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Effects of the drying process on the fatty acid content, phenolic profile, tocopherols and antioxidant activity of baru almonds (*Dipteryx alata* Vog.)

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SUMMARY: This study carried out a chromatographic and spectrophotometric characterization of the bioactive compounds, antioxidants, phenolics, tocopherols, sterols and fatty acids of baru almonds "in natura" and submitted to drying processes. It was determined that baru "in natura" almonds presented high levels of phenolic compounds, vitamin C, antioxidants, phenolics, sterols, total monounsaturated fatty acids and low thrombogenic, and atherogenic indexes. During the process of drying it at 65 °C for 30 minutes, a decrease was noted in the levels of caffeic acid, chlorogenic acid, anthocyanins, *p*-coumaric acid, ferulic acid, *o*-coumaric acid, quercetin, and polyunsaturated fatty acids. The same condition resulted in an increase in the levels of gallic acid, rutin, catechin, *trans*-cinnamic acid, vanillin, m-coumaric acid, tocopherols, monounsaturated fatty acids and antioxidant activity (ORAC and DPPH). When submitted to a temperature of 105 ºC for 30 minutes the same behavior was seen with a reduction in the vitamin C and ORAC contents and increased presence of flavonoids.

KEYWORDS*: Antioxidant activity; Bioactive compounds; Brazilian Cerrado; High-Performance Liquid Chromatography; Oilseeds*

RESUMEN: ***Efectos del proceso de secado sobre la composición en ácidos grasos, perfil fenólico, tocoferoles y actividad antioxidante de almendras barú (*Dipteryx alata *Vog.)*.** Este estudio realizó una caracterización cromatográfica y espectrofotométrica de la presencia de compuestos bioactivos, antioxidantes, fenólicos, tocoferoles, esteroles y ácidos grasos en almendras del tipo baru "in natura" y sometidos a procesos de secado. Se detectó, en la almendra de baru "in natura", altos contenidos de compuestos fenólicos, vitamina C, antioxidantes fenólicos, esteroles, ácidos grasos monoinsaturados totales y bajos índices de trombogénicos y aterogénicos. Durante el proceso de secado a 65 °C durante 30 minutos, se observó una disminución en los niveles de ácido cafeíco, ácido clorogénico, antocianinas, ácido *p*-cumárico, ácido ferúlico, ácido *o*-cumárico, quercetina y ácidos grasos poliinsaturados. De la misma manera se observó un aumento en los niveles de ácido gálico, rutina, catequina, ácido *trans*-cinámico, vanilina, ácido *m*-cumárico, tocoferoles, ácidos grasos monoinsaturados y actividad antioxidante (ORAC y DPPH). Cuando se sometió a una temperatura de 105 °C durante 30 minutos, presentó el mismo comportamiento, sin embargo, influyó en la reducción del contenido de vitamina C y ORAC y aumentó la presencia de flavonoides.

PALABRAS CLAVE*: Actividad antioxidante; Cerrado Brasileño; Compuestos bioactivos; Cromatografía líquida de alta resolución; Semillas oleaginosas*

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1. INTRODUCTION

Drying is one of the oldest processes used by man for preserving food. Due to reduced water activity, there is a minimization of microbial growth which results in an increased shelf-life of raw materials. It can facilitate and reduce the costs of packaging, transport and storage, in addition to bringing improvements in sensory aspects (especially when it comes to oleaginous seeds since the majority, when in natura, have a bitter and astringent taste due to the presence of tannins), inhibiting anti nutritional factors and providing raw material throughout the year (Igual *et al*., 2012).

Despite the benefits, it is estimated that drying can contribute to a reduction in the functional activity of food, because the vast majority of bioactive compounds exhibit heat-sensitive behavior (Lemos *et al*., 2012; Lemos *et al*., 2016). There is a high incidence of producers that improperly employ this technique in their raw materials. As a result, there is a distribution of products with compromised functional activity, and oilseeds are the most affected, as these employ this method of conservation.

In order to maintain the nutritional quality of food, the verification of bioactive compound changes during the drying process must be monitored. Recent research has linked a daily consumption of oilseeds with reduced incidence of chronic non-communicable diseases due to the presence of biologically active substances (Liu *et al*., 2019).

Despite the limited amount of information about the baru almond (*Dipteryx alata* Vog.), it stands out for its superior nutritional composition (Fraguas *et al*., 2014; Lemos *et al*., 2016). It contains high levels of lipids, proteins, amino acids, and minerals (such as calcium, iron, magnesium, potassium and zinc) and dietary fiber (Fraguas *et al*., 2014). Besides being found in regions with high sun exposure, the plant's defense system promotes protection from the sun through the biosynthesis of secondary compounds that present bioactive characteristics. These substances have the ability to minimize the action of free radicals through the interception of active oxygen (responsible for oxidative damage in cell membranes and DNA), and they can assist in the prevention of chronic non-communicable diseases (Lemos *et al*., 2012).

In spite of the aforementioned benefits, research is limited on the specific compounds that exhibit desirable characteristics for minimizing chronic non-communicable diseases, such as fatty acids and phenolics and tocopherols. Therefore, with the aim of obtaining complete and efficient use of baru almonds to meet their new functional properties, detailed research on the presence of different bioactive compounds such as phenolic metabolites, fatty acids, and tocopherols, among others is necessary. In addition, it is necessary to monitor the effectiveness of the two drying processes (65 and 105 °C for 30 minutes) which are the most often used by the producers of this food, because the vast majority reach the consumer in this condition.

