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Protein quality evaluation of amaranth in adult humans

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Abstract. This study was carried out to determine the nutritional quality of the protein of amaranth grain submitted to extrusion and popping processes, using cheese protein as reference. For the biological evaluation, the short-term nitrogen balance index method was followed with 12 experimental adult male human subjects. A Latin square series 3 × 3 was used (three periods, three subjects) as an experimental design balanced to minimize residual effects by randomly ordering treatments, columns and rows. The study consisted of three periods of nine days each.

The first period started by feeding all subjects a low nitrogen diet, followed by increases of the protein level every two days. The levels were 0.2, 0.4, 0.6/g protein/kg/day, keeping other nutritional elements constant and adequated, including calories, minerals and vitamins. All subjects received all their meals using as a sole source of protein extruded amaranth, popped amaranth or processed cheese. Water intake was kept at a rate of 0.8–1.0 ml per calories consumed. During the study, the subjects maintained regular physical activity. *Amaranthus cruentus* was utilized. The extruded amaranth was prepared with the Brady Crop Cooker under conditions previously established in other studies. The popped amaranth was prepared at a 250 °C temperature during 15–20 sec.

The extruded and popped amaranths were provided as a sweet puree and, as all the other foods conforming the diets of each subject, they were weighed with 0.1 g of accuracy. Diet samples, as well as faeces and urine, were collected daily, which were ordered according to period and level of protein, conforming pools to determine their nitrogen content by the Kjeldahl method. True digestibility results of the protein were 101.4, 89.8 and 85.5% for cheese, extruded amaranth and popped amaranth, respectively. The statistical analysis according to the Tukey test showed that the true digestibility of the protein was the same for the two products of amaranth and different than the digestibility of cheese. Nitrogen balance index values from the equation between nitrogen intake and nitrogen retained, were 0.97, 0.86 and 0.79 for cheese, extruded amaranth and popped amaranth, respectively. The respective values between nitrogen absorbed and nitrogen retained were 0.97, 0.98 and 0.96. The Tukey test indicated that for NI to NR cheese was statistically different for the two amaranth products, which were similar between them. For the relationship NA to NR all values were statistically the same. The calculation of nitrogen intake for nitrogen equilibrium indicated that the amaranth protein is among the highest in nutritive quality of vegetable origin and close to those of animal origin products.

Introduction

Amaranth is a pseudo-cereal appreciated very much among the Mayan, Aztec and Incan civilizations of the past before the Spanish conquest of the Americas [32]. Its cultivation was reduced signicantly after the conquest. Its small scale production has prevailed, however, mainly in Mexico [32], while in other regions the leaves have been used continuously as a vegetable [9]. The interest in the production and utilization of amaranth grain has been revived due to the relatively large amounts of protein it contains (around 15%), with a good essential amino acid pattern and high lysine content [6, 32]. Amaranth contains around 8% fat, which makes its energy content high as compared to that of cereals [2, 6, 32]. Agronomically, it is resistant to water stress and grows well under different environments [32]. For its consumption, amaranth grain is processed in different manners, although today the most common form is as expanded grain [32]. Other processes include cooking in water [23], extrusion cooked [24], roasted and as flakes [13]. The nutritional evaluations carried out with processed amaranth grain show that when the process is done under controlled conditions, an increase in protein quality is observed as compared with the raw grain value [11, 16, 24]. This observation suggests that the raw grain contains adverse physiological compounds not yet identified. The expansion process, using high temperatures for short periods of time, must be well controlled, since it may reduce the protein quality of the product [6]. The chemical score of the amino acid pattern of amaranth protein has suggested leucine to be its first limiting amino acid [10]. Extensive biological testing in growing rats has indicated, however, that threonine is the first limiting amino acid [10]. Processed amaranth grain is beginning to be used in a variety of foods, alone or in mixtures with cereal grain flours, some still at the laboratory level [7, 22, 31, 32], and others at a commercial scale [1]. The protein quality of amaranth has been evaluated in children by Morales, Lembcke and Graham [25].

These studies indicate that nitrogen balances of roasted, expanded and flaked amaranth were 30.3, 28.8 and 24.7% of nitrogen intake, with the quality of the roasted product being superior to that of the other two. These authors [25] also showed that 20–30% roasted amaranth grain improved the protein quality of maize flour. In the present study the protein quality of processed amaranth grain fed to adult humans as the sole protein source was evaluated [29].

Materials and methods

Selection and preparation of the raw material

Amaranthus cruentus variety GUA-17 was harvested at the Experimental Farm of the Institute of Nutrition of Central America and Panama (INCAP). It was processed by extrusion cooking and popping.

