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# Baru (*Dipteryx alata* Vogel) Oil Extraction by Supercritical-$CO_2$: Improved Composition by Using Water as Cosolvent

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**Abstract: Baru (*Dipteryx alata*) almond is an emerging nut from the Brazilian savannah, that presents unique flavor and an interesting specialty oil. In this study, we aimed at investigating the effects of pressure, temperature, type (alcohol and/or water), and concentration of polar cosolvent on the extraction yield and tocopherol contents of baru oil obtained by supercritical-$CO_2$ extraction (SC-$CO_2$); and to investigate the effect of temperature and pressure on phytosterol, phenolic, and volatile compounds' profile in the oil when $H_2O$ was the cosolvent. Baru oil extracted with SC-$CO_2$ using alcohol as a cosolvent showed a higher extraction yield (20.5-31.1%) than when using $H_2O$ (4.16-22.7%). However, when 0.3% $H_2O$ was used as cosolvent, baru oils presented the highest γ-tocopherol (107 and 43.7 mg/100 g) and total tocopherol (212 and 48.7 mg/100 g) contents, depending on the temperature and pressure used (50°C and 10 MPa or 70°C and 30 MPa, respectively). Consequently, the lowest pressure (10 MPa) and temperature (50°C) values resulted in baru oils with better γ/α-ratio, and the highest contents of β-sitosterol (107 mg/100 g) and phenolic compounds (166 mg/100 g). However, the highest pressure (30 MPa) and temperature (70°C) values improved the volatile profile of oils. Therefore, although alcohol as a cosolvent improved oil yield, small amounts of $H_2O$ provided a value-added baru oil with either high content of bioactive compounds or with a distinctive volatile profile by tuning temperature and pressure used during SC-$CO_2$ extraction.**

**Key words:** γ-tocopherol, volatile compounds profile, β-sitosterol, polar cosolvent, baru almond

## 1 Introduction

Baru (*Dipteryx alata* Vogel.) is a large tree belonging to the *Leguminosae Faboideae* family from the Brazilian Savannah, the second largest biome in South America. The picking of baru almonds depends on and concomitantly contributes to the preservation of this unique biome. Therefore, adding value to this native plant is an interesting means to contribute to the sustainable use of natural resources in the Brazilian Savannah[1)]. Baru almonds have a pleasant flavor and high oil (40%) content, that is concentrated in oleic acid, tocopherols, phenolic acids, and β-sitosterol[2)], providing an oil with promising bioactive[3)] and sensorial properties[1)]. Published works on baru oil (BO) extraction and chemical profile provided valuable data, but there are persisting knowledge gaps concerning the chemical profile of minor components and how it may be affected by the methods used for oil extraction. Minor components in plant oils contain antioxidants that impact on oil stability and on consumers' health, such as phytosterols that may help control consumers cholesterol levels in blood, and volatile and partially volatile compounds that impact on oil flavor, among other minor components that impact on oil value. Pressing, either by expeller or hydrau-

***Abbreviations*: ANOVA**; analysis of variance, **ASFE**; automatic supercritical fluid extractor, **BO**; baru oil, **CAR**; carboxen, **DVB**; divinylbenzene, **FAME**; fatty acid methyl ester, **GC**; gas chromatography, **HPLC**; high performance liquid chromatography, **LRI**; linear retention index, **MS**; mass spectrometry, **NIST**; National Institute of Standards and Technology, **PDA**; photodiode array detector, **PDMS**; polydimethylsiloxane, **PVDF**; polyvinylidene difluoride, **SC-$CO_2$**; supercritical carbon dioxide, **SI**; similarity index, **SPME**; solid phase microextraction

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lic press, was used previously to extract BO[1, 4, 5], but in terms of chemical profile, only fatty acid composition was assessed in these three works, combined with indices of chemical quality and other quality evaluations, but minor components were not analysed. Takemoto *et al.*[6] and Garcia *et al.*[7] assessed the fatty acid profile in BO, and in the first[4] the profile of tocopherols in BO was also determined, but in these two works the oil was extracted by a technique not representative of industrial technologies as they used, hexane in a Soxhlet extractor[6, 7]. More recently, Marques *et al.*[8] obtained pressed BO and determined the profile of phytosterols and terpenes, but phenolic compounds and volatiles were not analysed[8]. Even more recently, supercritical-$CO_2$ (SC-$CO_2$) extraction technology was investigated to obtain BO[9–11], but in neither of these three studies the chemical profile of minor components was determined, except for tocopherols in Fetzer *et al.*[10].

