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Original Article

Identification of terpenes and phytosterols in *Dipteryx alata* (baru) oil seeds obtained through pressing

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ABSTRACT

The oil from seeds of *Dipteryx alata* Vogel, Fabaceae, popularly known as baru, was extracted by hydraulic and continuous screw pressing. A total of eleven chemical constituents obtained by hydraulic pressing, including steroids, mono and sesquiterpenes and tocopherol derivatives were identified by gas chromatography–tandem mass spectrometry (GC–MS). Compounds limonene, β-elemene, γ-elemene, α-caryophyllene, β-caryophyllene, campesterol, stigmasterol, β-sitosterol and cycloartenol are being described for the first time in the baru oil.

Introduction

Baru (*Dipteryx alata* Vogel, Fabaceae) is a vegetal species of the cerrado whose seeds (nuts) present great nutritional value (Takemoto et al., 2001), a considerable content of phenolic compounds (568.9 mg/100 g) (Lemos et al., 2012), antioxidant activity (DPPH method) (Lemos et al., 2012), a preventive effect on iron-induced oxidative stress in rats (Siqueira et al., 2012) and the capacity to reduce cholesterol, triacylglycerides and lipid peroxidation in rats (Fernandes et al., 2012). Oil extracted from these seeds is popularly used as an anti-rheumatic agent and presents sudorific, tonic and menstrual regulatory properties (Sano et al., 2004). The baru oil also contains tocopherols and high content of unsaturated fatty acids (81.2%) (Takemoto et al., 2001). Interest in edible vegetable oils, especially those with high unsaturated fatty acid contents has increased due to its beneficial health effects, such as cholesterol reduction and atherosclerosis prevention (Gromadzka and Wardencki, 2011; Plat and Mensink, 2000; Ausman et al., 2005).

Among the most common compounds in vegetable oils there are fatty acids, hydrocarbons, tocopherols, tocotrienols, phenolic compounds, terpenes and phytosterols. The presence and amount of these substances are related to the quality, nutritional and functional values of such oils, and can vary depending on the species, cultivation climate conditions, oil extraction system and refining processes (Cert et al., 2000; Gromadzka and Wardencki, 2011). The extraction of vegetable oils is commonly performed using hydraulic pressing, mechanical screw pressing or solvents. Extraction through use of solvent is not recommended since it can generate toxic residues in the product. Although mechanical screw pressing is the most common method used in oilseed industries, hydraulic pressing is still used in the production of certain specialized oils (Kemper, 2005; Savoire et al., 2013).

However, despite the studies focusing on the functional potential of baru seeds, it has not been given enough attention to the knowledge of the chemical composition of the oil from the seed of baru. Given this background, the present study was to evaluate the chemical composition of the baru oil using gas chromatography–tandem mass spectrometry (GC–MS) and to analyze the influence of extraction processes (hydraulic and continuous screw pressing) on oil composition.

Materials and methods

Plant material and oil extraction

Baru seeds (*Dipteryx alata* Vogel, Fabaceae) were collected in August 2012 from different trees in Jussara, Goiás, Brazil (15°51′ South; 50°52′ West; 317 m altitude), identified by Dr. José Realino de Paula, and stored at freezer. The seeds were joined, stored for three months in a cool place and then the oil was obtained using both manual hydraulic pressing (manufacturer: Ribeiro®) and mechanical continuous pressing (MPE-40, Ecirtec®). Hydraulic

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pressing was performed with 50 g of seeds over which was applied a pressure of 1.2×10^7 to 1.4×10^7 Pa for three consecutive times. For continuous pressing, a frequency inverter was used operating at 50 cycles per minute, with 0.4 mm spacers. After pressing, using both methods, oil was submitted to centrifugation ($5000 \times g$ for 10 min).

The extraction yield was calculated as the ratio between the oil mass obtained and seed mass submitted to this process. Extraction efficiency was calculated as the ratio between the yield and the percentage oil present in the seeds, calculated by the Bligh & Dyer technique (Bligh and Dyer, 1959).