Extrusion. Seventy-five pounds of amaranth were submitted to extrusion cooking using the Brady Crop Cooker, previously heated with soybean, as was described by Mendoza-Montano and Bressani [24]. The grain was processed with its natural moisture. The extruder was maintained at a constant feeding rate equal to two with a cone opening not wider than 1 mm and at a temperature between 280–305 °F.

The flakes were allowed to cool and then ground in a hammer mill with an 80 mesh screen. A sample was obtained for chemical analysis and the remaining material was stored at 4 °C until the initiation of the biological trials.

Expansion. For this process, special equipment to pop the amaranth grain was used. The equipment consisted of a stainless steel sheet heated by an electric resistance. The amaranth grain remained in direct contact with the surface for 15–20 seconds at a temperature of 230–240 °C. The grain was constantly moved with a natural bristle brush, to avoid burning, and then removed [35]. The expanded grain was sieved to remove the toasted grain.

The expanded grain was ground in a hammer mill with an 80 mesh screen. A sample of the floor was taken for chemical analysis and the rest of the material was stored at 4 °C until used.

Biological evaluation

Preliminary activities. Previous to selection of the experimental subjects recruited by voluntary decision to participate in the study, they were interviewed individually to obtain general data, determine their food habits and to acquaint them with information regarding the study. They were then subjected to medical examination including clinical analysis. Twelve were selected out of 26 male persons interviewed, taking into consideration the homogeneity among them with respect to weight, height, age, and occupation, food habits and health status. The selected experimental subjects

Table 1. Physical characteristics of experimental subjects

Subject	Age	Height	Weight (Kg)	
			Initial	Final
1	29	156	47.73	45.45
2	28	157	47.73	46.50
3	32	152	50.00	49.00
4	19	165	58.64	57.60
5	22	170	60.45	59.25
6	24	163	61.36	60.22
7	31	169	64.54	60.54
8	26	162	64.54	60.54
9	28	161	60.00	61.50
10	31	154	62.27	60.20
11	31	154	65.17	64.50
12	34	169	66.82	65.30
$\bar{X}$	27.9	160.7	59.10	57.63
SD	±4.44	±5.56	±6.84	±6.79

$\bar{X}$ = Average.
SD = Standard Deviation.

were instructed in particular details and responsibilities of the study. Each subject was assigned within the study according to the statistical design to be described later. Table 1 presents the age, height and weight of the participating persons. The Short-Term Nitrogen Balance Index [15, 8], was used to evaluate the protein quality of the two amaranth grain flour processes.

Duration of the study. The study had a duration of 30 days distributed in three periods of nine days each with one day of rest between periods. Each period consisted of:

— Three days during which a low nitrogen content diet with the components listed in Table 2 was fed, followed by diets with protein increments performed every two days as follows:
— Two days in a diet providing 0.2 grams of protein/kg body weight/day of amaranth or cheese.
— Two days in a diet providing 0.4 grams of protein/kg body weight/day, and
— Two days in a diet providing 0.6 grams of protein/kg body weight/day.

Diets. The diets, given during the different periods when the subjects were receiving levels of 0, 0.2, 0.4 and 0.6 grams of protein/kg body weight/day

Table 2. Food ingredients in the low nitrogen content diet*

Ingredients	Amounts (g)	Calories	Proteins (g)
Hypoproteic bread (according to recipe)	550	1034	2.2
Cinnamon cookies (according to recipe)	400	1984	1.6
Chicken flavor soup (according to recipe)	480	158	0.5
Vanilla dessert (according to recipe)	460	593	0.5
Cucumber	100	32	0.4
Pineapple	200	104	0.8
Instant coffee	4	5	–
Refreshment flavoring	4	–	–
Sugar for coffee and refreshment	100	382	–
Marmelade	120	334	0.6
Margarine	20	147	0.1
Carbonated beverages	720	222	0.0
Candies	1.2	5	
Total/day		5000	6.7
Per kg weight/day		50	0.06

* Average Experimental Subject:
100 kg weight
50 kcal/kg body weight/day
0 g protein/kg body weight/day.

had the basic composition described in Table 3 for the processed cheese diets used as reference protein and Table 4 for the extrusion cooked or processed amaranth diets, respectively.

