The amaranth protein has a high concentration of recently been aroused due to its high nutritive value. It has the additional advantage that it can be planted as an intermediate crop between successive soybean crops. Extrusion is considered one of the best methods to maximize the nutritive value of amaranth. The objective of this study was to evaluate the protein quality of raw, toasted and extruded amaranth. Defatted amaranth flour was extruded at 150°C and at four different moisture levels (11%, 13%, 15% and 24%). The samples were extruded through a screw of 3.55:1 compression ratio, 20:1 L/D ratio at the speed of 200 rpm in a laboratory extrusion machine.

Individually housed, male Wistar rats 21 to 23 days old maintained under controlled conditions of light and temperature, were used for the protein bioassays. The protein bioassay experiments used a total of eighty rats and were done according to published AOAC methods (1995). The extruded amaranth diet at 24% moisture reached higher corrected protein efficiency ratio (corrected-PER) than all the other treatments. Each of the extruded amaranth diet reached greater net protein ratio (NPR) values than casein control and raw and toasted amaranth diets. On the other hand, the true digestibility (TD) was similar to the raw and all processed grains, extruded and toasted diet, and lesser than casein control diet. The differences between extruded and raw amaranth samples were probably due to destruction of toxic factors present in the raw grain, in combination with increasing palatability, improvement of the carbohydrate use and it was not due to the increasing protein digestibility. The results indicate that extruded amaranth contains very high quality protein.

**KEY WORDS:** extrusion, toasting, amaranth, nutritional value.

**INTRODUCTION**

Amaranth (*Amaranthus caudatus* L.) is a false cereal belonging to the dicotyledonous class in the family Amaranthacea. The interest in amaranth has recently been aroused due to its high nutritive value. The amaranth protein has a high concentration of essential amino acids, especially lysine which is a limited amino acid in other crops (Bressani *et al.* 1989, Teutonico & Knorr 1985). Amaranth protein quality is very high and it has a performance comparable to the cheese when amaranth is studied in humans (Bressani *et al.* 1993). The amaranth has higher concentration of soluble fiber than others cereals, such as low growing crops.
as wheat, corn or oats. Its lipid composition presents polyunsaturated acids fats and squalene (Becker 1989, Bressani et al. 1987a). Moreover, amaranth has many minerals, such as calcium, sodium, iron, magnesium and vitamin E (Teutonico & Knorr 1985, Yáñez et al. 1994).

The amaranth processed under conditions that do not damage its protein and its essential amino acids availability, like moist heat cooking and extrusion, presents good protein quality, similar to casein. Furthermore, the nutritional quality of extruded amaranth is frequently the same (Bressani et al. 1992) or superior to casein (Mendonza & Bressani 1987).

Extrusion cooking has been optimized for amaranth snack production recently (Chávez-Jáuregui et al. 2000). This product showed high acceptability compared to the commercial brands. However, information about the protein quality on the optimized point and about extrusion conditions effects on the amaranth protein quality have not been studied yet.

An important interfering factor on both technological and nutritional qualities of extruded products is the moisture of the feeding material (Harper & Jansen 1985, Prudêncio-Ferreira & Arêas 1993a, Prudêncio-Ferreira & Arêas 1993b, Sánchez-Marroquin et al. 1985).

The objectives of this study were to evaluate the protein quality of defatted amaranth flour submitted to the extrusion, and to compare the protein quality observed in the raw and toasted amaranth in rats (Bressani 1988, Early & Early 1987). The following extrusion conditions were studied in order to accomplish this research: the optimized extrusion point (150°C and 15% moisture content), extreme conditions of flour moisture before the extrusion (11% and 24%) and the intermediary point (13%).

MATERIAL AND METHODS

Amaranth

The amaranth species employed was Amaranthus caudatus L., variety CAC-43 A, Oscar Blanco, provided by CICA - Centro de Investigación en Cultivos Andinos, Facultad de Agronomía y Zootecnia da Universidad Nacional de San Antonio Abad del Cusco, in Peru.

The seeds were milled using a knife mill (Model Termomatic, Marconi, made in São Paulo, SP, Brazil), and then defatted with n-hexane in Soxhlet apparatus to a residual lipid concentration of less than 1% before extrusion. The defatted flour had its proximate composition determined by conventional methods for moisture, protein, ash and lipids, as described by AOAC (1990) and Instituto Adolfo Lutz (1985).

