Proteins of Bacuri almonds - Nutritional value and *in vivo* digestibility

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Abstract

Bacuri (*Scheelea phalerata* Mart.) is a type of palm fruit tree widely distributed in the Brazilian Cerrado. The objective of this paper was to study the almonds of bacuri, in their form *in natura* and processed, focusing on their nutritional value through the profile of amino acids, anti-nutritional factors and *in vivo* digestibility. Raw and toasted samples of the almond presented a high level of proteins and fiber. Proteins of raw bacuri almond showed no limiting amino acid when compared to the ones recommended by FAO/WHO, and histidine was the most limiting essential amino acid in the toasted almonds. The almond of bacuri does not present anti- nutritional factors. In an assay with rats fed with control (casein), tests (bacuri almond flours) and aproteic diets, we verified the quantity of ration ingested and body weight gain, determining the urinary and metabolic nitrogen. Rats treated with the test diets presented inferior values of True Digestibility (DV), (82.9 and 72.3%, respectively for the raw and toasted almonds) when compared to the control group (92.3%). The raw bacuri almond presented a superior nutritional value to the one found in the toasted almond.

Keywords: *Scheelea phalerata* Mart.; biological assay; anti-nutritional factors; amino acid profile.

1 Introduction

Several fruits from native species of the Brazilian Cerrado have high nutritional value and pleasing features such as color, flavor, and intense and peculiar aromas; however, some have still not been fully explored commercially (Vieira et al., 2008). Some native fruits of the Cerrado, such as araticum, buriti, cagaita and pequi are excellent food sources with nutrient levels equivalent to or even higher than those of fruits traditionally consumed by the Brazilian population, for example avocado, banana, and guava (Roesler et al., 2007).

Studies on alternative sources of nutrients, such as foods of plant origin, are necessary, since they can supplement the diet of needy individuals (Madruga et al., 2004; Denadai et al., 2007). In addition, the investigation of the chemical composition of native foods contributes to a better understanding of the relationship between nutrition and biodiversity, especially regarding production and processing of food for human consumption (Fernandes et al., 2010), and that between nutrition and human health since the consumption of Brazilian nuts have shown health benefits with respect to cardiovascular diseases and cancers (Yang, 2009).

Brazilian Cerrado almonds have been widely studied; and some such as baru (Fernandes et al., 2010; Guimarães et al., 2012; Czedera et al., 2012) and the bocaiuva (Hiane et al., 2006) have high content of quality protein. Bacuri (*Scheelea phalerata* Mart.), also known as acuri or acurizeiro, belongs to the Palmae family and is a widely distributed fruit in the States of Mato Grosso and Mato Grosso do Sul in Brazil. The color of the pulp of this fruit varies from yellow to orange due to the presence of carotenoids; some of these carotenoids are vitamin A precursor.

In addition, the pulp oil of is rich in saturated, unsaturated, and polysaturated fatty acids, especially the oleic acid, which is found in large quantities (Hiane et al., 2003).

From a technological point of view, the study of proteins comprises of a fundamental aspect for the incorporation of proteins in food systems due to their applications in food products (Sánches-Vioque et al., 1999).

The bio-availability of amino acids depends on the digestibility of proteins (Neves et al., 2006) and is a determinant of the protein quality of a diet (Pires et al., 2006). Low protein digestibility is usually found in nuts (Neves et al., 2006). Heat treatment can increase protein digestibility and reduce anti-nutritional factors present in foods (Silva et al., 2010; Naves et al., 2010). With the objective of making a better use of bacuri fruits, this study investigated the nutritional value of bacuri almond proteins through their profile of amino acids, presence of anti-nutritional factors, and *in vivo* digestibility assessed in rats.

2 Materials and methods

2.1 Raw material and flour preparation

Around 20 kilos of ripe bacuri fruits were collected from the regions of Campo Grande, Bodoquena, Bonito, and Santa Rita do Pardo in the State of Mato Grosso do Sul, Brazil. The fruits collected were newly fallen or were on the ground in perfect morphological condition.