These diets were prepared using extruded amaranth, popped amaranth and cheese, as the sole source of protein. The extruded amaranth and the popped amaranth were given as a sweet gruel. Sample menus were prepared in order to facilitate distribution of diets among the experimental subjects, to be described. Each dietary treatment covered the nutritional needs of the subjects with respect to calories, vitamins and minerals. The diets fed provided between 48–50 kcal/kg body weight/day and they were given a daily vitamin and mineral supplement (UNICAP-T and CA-C1000) containing the following:

Vitamin and mineral supplement composition table

UNICAP-T Tablet – Upjohn Laboratories	mg
Vitamin A (5000 I.U.)	1.5
Vitamin D (5000 I.U.)	12.5
Mononitrate of Thiamine (B1)	10.0
Riboflavin (B2)	10.0
Ascorbic Acid (C) as Sodium Ascorbate	300.0
Niacinamide	100.0
Chlorhidrate of Piridoxin (B6)	2.0
Pantothenate of Calcium	20.0
Vitamin B12 activity	4.0
Copper Sulfate (Copper 1 mg)	3.92
Ferrum Sulfate (Iron 10 mg)	31.3
Potassium Iodate (Iodate 0.15)	0.196
Calcium carbonate (Calcium 50 mg)	125.0
Manganese Sulfate (Manganese 1 mg)	3.08
Magnesium Sulfate (Magnesium 6 mg)	29.7
Potassium Sulfate (Potassium 5 mg)	11.42
CA-C1000 Tablet from the Sandoz Laboratories	
Ascorbic Acid	0.5 g
Calcium Lactogluconate	0.5
Calcium Carbonate	0.2

Water intake was calculated at a rate of 0.8–1.0 ml per calorie consumed per day. All foods conforming the diets of each subject were weighed with 0.1 gram of precision.

Sample collection of diets, urine and faeces

— Diets. During each of the three periods of the study a complete diet per subject per protein level was weighed and homogenized. Two aliquotes were taken which were analyzed for their nitrogren content.

— Urine. Daily each subject received a container marked with their personal data, containing 10 ml HCl 0.5 N. After 24 hr of collection the subjects left the container which was stored at 4 °C and the volume was measured. The two-day collection of the same level of protein was formed, and it was then homogenized and an aliquote was taken to determine its nitrogen content.

— Faeces. During each of the three stages of the study the subjects were provided in alternate days 300 mg/subject of Carmin and 300 mg/subject of vegetable activated carbon, to adequately separate the protein levels. Each subject collected the faeces in polyethylene bags, weighed, and labelled with their respective personal data. The contents were weighed

Table 3. Diet ingredients according to protein level intake[a] – cheese

	Protein level, g/kg weight/day								
	0.2			0.4			0.6		
	Wt. (g)	Cal.	Prot. (g)	Wt. (g)	Cal.	Prot. (g)	Wt. (g)	Cal.	Prot. (g)
Hypoprot bread	460	865	0.5	463	879	1.8	463	870	1.8
Cucumber	60	9	0.4	60	8	0.6	60	8	0.6
Pineapple or papaya	90	20	0.4	100	22	0.5	200	104[b]	0.8
Beverage flavor	4	–	–	4	–	–	4	–	–
Instant coffee	–	–	–	4	5	–	4	5	–
Sugar	140	539	–	100	382	–	100	382	–
Cinnamon cookies	450	2232	1.8	400	1984	1.6	400	1984	1.6
Carbonated beverage	220	222	–	720	222	–	720	222	–
Marmelade	100	253	0.3	150	417	0.8	150	417	0.8
Margarine	70	527	0.5	72	542	0.5	20	147	0.1
Candies	11	44	–	–	–	–	–	–	–
Processed cheese	72	286	18.0	138	548	34.5	217	861	54.3
Total/day	–	5001	21.9	–	5000	40.3	–	5000	60.0
Per kg wt/day	–	50	0.21	–	50	0.4	–	50	0.6

[a] Sample diet for subjects with a 100 kg body weight (50 Kcal/kg wt/day) and (0.2, 0.4, 0.6 g prot/kg wt/day).
[b] From pineapple.

and stored at −20 °C. The samples were then separated by periods, levels and dates according to the data on each label and the color given by the marker. The daily excretion was recorded and pooled per level, homogenized with the addition of HCl 0.1 N in an amount equal to the weight of the faeces. Aliquotes were taken to obtain the nitrogen content.

Controls. The subjects were weighed daily in the morning under fasting conditions and this weight was taken as the basis to calculate their respective diet intake. A daily record on food intake was followed, as well as the excretion of urine and faeces. The health status of the subject was controlled when needed at the Clinical Center of INCAP.