Extrusion

The extrusion was performed in a laboratory single-screw extruder L/D of 20:1 (Laboratory machine, Miotto Ltda., made in São Bernardo do Campo, SP, Brazil), 20 mm diameter, at 70g min.\(^{-1}\) feed rate, 200 rpm screw speed, 3.55:1 compression ratio, 150°C central zone temperature (±10°C) and 4 mm die diameter. In the extrusion process defatted soybean was used as the material between runs to keep stable the extruder conditions.

Moisture contents of flours were adjusted by a gravimetric method (Instituto Adolfo Lutz 1985) with the addition of distilled water. The added water content (\(W, \%\)) was obtained by using a stoichiometric calculation according the equation:

\[
W = w_1 - \left( \frac{w_2}{100 - w_2} \right) 100
\]

where:

\(w_1\): moisture content to be reached (in this case, 11%, 13%, 15% and 24%); \\
\(w_2\): initial moisture content (g) of sample.

Toasted grain procedure

The samples were toasted according to a domestic method used in Peru, described in detail in Bressani et al. (1987b) and Ferreira (1999). Intact amaranth grains were directly put in a metal sheet and heated in a domestic stove through a medium intensity flame. The grains were mixed with a typical cutlery during all the process. The end of the process was established after the expansion of the grains, like a popcorn, that in this case occurred after two minutes of toasting.

Animals and diets

The animal study used eighty male albino Wistar rats (Rattus norvegicus, var. albino Rodentia mammalia) 21 to 23 days old, weighing an average of 46 ± 1.0 g. The rats were housed into metal cages, and the laboratory conditions were kept at controlled temperature and moisture under 12h light and 12h dark. They received food and water ad libitum, and the experiment lasted 28 days, except the protein free and raw amaranth groups that lasted 14 days.

Groups of eight animals were arranged per treatments in randomized completed blocks. The bioassay experiments were carried out according to AOAC (1995). Diets were prepared containing 10%
Protein, except for the protein free diet. All the groups were fed with iso-nitrogenous and iso-caloric diets. Casein was used as protein reference and the other groups were fed by diets made of raw, toasted and extruded amaranth at 150ºC in four different moisture content (11%, 13%, 15% and 24%) (Table 1).

Diets were weighed everyday during the experiment and the rats were weighed on each seven days. The Table 2 presents the compositions of all diets. Diets were weighed every day and the rats on each seven days. Feces were collected from the 11th to the 14th days of experiment.

**Protein assay**

The biological indexes were determined from the results of the weight gain, the total amount of protein consumed, the total amount of diet consumed, and the total nitrogen excreted in the feces. The biological indexes calculated were: protein efficiency ratio (PER), corrected-PER, net protein ratio (NPR), apparent digestibility (AD) and true digestibility (TD).

**Statistical analysis**

A SAS (Statistical Analysis System) computer program was used for statistical calculations. All the results of the weight gain, the total amount of diet consumed, the protein consumed, the total amount of diet consumed, the total nitrogen excreted in the feces. The average values were compared to each other with the Tukey method at a statistical probability of 5%.

The proximate percent composition of extruded, toasted and raw amaranth flours is shown in Table 2. The composition of the experimental diets is in Table 3.

The amount of diet and protein consumed were statistically similar for the extruded amaranth diets, but were different from casein groups. Similar to other studies, the consumption of the extruded amaranth diet was greater than the consumption of the raw amaranth diet. The raw amaranth diet reduced the protein weight for the rats (Figure 1) relative to the control and other amaranth diets. In addition, the raw amaranth grain did not promote good growth of the animals in this experiment, similarly to the results of Bressani et al. (1987b) and Mendonza & Bressani (1987).

The product obtained from the higher moisture content during the amaranth extrusion process (24% moisture content) was statistically different (p<0.05) either from the other extruded samples (including the optimum point) or from the toasted amaranth sample, in terms of the biological index corrected-PER (Table 4), similar to other extruded proteins, such as bovine lung (Campos & Arêas 1993). The PER values for the extruded samples were lower than the casein ones. The greatest corrected-PER value was observed for the highest moisture extrusion condition (24% moisture). The toasted amaranth had the lower PER value.

