Ciência e Tecnologia de Alimentos
ISSN: 0101-2061
revista@sbcta.org.br
Sociedade Brasileira de Ciência e Tecnologia de Alimentos
Brasil

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Ciência e Tecnologia de Alimentos, vol. 35, núm. 2, abril-junio, 2015, pp. 326-330
Sociedade Brasileira de Ciência e Tecnologia de Alimentos
Campinas, Brasil

DOI: http://dx.doi.org/10.1590/1678-457X.6539

Annatto seed residue (*Bixa orellana* L.): nutritional quality

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Abstract

Considering that annatto seeds are rich in protein, the present work aimed to evaluate the biological quality of this nutrient in the meal residue originating from annatto seed processing. We determined the general composition, mineral levels, amino acid composition and chemical scores, antinutritional factors, and protein quality using biological assays. The following values were obtained: 11.50% protein, 6.74% moisture, 5.22% ash, 2.22% lipids, 42.19% total carbohydrates and 28.45% fiber. The residue proved to be a food rich in fiber and also a protein source. Antinutritional factors were not detected. The most abundant amino acids were lysine, phenylalanine + tyrosine, leucine and isoleucine. Valine was the most limiting amino acid (chemical score 0.22). The protein quality of the seed residue and the isolated protein showed no significant differences. The biological value was lower than that of the control protein but higher than that found in other vegetables. Among the biochemical analyses, only creatinine level was decreased in the two test groups compared to the control group. Enzyme tests did not indicate liver toxicity. The results showed favorable aspects for the use of annatto seed residue in the human diet, meriting further research.

Keywords: biological value; protein quality; annatto.

Practical Application: The evaluation of biological quality of the annatto seeds' protein showed favorable aspects for the use in the human diet.

1 Introduction

With the arrival of the Spanish conquerors in the New World, many plants whose extracts were used by the Mayans and Aztecs became known. One of these plants, annatto, occurring throughout tropical America was used as an extract to color fabrics and as body paint, and besides, it was used together with vanilla in the formulation of drinks made with cocoa (Giuliano, et al., 2003; Sandi & Cuen, 2003; Oliveira, 2005).

Annatto seeds are an important raw material to obtain the pigment bixin (represents more than 80% of the total fat-soluble carotenoids), norbixin and norbixinate, whose levels are variable according to seeds maturation (Dornelas et al., 2015; Mantovani et al., 2013). This is due to their characteristics as a non-toxic product with high dying power and ample color used for coloring food, products, cosmetics, pharmaceuticals and textiles, besides being used in cooking, as with the Brazilian spice colorau. Brazil is one of the largest producers and exporters of natural dye extracted from this plant (Mantovani et al., 2013; Anselmo et al., 2008). Annato seeds have been used to treat various diseases in popular medicine including for high lipid blood levels (Ferreira et al., 2013). Bacterial activity of annatto extracts was proportional to bixin content for various bacteria (Majolo et al., 2013).

The annatto seed contains cellulose, sucrose, oils, fragrances, and alpha-and beta-carotene (Paz et al., 2006). The dry meal residue from annatto seed processing has been found to consist of 13.5% crude protein, 45.7% neutral detergent fiber, 1.5% ether extract, 6.2% mineral matter and 63.8% nitrogen-free extract (Anselmo et al., 2008; Utiyama et al., 2002).

Therefore, since annatto seeds are rich in protein, this study aimed to evaluate the biological quality of this nutrient, the composition of the essential amino acids and other nutrients in the residue of annatto seeds, as well as evaluating the presence of some antinutritional factors.

2 Materials and methods

The annatto seed residue was donated in 2011 by a food industry, CHR HANSEN, located in Valinhos, São Paulo. The waste, consisting of wet annatto seeds and impurities (pieces of branches of the annatto tree), were dried in a forced ventilation oven at 50°C for 24 hours, then cleaned and milled to obtain a meal.

The composition of the annatto seed meal was determined using the methods described by the Adolfo Lutz Institute (Brasil, 2005) for moisture, fixed mineral residue (ash), carbohydrate, fiber, neutral detergent and lipids. Protein was determined by the micro-Kjeldahl method according to the Association of Official Analytical Chemists (1992).

