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Nutritional quality and bioactive compounds of partially defatted baru almond flour

Ana Paula Silva SIQUEIRA¹, Maria Teresa Bertoldo PACHECO², Maria Margareth Veloso NAVES^{3*}

Abstract

This study aimed to investigate the nutritional quality and bioactive potential of partially defatted baru (*Dipteryx alata* Vog.) almond flour (BAF). The flour's proximate and mineral compositions, total phenolic, tocopherols and carotenoids contents, antioxidant capacity, trypsin inhibitor and amino acid analyses were performed. An experiment was conducted with 24 male Wistar rats in order to evaluate the flour's protein quality. BAF has high protein, fiber and mineral contents (iron, zinc, magnesium and copper), and it is a source of calcium. BAF presented relevant amounts of total phenolics (625 mg/100g) and good antioxidant capacity (130 μ mol/Trolox eq). Autoclaved BAF showed essential amino acids profile, digestibility and protein quality better than *in natura* BAF. Autoclaved BAF might be used for human consumption as a source of quality protein and bioactive compounds, in healthy diets and processed foods.

Keywords: baru seed; protein; chemical composition; amino acids; antioxidants.

Practical Application: Defatted baru almond flour is a bioactive compound-rich raw material for healthy and innovative foods.

1 Introduction

Nuts and edible seeds contain high amounts of lipids, proteins, dietary fiber and ashes (minerals), and present a favorable essential amino acids profile, usually with slight lysine deficiency. These foods constitute protein and bioactive compounds sources for vegetable-based diets, since the consumption of nuts and edible seeds is associated with lower chronic diseases risk (Freitas & Naves, 2010).

Baru almond (*Dipteryx alata* Vog.) is an edible seed, native from the Brazilian Savannah. This exotic seed is rich in proteins (approximately 30 g/100g), lipids (around 40 g/100g) and fibers (12 g/100g). Moreover, baru almond is a mineral source, chiefly of calcium, iron and zinc (Czedler et al., 2012; Sousa et al., 2011). Besides its nutritional properties, the baru almond has become an important native fruit from Brazil because of its health benefits, as reported in a clinical trial (Bento et al., 2014), although studies on its bioactive potential are scarce.

The oil constitutes one of the baru almond's co-products, with social and commercial importance, including exportation. Baru oil extraction is handmade, performed in cooperatives by mechanical pressing, and generates the partially defatted baru almond flour (BAF), which is commercialized for animal feed. BAF presents nutritional relevance, mainly due to its high protein contents (49 g/100g) and ashes (5 g/100g) (Guimarães et al., 2012). However, there are no studies on bioactive potential and protein quality of this flour.

Considering the nutritive potential of baru almond, and the tendency of using nutritive co-products of the agroindustry for

human consumption, the objective of this study was to investigate the nutritional and biological quality of partially defatted BAF.

2 Materials and methods

2.1 Sample collection

The fruits were collected from a region of the Brazilian Savannah, located at southeast of Goiás State (coordinates: 17° 27' 51.83" S and 48° 12' 12.51" W, and altitude of 779 m), during harvest season, between August and September, from eight trees, at least 200 m distant. The collected fruits (total= 1200) were those recently fallen on the ground, in perfect morphological condition. The baru almonds were extracted using a guillotine and were stored at temperature between 5 °C and 10 °C until analysis, which were carried out in whole baru almond (*in natura*), *in natura* BAF and autoclaved BAF.

2.2 Partially defatted BAF processing

The oil of baru almonds (*in natura* and with peel) were extracted by hydraulic machine (Carver, model C, serie #24000-415, USA) without heating and with controlled pressure (9 ton). The co-product obtained was powdered (Skymssen, PA- 7SE processor, 60Hz and 0.33 CV/ 245w, Brazil) and sieved (Retsch, 10 mesh, Germany). The partially defatted BAF generated (Figure 1) was divided in two lots: *in natura* BAF and autoclaved BAF (131 °C for 30 min).

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Figure 1. Baru: (a) fruit; (b) almond and (c) partially defatted almond flour (BAF).