Supercritical-$CO_2$ extraction is a green technology suitable for extracting value-added oils rich in bioactive compounds, especially lipid-soluble and heat-sensitive substances, with potential use in the food, health, and cosmetic industries[12]. Moreover, the extraction efficiency and selectivity power of SC-$CO_2$ can be modulated by the pre-treatment of seed particles, by changing extraction parameters such as pressure and temperature and/or using polar cosolvents, favoring extraction of moderately polar compounds[12]. Recently, BO extraction by ultrasound assisted SC-$CO_2$[9] or by cold pressing combined with SC-$CO_2$[11] led to higher oil yield than by SC-$CO_2$ alone, although fatty acid composition and phytochemical profiles were similar. However, the extraction of some bioactive compounds in BO such as tocopherols and total phenolic compounds was improved by using ethanol as cosolvent in SC-$CO_2$[10]. Although these recent works brought new data on BO extraction by SC fluid technology, knowledge on BO composition on minor chemical components still lacks, especially concerning the profiles of phenolics and of volatiles compounds by chromatographic techniques.

Ethanol is the most commonly used cosolvent in SC-$CO_2$ because it is generally recognized as safe (GRAS) and is produced from renewable sources[12]. But water might also be used as polar cosolvent, it is ubiquitous and innocuous being safe for use in food and pharmaceutical products[13–16] and it provides more effective extraction of polar components into the oil. Hence, it is of interest to investigate the use of $H_2O$ as cosolvent in SC-$CO_2$ for the enhanced extraction of polar and moderately polar components from baru almonds, such as tocopherols, phenolics, phytosterols and volatile compounds. The use of water as cosolvent in SC-$CO_2$ to obtain BO concentrated in polar bioactive compounds has not been previously investigated. Additionally, the volatile compounds profile in BO obtained by SC-$CO_2$ extraction with polar cosolvent is uninvestigated, and this matter is of technological interest aiming future use in food and cosmetics industries.

Therefore, the present study aimed to investigate the effects of SC-$CO_2$ extraction conditions, namely type and concentration of cosolvent (alcohol and/or water), temperature, and pressure on the extraction yield and tocopherols profile in BO using a fractional factorial design ($2^{4-1}$). Besides, the BO most concentrated in tocopherols, used herein as target compounds and as markers of oils' global quality, were assessed for their profile of fatty acids, phytosterols, phenolic and volatile compounds.

## 2 Materials and Methods

### 2.1 Materials

Commercial standards (purity > 95%) of tocopherols (α-, β-, γ- and δ-), fatty acid methyl ester mix (37-FAME mix, Supelco), phenolic compounds (gallic acid, 5-CQA, di-OH-phenylacetic acid, caffeic acid, 2,4-di-OH-benzoic acid, hydroxycinnamic acid, rosmarinic acid, and naringenin) and phytosterols (β-sitosterol, stigmasterol, and cholestanol) were acquired from Sigma-Aldrich Chemical Co. (São Paulo, Brazil). Anhydrous reagent alcohol (90% ethanol, 5% methanol, 5% 2-propanol; v/v) was used in SC-$CO_2$ extractions and referred to simply as alcohol throughout this work. All solvents were HPLC grade (Tedia®, Rio de Janeiro, Brazil). The high purity $CO_2$ (purity 99.99%) used for supercritical extraction was purchased from AltaTec LTDA (Rio de Janeiro, Brazil). Ultrapure water (Milli-Q system, Millipore, Bedford, MA, USA) was used throughout the experiments.