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The almonds were removed from the fruits and ground using a Turratec grinder and sifted through a 60 mesh screen forming whole flour (10 kilos). A sufficient amount of the whole flour was separated for chemical composition analysis. The remainder was defatted (raw flour) with petroleum ether P.A. (30-60°C) by cold extraction, and half of the raw flour was dried in an oven at a temperature of 200 ° C (15 min) (Togashi & Sgarbieri, 1995) and homogenized resulting in a toasted almond flour.

2.2 Chemical composition

The moisture of the samples was determined by the gravimetric method (oven at 105°C) (Instituto Adolfo Lutz, 2005). The proteins were determined according to the micro Kjeldahl method (Association of Official Analytical Chemists, 1992) using the factor 6.25 for the conversion of nitrogen into protein. The total lipids were determined by the direct extraction method with petroleum ether in a Soxhlet apparatus) (Instituto Adolfo Lutz, 2005); ash (fixed mineral residue) was determined by the gravimetric method (oven at 105°C)) (Instituto Adolfo Lutz, 2005) and the carbohydrates according to the Lane-Eynon method, which determines reducing and non-reducing sugars) (Instituto Adolfo Lutz, 2005). The total dietary fiber was determined using the enzymatic-gravimetric technique (Prosky et al., 1988).

2.3 Composition and chemical score of amino acids

The composition of amino acids was determined in the raw and toasted samples of almond flour using a Pico-Tag amino acid analyzer (Waters system) (Henrikson & Merdeith, 1984). Proteins of the almond flour were extracted with 150ml of 4% NaCl for 1h. The samples were hydrolyzed in 6M HCl/1% phenol at 106˚C for 24h. The hydrolysate reacted with 20µL of fresh derivatization solution (metanol: triethylamine: water: phenylisothiocyanate, 7:1:1:1, v/v) for 1h at room temperature. After derivatization, phenylisothiocyanate (PTC) amino acids were identified on a reverse-phase HPLC by comparing their retention times to those of standard PTC amino acids (Pierce). Cysteine residues were quantified as cysteic acid. The Essential Amino Acids Score (EAE) was calculated according to the FAO/WHO standard of reference (Food and Agriculture Organization & World Health Organization, 2007).

2.4 Protein Digestibility Corrected Amino Acid Score (PDCAAS).

The calculation of PDCAAS was based on the chemical score of the limiting essential amino acid of the test protein, which was multiplied by true protein digestibility (Henley & Kuster, 1994).

2.5 Anti-nutritional factors

Hemagglutination activity and inhibitory activity of trypsin and chymotrypsin

Hemagglutination assay was performed in microtiter U-plates using serial dilutions (50µL) with 0.15 M NaCl; 50µL volume of 2% suspension of type A human erythrocytes was added, and after 1 hour at room temperature, the results were read. Hemagglutination titer is defined as the reciprocal of the highest dilution that produces one hemagglutination unit (HU) (Freire et al., 2002). A negative control was prepared using erythrocytes and a saline solution.

Bovine pancreatic trypsin and bovine pancreatic chymotrypsin were used in the enzymatic assays. Trypsin-like activities were assayed using Nα-benzoyl-DL-arginine *p* – nitroanilide (BApNA) as substrate. Chymotrypsin-like activities were assayed using N-benzoyl-L-tyrosine p-nitroanilide (BTpNA) as substrate. For the enzymatic assays, pancreatic bovine trypsin and chymotrypsin were used with BApNA as substrate.

A standard assay is a reaction mixture of 50µL of each enzyme extract, reaction buffer. (0.1-M Tris-HCl buffer, pH 8.0), and 50µL of 1-mM substrate reaching the final volume of 500µL. The reaction is stopped by adding 200µL of 30% acetic acid.

The release of *p*-nitroaniline groups was measured spectrophotometrically at 410nm.

The protease inhibitor was assayed by preincubating 50µL of each fraction at concentrations ranging from 25 to 200µg with 50 µL of protease and 350µL of reaction buffer at 37°C for 15 min. The reaction was started upon adding the substrate and was performed as described above. The remaining activity was expressed as the percentage of the enzymatic activity in the absence of inhibitor (Macedo et al., 2003). The presence of enzymatic activity was determined by the hydrolysis of the substrate and the consequent release of p-nitroanilide.