Therefore, in order to extend the knowledge about the functional potential of compounds and investigate the possible changes resulting from processing, the present study aimed to carry out a chromatographic and spectrophotometric characterization regarding the presence of bioactive compounds, antioxidants, sterols and phenolic profiles, tocopherols and fatty acids in baru almonds "in natura" and subjected to the drying process.

2. MATERIALS AND METHODS

2.1. Reagents, materials and experimental design

All chemicals, reagents and solvents used were of analytical grade or HPLC and obtained from Sigma-Aldrich (St. Louis, MO, USA). Deionized water (> 18 MΩ.cm) was obtained from a Milli-Q system (Millipore, Brussels, Belgium).

The buru almonds were obtained from the Cerrado Biome region, located in the city of Barra do Garças-MT, during the harvest season (between August and September 2016). The baru almond has the following centesimal composition (g/100g): 6.63 moisture, 22.96 protein, 31.73 lipid, 14.44 dietary fiber and it wieghs 1.55 grams.

For this work, the completely randomized experimental design was used with three replications. Three treatments were evaluated: baru almonds "in natura" (T1 – which was the control for the drying experiment), baru almonds submitted to drying at 65 °C for 30 min (T2) and baru almonds subjected to drying at 105 °C for 30 min (T3). The temperatures were chosen because they are the most commonly used by the producers of these oleaginous plants. In addition, it is known that the roasting process compromises part of the nutritional and bioactive value of the food, and therefore to use lower temperatures which are effective in drying the baru almond preserve its nutrients can become a new form of use and consumption for this food.

2.2. Drying process

The drying of the baru almonds was carried out in a forced air circulation oven (Marconi, MA0351, Piracicaba, Brazil). They were then ground (20 mesh - in experimental mill, Viti Molinos, VG 2000i, Itajaí, Brazil), packed in transparent polyethylene containers and stored at 25 °C (oven model Eletrolab, EL202). During the drying processes, the time and temperature were chosen because they are yet to be investigated. The moisture contents of the almonds were determined after the drying process (AOAC,

1990) and the averages were 6.63, 5.10 and 2.64 g·100 g^{-1} respectively for T1 T2 and T3. All the results of the analytical determinations described below (including moisture) were expressed on a wet basis.

2.3. Determination of bioactive compounds

The contents of total phenolic compounds, anthocyanins, flavonoids, tannins and vitamin C were evaluated. The hydroalcoholic extract was prepared according to the methodology adapted from Milardovic *et al.,* (2006).

Total phenolics were determined by the *Folin–Ciocalteu* reagent method, using gallic acid as the standard for the calibration curve. The absorbance was measured at 765 nm in a spectrophotometer (UV-Visible 50 Probe-Cary) and the results were expressed in mg of gallic acid equivalent (GAE) 100 g^{-1} (Lemos, 2012).

The total phenolic compounds were also evaluated by the use of diazonium salt Fast Blue BB, using standard gallic acid for the calibration curve. The absorbance was measured at 420 nm in a spectrophotometer (UV-Visible 50 Probe-Cary) and the results were expressed in milligrams gallic acid equivalent (GAE) per 100 g^{-1} (Palombini *et al*., 2016).

Monomeric anthocyanins were determined by the differential pH method described by Giusti and Wrolstad (2001), using a spectrophotometer (UV-Visible 50 Probe-Cary) for absorbance measurements of samples (510 and 700 nm) and the results were calculated as malvidin-3,5-diglucoside.

Flavonoids were determined following the methodology described by Fraguas *et al.,* (2014). The reading was held at 415 nm spectrophotometer (UV-Visible 50 Probe-Cary) using a 2% solution of aluminum chloride in methanol. The total flavonoid values were expressed as equivalents of catechin.

Tannins were measured by the colorimetric method according to the Association of Official Analytical Chemists (AOAC, 1990). The method was based on the intensity of the blue color produced in reducing the *Folin-Denis* reagent for phenols, and was then measured in the spectrophotometer (UV-Visible 50 Probe-Cary) to 760 nm expressed as equivalents of catechin.

Vitamin C was evaluated by the colorimetric method using 2,4-dinitrophenylhydrazine and the results were read on a spectrophotometer (UV-Visible 50 Probe-Cary) to 520 nm and expressed in equivalents of ascorbic acid (Milardovic *et al.*, 2006).

2.4. Individual identification of phenolic compounds by HPLC-DAD

The individual identification of phenolic compounds was made following the methodology described by Ramaiya *et al*., (2013). The quantification and identification of these phenols compounds were performed in a liquid chromatography (HPLC-DAD/UV-Vis) model Shimadzu (Shimadzu Corp., Kyoto, Japan) equipped with a gradient pump (2487 Serie), a valve injector with a loop of 50 µL, a degasser (Waters 200 Series) and an integrator-plotter with software (Total Chrom, Waters). Phenolic compounds were separated in a C18 reversed-phase column (150 mm × 4.6 mm I.D., 5 µM), (Phenomenex, CA, USA) with a C18 (20 mm × 4.6 mm I.D.) pre-column cartridge. The mobile phase consisted of 2% (v/v) acetic acid in deionized water (mobile Phase A) and 70:28:2 (v/v) methanol/water/acetic acid (mobile phase B), and phenolics were detected at 280 nm. Phenolic compounds were identified by comparison of retention times with standards (gallic acid, catechin, chlorogenic acid, caffeic acid, vanillin, *p*-coumaric acid, ferulic acid, *m*-coumaric acid, *o*-coumaric acid, *trans*-cinnamic acid, quercetin and rutin). The results were expressed as mg of phenolic compound in 100 g^{-1} of fresh weight.

