.3[a]	80.5[a]	83.3[a]
A. hypochondriacus	365[a]	343[a]	98[a]	78[a,b]	2.2[a]	2.1[a]	77.6[a]	80.3[a]
			Cooked Samples					
A. caudatus	380[b]	447[a]	103[a]	112[a]	2.6[a]	2.4[a]	82.2[a]	79.4[a]
A. cruentus	377[b]	447[a]	101[a]	114[a]	2.6[a]	2.5[a]	77.3[a]	75.4[a]
A. hypochondriacus	386[a,b]	391[a,b]	99[a]	96[a]	2.5[a]	2.4[a]	77.7[a]	77.6[a]
			Drum-Dried Samples					
A. caudatus	353[a]	357[a]	83[a,b]	81[a,b]	2.2[b]	2.2[b]	80.6[a]	80.8[a]
A. cruentus	373[a]	377[a]	99[a]	82[a,b]	2.7[a]	2.1[b]	80.3[a]	78.4[a]
A. hypochondriacus	331[a]	374[a]	72[b]	74[a,b]	2.6[a]	1.9[b]	82.3[a]	79.8[a]

[a] Key: N-D = nondefatted; D = defatted. a, b = statistical difference ($P < 0.05$) between values with different letters.

Table II. Species Differences of Nondefatted and Defatted Samples on Animal Performance[a]

species	food intake, g		weight gain, g		PER		digestibility, %	
	N-D	D	N-D	D	N-D	D	N-D	D
A. caudatus	345[a,b]	372[a]	85[a]	86[a]	2.3[a]	2.2[a,b]	80.5[a]	80.9[a]
A. cruentus	336[b]	388[a]	80[b]	93[a]	2.3[a]	2.3[a]	79.4[a]	79.0[a]
A. hypochondriacus	361[a]	369[a]	90[a]	83[a,b]	2.5[a]	2.1[b]	72.7[a]	79.2[a]

[a] Key: N-D = nondefatted; D = defatted. a, b = statistical difference ($P < 0.05$) between values with different letters.

Table III. Process Effect of Nondefatted Samples on Animal Performance[a]

process	food intake,[b] g		weight gain,[b] g		PER		digestibility,[b] %	
	N-D	D	N-D	D	N-D	D	N-D	D
raw	312	332	69	75	1.9[c]	2.2[b]	75.8	82.0
cooked	381	428	101	107	2.6[a]	2.4[a,b]	75.8	77.5
drum dried	352	369	85	79	2.5[a]	2.1[c]	81.1	79.6

[a] Key: N-D = nondefatted; D = defatted. a–c = statistical difference ($P < 0.05$) between values with different letters. [b] No statistical difference between values.

Table IV. Species Effects Independent of Fat Extraction and Processing[a]

species	av food intake, g	av weight gain, g	PER	app protein digestibility, %
A. caudatus	360.6[a]	85.3[a]	2.26[a]	80.7[a]
A. cruentus	361.6[a]	86.4[a]	2.30[a]	79.2[b]
A. hypchondriacus	364.9[a]	86.0[a]	2.29[a]	79.3[b]

[a] a, b = statistical difference ($P < 0.05$) between values with different letters.

liver oil, and corn starch to adjust to 100%. The rats were administered feed and water ad libitum and weighed every 7 days. Feces were collected during the last 7 days to determine the digestibility of the flours. A casein diet was used as control. PER values were calculated as weight/gram of ingested protein.

At the end of the PER assay, hemoglobin (Eilers, 1967), hematocrit (McInroy, 1954), and serum protein content (*Instruction Manual*, 1965) were determined in four rats (two males and two females) from groups fed each of the following flours: raw and cooked *A. cruentus*, raw defatted and cooked defatted *A. cruentus*, and casein. Macroscopic and microscopic analyses of damage to tissues were done on the organs of the same rats.

The statistical analysis included one-way and two-way analysis of variance followed by a media comparison using Tukey's student range at a level of 5% probability (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

The amaranth species *caudatus*, *cruentus*, and *hypochondriacus* used in this study contained 12.5, 15.0, and 14.4% protein and 6.7, 7.9, and 7.1% fat, respectively.

Results from the feeding studies for all the samples are shown in Table I. Raw samples for *A. caudatus* and *A. hypochondriacus* gave a statistically similar protein quality values for nondefatted and defatted flours; however, *A. cruentus* gave a significantly higher PER value for the fat-free flours. This observation may be partially due to

