Journal of Food and Nutrition Research (ISSN 1336-8672)

Effect of different extraction conditions on the antioxidant potential of baru almonds (*Dipteryx alata* Vog.): comparison to common nuts from Brazil

THAYS HELENA BORGES – NUNO RODRIGUES – ADRIANA MARQUES DE SOUZA – JOSÉ ALBERTO PEREIRA

Summary

The antioxidant properties and total phenols content of baru almonds (*Dipteryx alata* Vog.) were investigated using different solvents and extraction temperatures, and were compared with other types of nuts commonly consumed in Brazil. Although baru almonds are popular and widely consumed, few studies report on their biological properties and there are no data on optimum extraction conditions. For this reason, we tested five different extraction methodologies (water and methanol at boiling and room temperature, and water: methanol (1:1) at room temperature) on baru almonds, and evaluated the antioxidant properties based on scavenging activity, reducing power and total phenols content. The methanolic extraction at room temperature was the best extraction methodology providing the highest general antioxidant potential. The extraction method selected was applied to several dry fruits: baru almonds (three different samples), peanut, cashew nut, crude cashew nut, macadamia and Brazil nut. Among these nut fruits, baru had the highest antioxidant activity and total phenols content. The results obtained revealed that baru almonds are a good source of bioactive compounds, and have a higher bioactivity than many nut fruits widely distributed, consumed and more popular.

Keywords

baru almonds; extraction; antioxidant activity; dry fruits

The Brazilian biomes are rich in many types of exotic species and one of the interesting native species is *Dipteryx alata* Vog., commonly known as "barueiro". Barueiro is a tree from *Leguminosae* family, fruits of which (commonly known as baru) are characterized by a thin pale brown shell and a yellow pulp (mesocarp) with a sweet taste, that envelope a hard and edible seed [1]. Roasted baru almonds are employed in diverse products and, in local diet, are used as an ingredient for candies and are used in the regional gastronomy. The flavour of baru is similar to that of peanut, but it is milder to the palate. For these reasons, baru has become a popular product especially in the central-western region of Brazil [2].

The literature reports that baru almond has high contents of lipids (around 40%), a considerable amount of digestible proteins (30%) and ami-

no acids, and also are considered a good source of energy. Its mineral composition was also studied, revealing the presence of many minerals, such as zinc, iron and calcium [1–5]. Recent studies high-lighted the high total phenolic content in extracts of roasted baru almonds and the bioactive properties of this natural resource [6, 7].

According to many studies, almonds, walnuts and nuts have been identified as good sources of natural antioxidants with bioactive properties [8–13]. On the other hand, artificial and synthetic antioxidants used in industry, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ), are being gradually restricted due to suspicions about possible toxic and carcinogenic effects [14, 15]. Such facts increased the demand for new antioxidants from natural sources, which encouraged

Correspondence author:

Thays Helena Borges, Adriana Marques de Souza, School of Agronomy and Food Engineering, Federal University of Goiás, Campus Samambaia – Rodovia Goiânia / Nova Veneza, Km 0, CEP 74690-900, Goiânia, Brazil.

Nuno Rodrigues, José Alberto Pereira, Mountain Research Centre, School of Agriculture, Polytechnic Institute of Bragança, Campus de Sta Apolónia, Apartado 1172, 5301-855 Bragança, Portugal.

José Alberto Pereira, tel.: +351 273303277; fax: +351 273325405; e-mail: jpereira@ipb.pt

the scientific community towards the search for new natural bioactive substances [16].

A large variety of methods, solvents and temperatures of extraction were found in the literature to determine antioxidants in foods. The solvent choice for the extraction of antioxidants is due to the wide range of polarity of antioxidants. Some studies include comparison of several solvents, generally alcohol-based solvent mixtures, and it was concluded that methanol was the most suitable for the antioxidants extraction [17].

Studies with the determination of the antioxidant components using different solvents and extraction temperatures can contribute to the knowledge on the antioxidant potential of baru almonds. In this study, we attempted to develop the most suitable extraction methodology to enhance the extraction of antioxidant components and to correlate their levels with the antioxidant activity of the different extracts. Secondly, we estimated the in vitro potential of the extracts of baru almonds in comparison to other Brazilian nuts like: macadamia, peanuts, cashew nuts (with and without peels) and Brazil nuts.

MATERIALS AND METHODS

Standards and reagents

Methanol, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH*), and iron (III) chloride were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Sodium dihydrogen phosphate dehydrate and potassium hexacyanoferrate (III), trichloroacetic acid, and Folin-Ciocalteu's phenol reagent were obtained from Merck (Darmstadt, Germany). Hydrochloric acid, sodium carbonate anhydrous and di-sodium hydrogen phosphate dehydrate were obtained from Panreac (Barcelona, Spain).