2.3 Macronutrients and minerals determination

The following chemical composition parameters were determined in triplicate: moisture (Association of Official Analytical Chemists, 2010); nitrogen content by the micro-kjeldahl method (Association of Official Analytical Chemists, 2010) using a conversion factor 6.25; total lipids, by soxhlet (Association of Official Analytical Chemists, 2010); ashes at 550 °C (Association of Official Analytical Chemists, 2010) and total dietary fiber, according to the enzymatic-gravimetric technique (Prosky et al., 1988). The carbohydrate content was estimated: 100 - (moisture + protein + lipid + dietary fiber + ash). The energy was estimated using Atwater conversion factors of 4 kcal (protein and carbohydrate) and 9 kcal (lipid) (Merrill & Watt, 1973).

The minerals were quantified, in triplicate, after digestion of organic matter with nitric acid and nitrogen peroxide (Slavin et al., 1975), by ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry).

2.4 Bioactive compounds and antioxidant capacity analyses

In order to determine total phenolics, Folin-Ciocalteu (Genovese et al., 2008) method was adopted. Gallic acid was used as spectrophotometric standard (Varian, model Cary 50 Scan, USA) and total phenolic content was expressed as gallic acid equivalent (GAE/100g of fresh sample). Tocopherols were analyzed by High Performance Liquid Chromatography (HPLC) system (Shimadzu, model Luna/Phenomenex 4.6 × 250 mm, Japan); the peaks were identified through comparison with the retention times of authentic standards by DAD (Diode Array Detector) and the quantification was done by external calibration (American Oil Chemist's Society, 2009). Total carotenoids were determined as described by Higby (1962) and the reading was done by HPLC (Shimadzu, model Luna/Phenomenex 4.6 × 250 mm, Japan) (Ramos et al., 2001).

Antioxidant capacity was determined through 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging (Blois, 1958; Brand-Williams et al., 1995), using stable DPPH, which suffers reduction by antioxidants with color change from violet to yellow. The absorbance was read in a UV spectrophotometer (Varian, model Cary 50 Scan, USA) at 515 nm.

2.5 Trypsin inhibitor analysis

Trypsin inhibitor was determined in BAF extracts prepared with NaOH, and placed in the presence of trypsin, BAPA (Benzoyl-L-arginine-p-nitroanilide) and TCA (trichloroacetic acid). After centrifugation, the supernatant was collected and the absorbance was read in a spectrophotometer (Varian, model Cary 50 Scan, USA) at 410 nm (American Oil Chemist's Society, 2009).

2.6 Amino acids analysis

The amino acid analysis was performed, in triplicate, by acid hydrolysis of proteins and peptides (Moore & Stein, 1951). The hydrolyzed samples were placed in an automatic amino acids analyzer (Nicolas V, University of São Paulo Protein Chemistry Center, Ribeirão Preto-SP). After elution in the column and reaction with ninhydrin, the amino acids were quantified by a colorimetric assay. The results of this analysis were used to estimate the amino acid score (AAS) by using the following formula (World Health Organization, 2007): (mg of amino acid in 1 g of test protein/mg of amino acid in requirement pattern) × 100.

2.7 Biological assay

In order to evaluate the protein quality of BAF, a biological assay was carried out, after its approval by the Animal Use Ethics Committee of the Federal University of Goiás (Protocol n°153/2008). Twenty-four weanling male Wistar rats were distributed in four groups of six. Animals' body weight and food consumption were assessed throughout the experiment. Four diets were prepared, according to AIN-93G (Reeves et al., 1993), as following: reference (with casein); protein-free diet and two experimental diets - *in natura* and autoclaved BAF (Table 1). Diets were analyzed regarding protein and lipid contents, in order to confirm its isoproteic and isolipidic formulations (Table 1).

In natura and autoclaved BAF protein values were estimated through Net Protein Ratio (NPR) and Protein Digestibility-Corrected Amino Acid Score (PDCAAS) indexes. NPR and Relative NPR (RNPR) were calculated, as follows (Pellet and Young, 1980): $NPR = \frac{[\text{test-group weight gain (g)} - \text{protein-free group weight loss (g)}]}{\text{consumed protein by test group}}$; and $RNPR = \frac{[\text{test group NPR}]}{[\text{reference group NPR}]} \times 100$. True digestibility (%) was determined according to the Food and Agriculture Organization of the United Nations (1991), for *in vivo* tests. The

Table 1. Composition of experimental diets.