2.6 Nutritional assessment

Diets and biological assay

The nutritional assessment of bacuri almond proteins was performed using a biological assay. Four different types of ration composed the following treatment groups:

- Aproteic Group (Aproteic Ration = no addition of protein in the formulation);
- Casein Group (Standard/Control Ration = use of casein protein in the formulation);
- Test Group 1 (Test Ration 1 = use of raw defatted bacuri almond flour in the formulation);
- Test Group 2 (Test Ration 2 = use of toasted defatted bacuri almond flour in the formulation).

The diets were formulated according to the procedure described by the American Institute of Nutrition (AIN-93 diet) (Reeves et al., 1993) for growth and were adjusted according to the centesimal composition of the defatted flours (Table 1).

The diets were formulated to contain 10% protein, 8% fat, 9% fiber, 4% salt mixture, 1% vitamin mixture, 10% sucrose, 0.1% sodium benzoate (preservative action), and enough starch to make 100% of ration. Only cellulose fibers were used in the control and aproteic diets, while in the test 1 and test 2 diets, the

amount of cellulose fiber was complemented considering the bacuri almond fiber content. In the aproteic diet, protein was substituted for corn starch in a sufficient amount to complete the (100%) composition. The aproteic diet was used to estimate the endogenous and metabolic nitrogen of the rats.

Twenty- one day old male Wistar rats (average body weight of 50.06 g) were randomly divided into 4 groups (n = 7 each) and housed individually in metabolic cages under standardized environmental conditions (12-hour cycles of light and dark and temperature around 25°C). Each group was fed a different type of ration, and portable water was provided *ad libitum* for 23 days. Each animal as well as the remnants of the rations were weighed on alternate days allowing the determination of the total amount of feed consumed and the subsequent analysis of the N content that was ingested. Feces and urine were collected and identified separately for each group throughout the experiment and were later analyzed by the Kjeldahl method (Association of Official Analytical Chemists, 1992). Based on the data obtained, it was possible to calculate the indices that evaluated the protein quality of the feed.

At the end of the experiment, all animals were anesthetized with ketamine + xylazine before sacrifice in a CO_2 chamber. The present study was approved by the Ethics Committee for Use of Animal in Research (CEUA/UFMS), following the Regulations of the Brazilian College of Animal Experimentation (COBEA).

2.7 Statistical analysis

The statistical data were analyzed using analysis of variance (ANOVA) with a significance level of 1% ($p< 0.01$) and the Tukey test for comparison among the means. The results were expressed as means ± standard deviation.

3 Results and discussion

3.1 Chemical composition

The chemical composition of the flours is described in Table 2 below.

The most abundant components present in the whole bacuri almond flour are lipids and proteins. The extraction of oils and fats from the whole flour resulted in a flour with high content of proteins, close to that of bocaiuva almond flour (41.34%) (Hiane et al., 2006) and higher than that of baru almond (23.78%) (Fernandes et al., 2010) and sapucaia nuts (20,47%) (Denadai et al., 2007).

3.2 Amino acid profile

In Table 3, the amino acid profile was compared to the standard established by the FAO/WHO (Food and Agriculture Organization & World Health Organization, 2007) for children between 1 and 2 years old. The findings show that the levels of amino acid in the raw bacuri almond are in conformity to those previously established by the FAO/WHO. Proteins

Table 1. Formulation of Aproteic Ration, Standard/Control Ration (casein), Test Ration 1 (raw defatted bacuri almond flour), and Test Ration 2 (toasted defatted bacuri almond flour), expressed in g/100g of ration.

Components	Aproteic	Standard	Test 1	Test 2
Casein (86.67% protein)	-----	11.54	-----	-----
Bacuri Almond Flour	-----	-----	31.30**	28.64***
Fat****	8.0	8.0	7.87	7.79
Fiber****	9.0	9.0	0.68	0.64
Salt mixture	4.0	4.0	4.0	4.0
Vitamin mixture	1.0	1.0	1.0	1.0
Sucrose****	10.0	10.0	8.09	8.67
Starch	67.9	56.36	46.96	49.16
Sodium Benzoate	0.1	0.1	0.1	0.1

**Raw bacuri almond flour = 31. 95% of protein. ***Toasted bacuri almond flour = 34. 92% of protein. ****Adjusted to conform to the chemical composition of defatted bacuri almond.