### 2.2 Raw material

Baru almonds were produced in the year of 2015 in the municipality of Alto Paraíso de Goiás located in the Chapada dos Veadeiros, state of Goiás (Brazil), by a registered cooperative of producers, engaged in sustainable baru nut collection and preservation of the Brazilian savannah. In 2015 and 2016, the El Niño-Southern Oscillation (ENSO) phenomenon strongly influenced the weather conditions in the Midwest region that encompasses the savannah biome in Brazil[17], including the area where the baru almonds were collected. From April/2015 to March/2016, the surface temperature was anomalous from +0.78 to +2.95℃ (respectively, in April and November/2015). A mild to severe draught was recorded from June to December/2015[18]. The almonds were purchased in December 2015 in vacuum-packed aluminum foiled bags (three 1 kg bags) from the cooperative website and were stored at −20℃ until further processing. In the laboratory, almonds were ground, homogenized with distilled water (1:1, w/v) in an industrial blender (Tron®, São Paulo, Brazil) into a slurry, and freeze-dried (FreeZone 2.5 Liter; Labconco®, Kansas City, MO, USA) for 48 hours. The dried powder (2.30 ± 0.34

g/100 g moisture) was finely ground (MF 10 Basic analytical mill, 130 IKA®, São Paulo, Brazil), sieved (< 0.85 mm), and stored under vacuum in plastic bags at −20℃ until oil extraction.

### 2.3 Baru oil extraction by SC-$CO_2$

Oil extraction by SC-$CO_2$ was performed in an automatic supercritical fluid extractor system (MV-10 ASFE; Waters®, Massachusetts, USA) equipped with a high-pressure pump for $CO_2$, a chiller, a cosolvent pump, an oven, a back-pressure regulator, a temperature controller, and a heat exchanger. Extraction pressure, temperature, the flow of $CO_2$, and cosolvent were controlled by ChromScope v1.20 Software (Waters®).

Dried baru almond powder (18 ± 2 g) was loaded into 25 $cm^3$ extraction vessels by slowly adding the powder and concomitantly shaking and hitting the vessels on a hard surface to allow accommodation of the sample bed until it was firmly compacted, sealed and placed in the oven module. The pressure was adjusted to keep the system in static extraction for 5 minutes, followed by opening the valve to initiate the dynamic extraction step for 4 hours with $CO_2$ mass flow kept constant at 3 mL/min (time and flow necessary to exhaust BO extraction based on preliminary experiments). The operation conditions of the cosolvent mass flow rate (alcohol and/or water), pressure, and temperature were programed according to the experimental design (**Table 1**). The extracted oil was collected in 100 mL glass flasks and the water and alcohol used as cosolvents were removed by evaporation under a gentle nitrogen stream at 30℃ until constant weight. The oils were sealed under $N_2$, and stored at −20℃ until analysis.

### 2.4 Fractional factorial design: baru oil extraction by SC-$CO_2$

A fractional factorial design ($2^{4-1}$) was used to evaluate the effect of type and concentration of cosolvent, pressure, and temperature (at two levels) on extraction yield, individual (α- and γ-) and total (α, β, γ, and δ) tocopherol contents (**Table 1**). "Low (−)" and "High (+)" values of temperature and pressure were chosen based on Fetzer *et al.*[10], while the concentrations of the cosolvents were chosen based on Da Porto *et al.*[19], Silva *et al.*[20] and by preliminary tests. Extractions of eight combinations of all factors plus a center point (in triplicate) were carried out in random order. To avoid aliasing between the effects of lower hierarchies, we evaluated the significance of all main effects before ascertaining synergistic effects (**Table S1**). The experimental design was run in Statistica® 7.0 software (Statsoft Inc., Tulsa, OK, USA). The extraction yield (g of oil/100 g of baru seed) was calculated (Equation 1), and tocopherol composition (mg/100 g) was determined by HLPC as described in item *2.6.3*.

$$\textit{Extraction Yield}\,(g/100\ g) = \textit{mass of extracted oil}\,(g) \times 100 / \textit{mass of dried baru seed}\,(g) \quad \text{(Equation 1)}$$

Furthermore, BO extraction by pure SC-$CO_2$ and mechanical expeller (*item 2.5*) were performed aiming for comparisons with the responses of the factorial design (**Table 1**).

### 2.5 Baru oil extraction by continuous expeller press

Baru oil was extracted in a continuous screw expeller (Oekotec, CA59G, Germany) at 25 ± 2℃ in two batches that were combined. Crude oil was centrifuged at 5000 *g* × 15 min (Sorvall ST 16R, Thermo Scientific™, Osterode, Germany), filtered through a paper filter (Whatman N° 1), and stored at −20℃ until analysis[21].