Table V. Initial and Final Weights, Weight Gain, and Weight of Organs of Rats Fed Raw and Cooked, Defatted and Nondefatted *A. cruentus* Flours and Casein

group[a]	weight gain,[b] g	% total body weight						
		liver	kidney	heart	lungs	spleen	pancreas	testicles
1	109.7[a]	4.18 ± 1.70	0.77 ± 0.08	0.49 ± 0.09	0.81 ± 0.11	0.23 ± 0.08	0.26 ± 0.12	1.41 ± 0.05
2	44.7[b]	3.43 ± 0.44	0.96 ± 0.12	0.50 ± 0.11	0.80 ± 0.13	0.19 ± 0.04	0.21 ± 0.03	1.44 ± 0.48
3	110.5[a]	3.90 ± 1.54	0.84 ± 0.17	0.44 ± 0.13	0.74 ± 0.12	0.23 ± 0.13	0.17 ± 0.08	1.65 ± 0.19
4	74.2[a,b]	4.97 ± 1.28	1.07 ± 0.15	0.53 ± 0.08	0.94 ± 0.09	0.26 ± 0.07	0.21 ± 0.07	1.71 ± 0.51
5	117.2[a]	4.30 ± 0.45	1.36 ± 0.04	0.45 ± 0.06	0.79 ± 0.12	0.22 ± 0.05	0.21 ± 0.07	1.28 ± 0.42

[a] Groups: 1, casein; 2, raw *A. cruentus*; 3, cooked *A. cruetus*; 4, raw defatted *A. cruentus*; 5, cooked defatted *A. cruentus*. [b] Average initial weight 45.5 g. [c] a, b = statistical difference ($P < 0.05$) between values with different letters.

Table VI. Hemoglobin Content, Hematocrit, and Serum Proteins in Rats Fed Casein and *A. cruentus* Flours

group[a]	hemoglobin, g/dL	hematocrit, %	total serum proteins, g/dL
1	15.55 ± 0.64	44.25 ± 0.75	6.00 ± 0.47
2	15.30 ± 0.89	44.25 ± 0.50	6.30 ± 0.42
3	16.05 ± 0.41	47.00 ± 0.58	5.85 ± 0.92
4	15.45 ± 0.52	45.00 ± 1.22	6.30 ± 0.76
5	14.50 ± 0.62	44.00 ± 1.73	5.83 ± 0.40

[a] Groups: 1, casein; 2, raw *A. cruentus*; 3, cooked *A. cruentus*; 4, raw defatted *A. cruentus*; 5, cooked defatted *A. cruentus*.

the increased food intake recorded. However, protein digestibility also increased in the fat-free flours, suggesting an effect due more to a better balance of absorbed amino acids. All samples, with or without fat, had statistically equal protein digestibility. For *A. cruentus* and *A. hypochondriacus* the defatted drum-dried flours gave statistically lower PER values than nondefatted drum-dried flours. For *A. caudatus* both values were statistically similar. In the case of cooked flours, food intake was statistically higher for defatted flours of *A. caudatus* and *A. cruentus* than for nondefatted flours of the same species, whereas it was similar for *A. hypochondriacus*. Weight gain, PER, and apparent digestibility were statistically similar for defatted and nondefatted flours of the three species.

As shown in Table II, for defatted and nondefatted flours of *A. caudatus* and *A. hypochondriacus*, independently of whether they were processed or not, food intake and weight gain were statistically similar whereas for *A. cruentus* rats consumed and gained less with the nondefatted flours; apparent digestibility was statistically similar for the defatted and nondefatted flours of the three species; PER was statistically similar for *A. caudatus* and *A. cruentus*, defatted and nondefatted, while it was lower for defatted *A. hypochondriacus*.

In general, independent of the species used, drum-dried defatted flours gave statistically similar PER values to nondefatted raw flours, whereas nondefatted drum-dried flours were higher. As for food intake, weight gain, and apparent digestibility, there were no statistical differences (Table III). The results with drum drying were inconsistent probably due to the lack of appropriate processing conditions, which must be established if this technique is to be used in future studies.

A statistical comparison of the all results between species (Table IV) independent of whether they were processed or not, and whether they were defatted or nondefatted, shows that the three of them had similar food intakes, weight gains, and PER values; the apparent digestibility was higher for *A. caudatus* (80.7%) than for *A. cruentus* (79.2%) and *A. hypochondriacus* (79.3%).

The extraction process with hexane in general did not affect the nutritional properties of *A. candatus* and *A. hypochondriacus*, the exceptions being a higher food intake for cooked *A. caudatus* and a lower PER for drum-dried *A. hypochondriacus*. For *A. cruentus* the raw defatted flour showed a higher food intake, higher weight gain, and higher PER values than the nondefatted flour and were comparable to those of the cooked nondefatted flour.

These results suggest, therefore, that removal of oil did not influence the quality of *A. caudatus* and *A. hypochondriacus* and that whatever factor is responsible for the lower performance in the raw samples is still present in the defatted raw sample, which upon cooking improves animal performance. The exception was the defatted *A. cruentus* flour for reasons not as yet determined. Because of this finding, studies are under way with this particular oil.

The weight of the organs, hemoglobin, and hematocrit of animals fed *A. cruentus*, raw, defatted and processed, are shown in Tables V and VI. The weight of the organs is a reflection of the weight gained, and in this respect a certain tendency can be found. The organs of animals fed the raw whole grain weighed less than those of animals fed the raw defatted, and the same happened upon cooking of the grain. There were no differences in hemoglobin, hematocrit, or total serum proteins.

Table VII presents a summary of the results for the microscopic analysis of rats belonging to the groups fed raw, raw defatted, cooked defatted *A. cruentus* and casein.