Samples

Two samples of 1 kg of roasted baru almonds (*Dipteryx alata* Vog.) were obtained in the Brazilian market (Goiás): samples A and B. A third sample (sample C) was constituted by a mixture of samples A and B (1:1) in order to test the best extraction methodology.

Peanut (Arachis hypogaea L.), macadamia (Macadamia intergrifolia), Brazil nut (Bertholletia excelsa Humb. & Bonpl.), cashew nut crude and roasted (Anacardium occidentale L.), were obtained from a local market (Brazil). A sample of crude baru almonds (1 kg) was also obtained from local producers (Goiás).

Sample preparation and extraction conditions

Each sample (approximately 100g) were mashed, weighed and stored until analysis at 4 °C protected from light and from spoilage. For extraction, a fine dried powder (particle size approx. 1.3 mm) of sample C (5 g) was used in five different conditions as described by SOUSA et al. [18]. Extraction yields (E) of each extraction methodology were calculated according to the following equation and expressed as percent:

$$E = \frac{m_{\rm e}}{m_{\rm s}} \times 100 \tag{1}$$

where m_e is the extract quantity obtained in grams, and m_s is the quantity of sample in grams used to perform the extraction.

Aqueous extraction conditions

- H₂O bt Extraction with 250 ml of boiling water (boiling temperature bt) for 45 min and filtration. The aqueous extract was frozen and lyophilized;
- H₂O *rt* Stirring with 125 ml of water at room temperature (*rt*) at 7.5 Hz for 24 h and filtration. The residue was then extracted with two additional 62.5 ml portions of water, as described earlier. The combined aqueous extracts were frozen and lyophilized.

Methanolic extraction conditions

- MeOH bt Extraction using a Soxhlet extractor for 8 h with 250 ml of methanol (MeOH). The methanolic extract was evaporated at 40–50 °C to dryness;
- MeOH rt Stirring with 125 ml of methanol at room temperature at 7.5 Hz for 24 h and filtration. The residue was then extracted with two additional 62.5 ml portions of methanol, as described earlier. The combined methanolic extracts were evaporated at 40 °C to dryness.

Methanol: water extraction conditions

- MeOH:H₂O *rt* - Stirring with 125 ml of methanol:water (1:1) at room temperature at 7.5 Hz for 24 h and filtration. The residue was then extracted with two additional 62.5 ml portions of methanol:water (1:1), as described earlier. The combined methanolic extracts were evaporated at 40 °C to dryness and, additionally, the aqueous extracts were frozen and lyophilized.

All the obtained extracts were re-dissolved in the corresponding solvent at a concentration of 50 mg·ml⁻¹, and analysed for their total phenols content and antioxidant activity. The best extraction methodology was employed in the preparation of samples A and B of baru almonds and to the other Brazilian nuts.

Reducing power assay

The reducing power was determined by the method of BERKER et al. [19] with some modifications. The extract solution (1.0 ml) was mixed with 2.5 ml of 200 mmol·l⁻¹ sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 °C during 20 min. Then, 2.5 ml of a trichloroacetic acid aqueous solution (100 mg·ml⁻¹) was added and the mixture was centrifuged at 17 Hz during 8 min (Centurion K24OR-2003 refrigerated centrifuge, Century Scientific, West Sussex, United Kingdom). The upper layer (2.5 ml) was mixed with 2.5 ml of deionized water and 1 ml of 0.1% of ferric chloride, and the absorbance was measured spectrophotometrically at 700 nm using a Genesys 10UV spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 700 nm against extract concentration in the solution and a blank solution.

Scavenging effect assay

The capacity to scavenge the DPPH[•] free radical was monitored by the method of MALHEIRO et al. [20]. The extract solution (0.3 ml) was mixed with 2.7 ml of methanolic solution containing DPPH[•] radicals (6×10^{-5} mol·l⁻¹). The mixture was shaken vigorously and left to stand for 60 min in the dark (until stable absorbance values were obtained). The reduction of the DPPH[•] radical was measured by continuous monitoring of the decrease of absorption at 517 nm. DPPH[•] scavenging effect (*SE*) was calculated as a percentage of discolouration using the equation:

$$SE = \frac{(A_{\rm DPPH\bullet} - A_{\rm s})}{A_{\rm DPPH\bullet}} \times 100$$
(2)

in which A_s is the absorbance of the solution when the sample extract was added and A_{DPPH} . is the absorbance of the DPPH[•] solution.

The extract concentration providing 50% inhibition (EC_{50}) was calculated from the graph of scavenging effect (as percentage) against extract concentration in the solution.