Oil characterization and quality

Specific gravity (*Association of Official Analytical Chemists* – AOAC Official Method 920.212 – Pycnometer method AOAC, 2000), refractive index (AOAC, 2000), iodine absorption number (AOAC Official Method 920.158 – Hanus method AOAC, 2000) and saponification number (AOAC Official Method 920.160 AOAC, 2000) were applied to obtain oil characterization, while oil quality was analyzed by peroxide value (AOAC Official Method 965.33 AOAC, 2000) and free fatty acid value (modified AOAC Official Method 940.28 AOAC, 2000 – alcohol was substituted by an ether:alcohol (2:1) solution to improve oil solubilization). All analyses were performed in triplicate.

Chemical composition of baru oil analyzed by Gas chromatography–tandem mass spectrometry (GC–MS)

A baru oil solution (20% in hexane) was analyzed by a GCMS-Q2010 Plus system (Shimadzu®) with a RTX-5MS capillary column (5% diphenyl/95% dimethylpolysiloxane, 0.25 mm × 15 m, Restek®). An electron impact ionization of 70 eV. Helium (White Martins® 6.0) was used as a carrier gas at a flow rate of 0.66 ml/min. Injections (3.0 μl) were performed by an automatic injector (AOC-5000, Shimadzu®).

Two different methods, based on previous studies (Kadioglu et al., 2009; Lin et al., 2012), were applied to identify the compounds. A: Injection, interface and detector temperatures of 220 °C, 270 °C and 270 °C, respectively, and column oven temperature starting at 40 °C (holding for 1 min), rising to 220 °C at 10 °C/min (held for 30 min). B: Injection, interface and detector temperatures of 300 °C and column oven temperature starting at 150 °C (holding for 1 min), rising to 310 °C at 10 °C/min (held for 30 min).

Equipment control, as well as peak identification and area integration, was performed by GCMS solution software.

Compounds were identified by mass spectra analysis (35–500 *m*/*z* range) using the NIST 05 library and GCMS solution software to calculate the similarity index. Similarity indexes between 90 and 100% were considered acceptable (Torane et al., 2011). Relative area (%) of each identified compound was calculated by the ratio between the compound peak area and the sum of all peak areas of identified compounds.

Statistical analysis

Comparative data analysis was performed by a *t*-test using a significance level of 0.05. The *p*-values were calculated using Microsoft Office Excel 2007 software.

Results and discussion

Oil obtainment

The extraction yields calculated for hydraulic and continuous screw pressing were 7.99 and 25.0%, respectively. The lipid content found in baru seeds was 36.01 ± 1.40% (similar to earlier published data of 38.2 ± 0.4% (Takemoto et al., 2001)) resulting in extraction efficiencies of 22.19 and 69.43% for hydraulic and continuous screw pressing, respectively. Maciel Júnior (2010) obtained an extraction efficiency higher than 89% to baru oil seeds using continuous screw pressing. No study describing the hydraulic pressing of baru seeds has been found yet.

Due to the higher extraction efficiency provided by continuous screw pressing, hydraulic pressing in oil seed industries has gradually been replaced over the years by screw pressing (Savoire et al., 2013), although hydraulic press is still used in the industrial production of olive oil which is obtained in the absence of high temperatures, which increases the commercial value of the product (Kemper, 2005). It should be noted that the continuous screw pressing has others advantages as simplicity of operation, quickly and easily adapt to various types of oil seeds (Silva, 2009). Moreover continuous pressing have a high energy consumption, which is dissipated in friction and can substantially increase the product temperature, which increases the risk of thermal degradation of heat-sensitive substances (Guedes, 2006).

Oil characterization and quality

Table 1 summarizes the physical chemical properties and quality characterization results of baru seed oil. No statistical difference was observed between iodine values, saponification values, refractive index and relative density of oils obtained by the two extraction methods ($p > 0.05$).