2.5. Screening of antioxidant activity

Test for elimination of free radicals (DPPH•). The antioxidant activity was evaluated by the DPPH• scavenging ability of the antioxidant activity. Absorbance was measured using UV-Visible spectrophotometer (50 Probe–Cary) at 517 nm expressed as % of free radical sequestration (Milardovic *et al*., 1943).

Antioxidant activity via β-carotene/linoleic acid system. Antioxidant activity determination by β-carotene/linoleic acid system was conducted according to the methodology described by Miller (1971) and absorbance was measured at 470 nm with a spectrophotometer (UV-Visible 50 Probe-Cary). The results were expressed as percentage of inhibition of β-carotene oxidation.

Oxygen Radical Antioxidant Capacity (ORAC) assay. The ORAC method used, with fluorescein (FL) as the "fluorescent probe", was applied as described by Aazza *et al.,* (2011).

2.6. Determination of total sterols, tocopherols and fatty acids

The levels of total sterols, tocopherols and fatty acids present in baru almonds were determined. To carry out these analyses, it was necessary to extract the oil present in the baru almond.

2.6.1. Process of baru almond oil extraction

To extract the oil from the almonds, an Extractor Oster (yoda model 60 Hz) was used with the following specifications: rated power-400W, Turbo-electric heating mode. Subsequently, the oil was transferred to amber jars and kept under refrigeration (4 °C).

This method was chosen because it is a method that extracts lipids in the cold.

Total sterols. For the reading of the samples 0.8 mL of *Lieberman-Burchard,* reagent was added to 0.1 g of the sample and 3.1 mL^{-1} of chloroform, and left to stand for 12 min. The solution was ready in the 625 nm spectrophotometer (UV-Visible 50 Probe-Cary), using chloroform as white (Kenny, 1952).

Identification of tocopherols by HPLC-DAD. For the determination of tocopherols (α and γ), 0.08 g of the obtained oil was dissolved in 4.0 mL of 2-propanol. The analysis was performed by liquid chromatography (HPLC-DAD/UV-Vis) model Shimadzu (Shimadzu Corp., Kyoto, Japan) equipped with a gradient pump (2487 Series), a valve injector with a loop of 50 μL, a degasser (Waters 200 Series) and an integrator-plotter with software (Total Chrom, Waters). Vitamin E was separated on a C18 reversed-phase column (150 mm × 4.6 mm I.D., 5μM), (Phenomenex, CA, USA) with a C18 (20 mm × 4.6 mm I.D.) pre-column cartridge. The mobile phase consisted of a mixture of methanol (96%) and water (4%) using the isocratic solvent and system with a flow rate of 1.0 mL min. Detection was made at 292 nm. The quantification of α and γ tocopherol was performed using the external standard method. The results were expressed in mg of tocopherol·100 g^{-1} (Freitas *et al*., 2008).

Profile of fatty acids by CG-FID. For the analysis of fatty acid profile, the lipids were extracted according to the procedures described by Folch *et al*., (1957). The analysis was performed by gas chromatography on a Shimatzu CG 2010 chromatograph (Agilent Technologies Inc., Palo Alto, CA, USA), equipped with a flame ionization detector, split injection at the rate of 1:50 and capillary column SPTM-2560 Supelco, 100 m × 0.25 mm × 0.20 μm (Supelco Inc., Bellefonte, PA, USA). The initial temperature of the column was 140 °C, maintained for 5 min, changing to 240 °C with increments of 4 °C, maintained for 30 min for a total of 60 min. The injector and detector were kept at the temperature of 260 °C and helium was used as the carrier. The identified fatty acids were compared to the retention times presented by the chromatographic pattern SupelcoTM37 FAME Mix (Supelco Inc., Bellefonte, PA, USA) and expressed in percentage (%) of the total fatty acids. Later they were grouped according to saturated fatty acids (SFA), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). The atherogenic index (AI) and thrombogenic index (TI) were determined in accordance with those specified by Ulbricht and Southgate (1991), as in equations 1 and 2:

$$AI: [(C12:0+(4\times C14:0)+C16:0)]/(\Sigma MUFAs+\Sigma\Omega 6+\Sigma\Omega 3) \quad (1)$$

$$TI:(C14:0+C16:0+C18:0)/[(0.5\times\Sigma MUFAs)+(0.5\times\Sigma\Omega 6)+(3\times\Sigma\Omega 3)+(\Sigma\Omega 3/\Sigma\Omega\text{-}6)] \quad (2)$$

The *ratio* of fatty acids hypocholesterolemic and hypercholesterolemic (h/H) was calculated according to the formula described by Santos-Silva (2002) and shown in equation 3:

$$h/H:(C18:1+C18:2+C20:4+C18:3+C20:5+C22:5+C22:6)/(C14:0+C16:0) \quad (3)$$

2.7. Statistical Analysis

The results were submitted to analysis of variance (ANOVA) and test for comparison of averages (5% probability of error according to the Tukey test). Statistical calculations were performed using the program R® version 5.0. The differences were considered significant when $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Determination of bioactive compounds present in baru almonds

The results of the determinations of total phenolics, monomeric anthocyanins, flavonoids, tannins and vitamin C of baru almonds are displayed in Table 1.

It was observed that the drying processes did not alter the total phenolic or tannins because these compounds remained unchanged ($p > 0.05$), thus showing the thermal stability to these temperatures. When applied to the process of 65 °C for 30 min (T2), a significant reduction ($p < 0.05$) was detected only in the presence of monomeric anthocyanins, which proves these molecules are sensitive to such a drying process. When using 105 °C for 30 min (T3) they were reduced ($p < 0.05$) in addition to the anthocyanins and vitamin C, although the level of flavonoids increased ($p < 0.05$).