Total phenols content determination

The phenolic compounds concentration in the extracts was estimated by a colorimetric assay based on procedures described by SINGLE- TON and ROSSI [21]. Briefly, 1 ml of sample was mixed with 1 ml of Folin and Ciocalteu's phenol reagent. After 3 min, 1 ml of saturated Na₂CO₃ solution was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark during 90 min, after which the absorbance was read at 700 nm against a blank solution (Genesys 10UV spectrophotometer). Gallic acid (0.294–1.47 mmol·l⁻¹) was used to construct the standard curve:

$$y = 1.01x - 0.274 ; R^2 = 0.9993$$
(3)

The results were expressed as grams of gallic acid equivalents (GAE) per kilogram of extract and per kilogram of fresh fruit.

Statistical analysis

For each extraction method conditions, three assays were performed using sample C and optimized conditions. The differences between treatments (solvent and temperature conditions) in each parameter were analysed using one-way analysis of variance (ANOVA) followed by Tukey's HSD (honest significant difference) test with $\alpha = 0.05$. This treatment was carried out using SPSS 19.0 program (IBM, New York, New York, USA). All the assays were carried out in triplicate and the results are shown as mean values and standard deviation. The results with p < 0.05 were considered significantly different and were indicated by different superscripts.

RESULTS AND DISCUSSION

Effect of extraction conditions on total phenols content and antioxidant activity of baru almonds

In order to optimize the extraction conditions of antioxidant compounds from baru almonds, five different approaches were conducted: aqueous extractions at room and boiling temperatures, methanolic extractions at room and boiling temperatures and methanol:water (1:1) extraction at room temperature. Tests were done with sample C (mixture of two commercial samples, A and B, of baru almonds) and total phenols content and antioxidant activity were assessed to evaluate which extraction methodology was more suitable for this type of almond. Data concerning total phenols content are presented in Fig. 1. By an increasing order, total phenols content was as follows: H₂O *rt* (34 g·kg⁻¹ extract expressed as GAE) <MeOH bt (68 g·kg⁻¹) < MeOH rt (76 g·kg⁻¹) < $H_2O bt (111 \text{ g·kg}^{-1}) < MeOH: H_2O rt (117 \text{ g·kg}^{-1}).$ These results could be influenced by different factors: the selectivity of extracted compounds is dependent on the extraction methodology, the extraction time, polarity of the solvent, structure of the target analytes, temperature and pressure of the extraction process. Methanol can extract more polar compounds such as plant pigments and other fractions with antioxidant capacity, while H₂O at boiling temperature and MeOH:H₂O are capable to extract more compounds, such as phenolic compounds. Compounds with low polarity can be better extracted at increase temperatures [22]. In fact, high total phenols contents were reported in aqueous extracts of dried hazelnuts [23], fresh walnuts [13] and pistachio [9], as well as in aqueous extracts of canola meal [24].

Data concerning extraction yield and antioxidant potential are presented in Tab. 1. Highest extraction yields were obtained by extractions conducted at boiling temperatures (23.6% and 17.2% with MeOH and H₂O, respectively). In fact, the extraction using Soxhlet apparatus and MeOH provided significantly higher extraction yield compared to other extraction methodologies tested (p = 0.01). Lowest extraction yield was obtained by MeOH:H₂O at room temperature, 13.2%. Comparatively, the extraction yields obtained in our work were a little higher than for Brazil nuts [8].

Concerning the antioxidant potential, two different chemical assays were conducted, namely, DPPH[•] and reducing power. The DPPH[•] scavenging effect is one of the most extensively used methods in antioxidant assays for plant samples. The assays based on scavenging of DPPH[•] free radicals through the addition of antioxidants capable of transferring electrons, which causes discolouration of the DPPH[•] solution [25, 26]. Concerning the reducing power assay, it is a method that measures the reduction of an iron

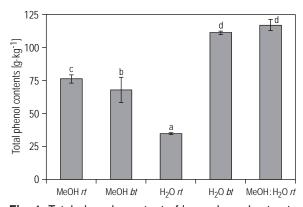


Fig. 1. Total phenols content of baru almond extracts obtained using water, methanol and water:methanol at boiling and room temperature.

Values are expressed as mean \pm standard deviation (as grams of gallic acid equivalents per kilogram of extract), n = 3.

MeOH – methanol, rt – room temperature, bt – boiling temperature, a–d – bars with different letters differ significantly (p < 0.05).

complex and a higher absorbance indicates the increased reducing power. The presence of reducers (i.e. antioxidants) causes the reduction of the Fe³⁺/ferricyanide complex to the ferrous form (Fe²⁺) monitored at 700 nm [18, 26].