Acid and peroxide values have been established as quality characteristics by Codex Alimentarius (2011). For cold pressed and virgin oils, the maximum acceptable levels for acid and peroxide are 4.0 mg KOH/g and 15 meq/kg, respectively. The levels for oils obtained by both extraction methods were approximately ten

Table 1
Physical–chemical properties and quality characterization of baru seed oil obtained by hydraulic and continuous screw pressing.

Results	Hydraulic pressing	Continuous screw pressing
Iodine value (g/100 g)	89.88 ± 4.42[a]	89.44 ± 2.59[a]
Saponification value (mg KOH/g)	159.92 ± 3.00[a]	156.41 ± 1.32[a]
Refractive index	1.468 ± 0.000[a]	1.469 ± 0.000[a]
Relative density	0.917 ± 0.038[a]	0.917 ± 0.014[a]
Acid value (mg KOH/g)	0.41 ± 0.01[b]	0.30 ± 0.01[b]
Peroxide value (meq/kg)	1.61 ± 0.05[b]	1.36 ± 0.05[b]

[a] No statistically significant difference for this parameter ($p > 0.05$).
[b] Statistically significant difference for this parameter ($p \leq 0.05$).

Table 2
Similarity index and relative area of compounds identified in baru seed oil obtained by hydraulic and continuous screw pressing.

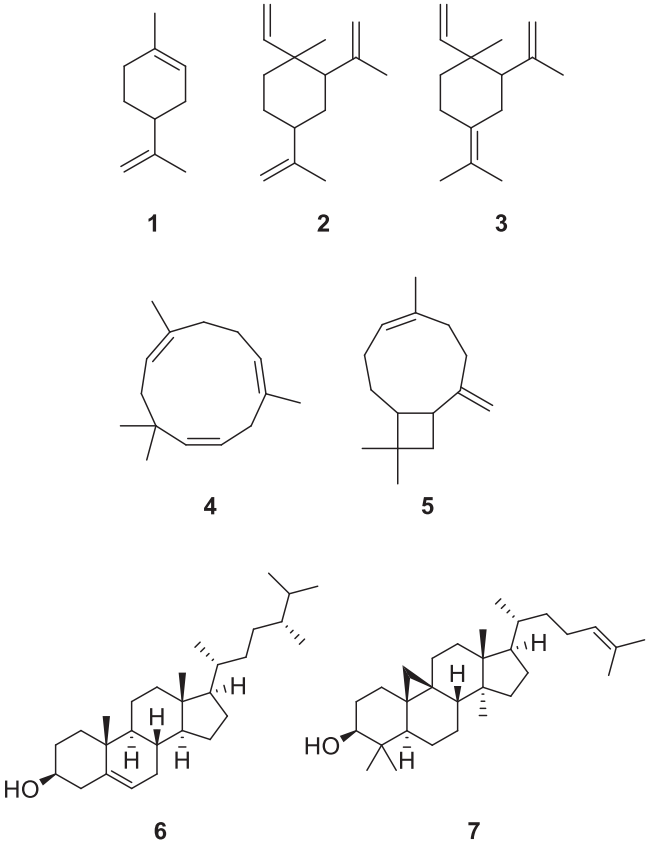
Compound	Similarity index	Relative area (%)	
		Hydraulic pressing	Continuous screw pressing
β-Sitosterol	89%	63.89	55.30
Stigmasterol	92%	14.21	17.74
α-Tocopherol	96%	7.43	7.72
Campesterol	92%	5.50	7.31
Cycloartenol	90%	4.61	6.43
γ-Tocopherol	91%	3.61	5.44
β-Caryophyllene	94%	0.25	ND[a]
γ-Elemene	90%	0.12	ND[a]
β-Elemene	95%	0.09	ND[a]
α-Caryophyllene	94%	0.08	ND[a]
Limonene	90%	0.03	ND[a]
Total		100%	100%

[a] Not detected.

times lower than the acceptance criteria (Table 1), which suggests considerable hydrolytic and oxidative stability for baru oil.