Content (g/100g)	Diet			
	Casein (reference)	<i>In natura</i> BAF	Autoclaved BAF	Protein- free
<i>Ingredient</i>				
Casein	12.50	-	-	-
BAF	-	36.20	35.08	-
Soybean oil	6.70	2.33	2.30	7.00
Mineral mix	3.50	3.50	3.50	3.50
Vitamin mix	1.00	1.00	1.00	1.00
Choline bitartrate	0.25	0.25	0.25	0.25
Corn starch	70.87	56.72	57.87	83.25
L-cystine	0.18	-	-	-
Fiber (cellulose)	5.00	-	-	5.00
<i>Chemical composition</i>				
Protein	10.72±0.26 ^a	10.39±1.24 ^a	11.65±0.06 ^a	0.24±0.03 ^b
Lipids	7.53±0.58 ^a	7.16±0.51 ^a	7.68±0.32 ^a	7.49±0.52 ^a

Diet formulation according to AIN-93G diet (Reeves et al., 1993), with 10% of protein, except protein-free diet; BAF-Baru Almond Flour; Casein: 80 g/100g protein and 3 g/100g lipids; (^{a,b}) means (± standard deviation of three replicates) with the same letter in the same row are not significantly different (Tukey test, $p<0.05$).

rats' feces were collected and marked during the experiment's second week for nitrogen analysis. PDCAAS was estimated as follows: PDCAAS= [test protein AAS (%) × test protein True Digestibility (%)]/100 (Food and Agriculture Organization of the United Nations, 1991; World Health Organization, 2007).

2.8 Statistical analysis

Results were submitted to variance analysis (ANOVA) followed by Tukey test, at 5% probability, except for comparison between two data groups, in which Student t-test was employed. It was performed a linear regression analysis between the animal's body weight and the days of experiment.

3 Results

The whole baru almond's moisture was approximately 7 g/100g, which was preserved in the autoclaved BAF. *In natura* and autoclaved BAF presented high protein, dietary fiber and ash contents, in addition to a residual lipid content of 13%. As a result of lipid extraction, BAF's energy was lower and the macronutrients contents were higher than those of whole baru almond (Table 2).

BAF is rich in iron, magnesium and zinc, and it is also calcium source. The flour presented higher contents of these minerals than those of whole baru almond (Table 3).

Regarding bioactive compounds (Table 4), BAF showed higher phenolic contents and antioxidant capacity than that of whole baru almond. Tannin and phytate contents were reduced by the flour's autoclaving process, since autoclaved BAF showed lower contents of these compounds than those of *in natura* BAF. Alpha- and gamma-tocopherol were the main isomers of the tocopherol found in baru almonds. BAF contain low amounts of tocopherols and carotenoids.

Table 2. Proximate composition and energy of whole baru almond and *in natura* and autoclaved baru almond flour (BAF).

Content	Whole baru almond	<i>In natura</i> BAF	Autoclaved BAF
<i>Proximate composition (g/100g)</i>			
Moisture	7.38±0.19 ^b	9.95±0.21 ^a	7.16±0.00 ^b
Protein	19.72±0.11 ^c	27.83±0.58 ^b	29.05±0.15 ^a
Lipids	38.37±0.07 ^a	12.59±0.28 ^b	12.97±0.13 ^b
Ashes	2.46±0.43 ^b	3.81±0.16 ^a	3.82±0.05 ^a
Dietary fiber	12.60±0.30 ^b	16.12±0.39 ^a	17.36±0.07 ^a
Carbohydrates	19.47±0.22 ^b	30.15±0.32 ^a	29.64±0.08 ^a
<i>Energy (kcal/100g)</i>	502.09±0.22 ^a	345.23±0.32 ^b	351.49±0.08 ^b

Values are mean ± standard deviation of three replicates; nitrogen conversion factor to raw protein: 6.25; (^{a,b}) means with the same letter in the same row are not significantly different (Tukey test, $p<0.05$).

Table 3. Mineral composition of whole baru almond and *in natura* baru almond flour (BAF) and relation to Estimated Average Requirements (EAR).