### Table 1. Ingredient composition of diets prepared from different protein sources

<table>
<thead>
<tr>
<th>Diets</th>
<th>V. M.</th>
<th>M. M.</th>
<th>cellulose</th>
<th>protein source</th>
<th>starch</th>
<th>oil</th>
<th>water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/100g</td>
<td>g/100g</td>
<td>g/100g</td>
<td>g/100g</td>
<td>g/100g</td>
<td>g/100g</td>
<td>g/100g</td>
</tr>
<tr>
<td>E A 11</td>
<td>1.0</td>
<td>2.9</td>
<td>-</td>
<td>68.5</td>
<td>15.8</td>
<td>7.9</td>
<td>4.3</td>
</tr>
<tr>
<td>E A 13</td>
<td>1.0</td>
<td>3.1</td>
<td>-</td>
<td>67.6</td>
<td>15.6</td>
<td>7.9</td>
<td>5.0</td>
</tr>
<tr>
<td>E A 15</td>
<td>1.0</td>
<td>3.2</td>
<td>-</td>
<td>63.0</td>
<td>24.7</td>
<td>7.7</td>
<td>3.4</td>
</tr>
<tr>
<td>E A 24</td>
<td>1.0</td>
<td>3.0</td>
<td>0.30</td>
<td>73.0</td>
<td>15.1</td>
<td>7.9</td>
<td>0.0</td>
</tr>
<tr>
<td>Toasted amaranth</td>
<td>1.0</td>
<td>3.0</td>
<td>0.30</td>
<td>63.0</td>
<td>23.0</td>
<td>4.1</td>
<td>5.6</td>
</tr>
<tr>
<td>Raw amaranth</td>
<td>1.0</td>
<td>2.9</td>
<td>-</td>
<td>73.5</td>
<td>20.7</td>
<td>2.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Casein</td>
<td>1.0</td>
<td>4.6</td>
<td>2.06</td>
<td>12.1</td>
<td>69.4</td>
<td>4.1</td>
<td>6.8</td>
</tr>
<tr>
<td>Protein free</td>
<td>1.0</td>
<td>5.0</td>
<td>2.06</td>
<td>0.0</td>
<td>79.8</td>
<td>8.0</td>
<td>8.1</td>
</tr>
</tbody>
</table>

1. E.A.11: extruded amaranth at 150ºC and 11% moisture content; E.A.13: extruded amaranth at 150ºC and 13% moisture content; E.A.15: extruded amaranth at 150ºC and 15% moisture content; E.A.24: extruded amaranth at 150ºC and 24% moisture content; w.b.: wet base; n =3.
2. protein content of casein > 85%
3. V. M.: Vitaminic Mix AOAC-1995 (g/Kg mixture): Vitamin A, 2000 UI; Vitamin D3 (colecalciferol), 200 UI; Vitamin E, 10 UI; Menadione, 0.5 mg/100g; Choline, 200; p-Aminobenzoic Acid, 10.0; Inositol, 10.0; Nicotinic Acid, 4.0; Calcium Pantothenate, 4.0; Riboflavin, 0.8; Thiamin-HCl, 0.5; Piridoxine-HCl, -0.5; Folic Acid, 0.2; Biotin, 0.04; Vitamin B12, 0.003 and Glucose q.s.p.1000.
4. M. M.: Mineral Mix AOAC-1995 (g/Kg mixture): NaCl, 139.3; KI, 0.79; KH,PO4, 389.0; MgSO4, 57.3; CaCO3, 381.4; FeSO4, 7H2O, 27.0; MnSO4.H2O, 4.01; ZnSO4.7H2O, 0.548; CuSO4.5H2O, 0.477; e CoCl2.6H2O, 6.023.
5. amount necessary to reach 10% protein content in the diet.
The PER test is regarded as one of the most important indexes for food quality protein evaluation, and it is standardized by the AOAC (Association Official Analytical Chemists). The obtained PER values for the casein control, 1.76 (Table 4), were smaller than those commonly reported in the literature, around 2.5 (De Angelis 1995). Thus, the corrected-PER index was used for comparative analyses of the protein quality of the studied treatments. This index is obtained by the percent relationship between the 2.5 value and PER value obtained for each treatment. Considering the corrected-PER, the highest protein efficiency was reached by the treatment based on amaranth 24% moisture.

The obtained value for NPR index of the casein control group, 3.2 (Table 4), were similar to those observed in Mendonza & Bressani (1987). Differently of PER index, NPR expresses the actual protein for the weight maintenance. Each of the extruded amaranth diet reached higher NPR values than raw and toasted amaranth and casein control diets (Table 4). On the other hand, the true digestibility (TD) was similar to all raw and processed grain, extruded and toasted samples, and lower than casein control diet (Table 5).