### 2.6 Determination of quality indices, fatty acids, bioactive and volatile compounds profile in selected baru oils

The SC-$CO_2$ extraction conditions that provided the BO most concentrated in total tocopherols were chosen to follow through with the determination of oil quality indices, fatty acid, and bioactive compounds (phytosterols and phenolic compounds), and volatile compounds profiles to disclose BO quality beyond tocopherols. Total tocopherol content was preferred over extraction yield as a selection criterion due to its nutritional and technological importance as antioxidants in vegetable oils[22].

#### 2.6.1 Oil quality indices

Oil quality was determined by peroxide value determined by iodometric titration (method Cd 8-53), acid value by potentiometric titration in an automatic titrator (Mettler Toledo, Greifensee, ZH, Switzerland) (method Cd 3d-63), and refractive index was determined in a portable digital refractometer (N-3E, ATAGO, Tokyo, Japan) as recommended by Codex Alimentarius Commission (method Cc 7-25)[23, 24].

#### 2.6.2 Fatty acid composition by GC-FID

Fatty acid methyl esters (FAMEs) were obtained by direct transesterification of total lipids[25] and analyzed in a GC-2010 system (Shimadzu®, Japan), equipped with split/splitless injector (operated with a split ratio of 1:200) and a flame ionization detector (FID). Samples and the standard mix in *n*-hexane were injected into an Omegawax-100 column (15 m × 0.1 mm i.d × 0.1 μm film thickness, Supelco CO., Belefonte, USA®) and analyzed as previously described[26]. Fatty acids peaks' identities in samples chromatograms were assigned by comparison with the relative retention times of those of commercial standards (37-FAME mix). Fatty acids contents were determined by internal normalization after correction of the peak areas by their respective theoretical correction factors of Ackman[27]. Results were expressed as g/100 g of total fatty acids.

#### 2.6.3 Tocopherol composition by HPLC-RF/PDA

The concentrations of α-, β-, γ- and δ-tocopherols were

**Table 1** Fractional factorial experimental matrix and response values for extraction yield (g/ 100 g) and tocopherol (T) contents (mg/100 g) of baru almond (*Dipteryx alata* Vog.) oil extracted by SC-$CO_2$ with cosolvents and by SC-$CO_2$ without cosolvents and mechanical expeller pressing as reference.

| Experimental Run[a] | Factors' levels (respectively) | | | | Independent factors (SC-$CO_2$ extraction conditions) | | | | Dependent variables (Responses)[b] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Temperature (℃) | Pressure (MPa) | Polar cosolvent | Cosolvent content (%) | Extraction yield (g/100 g) | α-T (mg/100 g) | γ-T (mg/100 g) | Total tocopherols (mg/100 g) |
| 1 | −1 | −1 | −1 | −1 | 50 | 10 | $H_2O$ | 0.3 | 4.16 | 102.79 ± 5.03 | 107.24 ± 5.96 | 212.07 ± 11.00 |
| 2 | +1 | −1 | −1 | +1 | 70 | 10 | $H_2O$ | 10 | 4.23 | 3.88 ± 0.03 | 15.47 ± 0.21 | 19.51 ± 0.16 |
| 3 | −1 | +1 | −1 | +1 | 50 | 30 | $H_2O$ | 10 | 18.49 | 0.44 ± 0.03 | 17.86 ± 0.91 | 19.25 ± 0.78 |
| 4 | +1 | +1 | −1 | −1 | 70 | 30 | $H_2O$ | 0.3 | 22.70 | 4.21 ± 0.02 | 43.69 ± 4.06 | 48.73 ± 4.42 |
| 5 | −1 | −1 | +1 | +1 | 50 | 10 | Alcohol | 10 | 11.69 | 3.17 ± 0.32 | 13.46 ± 1.14 | 17.54 ± 1.42 |
| 6 | +1 | −1 | +1 | −1 | 70 | 10 | Alcohol | 0.3 | 10.09 | 0.65 ± 0.04 | 29.64 ± 2.58 | 30.98 ± 2.52 |
| 7 | −1 | +1 | +1 | −1 | 50 | 30 | Alcohol | 0.3 | 31.06 | 1.29 ± 0.10 | 13.60 ± 0.23 | 15.11 ± 0.35 |
| 8 | +1 | +1 | +1 | +1 | 70 | 30 | Alcohol | 10 | 29.68 | 0.23 ± 0.03 | 2.49 ± 0.48 | 3.38 ± 0.57 |
| 9 | 0 | 0 | 0 | 0 | 60 | 20 | Alcohol:$H_2O$[d] | 5 | 16.15 | 3.49 ± 0.47 | 15.34 ± 0.65 | 18.92 ± 1.12 |
| 10 | 0 | 0 | 0 | 0 | 60 | 20 | Alcohol:$H_2O$ | 5 | 21.67 | 3.69 ± 0.03 | 16.77 ± 0.30 | 20.59 ± 0.32 |
| 11 | 0 | 0 | 0 | 0 | 60 | 20 | Alcohol:$H_2O$ | 5 | 20.47 | 4.62 ± 0.37 | 15.76 ± 0.90 | 20.47 ± 1.30 |
| Reference techniques[c] | | | | | Expeller pressing baru oil | | | | 4.61 | 4.26 ± 0.20 | 8.56 ± 0.53 | 13.00 ± 0.70 |
| | | | | | SC-$CO_2$ (50℃; 10 MPa) | | | | 4.03 | 18.13 ± 0.66 | 55.99 ± 1.42 | 74.72 ± 0.78 |
| | | | | | SC-$CO_2$ (50℃; 30 MPa) | | | | 36.93 | 6.59 ± 0.04 | 16.00 ± 0.04 | 23.41 ± 0.31 |
| | | | | | SC-$CO_2$ (70℃; 10 MPa) | | | | 2.72 | 2.20 ± 0.04 | 12.87 ± 0.13 | 15.19 ± 0.08 |
| | | | | | SC-$CO_2$ (70℃; 30 MPa) | | | | 29.85 | 5.52 ± 0.20 | 15.62 ± 0.26 | 21.91 ± 0.67 |