Mineral (mg/100g)	Whole baru almond	<i>In natura</i> BAF	EAR (mg/day)	% EAR of <i>in natura</i> BAF
Calcium	88±3.00 ^b	122±7.00 ^a	800	15
Cooper	1±0.04 ^a	1±0.04 ^a	0.7	143
Iron	3±0.20 ^b	5±0.30 ^a	6	83
Magnesium	107±3.00 ^b	136±6.00 ^a	350	39
Selenium (µg/100g)	0.1±0.01	-	45	-
Sodium	2±0.02 ^a	0.3±0.04 ^b	1500	0.02
Zinc	2±0.10 ^b	4±0.20 ^a	9.4	43

Values are mean ± standard deviation of three replicates; EAR for men with 31-50 years old (Institute of Medicine, 2014); (^{a,b}) means with the same letter in the same row are not significantly different (Student t- test, $p<0.05$); sodium value corresponding to Adequate Intakes (AIs).

Table 4. Bioactive compounds of whole baru almond and *in natura* and autoclaved baru almond flours (BAF).

Content	Whole baru almond	<i>In natura</i> BAF	Autoclaved BAF
<i>Bioactive compounds (mg/100g)</i>			
Phenolics	388.04±47.61 ^c	588.11±63.45 ^b	662.04±34.43 ^a
Tanins	562.87±3.57 ^c	992.51±3.95 ^a	648.26±3.15 ^b
Phytates	312.68±0.12 ^b	447.87±0.11 ^a	254.43±0.15 ^c
Tocopherol	11.61±0.08 ^a	1.46±0.08 ^b	1.50±0.08 ^b
Carotenoid (µg/100g)	11.40±0.40 ^a	1.43±0.40 ^b	1.47±0.40 ^b
Antioxidant capacity DPPH (µmol/Trolox eq)	67.00±6.31 ^b	130.93±3.27 ^a	130.46±7.18 ^a

Values are mean ± standard deviation of three replicates; Baru almond total tocopherol (mg/100g): alpha= 7.00; beta=0.35 and gamma= 3.5; (^{a,b}) means with the same letter in the same row are not significantly different (Tukey test, $p<0.05$).

The values of trypsin inhibitor were 12.84 ± 0.10 (UTI/mg) for whole baru almond, 12.89 ± 0.24 (UTI/mg) for *in natura* BAF and 0.46 ± 0.44 (UTI/mg) for autoclaved BAF.

In natura BAF is complete in essential amino acids, according to its AAS value (Table 5). Amino acids profile of autoclaved BAF

Table 5. Amino acid composition of *in natura* baru almond flour (BAF) and Amino Acid Score (AAS) according to the WHO/FAO/UNU requirement pattern.

Amino acid (mg/g protein)	WHO/FAO/UNU requirement pattern	<i>In natura</i> BAF
<i>Indispensable (Essential)</i>		
His	16.0	29.07
Ile	31.0	41.92
Leu	61.0	85.37
Lys	48.0	59.36
Met + Cys	24.0	27.36
Phe + Tyr	41.0	89.35
Thr	25.0	36.72
Trp	6.6	20.20
Val	40.0	47.74
TOTAL	292.6	416.89
AAS (%)	100	114
<i>Dispensable (Non-essential)</i>		
Asp	-	116.59
Glu	-	250.61
Ala	-	41.00
Arg	-	100.98
Gly	-	43.15
Pro	-	40.70
Ser	-	48.65
TOTAL	-	641.68

Values are mean of two replicates; WHO/FAO/ONU requirement pattern of essential amino acids for schoolchildren (World Health Organization, 2007); Trp of *in natura* BAF: according to Fernandes et al. (2010); bold value indicates the first limiting amino acid.