The results obtained showed that, in both methods, the activity obtained was related to the concentration of the extract tested (Fig. 2). Higher extraction yield does not necessarily mean higher antioxidant activity (Tab. 1). In general, lower EC_{50} values (concentration necessary to scavenge 50% of DPPH[•] radicals; and concentration necessary to achieve an absorbance of 0.5 in reducing power assay) were obtained by MeOH extraction at room temperature, which consequently means higher antioxidant potential (EC_{50} of 0.19 mg·ml⁻¹

	Extraction yield [%]	DPPH [•] scavenging effect [mg·ml ⁻¹]	Reducing power [mg·ml⁻1]
MeOH <i>rt</i>	14.9 ± 1.7^{a}	0.19 ± 0.01^{ab}	0.48 ± 0.05^{a}
MeOH bt	23.6 ± 5.6^{b}	0.30 ± 0.02^{b}	$0.86\pm0.13^{\text{b}}$
H ₂ O <i>rt</i>	15.5 ± 0.4^{a}	$0.42\pm0.12^{\circ}$	1.84±0.09°
H ₂ O bt	17.2 ± 1.3 ^{ab}	0.18 ± 0.02^{a}	0.58 ± 0.16^{a}
MeOH : H ₂ O <i>rt</i>	13.2 ± 0.8^{a}	0.19 ± 0.01^{ab}	0.59 ± 0.05^{a}

 Tab. 1. Extraction yield, reducing power and scavenging effect of baru almond (C) extracts obtained using different solvents and temperatures in the extraction.

Values are expressed as mean \pm standard deviation, n = 3. Means within a column with different letters differ significantly, p < 0.05.

DPPH[•] scavenging effect is expressed as EC_{50} (effective concentration at which 50% of DPPH[•] radicals are scavenged). Reducing power is expressed as EC_{50} (effective concentration at which the absorbance is 0.5).

MeOH - methanol, rt - room temperature, bt - boiling temperature.

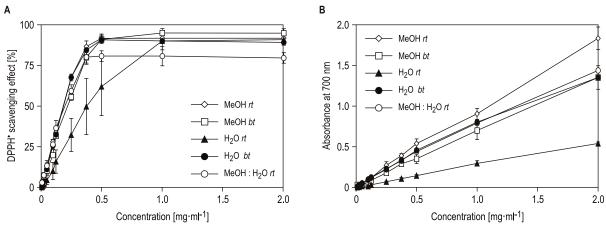


Fig. 2. Scavenging effect and reducing power of baru almond extracts obtained using water, methanol and water: methanol at boiling and room temperature. A – DPPH• scavenging effect, B – reducing power.

Values are expressed as mean \pm standard deviation, n = 3. MeOH – methanol, rt – room temperature, bt – boiling temperature.

and 0.48 mg·ml⁻¹ for DPPH[•] and reducing power assays, respectively). Such results did not significantly differ from those obtained by H₂O extraction at boiling temperature and by MeOH:H₂O at room temperature (p > 0.05). Samples extracted with water at room temperature were those that had significantly lower antioxidant activity (p < 0.001), with EC_{50} values of 0.42 mg·ml⁻¹ in DPPH[•] and 1.84 mg·ml⁻¹ in reducing power assays, respectively. The results obtained for the antioxidant activity can be related with the total phenols content of the different extracts.

In fact, values of total phenols content and antioxidant potential determined previously by various extraction methodologies with different walnut varieties [13] were similar to the data obtained in this study with baru almonds. However, and contrary to the results reported in this study, the best extraction methodology to extract phenolic compounds with bioactivity was the one with water at boiling temperature. Highest antioxidant potential was achieved by aqueous extractions also in study of HASSAS-ROUDSARI et al. [24]. Methanol has been listed as one of the best solvents for antioxidant extraction in diverse of food and plant matrices [8, 22, 27-29]. Baru almonds were studied for the antioxidant potential and total phenols content in two recent studies. LEMOS et al. [6] evaluated the antioxidant properties of baru almonds extracted at room temperature with methanol, while SIQUEIRA et al. [7] referred that ethyl acetate and water were appropriate solvents to obtain extracts rich in phenolic compounds, with good activity against the free radicals of DPPH.

However, the combination of solvents and temperatures has never been tested with this matrix and neither their influence on the bioactivity and total phenols content.

Considering the results obtained on the antioxidant activity and total phenols content of the different extracts of baru almonds, the methanolic extraction at room temperature was found to be the best methodology, not only for the results obtained but also due to being a quick, and easily applicable extraction method. This methodology was also selected to compare the antioxidant activity and total phenols content of baru almonds with other types of nuts from Brazil.