Chemical analyses

The chemical analysis of the raw and processed amaranth and cheese was carried out according to the AOAC methodology [1]. Calories were deter-

Table 4. Diet ingredients according to the level of protein intake[a] amaranth (protein level g/kg body weight/day)

Ingredients	0.2			0.4			0.6		
	g	Cals.	Prot. (g)	g	Cals.	Prot. (g)	g	Cals.	Prot. (g)
Hypoproteic bread	440	827	0.4	200	376	0.8	260	489	1.0
Cucumber	47	7	0.3	60	8	0.6	60	8	0.6
Pineapple papaya	90	20	0.4	100	22	0.5	200	104[b]	0.8
Flavoring	4	–	–	4	–	–	4	–	–
Instant coffee	3	4	–	4	5	–	4	5	–
Sugar	140	538	–	100	382	–	100	382	–
Cinnamon cookies	195	967	0.7	193	957	0.8	130	645	0.5
Carbonated beverages	720	222	–	720	222	–	720	222	–
Marmelade	70	194	0.4	80	222	0.4	22	61	0.1
Margarine	70	527	0.5	–	–	–	–	–	
Candies	15	60	–	–	–	–	135	54	–
Amaranth[c]	1100	1639	18.7	2176	2807	37.0	3000	3000	57.0
Total/day	–	5001	21.4	–	5001	40.1	–	5000	60.0
Total/kg wt/day	–	50	0.21	–	50	0.40	–	50	0.60

[a] Sample diet for a subject with a body weight of 100 kg (50 kcal/kg weight/day) and (0.2, 0.4, 0.6 g prot/kg weight/day).

[b] From pineapple.

[c] Porridge prepared with extruded amaranth expanded or processed, prepared with 5 ml vanilla, 2 g salt, 40–46 g sugar, 0–25 g margarine, 255 g water added to 40 g of amaranth flour. The mixture was cooked during 10–15 minutes.

mined by calorimetric bomb and available lysine by dinitroflorurobenzene [21]. Nitrogen analysis was carried out in diets, and urine and faeces by the Kjeldhal method.

Experimental design

Statistical design

A series of Latin Square designs 3 × 3 (three periods, three subjects) balanced to minimize residual effects were used, as indicated below.

Subjects	Periods		
	1	2	3
1	A	B	C
2	B	C	A
3	C	A	B
4	A	C	B
5	B	A	C
6	C	B	A
7	B	A	C
8	A	C	B
9	C	B	A
10	B	C	A
11	A	B	C
12	C	A	B

The letters A, B and C, represent cheese as control, popped amaranth and extruded amaranth, assigned at random.

Ther columns and rows were aligned at random, according to Cochram and Cox [20], and the treatments grouped in replicates in two different forms, the effect of which is to eliminate the errors of the differences between rows, as well as all differences between columns. The design also permits elimination of the residual effects due to the application in sequence of the treatments to the same subject in successive periods.

To minimize the residual effect between treatments a resting period of one day between the first and second period was permitted, and two days between the second and third period. In the design used, the columns represent the successive feeding periods in sequence of the different treatments to the same subject and the rows represent the subjects.

Results and discussion

Raw material

Chemical analyses. Table 5 shows the partial chemical composition of the foods used. The results fall within the range of values reported in the literature [6, 11, 30, 32]. The protein content was slightly higher in the processed amaranth (16.2 and 17.0%) in comparison with the raw sample (13.8 g%) due to a decrease in the moisture content of the grain when it is processed. Moisture alone, however, does not explain all of the increase.

Table 5. Chemical composition of foods used in the study

Raw material	Moisture	Ether extract	Crude fibre	Protein	Energy kcal (%)	Available lysine (g/16 g N)
	(g/100 g)					
Raw amaranth	12.5	5.6	5.0	13.8	479	5.61
Extruded amaranth	6.4	8.1	5.4	16.2	491	5.79
Popped amaranth	7.9	5.4	8.6	17.0	471	4.57
Yellow processed cheese	ND	18.3	ND	22.0	ND	ND
White processed cheese	ND	18.5	ND	22.6	ND	ND

ND = Not determined.

With respect to the protein content of the extruded amaranth grain, the figure found is equal to the protein content of the popped material, which may be taken as indicative that there was no contamination with the soybean utilized to adjust the temperature of the equipment. The value of 8.1 g% fat for extruded amaranth is probably due to the oil which remains in the equipment from soybeans and is absorbed by the amaranth when this is introduced during the extrusion process.

The popping process induced a decrease of 19% of the available lysine (4.57 g/16 g N) when compared with that of the raw sample (95.61 g/16 g N), a result similar to those reported by other studies [13, 16]. The extrusion process, however, did not affect available lysine values.

Calories determined by bomb calorimetry was highest for the extruded amaranth (491 kcal/100 g) followed by the raw amaranth (479 kcal/100 g) and popped amaranth (471 kcal/100 g).

With respect to cheese, the protein value obtained (22.0 and 22.6 g/100 g of product) are similar to the values reported by the USDA Composition of Foods [Handbook No. 8, 1976].

Diets. The food composition of the diets offered to the subjects according to the level and source of protein are shown in Tables 2–4.

The results in Table 6 show that for 100 g of diet consumed, more than 90% of the total protein given to the subjects was contributed by the protein source being evaluated.