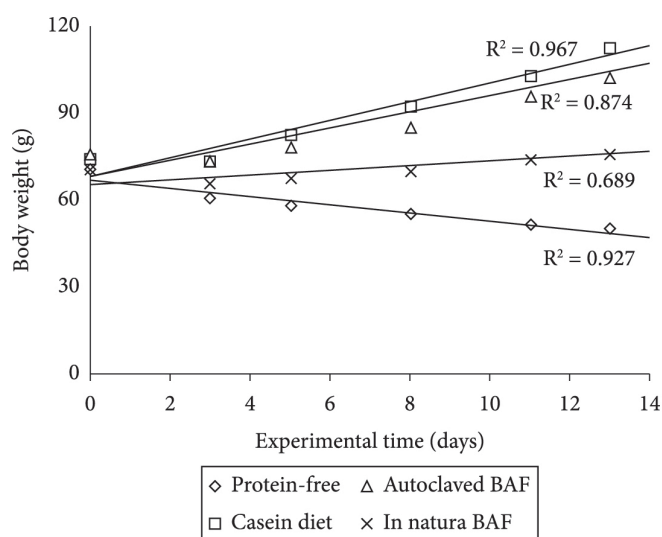
can be considered similar to that of *in natura* BAF. Throughout the 14 days of experiment, body weight gain of the animals was positive and linear, except for the protein-free diet group, which showed body weight loss (Figure 2). Determination coefficient (R^2) values ranged from 0.69 to 0.97 for the body weight curves. The *in natura* BAF diet group showed a very low weight gain, and the autoclaved BAF diet group's weight gain was similar to that of the reference group.

True digestibility of *in natura* BAF was approximately 50%, and the autoclaving process resulted in a 50% increase of autoclaved BAF protein digestibility. As a result, protein quality of autoclaved BAF, evaluated through PDCAAS and NPR, was higher than that of *in natura* BAF (Table 6).

4 Discussion

The present study's data indicate that BAF is a co-product with high nutritive value and antioxidant capacity. There is no previous research about the *in vivo* true digestibility and protein quality, total dietary fibers content and bioactive compounds content of the autoclaved BAF.

The autoclaved BAF produced in this study was partially defatted and its lipid content is higher than the value reported by Guimarães et al. (2012) that carried out a preliminary study

**Figure 2.** Body weight curves of Wistar male rats treated with different diets (casein, *in natura* and autoclaved baru almond flour-BAF, and protein-free) during 14 days of experiment.**Table 6.** Body weight gain, food and protein intakes, Net Protein Ratio (NPR), Relative NPR (RNPR) and Protein Digestibility-Corrected Amino Acid Score (PDCAAS) of Wistar male rats during 14 days of experiment.

Parameter	Diet		
	Casein	<i>In natura</i> BAF	Autoclaved BAF
Body weight gain (g)	44.00 ± 11.71 ^a	9.80 ± 1.72 ^b	36.73 ± 3.70 ^a
Total food intake (g)	150.30 ± 13.84 ^b	137.20 ± 7.82 ^c	179.10 ± 21.96 ^a
Total protein intake (g)	16.11 ± 1.23 ^b	14.25 ± 0.81 ^c	20.86 ± 2.55 ^a
True digestibility (%)	95.38 ± 1.91 ^a	47.95 ± 3.16 ^c	71.24 ± 3.53 ^b
NPR	4.00 ± 0.48 ^a	2.27 ± 0.17 ^c	2.87 ± 0.41 ^b
RNPR (%)	100	54.96 ± 0.17 ^b	69.52 ± 0.41 ^a
PDCAAS (%)	-	55.00 ± 0.17 ^b	81.00 ± 0.41 ^a

BAF-Baru Almond Flour; (^{a,b}) means (± standard deviation of six animals) with the same letter in the same row are not significantly different (Tukey test, $p < 0.05$).

on proximate composition and protein properties of BAF. The autoclaved BAF's residual oil aggregates nutritional value to BAF, since baru oil is rich in unsaturated fatty acids, especially monounsaturated acids (Bento et al., 2014), and contains tocopherols and carotenoids (Table 4).

The autoclaved BAF is rich in total dietary fiber (Table 2), mainly insoluble fibers according to the literature (Czedler et al., 2012). This fact reinforces the potential use of BAF due to the physiological effects of insoluble dietary fibers, such as the increase of fecal volume and the reduction of intestinal transit time (Roberfroid et al., 2010).