[a] All experiments were run in random order. [b] Mean ± standard deviation values of tocopherols analytical experiments performed in triplicate. [c] These extractions were conducted and are used herein as references for comparisons with the experimental runs from the fractional experimental design. [d] Alcohol:$H_2O$ = 50:50, v/v. Standard deviation of extraction yield was 0.93 by fractional factorial design. The statistical significance was based on the total error criteria with a confidence level of 95%. Average coefficient of variation among experimental triplicates was lower than 10%.

determined on an HLPC system, equipped with a quaternary pump (LC-20AT), system controller (CBM-20A), degasser (DGU-20A5), and a fluorescence (RF-10AXL) and a photodiode array (PDA; SPD-10A) detectors (Shimadzu®, Japan) after separation in a normal phase unmodified silica column (ZORBAX® Rx-Sil, 5 μm, 4.6 mm × 250 mm; Agilent Technologies®, CA, USA) using a binary isocratic mobile phase of *n*-hexane:2-isopropanol (99:1, v/v) at 1.0 mL/min flow rate as previously described[28]. Tocopherols were monitored between 390 and 700 nm by the PDA detector, and at 290 nm (excitation) and 330 nm (emission) by the RF detector. Peak identity in samples was assigned by comparing retention time and peak PDA-spectra with those of commercial standards of α-, β-, γ- and δ-tocopherols. Contents in samples were determined by external calibration (concentration range from 0.5 to 3.0 μg/mL; $R^2 > 0.9946$; $p < 0.01$; $LOD_{max} = 0.48$ μg/mL; $LOQ_{max} = 1.60$ μg/mL). Results were expressed as mg/100 g oil.

2.6.4 Phytosterol composition by HPLC-PDA

Phytosterols were extracted from oils by cold saponification with a 0.5 N KOH solution and the unsaponifiable fraction was separated using liquid-liquid partitioning in 10 mL of distilled water with 10 mL of *n*-hexane. The upper phase was carefully removed, evaporated, and reconstituted with 1.5 mL of acetonitrile:isopropanol (98:2, v/v), and the extract filtered through a polyvinylidene difluoride (PVDF) membrane (0.45 μm). Sample extracts and phytosterols' commercial standards were analyzed in the same HPLC system described in *2.6.3*, equipped with a reversed-phase C18 column with di-isobutyl side chains (5 μm, 50 mm × 2.1 mm i.d, Phenomenex®, Torrance, CA, USA) eluted with an isocratic mobile phase (acetonitrile:isopropanol, 98:2, v/v) at 0.4 mL/min flow rate[29]. Phytosterol peaks were monitored at 210 nm and peak identity was assigned by comparing retention times and UV-spectra with those of commercial standards of stigmasterol and β-sitosterol. Contents were determined by external calibration (concentration range from 0.05 to 1.5 mg/mL; $R^2 > 0.9974$; $p < 0.0001$; $LOD_{max} = 0.02$ mg/mL; $LOQ_{max} = 0.07$ mg/mL, for both standards).