Subjects

Table 1 presents the physical characteristics of the experimental subjects. They average 27.9 years of age, with an average height of 160.7 cm. It was observed that at the end of the study there was a slight decrease with respect to weight, although efforts were made to maintain caloric intake of the

Table 6. Nitrogen contribution by the best ingredients to the diets fed[a]

Type of diet	Nitrogen content (g/100 g diet)					
	Total		Basal[b]		Source of nitrogen[c]	
	(g)	(%)	(g)	(%)	(g)	(%)
Low nitrogen content	0.1	100	0.1	100	0.0	0.0
Extruded amaranth	2.5	100	0.2	8.0	2.3	92.0
Popped amaranth	2.3	100	0.2	8.6	2.1	91.3
Cheese	22.3	100	0.3	2.0	22.0	98.0

[a] Nitrogen supply determined by the Kjeldahl method.
[b] Nitrogen content supplied by all the foods except the protein source.
[c] Nitrogen content of the protein being evaluated and given as extruded amaranth puree. Popped amaranth, puree or cheese.

experimental subjects, adequate and constant. The decrease was probably due to the ordinary physical activity conducted developing the usual working activities and participating in heavy sport two to three times weekly. It was observed that one of the subjects whose working activity was not very active maintained his weight during the entire experimental period. Another possible explanation for the weight loss may be that the calories contributed by the foods may not be 100% available.

Biological evaluation

Endogenous nitrogen excretions. In Table 7 endogenous nitrogen excretion per subject, per period is shown. Urine nitrogen excretions (63.07 mg N/kg/day) as well as faecal nitrogen excretions (25.39 mg N/kg/day) were found in higher amounts during the first part of the study as compared with the excretions for periods II and III which were found to be more homogeneous. For period II the respective values are 49.00 and 21.61 for urine and faecal excretion, respectively, and 48.23 and 21.22 mg N/kg/day for period III. In the three periods the nitrogen intake from the low nitrogen content diet was similar.

The values obtained are higher than those reported in the study carried out by Huang et al. [33] evaluating the obligatory losses of urine and faecal nitrogen of 85 adult males receiving a free nitrogen diet during 14 days. They found mean values of 37.2 mg N/kg/urine weight in 8.76 mg N/kg/faeces weight; values that as can be observed, are significantly lower than those obtained in this study. Using the Nitrogen Balance Index – Short Method, however, Navarete et al. [26–28], and Bressani et al. [8, 14, 15, 17, 18] in different assays with humans found values for urine and faecal nitrogen excretion above the values of those reported in long-term studies

Table 7. Excretion of endogenous nitrogen by subject, by study period (mg N/kg/day)

	Periods								
	I			II			III		
Subject	NI	NU	NF	NI	NU	NF	NI	NU	NF
1	15.15	47.70	23.53	19.69	44.70	16.45	15.15	40.00	21.09
2	14.12	76.40	33.82	16.38	62.40	15.15	15.19	55.20	32.55
3	15.57	60.50	35.92	15.80	48.50	29.76	15.03	50.80	18.58
4	14.08	68.00	16.29	15.02	45.10	14.83	16.75	46.40	14.25
5	14.75	61.90	18.51	14.91	40.70	25.95	16.22	43.20	23.62
6	16.72	66.80	30.71	13.21	48.40	13.78	10.85	55.20	19.90
7	13.53	77.00	21.37	14.08	49.00	22.98	16.47	55.46	22.38
8	15.08	67.30	16.05	19.39	56.80	15.26	15.05	57.20	21.21
9	15.41	64.30	23.02	13.76	58.60	23.23	15.11	60.20	20.44
10	15.24	60.90	34.33	13.70	53.20	20.38	15.29	38.30	25.30
11	14.89	45.67	25.15	13.63	39.90	30.48	14.02	37.20	12.00
12	17.85	51.40	26.08	14.89	40.80	30.34	16.92	39.60	23.37
$\bar{X}$	15.23	63.07	25.39	14.95	49.00	21.61	15.24	48.23	21.22
SD ±	1.14	10.27	6.96	1.78	7.41	6.42	1.61	7.87	5.22

$\bar{X}$ = Average.
SD = Standard Deviation.

[26, 27, 34]. This tendency was also observed in the present study although with figures slightly higher than those reported by them, probably due to the fact that faeces of three days of free nitrogen diets were homogenized, and not only those of the last two days as established by the method.

Apparent nitrogen balance. Data on mean nitrogen intake, urine and faecal excretion per protein level are presented in Table 8 for balance studies with cheese and expanded amaranth proteins and also with the protein of amaranth processed by extrusion. In this Table it is observed for all protein sources fed that as the protein intake level increases, there is also an increase in the urine and faecal nitrogen excretion, resulting in a nitrogen balance which was negative at the 0.2 g protein/kg/weight level and becoming positive when the diets provided 0.6 g protein/kg/weight.