Table 2. Chemical composition of the whole flour, raw defatted flour, and toasted defatted flour of bacuri almonds, expressed in dry and wet basis (%).

Components	Whole Flour		Raw Flour		Toasted Flour	
	WB	DB	WB	DB	WB	DB
Moisture	13.14 ± 0.090	--	11.14 ± 0.061	--	1.39 ± 0.085	--
Ashes	1.70 ± 0.002	1.96	5.85 ± 0.058	6.58	6.60 ± 0.071	6.69
Lipids	61.64± 0.164	70.96	0.41 ± 0.046	0.46	0.74 ± 0.055	0.75
Proteins	9.21 ± 0.454	10.60	31.95 ± 0.538	35.95	34.92 ±1.379	35.41
Sucrose	1.63 ± 0.106	1.88	5.30 ± 0.172	5.96	4.25 ± 0.115	4.31
Starch	5.55 ± 0.042	6.39	18.76 ± 1.050	21.11	22.91 ±0.891	23.23
Fibers	7.13 ± 1.061	8.21	26.59 ± 0.668	29.92	29.19 ± 1.025	29.60
Dry Matter	86.86	100.00	88.86	99.98	98.61	99.99

Mean values of the determinations in triplicate ± standard-deviation. WB = wet base/DB = dry base.

Table 3. Amino acid profile of the raw and thermally processed (toasted) bacuri almond, expressed in mg/g of protein in comparison to the standard established by FAO/WHO.

Amino acids	Raw Almonds	Toasted Almonds	FAO/WHO Standard[1]
Aspartic Acid	89.1	67.3	
Glutamic Acid	141.2	213.6	
Alanine	67.7	56.1	
Arginine	112.1	101.7	
Glycine	79.1	94.0	
Proline	42.0	37.6	
Serine	59.5	70.0	
Histidine	**21.8**	**9.1**	**18**
Isoleucine	**39.0**	**30.9**	**31**
Leucine	**83.2**	**79.2**	**63**
Lysine	**57.4**	**42.6**	**52**
Methionine	**27.1**	**17.2**	**26***
Cysteine	6.8	4.5	
Phenylalanine	**50.9**	**70.2**	**46****
Tyrosine	19.2	18.8	
Threonine	**30.2**	**22.6**	**27**
Tryptophan	**ND**	**ND**	**7.4**
Valine	**73.6**	**64.7**	**42**

ND= Not detected; *Methionine + cysteine (SAA); **Phenylalanine + tyrosine (AAA). [1]Amino acid scoring patterns: Theoretical standard of WHO/FAO – indispensable amino acids for children between 1 and 2 years old (Food and Agriculture Organization & World Health Organization, 2007).

of raw bacuri almond showed no limiting amino acid when compared to the one considered as a reference (Food and Agriculture Organization & World Health Organization, 2007). The content of all essential amino acids was higher than those recommended by FAO/WHO. Sulphurated Amino Acids (SAA) are limiting factors in many food plants such as soy (Friedman & Brandon, 2001), beans, other vegetables (Sgarbieri, 1987), and nuts (Fernandes et al., 2010; Czedera et al., 2012). The level of sulphurated amino acids (methionine + cysteine) of the raw bacuri almond protein was 33.9 %; this value is higher than that established by the FAO/WHO and those found for beans (19.95 %), wheat (18.12 %), corn (22.21 %), soy (18.65 %), and it was close to that found for beef (35.59 %) (Pires et al., 2006).

The toasted almond protein was deficient in hystidine, isoleucine, threonine, methionine + cysteine, and lysine. Hystidine was the most limiting amino acid (9.1 %) compared to that established by the FAO/WHO (18.0 %). It is possible that the thermal processing applied to obtain the toasted flour was responsible for the considerable loss in the levels of almost every amino acid in the proteins. Such loss can probably be attributed to the irreversible formation of complex carbohydrates and amino acids in the Maillard reaction mechanisms (Özdemir et al., 2001; Shibão & Bastos, 2011). This justifies the reduced growth of the animals treated with the ration prepared with the toasted bacuri almond flour.