In reference to the means obtained from the determination of phenolic compounds, it the predominance of these substances in baru almonds was demonstrated, which in turn, exceeded the values reported for other nuts (with an averages between 32 and 420 mg·100 g^{-1} for macadamias, cashews, walnuts, hazelnuts and peanuts) (Kornsteiner *et al*., 2006). The process of drying was found not to influence this content and this behavior can be associated with the absence of oxidative reactions by reactive oxygen species. Discrepant behavior was found by Lin *et al.,* (2016), who studied the effect of drying on almonds (150 °C for 20 min) and found that the phenolic compounds were reduced. Pasqualone *et al*., (2018), when studying the drying process of almond by-products, also found a significant reduction in the presence of phenolic compounds in these foods.

Table 1. Average values of total phenolics (measured by *Folin Ciocalteau* methods and Fast Blue BB), monomeric anthocyanins, total flavonoids, tannins, vitamin C, ORAC, DPPH and β-carotene/linoleic acid present in baru almonds submitted to different drying processes[1]

	Treatments[2]			
Analytical Determinations	**T1**	**T2**	**T3**	**P**
TPC (*Folin Ciocalteau*) GAE [3] (mg 100 g^{-1})	1254.12±39.71	1175.23±35.15	1306.34±33.18	0.6229
TPC (Fast Blue BB) GAE [3] (mg 100 g^{-1})	179.14±1.54	167.85±1.42	186.56±1.26	0.5698
Monomeric Anthocyanins (mg 100 g^{-1})	0.38±0.02^{a}	0.32±0.01^{b}	0.25±0.02^{c}	0.0006
Total Flavonoids QE [4] (mg 100 g^{-1})	9.17±0.45^{b}	8.01±1.46^{b}	15.73±0.45^{a}	0.0001
Tannins CE [5] (g 100 g^{-1})	1.51±0.87	1.62±0.65	1.67±0.89	0.1567
Vitamin C [6] (mg 100 g^{-1})	39.14±0.43^{a}	37.85±0.79^{a}	35.23±0.31^{b}	0.0004
ORAC [7] (uM g^{-1})	4.06±0.76^{a}	3.43±0.98^{b}	2.96±0.45^{c}	0.0029
DPPH• (% SRL)	69.02±2.86^{b}	79.68±3.78^{b}	84.38±3.98^{a}	0.0056
β-carotene/linoleic acid system (% protection)	91.72±3.35	89.94±10.52	86.10±1.42	0.5738

[1] Mean values ± standard deviation (in quadruple, n = 4). Values followed by different letters in the same line show differences by Tukey's test at 95% significance ($p < 0.05$); [2] Treatments: T1 (Almond "in natura"), T2 (Almond subjected to drying at 65 °C for 30 minutes) and T3 (Almond subjected to drying at 105 °C for 30 minutes); [3] TPC: Total Phenolic Compounds; GAE: Gallic acid equivalent; [4] Equivalent of rutin; [5] Equivalent of catechin; [6] Expressed in mg of ascorbic acid; [7] Equivalent of trolox; *p: p*-value.

On the other hand, the use of the Fast Blue BB method provided a reduction in the detection of these phenolic substances, demonstrating a difference between the two processes. This evidence is attributed to the fact that this procedure is specific and unique to the quantification of phenols, for pure reagents which are not mixed with other compounds such as proteins, sugars or ascorbic acid reducers (as occurs in the *Folin Ciocalteau's* method) (Naczk and Shahidi, 2004). It was noted that this methodology presented greater precision for determining these substances.

Concerning the quantification of monomeric anthocyanins in baru almonds, a considerable reduction ($p < 0.05$) was observed (15.78% and 34.21%, respectively in T2 and T3). It was observed that the higher the temperature of the drying process, the greater the degradation of these substances. A negative effect of the temperature/oxygen interaction was evidenced, which was detrimental to the maintenance of these molecules.

Higher flavonoid contents were found in treatment T3 when compared to T1 and T2, indicating that the process of drying at 105 °C for 30 min increased ($p < 0.05$) the presence of these molecules by 72%. The increase in bioactive compounds, such as flavonoids, may be related not only to the degradation of polymeric polyphenols and flavonoid glycosylated hydrolysis (Lemos *et al*., 2012) but also to the *Maillard* products reaction (Liu, Kitts, 2011). In addition, the intracellular water evaporation could have changed the lignocellulosic structure, in addition to promoting the denaturation of proteins, resulting in an increased availability of active compounds in the array (Lemos *et al*., 2012). Similar behavior was found by Lin *et al.,* (2016), who concluded that the roasting process (200 °C for 20 min) contributed to the increase of 124% in the content of flavonoids in almonds.

With respect to tannins, a considerable presence was observed in baru almonds and the drying processes were found not to promote a significant reduction ($p > 0.05$) thereof. The abundant consumption of this substance should be controlled because the exploitation of minerals and proteins causes antinutritional effects. However, as the consumption of "in natura" baru almonds is not a common practice, mostly due to its sensory characteristics in this condition (bitter and astringent), the application of heat becomes an important feature that aims to improve the sensory aspects and inactivate the antinutritional substances. Recent researches has indicated that these substances present anti mutagenic potential, which, in turn, are related to their antioxidant potential, and are effective in protection from oxidative damage (Macáková *et al*., 2014).