Digestibilities. The apparent and true protein digestibilities per subject per diet are presented in Table 9 for the intake level of 0.6 g protein/kg/day. When the apparent digestibility per subject per diet is analyzed, it is observed that with the control diet the percentages obtained fall within the range of 70.3 to 88.5%. These values are higher than those obtained with the extruded amaranth where the range was from 59.5 to 80.8% and for the popped amaranth, which ranged from 57.3 to 84.1%. The overall mean

Table 8. Apparent nitrogen balance per diet (mg N/kg b.wt./day)

Protein source level (g/kg)	Intake	Urine	Faecal	Absorbed	Retained
			$\bar{X} \pm SD$		
Cheese					
0.2	47.64 ± 9.04	47.19 ± 11.29	22.71 ± 14.61	24.93 ± 10.61	−22.25 ± 17.62
0.4	67.59 ± 8.39	44.94 ± 8.47	20.18 ± 5.81	47.41 ± 9.16	2.47 ± 13.44
0.6	105.72 ± 13.09	52.1 ± 9.74	20.60 ± 4.82	84.64 ± 13.29	31.86 ± 9.42
Extruded Amaranth					
0.2	51.12 ± 4.96	47.22 ± 11.18	25.64 ± 12.07	25.48 ± 15.14	−21.73 ± 22.50
0.4	76.38 ± 16.99	46.54 ± 8.81	27.83 ± 9.64	48.54 ± 18.90	2.00 ± 20.29
0.6	126.50 ± 11.16	55.87 ± 15.94	34.44 ± 8.49	92.04 ± 11.15	36.17 ± 15.66
Popped Amaranth					
0.2	54.28 ± 6.09	44.04 ± 10.91	25.16 ± 9.84	29.11 ± 10.77	−14.94 ± 20.03
0.4	93.07 ± 12.48	47.88 ± 9.55	31.62 ± 10.14	61.46 ± 19.77	13.60 ± 23.41
0.6	153.71 ± 26.49	58.59 ± 11.12	45.3 ± 16.31	108.40 ± 24.46	49.82 ± 18.18

Table 9. Percentage of apparent and true digestibility, per subject, per diet at a 0.6 grams protein level per kilo per day. Biological evaluation of the amaranth grain

Subject	Diet					
	Cheese control		Extruded amaranth		Popped amaranth	
	AD	TD	AD	TD	AD	TD
1	82.14	107.30	67.76	86.14	74.92	85.41
2	82.44	97.59	72.09	83.39	76.37	95.06
3	80.04	104.63	59.46	85.66	57.30	66.44
4	85.15	98.22	66.97	80.82	84.08	91.29
5	88.54	109.51	80.81	99.80	70.05	83.99
6	82.42	102.56	72.54	94.44	76.65	86.68
7	70.32	92.70	74.09	89.96	70.05	83.99
8	83.20	99.11	77.92	90.67	71.48	88.33
9	75.30	96.51	78.60	94.94	75.07	92.36
10	76.83	101.08	72.30	88.87	62.03	85.59
11	77.17	104.10	79.53	89.68	68.48	92.90
12	75.11	104.07	72.10	93.33	74.01	90.39
$\bar{X}$	79.89	101.44	72.76	89.90	70.43	85.52
SD ±	4.86a	4.82a	6.11ab	5.34b	8.57b	9.24b

$\bar{X}$ = Average.
SD ± = Standard Deviation.

Table 10. Statistical analysis of the digestibility data

Source	G.L.	Variance analysis digestibility			
		Apparent		True	
		C.M.	S	C.M.	S
Subjects	11	40.432	NS	26.751	NS
Periods	2	25.685	NS	3.169	NS
Diets	2	291.246	**	811.703	**

Diet	Tukey test – diets digestibility	
	Apparent	True
Cheese	79.9 a	101.4 a
Extruded amaranth	72.8 ab	89.8 b
Popped amaranth	79.4 b	85.6 b

NS = Not significant ($P < 0.05$).
** = Significant ($P < 0.05$).