The extract used in the determination of mineral was obtained by the method described by Moraes & Rabelo (1986) and Salinas et al. (1985). The minerals cupper, zinc, manganese, calcium, iron and magnesium were analyzed by flame atomic

Received 15 Dec., 2014
Accepted 08 Mar., 2015
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absorption spectrometry and the calcium in flame photometer, 589nm (Varian model Spectra AA 220FS). The quantification was done by standard curve of each mineral.

Tannins were determined according to the method described in the Association of Official Analytical Chemists (1984). Phytic acid content was analyzed by the method of Latta & Eskin (1980). Trypsin inhibitory activity was evaluated by the method of Erlanger et al. (1961), and lectins were determined according to Witisuwannakul et al. (1998).

The solubility of the protein the annatto seed residue meal according to pH was determined by the methods described by Glória & Regitano-D'Arce (2000). Proteins of the residue meal extract were assayed according to Macedo & Damico (2000), and amino acid analysis was performed by the method of Heinrikson & Meredith (1984), using the analyzer Pico-Tag (Waters System) and identification in HPLC reverse phase column, comparing the retention times of the amino acids of the sample with the standards (Pierce).

The biological assay was carried out in 40 rats, males, age of 21 days, (10 per group), Wister, *Rattus norvegicus*, in a clean environment with controlled temperature of 25ºC and appropriate light, with a 12-hour light/dark cycle, lasting 28 days. The use of the animals was approved in the ethics committee, n. 267, by the Ethic Committee in Animal use CEUA/UFMS.

The diets offered to the animals were prepared in accordance with AIN93-G (Reeves et al., 1993) modified for the following groups: control (casein), non-protein, test 1 (annatto seed residue) and test 2 (isolated protein). During the experiment, feces and urine were collected from the animals in each group for quantification of excreted nitrogen by the Kjeldahl method as described by the Association of Official Analytical Chemists (1992). From these results, the indices that provide information on the quality of the protein were calculated. Protein efficiency rate (PER) and biological value (BV) were determined according to the methods described by Hiane et al. (2006) and Tirapegui et al. (2012). Coefficient of feeding efficiency (CFE) was calculated according to Souza (2003) and true digestibility (TD) using the method of Cruz et al. (2005) and Tirapegui et al. (2012). Nitrogen balance (NB) was calculated according to the method of Hiane et al. (2006). The animals' weight was monitored weekly.

At the end of the experiment, after fasting for 12 hours, blood was collected and glucose, total cholesterol, creatinine, triglycerides, very low density lipoprotein (VLDL), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using specific kits for each sample and using an automated 400 Plus Cobas Integra System (Roche) according to methods described in the instrument manual.

3 Results and discussion

The composition of the seed residue meal showed a high levels of protein (11.5%), fixed mineral residue (5.22%), carbohydrates (42.2%) and fiber (28.48%) (Table 1). The protein content here was slightly higher than that recorded by Carvalho et al. (1991) (10.8%) and similar to that obtained by Pedrosa et al. (1999) (12.67%) for annatto seeds, compared to 11.56% for wheat flour reported by Pires et al. (2006).

Table 1. Proximate composition of the annatto seed residue expressed in g.100 g^{-1} of whole sample*.

Parameter	Means ± SD*	%DRI	DRI (g)**
Moisture	6.75 ± 0.089	-	-
Fixed mineral residue	5.22 ± 0.35	-	-
Total lipids	2.23 ± 0.11	4.05	60
Protein	11.50 ± 0.06	15.33	75
Total carbohydrate	42.2 ± 0.69	14.06	300
Sucrose	11.78 ± 0.02	-	-
Starch	30.42 ± 0.58	-	-
Fiber	28.48 ± 0.91	113.92	25
Total caloric value (kcal/100g**)	234.46	11.72	2000

*Mean values of 3 determinations ± standard deviation. **Dietary reference intake based on a diet of 2000 kcal (Brasil, 2003).