The concentration of vitamin C was fond to decrease ($p < 0.05$) by 9.98% due to the drying process at 105 °C for 30 min in T3, which can be assigned to a likely oxidation of this vitamin, which by its biologically active nature, is unstable and reversibly oxidized to Ldehidroascórbic acid. Even with this, it was found that the treatments corresponded to a high content of vitamins. According to the Dietary Guidelines for Americans (McGuire, 2011), the recommendation of daily vitamin C intake is 75 mg for women and 90 mg for men over 30 years old. These levels are based on their physiological functions and antioxidants needed to benefit the physiological organism. In this regard, when 100 g of baru almonds are consumed, treatments T1, T2 and T3 may represent 52.2, 46.9 and 50.5%, respectively, of the recommendation for women, and 41.4, 43.5 and 39.2% for men. It is found that this almond is a food

with high levels of this nutrient. It is important to note that Fatin and Azrina (2017) have found lower results for vitamin C in fresh lime (27.78 mg·100 g^{-1}), which is one of the referenced foods for this vitamin. Therefore, the consumption of baru almonds may be beneficial in the case of possible vitamin deficiencies.

3.2. Screening for antioxidant activity

Test of elimination of free radicals (DPPH•). The results obtained for the total antioxidant levels analyzed by the DPPH• method were 69.02, 79.68 and 84.38% free radical scavenging capacity for T1, T2 and T3, respectively (Table 1). The drying process at 105 °C for 30 min promoted a significant reduction ($p < 0.05$) in T3, decreasing by 36.05% the DPPH• radical scavenging capacity in comparison with baru almonds "in natura". However, almond T2 remained stable when compared to T1 (control), therefore, it is reasonable to conclude that the drying process held at 65 °C for 30 min did not reduce the presence of antioxidants in almonds. It was found that more intense heat treatments could be responsible for the loss in antioxidants. Lemos *et al*., (2012), explained that during heating, part of the moisture was lost through evaporation and the *Maillard* reaction increased the antioxidant capacity of the almonds. Higher values for free radical scavenging capacity indicate a lower antioxidant activity. Some compounds do not react against the DPPH• free radical because they are lipophilic and, therefore, it is necessary to apply different methodologies that seek to characterize such components.

Antioxidant activity via β-carotene/linoleic acid system. The total antioxidant activity was also measured by the β-carotene/linoleic acid system and the average value obtained among treatments was 89.24% protection without any statistical differences ($p > 0.05$) (Table 1). These results indicated that baru almonds presented high levels of these components and heat-resistant behavior. These data present promising results because oxidative processes can be avoided through the use of antioxidants with prevention or decrease in triggering oxidative reactions. Moreover, this method is suitable for the investigation of lipophilic antioxidants and fits the constitution of baru almonds especially due to their high lipid content.

Oxygen radical activity capacity (ORAC) assay. In the ORAC method it was observed that baru almond "in natura" showed significantly ($p > 0.05$) more antioxidant capacity when compared to the other treatments (Table 1). As they are heat-sensitive substances, the drying processes have contributed to the reduction in this attribute. As this essay is based on a hydrogen atom transfer reaction using a free radical source which is predominant in human biology (peroxyl radical), the ORAC test is relevant to express the antioxidant capacity of a given substance under *in vivo* conditions.

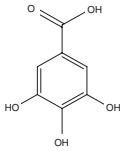
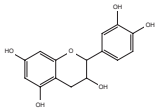
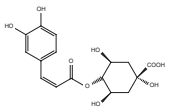
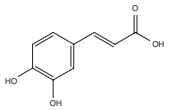
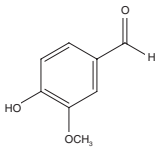
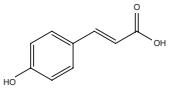
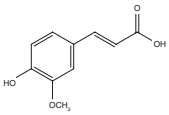
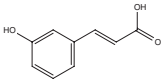
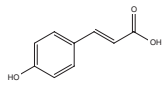
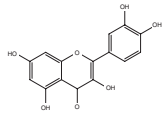
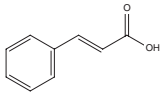
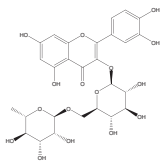
3.3. Individual identification of phenolic compounds by HPLC-DAD

A total of twelve phenolic compounds were detected in the three treatments, and the results associated with the identification and quantification of these molecules are shown in Table 2.

Among the phenols identified by high-performance liquid chromatography, gallic acid was the main phenolic compound in baru almonds. It should be noted that the high contents of these substances in food, more specifically in almonds, are desirable because they provide health benefits. Gallic acid, which has a strong free radical capacity, is effective in preventing disease, and research indicates that it is able to induce apoptosis and cytotoxic and antiproliferative effects among different strains of tumor cells (Guimarães *et al*., 2007). As the drying process increased these substances (which was an increase of 1.07 times), we can infer that the benefits to be achieved through the ingestion of these molecules can be enhanced. Lin *et al*., (2016) have also observed a significant increase in the presence of gallic acid in almonds dried at 150 °C for 30 min. However, this increase was 13 times greater (when compared to raw almonds) and the end content of this substance was 52 mg·100 g^{-1}.