The definitions of endogenous and exogenous nitrogen are arbitrary with some crossover between categories. However, they are useful for evaluation of the biological quality of dietary protein. Endogenous nitrogen comes from the breakdown of the body tissue and represents nitrogenous compounds produced as a result of one-way reactions. Regardless of the dietary protein intake, the excretion of these compounds by normal individuals is relatively constant. In contrast,

Table 3. Chemical composition of the experimental diets fed to the animals

<table>
<thead>
<tr>
<th>Diets 1</th>
<th>moisture g/100g</th>
<th>ash g/100g</th>
<th>protein g/100g</th>
<th>lipids g/100g</th>
<th>crude fibre g/100g</th>
<th>NFF 2 g/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.A.11</td>
<td>10 ± 0.1 2/</td>
<td>4.4 ± 0.2</td>
<td>9.6 ± 0.2</td>
<td>8.9 ± 0.1</td>
<td>2.1 ± 0.03</td>
<td>64.2</td>
</tr>
<tr>
<td>E.A.13</td>
<td>10 ± 0.1</td>
<td>4.3 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>9.6 ± 0.3</td>
<td>2.0 ± 0.02</td>
<td>64.3</td>
</tr>
<tr>
<td>E.A.15</td>
<td>10 ± 0.2</td>
<td>4.1 ± 0.1</td>
<td>10.1 ± 0.2</td>
<td>8.4 ± 0.2</td>
<td>2.1 ± 0.03</td>
<td>65.6</td>
</tr>
<tr>
<td>E.A.24</td>
<td>10 ± 0.2</td>
<td>4.3 ± 0.1</td>
<td>9.5 ± 0.2</td>
<td>7.3 ± 0.2</td>
<td>2.1 ± 0.06</td>
<td>66.2</td>
</tr>
<tr>
<td>Toasted amaranth</td>
<td>10 ± 0.0</td>
<td>4.6 ± 0.2</td>
<td>9.5 ± 0.2</td>
<td>6.4 ± 0.1</td>
<td>2.1 ± 0.01</td>
<td>66.9</td>
</tr>
<tr>
<td>Raw amaranth</td>
<td>11 ± 0.0</td>
<td>4.1 ± 0.1</td>
<td>9.5 ± 0.2</td>
<td>7.2 ± 0.3</td>
<td>2.1 ± 0.01</td>
<td>65.8</td>
</tr>
<tr>
<td>Casein</td>
<td>12 ± 0.1</td>
<td>3.9 ± 0.1</td>
<td>9.7 ± 0.2</td>
<td>8.7 ± 0.3</td>
<td>2.1 ± 0.05</td>
<td>63.6</td>
</tr>
<tr>
<td>Protein free</td>
<td>12 ± 0.2</td>
<td>4.5 ± 0.2</td>
<td>0.0</td>
<td>8.3 ± 0.2</td>
<td>2.2 ± 0.02</td>
<td>73.0</td>
</tr>
</tbody>
</table>

1. E.A.11: extruded amaranth at 150°C and 11% moisture content; E.A.13: extruded amaranth at 150°C and 13% moisture content; E.A.15: extruded amaranth at 150°C and 15% moisture content; E.A.24: extruded amaranth at 150°C and 24% moisture content;
2. Average ± standard deviation; 3. Nitrogen fraction free; n =3.

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intestinal flora, desquamated intestinal cells, and portion. Fecal nitrogen also represents the nitrogen of amino acids are consumed. The exogenous nitrogen fraction is urea, which is produced when an excess of exogenous nitrogen fluctuates in response to the parameter and this corrupts the values of excreted nitrogen (Berdanier 1995). Currently, one protein free group is used for the calculations of the excreted endogenous.

In this research, it was observed that the true digestibility (TD) for experimental groups of rats fed amaranth ranged from 88.0 to 89.6 and the apparent digestibility (AD) ranged from 86.8 to 88.5. These results were not statistically different (p<0.05). However, there were statistically differences between experimental and casein control groups, with an average AD value of, 96.46 and TD, 97.25. Then, it may be that the experimental groups of raw and toasted amaranth had a super estimation for endogenous nitrogen because of the amount of diet consumed. In the research reported by Pedersen et al. (1987), the protein digestibility of the toasted amaranth samples was equal or lesser than the protein digestibility of the raw grain samples.

The explanation for the different results obtained by the extruded and raw amaranth samples can be: i) an increase in the palatability; ii) inactivation of the antinutritional factors; iii) improvement of the protein texture; iv) an improvement of the carbohydrate availability, similar to the increase that determination of protein quality based on the loss of endogenous nitrogen super estimate this value. This greatly affects the values obtained for poor quality protein sources, since inconsistent values for this parameter are often obtained. In these cases, body proteins are mobilized to support needed protein syntheses. The rest of the protein chain is excreted and this corrupts the values of excreted nitrogen.