Total phenols content and antioxidant potential of baru almonds in relation to other Brazilian nuts

The total phenols content and antioxidant activity of baru almonds (samples A and B and crude sample) were compared with other types of Brazilian nuts, namely, peanut (Arachis hypogaea L.), macadamia (Macadamia intergrifolia), Brazil nut (Bertholletia excelsa Humb. & Bonpl.), roasted cashew nut and crude cashew nut (Anacardium occidentale L.), all extracted by the methanolic extraction at room temperature. The results obtained on the total phenols content and extraction yield with different types of nuts are presented in Tab.2. Highest extraction yields were obtained with samples of crude baru (20.2%), baru sample A (18.3%) and roasted cashew nuts (18.0%). Significantly lower extraction yield was achieved with peanuts compared to the previously mentioned samples (p < 0.001). However, as mentioned before, samples with high extraction yield did not always exhibit higher antioxidant content and higher bioactivity.

Baru samples, both roasted and crude, had higher bioactivity and higher content of total phenols content than the remaining samples studied. Baru samples had EC_{50} values 17 to 25 times lower, and between 5 to 7 times lower at reducing power assay, compared to peanuts (Fig. 3), which reveals their high potential. Among the assessed samples, only crude baru cannot be consumed, since they contain antinutritional components when crude, such as protease inhibitors, mainly trypsin inhibitors. These inhibitors are mainly related to the levels of phytic acid identified in baru almonds [4, 30]. Phytic acid (10.7 g·kg⁻¹ of baru almonds), while common in leguminous plants, has to be inactivated by heating, because otherwise it can reduce bioavailability of proteins, minerals and vitamins [31]. The evaluation of samples of crude baru almonds was justified, on one hand, by dealing with *Dipteryx alata* seeds, and knowledge about extraction methodologies, total phenols content and antioxidant activity of this matrix is scarce in literature. On the other hand, some authors claim that, during the roasting process, some phytochemicals suffer degradation and, consequently, their antioxidant properties are reduced [4, 6]. However, in the present work, the roasting process was not found to affect the antioxidant ac-

 Tab. 2. Extraction yield and total phenols content of baru almonds and other nuts obtained using methanolic extraction at room temperature.

	Extraction yield [%]	Total phenols in extract [g·kg ⁻¹]	Total phenols in sample [g·kg ⁻¹]
Baru almonds A*	18.3 ± 0.1^{cd}	$115.8 \pm 5.4^{ f}$	21.2 ± 1.2^{d}
Baru almonds B*	16.7±0.3°	105.0 ± 3.1 °	17.5±0.5°
Baru almonds crude*	20.2 ± 0.2^{d}	111.3 ± 4.7^{f}	$22.5\pm0.8^{\text{d}}$
Peanut*	12.1 ± 1.5ª	41.6±1.5 ^d	$5.0\pm0.8^{\mathrm{b}}$
Cashew nuts roasted	$18.0 \pm 1.5 ^{cd}$	15.7 ± 2.1 ª	2.8 ± 0.5^{a}
Macadamia	13.6±1.9 ^{ab}	19.0 ± 0.4^{ab}	2.6 ± 0.3^{a}
Cashew nuts crude	15.6 ± 0.6^{bc}	23.8±1.7°	3.7 ± 0.3^{ab}
Brazil nuts*	12.7 ± 0.7^{ab}	$20.4\pm2.2^{\text{bc}}$	2.6 ± 0.4^{a}

Means within a column with different letters differ significantly, p < 0.05.

* - baru almonds (A, B and crude), peanuts and Brazil nuts were analysed with peels.

Total phenols are expressed in grams of gallic acid equivalens per kilogram of extract and fresh fruit sample.

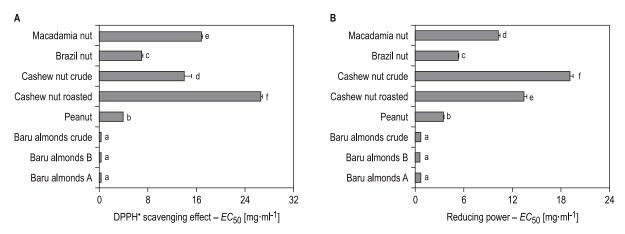


Fig. 3. Antioxidant potential of different nuts compared to baru almonds assessed by DPPH[•] and reducing power chemical assays.

A – DPPH[•] scavenging effect, B – reducing power.

Values are expressed as mean \pm standard deviation, n = 3. Means within each figure with different letters differ at p < 0.05. a-f - in each graph, bars with different letters differ significantly (p < 0.05). DPPH• scavenging effect is expressed as EC_{50} (effective concentration at which 50% of DPPH• radicals are scavenged).

DPPH[•] scavenging effect is expressed as EC_{50} (effective concentration at which 50% of DPPH[•] radicals are scavenged). Reducing power is expressed as EC_{50} (effective concentration at which the absorbance is 0.5). tivity of baru almonds, since no significant differences were observed between crude and roasted samples (p > 0.05).