It was found that other components, such as catechin, vanillin, *trans*-cinnamic acid, *m*-coumaric acid and rutin, were increased ($p < 0.05$) with the drying process (when compared to the "in natura" treatment), and this increase was 1.22, 1.34, 1.19, 1.17 and 1.03%, respectively. One possible explanation for this increase is associated with the changes that might have occurred in the protein of phenolic compounds, causing their exposure and the consequent increased availability (Lemos *et al*., 2012). Thermal processing is responsible for increasing the phenolic content due to the increase in the number of free phenolic groups resulting from the hydrolysis of glycosylated flavonoids that are released from the phenolic cell walls (D'archivio *et al*., 2010). Rodríguez-Bencomo *et al.,* (2015), when studying the effects of the roasting process (160 °C for 20 min) in pistachios, found an increase of 17, 79 and 81% in the presence of chlorogenic acid, rutin and catechin. It was also found that the drying (when comparing T1 to T3) promoted a reduction ($p < 0.05$) in the presence of chlorogenic acid, caffeic, *p*-coumaric acid, quercetin, ferulic and *o*-coumaric acid of 7.2, 41.3, 30.2, 9.4, 74.3 and 7.57%, respectively. The decreases in these substances can be attributed to the effects of heat, which may have caused possible protein denaturation resulting from the disruption of covalent links (Shahidi and Yeo, 2016).

TABLE 2. Identification and quantification via HPLC-DAD/UV-Vis of phenolic compounds present in baru almonds submitted to different drying processes[1]

Phenolic Compound	Chemical Structure	Treatments (mg·100 g^{-1})[2]			
		T1	T2	T3	P
Gallic Acid		45.83±0.69^{c}	47.18±1.16^{b}	48.90±1.28^{a}	0.0001
Catechin		9.01±1.40^{c}	9.98±0.52^{b}	11.06±0.57^{a}	0.0001
Chlorogenic Acid		7.33±0.87^{a}	7.23±0.62^{b}	6.80±0.98^{c}	0.0001
Caffeic Acid		19.89±1.17^{a}	11.94±1.09^{b}	11.68±1.62^{c}	0.0001
Vanillin		7.56±0.01^{c}	9.18±0.33^{b}	10.17±0.50^{a}	0.0001
P-coumaric		0.43±0.23^{a}	0.32±0.22^{b}	0.30±0.02^{c}	0.0001
Ferulic Acid		1.17±0.62^{a}	0.98±0.31^{b}	0.30±0.02^{c}	0.0001
M-coumaric		0.86±1.09^{c}	0.93±1.22^{b}	1.01±1.35^{a}	0.0002
O-coumaric		9.24±2.01^{a}	8.78±0.98^{b}	8.54±0.20^{c}	0.0001
Quercetin		1.60±0.25^{a}	1.48±0.19^{b}	1.45±0.22^{b}	0.0002
Trans-Cinnamic Acid		9.12±0.27^{c}	9.41±1.44^{b}	10.12±1.70^{a}	0.0001
Rutin		17.81±1.40^{c}	17.89±1.04^{b}	18.40±0.74^{a}	0.0001

[1] Mean values ± standard deviation (in quadruple, n = 4). Values followed by different letters in the same line show differences by Tukey's test at 95% significance ($p < 0.05$); [2] Treatments: T1 (Almond "in natura"), T2 (Almond submitted to 65 °C for 30 minutes) and T3 (Almond submitted to 105 °C per 30 minutes); *p*: *p*-value.

TABLE 3. Average of total sterols and tocopherols present in the baru almond submitted to different drying processes[1]

	Treatments[2]			
Analytical Determinations	**T1**	**T2**	**T3**	**P**
Total sterols (mg·100 g^{-1})	427.34±14.27	432.76±13.59	439.94±24.68	0.7227
α- tocopherol (mg·kg^{-1})	0.50±0.02^{c}	0.82±0.23^{b}	0.94±0.04^{a}	0.0001
γ- tocopherol (mg·kg^{-1})	1.47±0.32^{b}	1.33±0.02^{b}	2.33±1.75^{a}	0.0025
∑ α + γ tocopherol (mg·kg^{-1})	1.97±0.23^{c}	2.15±0.35^{b}	3.27±0.10^{a}	0.0001

[1] Mean values ± standard deviation (in quadruple, n = 4). Values followed by different letters in the same line show differences by Tukey's test at 95% significance ($p < 0.05$); [2] Treatments: T1 (Almond "in natura"), T2 (Almond submitted to 65 °C per 30 minutes) and T3 (Almond submitted to 105 °C per 30 minutes); p: *p*-value.

3.4. Determination of total sterols, tocopherols and fatty acids

Total sterols. The determination of the total sterol contents of baru almonds subjected to different drying processes of drying is shown in Table 3.

The total sterol content of baru almonds was measured at an average of 432.66 mg·100 g^{-1} without statistical difference ($p > 0.05$) among the treatments, and these results may demonstrate that the drying process does not influence these molecules. For health benefits, it is recommended to eat between 1 to 2 g of plant sterols a day. In this regard, the ingestion of 100 g of baru almonds can meet more than 40% of this recommendation (considering the consumption of 1 g/day). This result is favorable since these components have different physiological effects mainly in the treatment of hypercholesterolemia, as well as in the control of cholesterol by secondary causes in diabetics and patients with metabolic syndrome (Lemos *et al*., 2016). In addition, baru almonds present total sterol values greater than other oilseeds considering that Brazil's almonds, walnuts, cashews, hazelnuts, macadamia nuts, pistachios and walnuts have average total sterols of 192, 160, 154, 132, 105, 189 and 197 mg·100 $g^{-1,}$ respectively (Miraliakbari and Shahidi, 2008).