3.3 Chemical score of essential amino acid and PDCAAS

The value of PDCAAS for the proteins of the raw bacuri almond was closer to the casein level (Table 4), and higher than soybean (Pires et al., 2006). Bacuri almond showed better score of amino acids than that of soybean since it had methionine + cysteine (SAA) lower than 1.0. Moreover, the digestibility found for soy was 71.76 % (Pires et al., 2006), while the raw bacuri protein is 82.87 % since the PDCAAS takes into consideration the true digestibility of proteins. This value was higher than that found for beans (78.7%) (Pires et al., 2006) and baru almond (73.10%) (Fernandes et al., 2010). The protein of the raw bacuri almond also had PDCAAS higher than that of wheat (0.4025), corn (0.3707), and beans (0.6296) (Pires et al., 2006).

Table 4. Chemical score of essential amino acids and chemical score corrected by the proteic digestibility (PDCAAS) of the protein in the Casein, Test 1 (containing raw bacuri almonds) and Test 2 (containing toasted bacuri almonds) Groups.

Essential Amino Acids	Casein*	Test 1	Test 2
Hystidine	1.00	1.21	0.50
Isoleucine	1.68	1.26	0.99
Leucine	1.41	1.32	1.26
Lysine	1.36	1.10	0.82
Methionine + cysteine	1.21	1.30	0.83
Phenylalanine + Tyrosine	1.74	1.52	1.93
Threonine	1.27	1.19	0.84
Tryptophan	ND	ND	ND
Valine	1.57	1.75	1.54
PDCAAS	0.9272	0.9116	0.3616

* Chemical score of essential amino acids of casein (Sgarbieri, 1987). ND: Not determined.

However, the thermally treated protein of bacuri had five of the nine essential amino acids, i.e., hystidine (0.50), threonine (0.84), isoleucine (0.99), methionine + cysteine (0.83), and lysine (0.82), with a chemical score below 1.00. The PDCAAS value was lower than that found for the casein and raw bacuri presented in the present study, and also for soy, wheat, beans, and corn (Pires et al., 2006).

3.4 Anti-nutritional factors

Inhibitory activity of trypsin and chymotrypsin of up to 10mg/ml (high concentration of proteins) was not detected in the bacuri almond; moreover, there was no hemagglutination activity of lectins in dilutions of up to 50μg of protein.

3.5 Biological assay

The weight gain of the animals in test 1 and test 2 groups was lower than that of the animals in the casein group, with a significant difference of 1%. As for the tests groups, there was no significant difference in the weight gain between test 1 and test 2 groups at a level of 1% ($p<0.01$).

There was no significant difference at a level of 1% in the quantity of ingested diet, ingested protein, and ingested nitrogen, and therefore it can be said that the groups ingested similar quantities of protein and consequently of nitrogen.

Table 5 shows the data related to the Protein Efficiency Ratio (PER) and Net Protein Ratio (NPR).

Table 5. Protein Efficiency Ratio (PER), Net Protein Ratio (NPR), and Nitrogen Balance (NB) in rats submitted to the Control (casein), Test 1 (containing raw bacuri almond) and Test 2 (containing toasted bacuri almond) diets for 23 days.

Diet	PER*	NPR*	NB**
Casein	2.749 ± 0.271	3.652 ± 0.290	2.447 ± 0.809
Test 1	1.729 ± 0.546	2.757 ± 0.439	1.936 ± 0.319
Test 2	0.969 ± 0.295	2.137 ± 0.237	1.489 ± 0.469

Values mean ± standard-deviation. *Values with significant differences between the three groups at 1% probability level ($p<0.01$). **Values with no significant differences between the three groups at 1% probability level ($p<0.01$).

Table 6. True Digestibility (TD), Biological Value (BV), and Food Efficacy Coefficient (FEC) values of the proteins in raw bacuri almond flour (Test 1) toasted bacuri almonds flour (Test 2) and the standard protein (Casein).

Diet	TD (%)*	BV (%)**	FEC*
Casein	92.32 ± 1.758	87.82 ± 2.982	0.269 ± 0.027
Test 1	82.87 ± 2.249	91.29 ± 1.721	0.167 ± 0.053
Test 2	72.33 ± 5.680	91.81 ± 2.343	0.096 ± 0.029

Values mean ± standard deviation. *Values with significant difference between the groups at 1% probability level ($p<0.01$). **Values with no significant differences among the groups at 1% probability level ($p<0.01$).