Concerning the increasing antiradical activity (which means lower EC_{50} values), the results were as follows: roasted cashew nuts (26.54 mg·ml⁻¹) < macadamia (16.80 mg·ml⁻¹) < crude cashew nuts (13.93 mg·ml⁻¹) < Brazil nuts (6.94 mg·ml⁻¹) < peanuts (3.90 mg·ml⁻¹) < baru sample A (0.23 mg·ml⁻¹) < baru crude (0.22 mg·ml⁻¹) < and baru sample B (0.16 mg·ml⁻¹). The results obtained by the reducing power assay were not exactly the same as those observed by the DPPH[•] method, but a similar trend was observed – baru samples possessed higher reducing potential than the remaining nut types (Fig. 3). By both antioxidant methods tested, baru samples had significantly higher antioxidant potential (p < 0.001 for both methods).

Concerning total phenols content, extracts of baru samples had significantly higher values (115.75 g·kg⁻¹, 111.31 g·kg⁻¹, and 104.96 g·kg⁻¹ for baru sample B, baru crude and baru sample A, respectively; p < 0.001), followed by peanuts (41.60 g·kg⁻¹ extract). The extract of roasted cashew nuts had the lowest total phenols content, 15.74 g·kg⁻¹. When total phenols content was converted to fresh sample (expressed in grams per kilogram of sample), the same tendency was observed, i.e. baru almond samples had the highest total phenols content (Tab. 2). In order to ingest the same quantity of beneficial phenolic compounds, consumers need to eat less baru almonds than other common nuts.

The total phenols content may be responsible for a part of the antioxidant potential displayed by the samples. Recently, LEMOS et al. [6] revealed the high antioxidant activity and total phenols content of baru almonds raw, roasted and with peels. The same authors also characterized the individual phenolic composition of the above mentioned baru almonds and observed the prevalence of gallic acid in all baru almonds [6]. Besides phenols, other compounds may be responsible for the antioxidant activity displayed by the different nut types, such as tocopherols [12] and minerals [2].

Concerning peels, they were present in the three baru samples, in peanut and Brazil nut samples. Comparatively to the samples without peels, samples with peels exhibited higher antioxidant activity and, in general, had higher total phenols content (Tab. 2). It seems that peels are an important factor in the antioxidant activity and phenolic composition of nuts. LEMOS et al. [6] also stated that raw and roasted baru almonds with peels had higher total phenols content and higher antioxidant activity than raw and roasted baru almonds without peels. These authors observed that removing peels caused a loss of more than 50% of total phenols content in raw baru almonds. These data are also in accordance with those of BLOM-HOFF et al. [32] for other nut types. Regarding roasted baru almonds, LEMOS et al. [6] reported even higher loss of total phenols, reaching a loss of almost 80% of the compounds. The same tendency was observed in the antioxidant potential, decreasing the bioactivity about 10 times. These authors declared the importance of the presence of peels in nuts. This could also explain the results of our study for the most bioactive nut types (Tab. 2).

Another fact that affected the bioactivity of the nuts was the process of cooking or roasting. Cashew nuts were evaluated without peels and the roasting process increased to the double the EC_{50} value obtained by the DPPH[•] method (crude cashew 13.93 mg·ml⁻¹; roasted cashew $26.54 \text{ mg}\cdot\text{ml}^{-1}$), which means that the antiradical potential decreased to a half (Tab. 2). However, roasted cashew nuts had higher reducing power than the crude fruits. It appears that the roasting process was capable to slightly increase the capability of the methanolic extracts of cashew to reduce the Fe³⁺/ferricyanide complex. In accordance with our results, ACAR et al. [33] also observed that higher roasting time increased slightly the total antioxidant capacity of cashew nuts. Total phenols content of cashew nuts decreased significantly (p < 0.001) from 23.83 g·kg⁻¹ of extract to 15.74 g·kg⁻¹ of extract after the roasting process. Besides the presented data for cashew nuts, similar results were obtained for reducing power and total phenols content by MISHRA et al. [34].

CONCLUSIONS

From the different extraction methodologies tested, methanolic extraction at room temperature was the most suitable to extract antioxidant compounds from baru almonds, which were compounds with high antiradical and reducing power activity. Compared to other common popular nuts from Brazil, baru almonds had higher antioxidant potential and higher total phenols content. Baru almonds were found to be a rich source of bioactive compounds with exceptional antioxidant activity. In the light of these results, new research may open in the pharmaceutical, medicinal and food industrial sector regarding baru almonds.