Table 11. Regression equations of nitrogen intake *vs* nitrogen retained per subject per diet, and global equations per diet

Subject	Diets		
	Cheese-control	Extruded amaranth	Popped amaranth
1	NR = −69.22 ± 1.12 NI	NR = −56.99 ± 0.80 NI	NR = −47.33 ± 0.68 NI
2	NR = −76.22 ± 0.84 NI	NR = −72.77 ± 0.73 NI	NR = −99.26 ± 0.94 NI
3	NR = −75.03 ± 1.07 NI	NR = −92.46 ± 0.95 NI	NR = −55.99 ± 0.67 NI
4	NR = −79.73 ± 1.12 NI	NR = −63.08 ± 0.81 NI	NR = −36.76 ± 0.70 NI
5	NR = −60.59 ± 0.94 NI	NR = −70.25 ± 0.99 NI	NR = −63.28 ± 0.69 NI
6	NR = −63.49 ± 0.95 NI	NR = −79.11 ± 0.78 NI	NR = −52.70 ± 0.71 NI
7	NR = −66.43 ± 0.88 NI	NR = −68.96 ± 0.96 NI	NR = −91.42 ± 0.96 NI
8	NR = −82.24 ± 1.07 NI	NR = −71.08 ± 0.86 NI	NR = −74.0 ± 0.89 NI
9	NR = −71.21 ± 1.07 NI	NR = −82.67 ± 0.94 NI	NR = −76.48 ± 0.81 NI
10	NR = −58.01 ± 0.93 NI	NR = −66.93 ± 0.85 NI	NR = −90.56 ± 0.93 NI
11	NR = −80.54 ± 0.85 NI	NR = −42.31 ± 0.98 NI	NR = −60.34 ± 0.87 NI
12	NR = −63.17 ± 1.06 NI	NR = −63.85 ± 0.87 NI	NR = −51.96 ± 0.76 NI
Global equation	NR = −69.02 ± 0.97 NI	NR = −68.64 ± 0.86 NI	NR = −66.49 ± 0.79 NI

Table 12. Regression equations of nitrogen absorbed *vs* nitrogen retained per subject per diet, and global equations per diet

Subject	Diets		
	Cheese-control	Extruded amaranth	Popped amaranth
1	NR = −43.72 ± 10.3 NA	NR = −43.22 ± 0.96 NA	NR = −39.41 ± 0.85 NA
2	NR = −54.32 ± 0.82 NA	NR = −59.79 ± 0.88 NA	NR = −68.27 ± 1.01 NA
3	NR = −43.43 ± 1.00 NA	NR = −54.25 ± 1.07 NA	NR = −45.69 ± 1.02 NA
4	NR = −63.45 ± 1.15 NA	NR = −51.20 ± 1.05 NA	NR = −31.23 ± 0.79 NA
5	NR = −39.48 ± 0.83 NA	NR = −46.69 ± 1.09 NA	NR = −53.66 ± 1.16 NA
6	NR = −46.07 ± 0.93 NA	NR = −56.56 ± 0.86 NA	NR = −43.21 ± 0.84 NA
7	NR = −47.26 ± 0.94 NA	NR = −53.51 ± 0.86 NA	NR = −72.07 ± 1.11 NA
8	NR = −64.04 ± 1.08 NA	NR = −48.75 ± 0.89 NA	NR = −54.12 ± 1.02 NA
9	NR = −53.79 ± 1.15 NA	NR = −60.50 ± 1.02 NA	NR = −53.83 ± 0.88 NA
10	NR = −37.10 ± 0.93 NA	NR = −47.31 ± 0.96 NA	NR = −60.96 ± 1.10 NA
11	NR = −56.40 ± 1.10 NA	NR = −36.22 ± 1.11 NA	NR = −37.18 ± 0.98 NA
12	NR = −38.87 ± 1.02 NA	NR = −42.93 ± 0.96 NA	NR = −35.94 ± 0.90 NA
Global equation	NR = −47.99 ± 0.97 NA	NR = −50.28 ± 0.98 NA	NR = −49.85 ± 0.96 NA

Table 13. Statistical analysis of the nitrogen intake to nitrogen retained relation and the nitrogen absorbed to nitrogen relation

Source	G.L.	Analysis of variance relation			
		NI to NR		NA to NR	
		C.M.	S	C.M.	S
Subjects	11	0.0041	NS	0.0087	NS
Period	2	0.0227	NS	0.0513	*
Diet	2	0.1138	*	0.0025	NS

Diet	Tukey test – diet relation	
	NI to NR	NA to NR
Cheese	0.992 a	0.998 a
Extruded amaranth	0.862 b	0.976 a
Popped amaranth	0.801 b	0.972 a

NS = Not significant ($P < 0.05$).
* = Significant ($P < 0.05$).

values for apparent protein digestibilities for cheese was of 79.9 ± 4.86, extruded amaranth 72.8 ± 6.11 and for popped amaranth of 70.4 ± 8.57. True protein digestibility followed the same tendency with a mean overall value for cheese of 101.4 ± 4.82, extruded amaranth 89.9 ± 5.34, and for popped amaranth of 85.5 ± 9.24. Analysis of variance for apparent and true protein digestibilities are presented in Table 10. Significative differences ($P < 0.05$) between diets were obtained. Tukey range test showed that the

Table 14. Minimum nitrogen requirements per diet to obtain nitrogen equilibrium: Biological evaluation of the amaranth grain

Diet	NI (mg N/kg/day)	NA (mg N/kg/day)
Cheese	71	49
Extruded amaranth	80	51
Popped amaranth	84	52
Other studies		
Milk	75–86	48–60
Casein	94	69
Texturized soy protein	97	70
Soybean isolate	87	62
Meat	85	64
Soybean/meat	92	68
Maize, beans	97	67
Beans	116	71
Rice/beans	96	
Plantain 'beans	112	

cheese diet is statistically equal ($P < 0.05$) to diet 2. Diets 2 and 3 are similar but diet 3 is different from diet 1.