BAF's mineral composition is relevant, since zinc is especially important for normal nervous system activity (Stelmashook et al., 2014). The low sodium content of baru almond and BAF (Table 3)

is favorable to health because the high consumption of sodium is an important dietary factor that increases chronic diseases risk (Freitas & Naves, 2010). Other oilseeds, such as almond, peanut and Brazil nut are mineral sources, mainly calcium, iron and zinc (Freitas & Naves, 2010).

Concerning bioactive compounds, autoclaved BAF showed higher phenolic contents than *in natura* BAF, which could be partially explained by the reduction of the autoclaved flour's moisture (Table 2). Both BAF have higher contents of phenolic compounds than those reported in the literature for cashew nut (106 mg/100g) and Brazil nut (381 mg/100g). The high phenolic content in nuts is related to high antioxidant capacity (Abe et al., 2010), as observed in the present study for BAF compared to whole baru almond (Table 4).

Tannin and phytate values were reduced in autoclaved BAF compared to *in natura* BAF (Table 4). It is valid to add that the inactivation of these compounds increases the availability of some minerals for absorption (Weaver & Kannan, 2002), such as iron and zinc, which are present in high amounts in BAF. Phytates and tannins can bind to minerals, which generates insoluble complexes and unavailable biologically for humans under normal physiological conditions (Raes et al., 2014). In a previous study with baru almond (Togashi & Sgarbieri, 1994), the authors did not find significant amounts of phytate and tannin in the raw baru almond. These compounds were inactivated after heating treatment, as observed in the present study for BAF.

Regarding the baru almond's alpha-tocopherol content, it is higher than that observed by Takemoto et al. (2001). According to the Institute of Medicine (Institute of Medicine, 2000), the daily recommended intake (DRI) for vitamin E is 15 mg per day, thus the consumption of one portion (20 g) of baru almond (Bento et al., 2014; Fernandes et al., 2010) provides considerable amounts of vitamin E per day (15%). It should be stressed that vitamin E is an important *in vivo* antioxidant (Niki, 2014), so it may contribute to the high BAF antioxidant capacity. Additional *in vivo* studies are required to highlight the biological antioxidant activity of the autoclaved BAF, since the DPPH is an *in vitro* method used for initial evaluation of the antioxidant properties (Alves et al., 2010).

In the present study, the analysis of the amino acid profile was performed only in the *in natura* BAF sample (Table 5), since the amino acid contents do not change significantly with heating (roasting or autoclaving) (Freitas et al., 2012). On the other hand, studies showed that the amino acids profile of the baru almond may be influenced by the native area of the fruit (Czedler et al., 2012; Fernandes et al., 2010).

BAF has high good quality protein contents. Studies with baru almonds from different region of the Brazilian Savannah reported AAS values from 75% (Freitas et al., 2012) to 89% (Czedler et al., 2012), lower than that observed in this study for BAF (Table 5). However, Fernandes et al. (2010) reported AAS values around 100% for baru almond, which is comparable to that found in the present study. Autoclaved BAF showed protein efficiency approximately 30% higher than that of *in natura* BAF. The improved protein quality of autoclaved BAF, estimated by NPR and PDCAAS, is a result of the protein

digestibility improved by the heating process (autoclaving), which induces the inactivation of antinutrients and the desnaturation of proteins (Muzquiz et al., 2012). Czedler et al. (2012) investigated the protein quality of roasted baru almonds from the southeast area of Goiás State by NPR, and the authors found a value of 2.76, which is comparable to that of autoclaved BAF (Table 6). PDCAAS value of autoclaved BAF (81%) was higher than those found in the literature for whole baru almond, from 65% to 73% (Czedler et al., 2012).

The overall results of the present study suggest greater advantages on the consumption of autoclaved BAF, when compared to baru almond intake, as source of good quality protein and bioactive compounds. Therefore, this co-product of the baru almond may be used as a raw material with nutritional and health benefits in the formulation of several products, as granola, breakfast cereals, cereal bars and cookies.

5 Conclusion

The flour of partially defatted baru almond is a source of good quality protein, calcium and bioactive compounds, and it is rich in dietary fiber, iron and zinc. Autoclaved BAF can be used for human consumption, as a source of complementary protein and bioactive compounds in healthy diets, and as raw-material for processed foods with nutritional advantages.

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