Acknowledgments

We acknowledge Federal University of Goiás, (Goiania, Brazil), Mountain Research Centre (CIMO,

Bragança, Portugal) and the Polytechnic Institute of Bragança (Bragança, Portugal) for the scholarship support to the first author.

REFERENCES

- Da Cruz, K. S. Da Silva, M. A. De Freitas, O. Neves, V. A.: Partial characterization of proteins from baru (*Dipteryx alata* Vog) seeds. Journal of the Science of Food and Agriculture, *91*, 2011, pp. 2006–2012.
- Fernandes, D. C. Freitas, J. B. Czeder, L. P. Naves, M. M. V. : Nutritional composition and protein value of the baru (*Dipteryx alata* Vog.) almond from the Brazilian Savanna. Journal of the Science of Food and Agriculture, 90, 2010, pp. 1650–1655.
- 3. De Oliveira, A. G. S. Fernandes, D. C. Alves, A. M. – Freitas, J. B. – Naves, M. M. V.: Nutritional quality and protein value of exotic almonds and nut from the Brazilian Savanna compared to peanut. Food Research International, *44*, 2011, pp. 2319–2325.
- Marin, A. M. Siqueira, E. M. A. Arruda, S. F.: Minerals, phytic acid and tannin contents of eighteen fruits from the Brazilian Savanna. International Journal of Food Science and Nutrition, 60, 2009, pp. 180–190.
- Czeder, L. P. Fernandes, D. C. Freitas, J. B. Naves, M. M. V.: Baru almonds from different regions of the Brazilian Savanna: implications on physical and nutritional characteristics. Agricultural Sciences, *3*, 2012, pp. 745–754.
- Lemos, M. R. B. Siqueira, E. M. A. Arruda, S. F. Zambiazi, R. C.: The effect of roasting on the phenolic compounds and antioxidant potential of baru nuts [*Dipteryx alata* Vog.]. Food Research International, 48, 2012, pp. 592–597.
- Siqueira, E. M. A. Marin, A. M. F. Arruda, S. F. Cunha, M. S. B. – Fustinoni, A. M. – Dourado, L. P.: Consumption of baru seeds [*Dipteryx alata* Vog.], a Brazilian savanna nut, prevents iron-induced oxidative stress in rats. Food Research International, 45, 2012, pp. 427–433.
- André, C. Castanheira, I. Cruz, J. M. Paseiro, P. – Sanches-Silva, A.: Analytical strategies to evaluate antioxidants in food: a review. Trends in Food Science and Technology, 21, 2010, pp. 229–246.
- Arcan, I. Yemenicioğlu, A.: Antioxidant activity and phenolic content of fresh and dry nuts with or without the seed coat. Journal of Food Composition and Analysis, 22, 2009, pp. 184–188.
- Dinis, L. T. Oliveira, M. M. Almeida, J. -Costa, R. - Gomes-Laranjo, J. - Peixoto, F.: Antioxidant activities of chestnut nut of *Castanea sativa* Mill. (cultivar 'Judia') as function of origin ecosystem. Food Chemistry, *132*, 2012, pp. 1–8.
- Kamath, V. Rajini, P. S.: The efficacy of cashew nut (*Anacardium occidentale* L.) skin extract as a free radical scavenger. Food Chemistry, *103*, 2007, pp. 428–433.
- 12. Kornsteiner, M. Wagner, K. H. Elmadfa, I.:

Tocopherols and total phenolics in 10 different nut types. Food Chemistry, *98*, 2006, pp. 381–387.