Identification of tocopherols by HPLC-DAD. The total contents of tocopherols (α and γ) (Table 3) obtained among the treatments showed significant differences ($p < 0.05$) indicating that the drying process (65 and 105 °C for 30 min) can contribute to an increase of 8.12 and 63.95% in these compounds, respectively. When evaluated individually, the tocopherols also presented the same behavior, and these data indicate that these substances may be enhanced through the use of heat. γ-tocopherol was the most abundant, with corresponding values for T1, T2 and T3 of 74.6, 71.2 and 61.9%, respectively of total tocopherol content. α-tocopherol presented values of 25.3, 38.1 and 43.7% for the same treatments. Factors such as the composition in fatty acids, the presence of compounds with antioxidant activity (especially those from lipophilic), variety, degree of ripeness and care in almond production can be responsible for the maintenance of these molecules (Lemos *et al*., 2016). The quantification of the levels of tocopherols, specifically in food, becomes necessary due to the action of this vitamin in preventing oxidative damage to DNA, which act as antioxidant structures and assist in peroxidation inhibition of lipids (Lemos *et al*., 2016).

Profile of fatty acids by CG-FID. Table 4 presents the identification, quantification and relations among the saturated, polyunsaturated and unsaturated fatty acids present in baru almond subjected to different drying temperatures, as well as the atherogenic index (AI) and thrombogenic index (TI), and the hypocholesterolemic and hypercholesterolemic fatty acids (h/H).

It was found that the unsaturated fatty acids, including oleic and linoleic, were predominant. The high proportion of monounsaturated fatty acids and polyunsaturated was also observed. The Dietary Guidelines for Americans (AHA, 2016) states that the daily intake of PUFAs is 20 g^{-1} and, in this sense, it is recommended to ingest 63.5, 65.1 and 64.1 g^{-1} baru almonds corresponding to T1, T2 and T3 to provide 100% of this recommendation.

Among the saturated fatty acids, palmitic acid was the major representative in all treatments. The relation between PUFA/SFA was higher than 1.9, 1.8 and 1.8 respectively for T1, T2 and T3, and T2 and T3 are statistically similar ($p < 0.05$). For a balanced intake of these elements, this value must be at least 0.45. In relation to this profile (low levels of SFA and high of PUFAs), it is estimated that baru almonds can be effective in the control of the traditional risk factors for atherosclerotic cardiovascular disease. This is because the presence of polyunsaturated fatty acids may promote a hypocholesterolemic effect.

As to the atherogenic index (AI) and thrombogenic index (TI) and the hypocholesterolemic fatty acids and hypercholesterolemic (h/H), which indicate the potential for stimulating the aggregation platelet and coronary artery disease, the values obtained for T1, T2 and T3 were respectively 0.28, 0.09, 11.31, 0.29, 0.08, 12.01, and 0.27, 0.07, 12.58

TABLE 4. Fatty acid profile of baru almonds submitted to different drying temperatures[1]

		Treatments[2]			
Fatty Acids (g·100 g^{-1})	**Chemical Structure**	**T1**	**T2**	**T3**	**P**
Methyl Hexanoate	C6:0	0.03±0.00^{a}	0.03±0.00^{a}	0.01±0.01^{b}	0.0271
Methyl Octanoate	C8:0	0.03±0.00^{b}	0.03±0.00^{b}	0.06±0.17^{a}	0.0001
Methyl Decanoate	C10:0	0.03±0.02^{a}	0.02±0.07^{a}	0.00±0.09^{b}	0.0001
Methyl Laurate	C12:0	0.24±0.06^{a}	0.13±0.01^{b}	0.08±0.06^{c}	0.0001
Methyl Myristate	C14:0	0.16±0.03^{a}	0.11±0.23^{b}	0.05±0.02^{c}	0.0001
Methyl Pentadecanoate	C15:0	0.22±0.03^{a}	0.18±0.21^{b}	0.18±0.06^{b}	0.0001
Methyl Palmitate	C16:0	6.59±0.04^{a}	6.28±0.03^{b}	6.16±0.01^{c}	0.0001
Methyl Heptadecanoate	C17:0	0.09±0.03^{a}	0.08±0.13^{b}	0.08±0.01^{b}	0.0076
Methyl Stearate	C18:0	4.39±0.21^{a}	5.32±0.08^{a}	5.22±0.12^{b}	0.0001
Methyl Arachidate	C20:0	1.06±0.07^{c}	1.18±0.09^{b}	1.21±0.16^{a}	0.0001
Methyl Behenate	22:00	3.47±0.25^{c}	3.67±0.54^{b}	3.73±0.43^{c}	0.0001
∑ SFA[3]	-	16.32±0.71^{c}	17.04±0.53^{a}	16.79±0.65^{b}	0.0001
Cis-10-pentadecanoic acid methyl ester	C15:1	0.031±0.01^{a}	0.07±0.03^{b}	0.02±0.01^{b}	0.0041
Methyl Palmitoleate	C16:1	0.08.±0.01^{a}	0.07±0.01^{b}	0.07±0.02^{b}	0.0012
Cis-10-heptadecanoic acid methyl ester	C17:1	0.15±0.02^{a}	0.13±0.76^{b}	0.13±0.16^{c}	0.0001
Cis-9-oleic acid methyl ester	C18:1Ω9	48.99±0.07^{c}	50.15±1,98^{b}	51.01±0.62^{a}	0.0001
Methyl cis-11-Eicosenoate	C20:1	2.40±0.87^{c}	2.52±0.54^{b}	2.56±0.19^{a}	0.0001
Methyl Erucate	22:1Ω9	0.26±0.06^{c}	0.28±0.01^{b}	0.29±0.05^{a}	0.0001
∑ MUFA[4]	-	51.91±1.98^{c}	53.18±1.34^{b}	54.08±1.54^{a}	0.0001
Methyl Linoleate	C18:2Ω6	27.28±0.23^{c}	26.46±0.35^{b}	26.89±0.04^{a}	0.0001
Methyl Linolenate	C18:3Ω3	0.14±0.01^{a}	0.13±0.03^{b}	0.12±0.05^{c}	0.0001
Cis-11,14-eicosadienoic acid methyl ester	C20:2Ω6	0.08±0.05^{a}	0.06±0.07^{b}	0.05±0.22^{c}	0.0001
Methyl cis-5,8,11,14,17-eicosapentaenoate	C20:5Ω3	0.07±0.09^{a}	0.06±0.06^{b}	0.05±0.32^{c}	0.0004
Cis-13,16-docasadienoic acid methyl ester	C22:2	3.93±0.35^{c}	4.04±1.78^{b}	4.13±0.97^{a}	0.0001
∑ PUFA[5]	-	31.50±1.04^{a}	30.74±0.75^{c}	31.25±0.69^{b}	0.0001
PUFA/SFA[6]	-	1.93±0.14^{a}	1.80±0.45^{b}	1.82±0.76^{b}	0.0001
MUFA/SFA[7]	-	3.180±0.53^{a}	3.12±0.25^{b}	1.87±0.34^{c}	0.0001
TI[8]	-	0.28±0.21^{b}	0.29±0.08^{a}	0.28±0.21^{b}	0.0003
AI[9]	-	0.09±0.09^{a}	0.08±0.76^{b}	0.08±0.34^{c}	0.0001
h/H[10]	-	11.32±0.24^{c}	12.02±1.12^{b}	12.59±0.43^{a}	0.0001