The percentage of fiber, however, was lower than the 36.8% obtained by Utiyama et al. (2002) and much higher than the 16% found by Bressani et al. (1983). Comparing the protein and fiber levels with the daily reference intake values established by Brazilian Resolution RDC No. 360/2003 (Brasil, 2003) and with classification of food as rich or sources of nutritious food when providing 30 or 15% of the recommended intake in 100 g, respectively, according to current Brazilian law (Brasil, 1998), the annatto seed residue can be classified as rich in fiber and a protein source.

The total carbohydrate was high (42.2%), but lower than that obtained by Moraes (2007) (78.11%). The percentage of minerals was similar to the 5.05%, found by Moraes (2007) and comparable to the 6.32 and 6.2%, obtained respectively by Pereira et al. (2009) and Utiyama et al. (2002).

The pH at which the highest protein solubility was seen in the studied sample (pH 12) was similar to that found by Glória & Regitano-D'Arce (2000). According to the used assays, tannins, phytic acid and protease inhibitors were not detectable in the sample.

The amino acid composition of the sample showed high levels of lysine (76.7 $mg.g^{-1}$), isoleucine (59.3 $mg.g^{-1}$), leucine (83.9 $mg.g^{-1}$) and phenylalanine + tyrosine (100.2 $mg.g^{-1}$) (Table 2). These levels higher than intake levels were for adults recommended by FAO/WHO (Food and Agriculture Organization of the United Nations, 2007), which are 45, 30, 59 and 30 $mg.g^{-1}$ protein, respectively. The amino acid valine was the most limiting with a chemical score of 0.22.

The minerals in annatto seed showing the highest values were sodium (35.61 $mg.g^{-1}$) and potassium (70.77 $mg.g^{-1}$), followed by manganese (0.25 $mg.g^{-1}$), calcium (0.11 $mg.g^{-1}$) and copper, iron and magnesium (0.03 $mg.g^{-1}$). The values found for potassium, zinc, copper and manganese were higher than those found by Ferreira & Falesi (1989), respectively, 19.2, 0.0354, 0.0046 and 0.023 $mg.g^{-1}$, while these authors reported a higher magnesium level (2.22 $mg.g^{-1}$). Calcium and iron levels here were higher than those reported for annatto seeds from

EMEPA (0.07 and 0.008 mg.g^{-1}, respectively) by Anselmo et al. (2008), but calcium was lower than that found by Ferreira & Falesi (1989) (1.82 mg.g^{-1}).

During the 28-day experiment, it was observed that there was a decrease in weight of the animals in the group without protein; the casein and test 2 (isolated protein) groups showed higher weight gain, 64.87 and 65.74 g, respectively; although, there was no statistically significant difference ($p > 0.05$) between these groups and test 1 (annatto seed residue) (55.08 g). As to the food that was consumed, there was no significant difference between the casein (392 g), test 1 (384.74 g) and test 2 (415.27 g) groups, indicating that the protein quality of the three diets was the same.

Table 2. Composition and the chemical score (CS) of the essential amino acids of the isolated protein (IP) of the annatto seed residue and the comparison with the FAO/WHO requirements for adults.

	IP (mg.g^{-1} protein)	CS	FAO* (mg.g^{-1} protein)
Lysine	76.7	1.70	45
Histidine	7.2	0.48**	15
Threonine	10.9	0.47**	23
Valine	8.5	0.22**	39
Methionine	12.8	0.8**	16
Methionine + cysteine	12.8	0.58**	22
Isoleucine	59.3	1.98	30
Cystine	Nd	Nd	6
Leucine	83.9	1.42	59
Phenylalanine + Tyrosine	100.2	3.34	30

*Food and Agriculture Organization of the United Nations (2007). **Limiting amino acids; nd: not determined.

Considering the protein quality indices (Table 3), there was no significant difference between the annatto residue group and the isolated protein group in relation to PER ($p > 0.05$), but both differed from the casein group. Although protein quality is low in vegetables, the PER found for both groups was higher than that found by Pires et al. (2006) in wheat flour (0.98 g) and soy flour (1.75 g) and also the values obtained by Hafez et al. (2009) in bread with 10% salt (0.94 g) and 15% (1.45 g) linseed flour. Tirapegui et al. (2008), obtained a PER value of 2.1 g for soybeans and 1.5 g for wheat.