The microscopic analysis of the organs did not show any damage, except for a slight fatty degeneration in the liver especially in the rats of the group fed the cooked flour and a slight congestion of the kidney in the rats of the casein group. Damage to the kidney was characterized by hydropic degeneration in tubular cells and intertubular edema. Damage to the cells of the hepatic tissue was present in all diets. Congestion of the kidney was possibly caused by the death of the animals, and turbid tumefaction might have occurred because the fixation of organs in formaldehyde was done about 2 h after the rat was sacrificed. Other damage to the kidney was probably caused by oxalate content of amaranth seeds as reported earlier (Cheeke and Bronson, 1980; Cheeke et al., 1981; Marshall et al., 1967; Osweiler et al., 1969; Stuart, 1975).

The data show that oil removal from amaranth grain does not alter its protein quality when fed raw and that a wet-cooking process increases the quality substantially in all the species studied. Whatever factor is responsible for the observations reported is not present in the oil and remains in the oil-free meal. However, it should be pointed out that the oil-free raw flour from *A. cruentus* gave a PER value significantly higher than for the nonextracted raw flour. This must be confirmed in future studies. As already reported, the factor is sensitive to heat, since in all situations heat-processed samples induced higher food intake, weight gain, and protein quality. The tissue weight and alterations cannot be ascribed to any known common antiphysiological factor. The weights were more a reflection of diet consumed than toxic effects.

Table VII. Microscopic Changes in Organs of Rats Fed Raw and Cooked, Defatted and Nondefatted *A. cruentus* Flours and Casein

raw *A. cruentus*	hepatic tissue with generalized turbid tumefaction; renal tissue with glomerular edema; protein hyaline cylinders and hydropic degeneration of tubular cells; slight turbid tumefaction in the heart; spleen normal
cooked *A. cruentus*	turbid tumefaction and slight fatty degeneration in the liver; in the kidney, slight glomerular edema and hydropic degeneration of tubular cells; muscles and spleen normal
raw defatted *A. cruentus*	in the kidney, turbid tumefaction, hydropic degeneration of cells in the proximal tubes and intertubular edema; slight interstitial edema in the muscle fibers of the heart; spleen normal
cooked defatted *A. cruentus*	turbid tumefaction and slight fatty degeneration in the liver; turbid tumefaction and hyaline cylinders in the kidney; interstitial edema in the heart
casein	hepatic, muscle, and renal tissues normal

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Radial Distribution of Amino Acids in the Milled Rice Kernel

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Radial distribution of all amino acids except Trp and Cys was determined for five varieties of rice. Consecutive fractions from the outer layer to the center of the rice kernel were obtained by abrasive milling. Five patterns of distribution of amino acid contents were observed. The patterns are as follows: (1) no change for Ile, Val, Arg, and Pro; (2) low in Gly; (4) low in the outer layer, increases in the middle layer, and no change in the central layer for Met and Ser; (5) the mirror image of (4) for Thr, His, Ala, and Asp. The data of Lys, the first limiting amino acid of rice proteins, suggest that the nutritive value of rice proteins may be reduced toward the middle layer and it may be elevated slightly in the kernel center.

The composition of rice proteins such as albumin, globulin, prolamin, and glutelin varies with the radial location in the kernel (Yoshizawa and Kishi, 1985). Moreover, it has been reported that there is a marked difference in the amino acid composition among their proteins (Juliano, 1985; Taira, 1962). These findings suggest that the amino acid composition may change with the portion of the rice kernel.

Previous studies (Hayakawa et al., 1985) have shown that there is a difference in the lysine content of milled rice between the outer layer and the inner layer, and the nutritive value of their layers depends on the limiting amino acid content. On the basis of elucidating the changes in the nutritive value of rice protein with processing, it is important to determine the radial distribution of amino acids. However, there has been very little work on the amino acid composition of consecutive layers of the kernel.

In the present study, we have examined the amino acid composition of each layer, obtained by abrasive milling of brown rice of five varieties, and have discussed the radial nutritive value of rice.

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MATERIALS AND METHODS

Rice Supplies. The brown rices of five varieties [Nipponbare, Koshihikari, Sasanishiki, Ohzora, Aoisora (*Oryza sativa* L. subsp. *japonica* Kato)] were prepared. These are the major varieties in Japan today. All rices were harvested in 1984 from Ibaraki prefecture, Japan.

Analytical Samples. A 5-kg sample of each brown rice was abrasively milled 25–35 consecutive times by passage through a Nakano type rice-whitening machine obtained from Sanyo Kosaku, Co., Ltd., Tokyo, Japan. The flour and particles produced from the kernels were collected after milling and sieved through a 32-mesh screen to remove the flour from the particles. The milling out-turn percent of rice was calculated from the weight of 1000 particles of milled rice divided by that of brown rice. The flour of 13–15 portions in 25–35 portions was subjected to the amino acid analysis and nitrogen determination.

Amino Acid Analysis and Nitrogen Determination. The samples (ca. 4 mg of protein) were analyzed for amino acid content by hydrolyzing for 22 h at 110 °C with 6 N HCl (containing 0.02% 2-mercaptoethanol) in evacuated

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