[1] Mean values ± standard deviation (in quadruple, n = 4). Values followed by different letters in the same line show differences by Tukey's test at 95% significance (p < 0.05); [2] Treatments: T1 (Almond "in natura"), T2 (Almond submitted to 65 °C for 30 minutes) and T3 (Almond submitted to 105 °C for 30 minutes); [3] Total saturated fatty acids; [4] Total unsaturated fatty acids; [5] Total of polyunsaturated fatty acids; [6] Relationship between saturated and polyunsaturated fatty acids; [7] Relationship between saturated and unsaturated fatty acids; [8] Thrombogenic index; [9] Atherogenic index; [10] hypocholestrolemic/ hypercholesterolemic potential; *p*: *p*-value

with statistical difference ($p < 0.05$) among the treatments. Although there is no established parameter for these indices, the smaller the result for AI and TI and greater for h/H, the less likely are the changes as mentioned above and the healthier the food is. This is due to the greater concentration of anti-atherogenic fatty acids (Ω3 and Ω6) (Turan *et al*., 2007). Foods such as cheeses and meats that are significant sources of fatty acids, have indexes of AI, TI and respective h/h 2.32, 3.11, 1.23 and 0.54, 1.15, 1.76 (Faria *et al*., 2015), and comparing these figures with those of baru almonds, the superiority and greater possibility of cardiovascular protection on the part of the almond is demonstrated.

With regards to the drying process (T3), it was found that heat promoted a reduction ($p < 0.05$) in different fatty acids (relative to T1) in C16:0 and C18:2 of 1.4 and 6.6%. By analyzing all the reductions and correlating them to the degree of unsaturation, one can infer that polyunsaturated fatty acids were the most affected. This may be due to the fact that this compound has a greater number of double bonds, which results in increased susceptibility to oxidation.

Conversely, this same process of drying promoted increases ($p < 0.05$) in different fatty acids, C18:0, C18:1, C22:0 and C22:2 with expansion of 18.9, 4.1, 4.9 and 7.5%, respectively. The saturated fatty acids were the most favored by the drying process (when compared to T1 with T3), because they represented together a total increase of 134.6%. This occurs because heating may increase the oxidation rate of these molecules. Rodríguez-Bencomo *et al*., (2015), when analyzing pistachio oil subjected to heating (160 °C for 20 min), found that heat is not promoted and there is no significant increase in the levels of SFA, PUFA and MUFA.

4. CONCLUSIONS

High levels of phenolic compounds, vitamin C, antioxidants (measured by the β-carotene/linoleic acid systems), gallic acid, caffeic acid, rutin, sterols, total monounsaturated fatty acids and low thrombogenic, atherogenic index were found in "in natura" baru almonds. During the process of drying at 65 °C for 30 min, a decline in the levels of caffeic, chlorogenic acid, anthocyanins, *p*-coumaric acid, ferulic, *o*-coumaric acid, quercetin, polyunsaturated fatty acids and the free radical scavenging capacity was observed. Under the same conditions, an increase in the levels of gallic acid, rutin, catechin, *trans*-cinnamic acid, vanillin, *m*-coumaric acid, tocopherols and monounsaturated fatty acids was observed. The temperature of 105 °C presented the same behavior as above, however, it caused a reduction in vitamin C content and the increase in the presence of flavonoids. The drying temperature did not affect the levels of total phenolics, tannins, β- carotene/linoleic acid system, or sterols. The chromatographic and spectrophotometric quantifications carried out in this work contributed to an increase in the scientific knowledge about the properties of baru almonds submitted to different drying processes in relation to the work already done and published in the scientific community. The almonds of this study present functional features that place it above other oilseeds. Its consumption promotes the plant biodiversity of the Cerrado Biome and contributes to a new generation of foods that can benefit the health of consumers. The bioactive properties of the molecules that are intensified by processing suggest great potential for application of baru almonds ("in natura" and/or submitted to different drying processes) in new products such as oils, cereal bars, bakery, chocolate, among others. However, their application depends on the adequacy of the industrial scale.

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