Table 4. Biological indices of protein quality observed after 14 and 28 days

<table>
<thead>
<tr>
<th>Treatments</th>
<th>diet feeding (g)</th>
<th>weight gain (g)</th>
<th>protein feeding (g)</th>
<th>NPR</th>
<th>PER</th>
<th>corrected-PER (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14 days</td>
<td>28 days</td>
<td>14 days</td>
<td>28 days</td>
<td>14 days</td>
<td>28 days</td>
</tr>
<tr>
<td>E.A.11</td>
<td>182 ± 14ab</td>
<td>413 ± 39a</td>
<td>61 ± 4.1a</td>
<td>101 ± 16a</td>
<td>17.4 ± 1.3^a</td>
<td>39.5 ± 3.7^ab</td>
</tr>
<tr>
<td>E.A.13</td>
<td>174 ± 20a</td>
<td>393 ± 51^ab</td>
<td>60 ± 10a</td>
<td>96 ± 15^ab</td>
<td>17.8 ± 2.1^a</td>
<td>40.2 ± 5.2^a</td>
</tr>
<tr>
<td>E.A.15</td>
<td>167 ± 26a</td>
<td>402 ± 46^ab</td>
<td>46 ± 12^ab</td>
<td>93 ± 15^ab</td>
<td>17.1 ± 2.6^a</td>
<td>40.9 ± 4.2^a</td>
</tr>
<tr>
<td>E.A.24</td>
<td>164 ± 21a</td>
<td>414 ± 49a</td>
<td>54 ± 18a</td>
<td>122 ± 15a</td>
<td>15.5 ± 2.0a</td>
<td>39.1 ± 4.6a</td>
</tr>
<tr>
<td>Toasted amaranth</td>
<td>176 ± 47a</td>
<td>357 ± 53^b</td>
<td>38 ± 9c</td>
<td>69 ± 10c</td>
<td>17.5 ± 4.7^a</td>
<td>35.7 ± 5.3^ab</td>
</tr>
<tr>
<td>Raw amaranth</td>
<td>111 ± 11b</td>
<td>–</td>
<td>15 ± 4</td>
<td>–</td>
<td>10.5 ± 1.0^b</td>
<td>–</td>
</tr>
<tr>
<td>Casein</td>
<td>162 ± 26a</td>
<td>318 ± 49b</td>
<td>43 ± 9^ab</td>
<td>59 ± 13^bc</td>
<td>17.3 ± 2.7^a</td>
<td>34.1 ± 5.3^ab</td>
</tr>
<tr>
<td>Protein free</td>
<td>87 ± 9</td>
<td>–</td>
<td>-5.9 ± 2</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

1- E.A.11: extruded amaranth at 150ºC and 11% moisture content; E.A.13: extruded amaranth at 150ºC and 13% moisture content; E.A.15: extruded amaranth at 150ºC and 15% moisture content; E.A.24: extruded amaranth at 150ºC and 24% moisture content; w.b.: wet base, n = 8, NPR: net protein ratio, PER: protein efficiency ratio; \(^2\): Average ± standard deviation; n=8, different letters in the same column indicated significant statistical differences between the averages (Tukey, \(\alpha = 0.05\))

Figure 1. Growth curves of rats 21 to 23 days old (n = 8) on diets containing 10% protein form E.A.11 (extruded amaranth at 150ºC and 11% moisture content), E.A.13 (extruded amaranth at 150ºC and 13% moisture content), E.A.15 (extruded amaranth at 150ºC and 15% moisture content) E.A.24 (extruded amaranth at 150ºC and 24% moisture content), Raw A (raw amaranth) and without protein (protein free)
occurs with soybean upon processing (Mendonza & Bressani 1987). In addition, these results were not due to increasing protein digestibility.

Although the raw amaranth grain presents an excellent amino acids profile, that is not reflected in protein bioavailability. In this case, the protein quality can be improved by mild extrusion processing conditions (Bressani et al. 1989), like that ones of this research.

CONCLUSIONS

1. The experimental diets based on the extruded amaranth samples had the better biological indexes of the protein quality than the raw or toasted amaranth samples.

2. The product obtained from the highest moisture content (24%) during amaranth extrusion had better protein quality, for the most of biological indexes evaluated, than the samples obtained at other extrusion conditions. In addition, the extrusion treatments were also better than others treatments (raw and toasted amaranths and casein).

3. The results showed that extruded amaranth can be regarded as a product with high protein bioavailability.

REFERENCES