- Pereira, J. A. Oliveira, I. Sousa, A. Ferreira, I. C. F. R. – Bento, A. – Estevinho, L.: Bioactive properties and chemical composition of six walnut (*Juglans regia* L.) cultivars. Food and Chemical Toxicology, 46, 2008, pp. 2103–2111.
- Prasad, K. Yang, B. Yang, S. Chen, Y. Zhao, M. – Ashraf, M. – Jiang, W.: Identification of phenolic compounds and appraisal of antioxidant and antityrosinase activities from litchi (*Litchi sinen*sis Sonn.) seeds. Food Chemistry, 116, 2009, pp. 1–7.
- Sasaki, Y. Kawaguchi, S. Kamaya, A. -Ohshita, M. - Kabasawa, K. - Iwamaa, K. - Taniguchi, K. - Tsuda, S.: The comet assay with 8 mouse organs: Results with 39 currently used food aditives. Mutation Research, 519, 2002, pp. 103–119.
- Ayala-Zavala, J. F. Vega-Vega, V. Rosas-Domínguez, C. – Palafox-Carlos, H. – Villa Rodriguez, J. A. – Wasim Siddiqui, M. D. – Dávila Aviña, J. E. – González-Aguilar, G. A.: Review: Agro-industrial potential of exotic fruit byproducts as a source of food additives. Food Research International, 44, 2001, pp. 1866–1874.
- 17- Perrin, C.- Meyer, M.: Quantification of synthetic phenolic antioxidants in dry foods by reversedphase HPLC with photodiode array detection. Food Chemistry, 77, 2002, pp. 93–100.
- Sousa, A. Ferreira, I. C. F. R. Barros, L. Bento, A. – Pereira, J. A.: Effect of solvent and extraction temperatures on the antioxidant potential of traditional stoned table olives "alcaparras'. LWT – Food Science and Technology, *41*, 2008, pp. 739–745.
- Berker, K. Güçlü, K. Tor, I. Apak, R.: Comparative evaluation of Fe(III) reducing powerbased antioxidant capacity assays in the presence of phenanthroline, batho-phenanthroline, tripyridyltriazine (FRAP) and ferricyanide reagents. Talanta, 72, 2007, pp. 1157–1165.
- Malheiro, R. Sá, O. Pereira, E. Aguiar, C. -Baptista, P. - Pereira, J. A.: *Arbutus unedo* L. leaves as source of phytochemicals with bioactive properties. Industrial Crops and Products, *37*, 2012, pp. 473–478.
- Singleton, V. L. Rossi, J. A.: Colorimetric of total phenolics with phosphomolybdic–phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16, 1965, pp. 144–158.
- Yuliana, M. Tran–Thi, N. Y. Ju, Y. H.: Effect of extraction methods on characteristics and composition of Indonesian cashew nut shell liquid. Industrial Crops and Products, 35, 2012, pp. 230–236.
- Oliveira, I. Sousa, A. Ferreira, I. C. F. R. Bento, A. – Estevinho, L. – Pereira, J. A.: Total phenols, antioxidant potential and antimicrobial activity of walnut (*Juglans regia* L.) green husks. Food and Chemical Toxicology, 46, 2008, pp. 2326–2331.
- Hassas-Roudsari, M. Chang, P. R. Pegg, R. B. Tyler, R. T.: Antioxidant capacity of bioactives extracted from canola meal by subcritical water, ethanolic and hot water extraction. Food Chemistry, *114*, 2009, pp. 717–726.

- Krishnaiah, D. Sarbatly, R. Nithyanandam, R.: A review of the antioxidant potential of medicinal plant species. Food and Bioproducts Processing, 89, 2011, pp. 217–233.
- Malheiro, R. Sousa, A. Casal, S. Bento, A. Pereira, J. A.: Cultivar effect on the phenolic composition and antioxidant activity of stoned table olives. Food and Chemical Toxicology, 49, 2011, pp. 450–467.
- Ganesan, P. Kumar, C. S. Bhaskar, N.: Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. Bioresource Technology, 99, 2008, pp. 2717–2723.
- Razali, N. Mat-Junit, S. Abdul-Muthalib, A. F. Subramaniam, S. – Abdul-Aziz, A.: Effects of various solvents on the extraction of antioxidant phenolics from the leaves, seeds, veins and skins of *Tamarindus indica* L. Food Chemistry, *131*, 2012, pp. 441–448.
- 31. Siddhuraju, P: Antioxidant activity of polyphenolic compounds extracted from defatted raw and dry heated *Tamarindus indica* seed coat. LWT Food Science and Technology, *40*, 2007, pp. 982–99.
- 32. Kalume, D. R. Sousa, M. V. Morhy, L.: Purification, characterization, sequence determina-

tion and mass spectrometric analysis of a trypsin inhibitor from seeds of the Brazilian tree *Dipteryx alata* (*Leguminosae*). Journal of Protein Chemistry, 14, 1995, pp. 685–693.

- Silva, M. R. Silva, M. A. A. P.: Aspectos nutricionais de fitatos e taninos. Revista de Nutriçao, *12*, 1999, pp. 21–32.
- 34. Blomhoff, R. Carlsen, M. H. Andersen, L. F. Jacobs, D. R.: Health benefits of nuts: potential role of antioxidants. British Journal of Nutrition, 96, 2006, pp. S52–S60.
- Açar, Ö. Ç. Gökmen, V. Pellegrini, N. Fogliano, V.: Direct evaluation of the total antioxidant capacity of raw and roasted pulses, nuts and seeds. European Food Research and Technology, 229, 2009, pp. 961–969.
- Mishra, N. Dubey, A. Mishra, R. Barik, N.: Study on antioxidant activity of common dry fruits. Food and Chemical Toxicology, 48, 2010, pp. 3316–3320.

Received 23 March 2013; 1st revised 13 June 2013; 2nd revised 19 August 2013; 3rd revised 21 October 2013; accepted 12 November 2013; published online 20 May 2014.