Chemical composition of baru oil by GC–MS

The analysis of baru seed oil by GC–MS resulted in the identification of eleven chemical constituents, nine of them have not been described yet for this oil, such as limonene (**1**), β-elemene (**2**), γ-elemene (**3**), α-caryophyllene (**4**), β-caryophyllene (**5**), campesterol (**6**), stigmasterol, β-sitosterol and cycloartenol (**7**).



The similarity index and relative area of these compounds in the two extraction methods (Table 2) shows that β-sitosterol presented a similarity index lower than 90% in the NIST 05 library and its identity was confirmed by comparing it with a commercial standard (purity ≥95%, Sigma), which resulted in a similarity index of 97%.

Of the compounds identified, there are mono and sesquiterpenes with well-known biological activities. Limonene (**1**) presents antioxidant activity (Yang et al., 2010) and exerts a protective effect on gastric mucosa (Moraes et al., 2009). β-Caryophyllene (**5**) is known for its anti-inflammatory (Awad and Fink, 2000; Sousa et al., 2008), antibiotic, antioxidant, and anti-carcinogenic activities (Adorjan and Buchbauer, 2010). Elemene has anti-tumor activity (Yang et al., 1996). Tocopherols, recognized antioxidants, had already been identified in baru oil in previous studies (Takemoto et al., 2001). In addition, phytosterols, including campesterol, stigmasterol, β-sitosterol and cycloartenol (**7**) found in baru oil, present antioxidant (Yoshida and Niki, 2003; Ju et al., 2004), hypocholesterolemic (Sposito et al., 2007; Bartnikowska, 2009), anti-carcinogenic (Awad and Fink, 2000; Moreau et al., 2002), anti-inflammatory (García et al., 1999; Hänninen and Sem, 2008) and estrogenic activities (Ju et al., 2004, Malini and Vanithakumari, 1993). β-Sitosterol, stigmasterol and campesterol are the most common phytosterols in vegetables (Moreau et al., 2002) and in herbal medicines (Ye et al., 2010).

It was observed that β-sitosterol was the major compound found in the baru oil obtained by both methods, while the essential oil fraction was found in considerably lower amounts, and was not detected in the baru oil obtained by continuous screw extraction. The temperature of the press increases in the crushing process of seeds reaching around 60 °C. This may be due the increase in oil temperature during continuous screw extraction, which can degrade essential oil compounds or facilitate their volatilization (Savoire et al., 2013).

The popular use of baru oil as a menstruation regulator and an anti-rheumatic agent (Sano et al., 2004) can be related with the estrogenic effect of phytosterols and the anti-inflammatory effects of caryophyllene and phytosterols. In addition, the association of phytosterols with high unsaturated fatty acid contents (81.2%, Takemoto et al., 2001) is an indicator that the oil could have a hypocholesterolemic effect (Gromadzka and Wardencki, 2011; Hänninen and Sem, 2008). This theory is supported by the fact that the consumption of baru nuts led to a cholesterol, triacylglyceride and lipid peroxidation reduction in rats (Fernandes et al., 2012). These findings suggest that baru oil could be used both as a functional food and for medicinal purposes.

Authors' contributions

FGM contributed to collecting the plant sample, carrying out laboratory work, conducting chromatographic analysis, data analysis and initial paper drafting. JRON contributed to chromatographic analysis. LCC contributed to the analysis and interpretation of the GC–MS data. JRP contributed to collecting and identifying the plants, and final approval of the article. MTFB contributed to the conception and design, supervision of laboratory work, provision of study materials, drafting of the paper and critical reading of the manuscript. All authors have read the final manuscript and approved its submission.

Conflicts of interest

The authors declare no conflicts of interest.

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