There was a significant difference ($p<0.01$) in the PER and NPR values between the groups tested. PER values (weight gain/quantity of ingested protein) showed a significant difference between the groups, which can be attributed to a different balance of amino acids of the ingested proteins; among other factors, the difference in digestibility between the groups can influence the animals' weight gain since the amount of proteins and nitrogen ingested did not have a significant difference between the groups.

In the protein of the algaroba seed (*Prosopis julifora*) associated to soy milk (*Glycine hispide*) a significant difference in the PER and NPR values was observed between the control protein (casein) and tested protein (Vieira & Bion, 1998). The proteins of the baru almond (raw and toasted) showed similar result to the one described above for PER, which is attributed to the deficiency of sulphurated amino acid in the baru almond (Naves et al., 2010).

Although PER value of the raw bacuri almond found was lower than the standard (casein) value, this value is higher than those found (Pires et al., 2006) for wheat flour (0.98) and corn (cornmeal) (0.68), and it is very close to that of soy flour (1.75). The NPR values of the test rations found were also significantly lower than that of the control group (casein) but similar to that reported for the proteins of baru almond (2.76) (Czedera et al., 2012).

Nitrogen balance values had no significant difference between the three groups at the 1% probability level ($p<0.01$) level, i.e., the balance between the amount of nitrogen ingested and the amount excreted in the feces and urine was not different among the groups.

The true digestibility values of the proteins of bacuri almonds raw (test 1), and toasted (test 2) and the standard protein (casein) found are statistically different between each other at the 1% level of significance (Table 6).

The true digestibility of the test group 1 was 10% lower than that of the control group. This shows that there was no efficient absorption of amino acids in test group 1, i.e., there was no satisfactory hydrolysis of proteins in this group. This is confirmed by a higher elimination of nitrogen in the feces when compared to that of the control group.

The true digestibility values of the proteins of raw bacuri almonds (82.87%) is similar to that reported for the proteins of bocaiúva almonds (83.51%) (Hiane et al., 2006) and higher than those found for corn protein (82.38%), soy flour (71.76 %), beans (78.70 %) (Pires et al., 2006), and baru almond (79.43%) (Fernandes et al., 2010) .

Test group 2 had a lower true digestibility than the casein group and test group 1. This means that the absorption of nitrogen was even lower than that of test group 1. There was a higher elimination of nitrogen in the feces when compared to that of the control and test 1 groups.

Probably, the true digestibility results obtained in the present study on toasted bacuri almonds is due to the fact that the amino acids are less available for absorption, i.e., a deficient balance of amino acids due to the intense thermal treatment it was submitted to, and possible chemical reactions between the amino acids and sugars could have occurred. Therefore, the thermal treatment under the conditions evaluated was not effective in increasing the protein digestibility of bacuri almond proteins.

The biological values of the test groups (Table 6) were similar to that found for the casein standard, i.e., the retention/absorbed nitrogen ratio in the test groups was similar to that of the control group. It is likely that between the absorbed protein fractions of the test groups, the one with the best amino acid balance was retained.

The biological values of the proteins of bacuri almond (raw and toasted) were close to that the proteins of sapucaia nuts (92.86%) (Denadai et al., 2007), higher than that of bocaiuva almonds (81.10 %) (Hiane et al., 2006) and baru almonds (79.43%) (Fernandes et al., 2010). As for the food efficiency coefficient (FEC) (weight gain/food intake), a significant difference between the groups ($p< 0.01$) was observed. This difference can be explained by the quality of ingested protein since the diets, in general, had differences between each other only in terms of the type of protein ingested and not in terms of quantity ingested. In addition, there was no significant difference in the diet intake ($p<0.01$) between the groups, and the diets proved balanced regarding the other compounds and total caloric value.

4 Conclusion

The raw bacuri almond showed higher protein nutritional value than that of toasted bacuri almond. The thermal treatment applied reduced the levels of almost all essential amino acids

resulting in unsatisfactory biological indexes in relation to the non-processed food; in addition, it was not effective in increasing protein digestibility. Bacuri almond can be used *in natura* as an alternative protein source in diets with low nutritional value since it does not have limiting essential amino acids and anti-nutritional factors that could prevent its use, from a nutritional standpoint, as a source of nutrients.

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