Although the biological values observed in the annatto residue group (94.89%) and isolates protein group (95.95%) were lower than in the casein group (99.01%), they were higher than those obtained by Silva et al. (2006) for soybean grain (87%) and soybean residue (88,1%).

In relation to the biochemical parameters (Table 4), only the data obtained for the total cholesterol for the casein group (75 mg.dL^{-1}) was similar to that found by Santos et al. (2010) (75 mg.dL^{-1}), while both test groups (test 1 and test 2) had higher values when compared to the data from the same authors (82.5 and 94.83 mg.dL^{-1}, respectively).

The triglyceride levels showed no significant differences between the different groups studied, although the test groups were slightly higher than the casein and also above the reference value for BALB/c and C57BL6 mice, from 21.11 to 29.68 mg.dL^{-1} as evaluated by Almeida et al. (2008) but close to that found by Santos et al. (2010) (47.6 mg.dL^{-1}). There were statistically significant differences in glucose levels in both groups (Test 1 and Test 2); however, they were lower than that found by Santos et al. (2010) (85 mg.dL^{-1}). The creatinine values showed a significant difference only with the control casein group; in the test groups the values were lower, indicating that the proteins in the annatto seed residue caused a decrease in creatinine levels.

Table 3. Protein quality indices of the casein (CA), annatto residue (test 1) and isolated protein (test 2) groups.

Protein quality index	CA*	Test 1*	Test 2*
Protein efficiency rate (PER) (g)	2.57 ± 0.25a	1.71 ± 0.41b	2.10 ± 0.33b
Coefficient of feeding efficiency (CFE) (g)	0.17 ± 0.02a	0.14 ± 0.03a	0.16 ± 0.02a
Biological value (BV) (%)	99.01 ± 0.22a	94.89 ± 1.24b	95.95 ± 0.52b
Nitrogen balance (NB) (g)	3.64 ± 0.67a	4.08 ± 0.94a	4.39 ± 0.82a
True digestibility (TD) (%)	96.43 ± 0.76a	87.99 ± 2.53c	92.63 ± 1.14b

*Values followed by the same letter in a row are not significantly different by Tukey's test at 5% probability.

Table 4. Biochemical parameters (mean ± sd, N = 10/group) of the casein (CA), annatto seed residue (test 1) and protein isolate (test 2) groups at the end of the biological assay.

Parameter (mg.dL^{-1})	CA*	Test 1*	Test 2*
Glucose	72.40 ± 14.64a	59.33 ± 12.72a	70.20 ± 15.29a
Total cholesterol	75.00 ± 10.65b	82.5 ± 5.96ab	94.83 ± 9.43a
Creatinine	0.3 ± 0.00a	0.2 ± 0.00b	0.23 ± 0.05b
Triglycerides	47.60 ± 19.48a	48.83 ± 16.58a	68.17 ± 17.89a
VLDL[1]	12.17 ± 7.68a	9.83 ± 3.37a	13.67 ± 3.72a
ALT[2]	24.17± 6.82a	32.17± 5.49a	27.67 ± 5.65a
AST[3]	155.5 ± 24.94a	149.33 ± 45.22a	152.33 ± 48.19a

*Values followed by the same letter within a row are not significantly different by the Tukey's test at 5% probability. [1]VLDL-very low density lipoprotein. [2]ALT-alanine aminotransferase. [3]AST-aspartate aminotransferase.

4 Conclusion

Annatto seed residue was found to have high levels of protein, carbohydrates, fiber and minerals, and considered to be a food rich in fiber and protein. The most abundant minerals found were potassium and sodium. Substantial concentrations of the amino acids lysine, isoleucine, leucine and phenylalanine + tyrosine were found, while valine was the limiting amino acid with a chemical score of 0.22. The antinutritional factors tannins, phytic acid and protease inhibitors were not detected.

The proteins of the annatto seed residue and from the isolated protein showed a lower biological value compared to the control protein. Among the biochemical parameters evaluated in the blood samples of the animals at the end of the experiment, only total cholesterol showed a significant difference between the groups; the creatinine level in the test groups was reduced, and the enzyme assays did not indicate liver toxicity.

Although the results showed favorable aspects for the use of the seed residue in food, further studies are needed to confirm these findings.

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