The lower digestibility of the popped amaranth may be due to the high temperature used in its preparation, which also reduced available lysine as shown previously. Taking into consideration the advantages of popping with respect to acceptable organoleptic characteristics of the product [35]. it would be necessary to study how to control the conditions of the process to obtain an acceptable product without damaging its nutritional quality.

Nitrogen Balance Index. The relationship between nitrogen intake to nitrogen retained is shown in Table 11 per subject, as well as the global equation for all subjects per each diet. From these equations, the mg of N per kg body weight per day needed for nitrogen equilibrium for each source of protein were 71.1 for cheese, 79.6 for extruded amaranth and 84.2 mg N/kg/day for expanded amaranth. In these equations the slope (b value) indicates the net protein utilization [8. 15] per subject per diet. The higher values were observed when the cheese diet was fed (0.84–1.12) followed by the extruded amaranth diet (0.73–0.99) and then for the popped amaranth (0.67–0.96). The average value for all subjects on the cheese diet was 0.97 followed by the extruded amaranth diet with a value of 0.86 and for the popped amaranth with 0.79. Analysis of variance of the slopes of the regression equations of NI *vs* NR show (Table 13) significant differences between diets ($P < 0.05$), but not between periods and subjects. With the Tukey statistical test differences among diets were established, with the control diet being different than diets 2 and 3, representing the extruded and popped amaranth diets, respectively. The test also indicates no significant differences among diets, with the control diet being different than diets 2 and 3, representing the extruded and popped amaranth diets. respectively. Likewise the test does not indicate significant differences among amaranth based diets (diets 2 and 3). The statistical analysis was unable to establish significant differences between the extruded and the popped amaranth; the protein utilization for extruded amaranth, however, is higher (0.86) than that of the popped amaranth (0.79). The difference is probably due to the apparent digestibility of each of the products.

Table 12 presents the regression equations for the relationship between nitrogen retained *vs* nitrogen absorbed per subject per diet, as well as the overall equations for each diet. The slope of the line provides an index of the biological value of the protein [5]. According to the data, the values of the slopes per subject for the cheese diet varied between 0.82 and 1.15; with extruded amaranth from 0.88 to 1.11 and for popped amaranth between 0.79 and 1.16. The overall equation showed that the biological value indices

were similar between diets; the extruded amaranth with a value of 0.98 followed by cheese with 0.97 and popped amaranth with 0.96.

The regression analysis of the nitrogen balance data provides an index of biological value represented by the regression coefficient, which according to the literature [4, 5, 8, 15], values of approximately 1 show the higher efficiency. In this case, it could be stated that the three diets show similar protein efficiency which is confirmed by the statistical analysis of latin squares of the slopes obtained in the regression equations of the relation NR *vs* NA (Table 13). Such analysis does not indicate significant differences between subjects and diets, but difference between periods. To corroborate this result the Tukey test was carried out among diets, resulting similar for the three diets.

The results obtained suggest that the biological value of amaranth protein is comparable to that of cheese. The differences found in the NI *vs* NR case are probably due to the effect of the type of processing on the digestibility of the popped amaranth and on its lysine content where there was a decrease of around 19%.

The extrusion, on the contrary, caused no detrimental effect in the protein quality of amaranth grain, since the statistical analysis was not able to detect significant differences in the protein quality of the extruded amaranth, as compared to the protein of cheese.

Estimate of the minimum nitrogen requirement for nitrogen equilibrium. The NI value for nitrogen equilibrium was calculated using the global regression equations between NI *vs* NR. This value represents the nitrogen intake necessary to maintain nitrogen equilibrium (50.57.57). In Table 14 it can be observed that for cheese, this value is 71 mg N/kg/day (0.44 g protein/kg/day), for extruded amaranth it is of 80 mg N/kg/day (0.5 g protein/kg/day), and for popped amaranth it is 84 mg N/kg (0.52 g protein/kg/day).

The Table also shows results of other studies using the same nitrogen balance methodology of [1, 3, 8, 12, 16, 19, 20, 27]. The information indicates that the protein quality of the amaranth grain is as good as that derived from animal origin.

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