2.6.5 Phenolic composition by HPLC-PDA

Phenolic acid compounds were extracted from the BO by solid phase extraction (SPE) using diol cartridges (Bond Elut 2-OH, 3 mL, 500 mg; Agilent Technologies; Santa Clara, CA, USA)[30]. The final extracts and phenolic compounds standard solutions were analyzed by the same HPLC system described in *2.6.3* using a reversed-phase C18 column (5 μm, 4.6 μm × 150 mm, Kromasil® Bohus, Ale, Sweden) and the mobile phase consisted of a 0.3% aqueous formic acid, methanol and acetonitrile[31]. Phenolic compounds were monitored by the PDA detector from 190 to 370 nm and were identified by comparing their retention times and UV-spectra with those of commercial standards of phenolic compounds, and by exact peak co-elution (sample spiking) with these same standards. Quantitative analysis was based on external calibration (concentration range from 1.0 to 20.0 μg/mL which were linear for all phenolic compounds' standards; $R^2 > 0.9909$; $p < 0.0001$; $LOD_{max} = 0.19$ μg/mL; $LOQ_{max} = 1.29$ μg/mL).

2.6.6 Volatile and semi-volatile compounds profile by SPME-GC-MS

The volatile compounds were extracted by solid phase microextraction (SPME) using a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco®, PA, USA) and analyzed by GC-MS). Analysis was performed by GC-MS (GC-17A coupled to a QP5050A quadrupole mass spectrometer; Shimadzu®, Japan) equipped with a split/splitless injector and a fused silica column of 5% phenyl/95% methylpolysiloxane (30 m × 0.32 mm i.d., 3 μm film thickness, 007-5, Quadrex®, Bethany, CT, USA). A mixture of $C_7$-$C_{30}$ hydrocarbons was analyzed in parallel under the same conditions to allow calculation of the linear retention index (LRI) values for the volatile compounds[32]. Peaks identities were tentatively assigned by comparing mass spectra with those of the National Institute of Standards (NIST) library and calculating similarity indices by the instrument software (Lab Solutions GC-MS; Shimadzu Co., Kyoto, Japan), as well as by comparing LRI values with published data. Aroma notes were taken from previous works (Table 4).

### 2.7 Statistical analysis

Results were expressed as mean ± standard deviation of triplicates. One-way ANOVA followed by Tukey's post-test was used to compare mean values, and *p*-values < 0.05 were considered significant. Statistical analyzes were performed using Prism software version 7 (GraphPad® Inc., San Diego, CA, USA).

## 3 Results and Discussion

### 3.1 Baru oil extraction by SC-$CO_2$: Tuning extraction yield and tocopherols contents by a fractional factorial design

Baru oils extracted by SC-$CO_2$ with polar modifiers showed extraction yields ranging from 4.16 g/100 g (run *1*) to 31.06 g/100 g (run *7*) and were similar to those observed by Fetzer *et al.*[10] BO extraction yields by pure SC-$CO_2$ ranged from 4.03 to 36.93 g/100 g, and by mechanical expeller pressing was 4.61 g/100 g. Therefore, in most cases, SC-$CO_2$ (with or without cosolvents) provided higher extraction yields than mechanical pressing, especially under the highest pressure (30 MPa). Furthermore, combining alcohol to SC-$CO_2$ was more critical to enhancing BO extraction yield at the lowest pressure value (10 MPa) than at the highest value (30 MPa) (Table 1).

The fractional factorial design showed that the main

effects (first-order effects) of pressure and type of cosolvent (alcohol) were significant to extraction yield (Fig. 1A and Fig. S1A), with runs *7, 8* and *4*, showing the highest oil yields (Table 1). Pressure and type of cosolvent contributed significantly with 56.1% and 25.7% to the model variation (adjusted $R^2 = 0.88$), respectively, with no interaction (second-order effect) between these variables (Table S2). High pressure was a major determinant of oil yield, and alcohol had a secondary impact, increasing yield, independently of the concentration used (Fig. 1A).

The solvation power of SC-$CO_2$ is directly associated with pressure. Pressure increases supercritical fluid density, favoring mass transfer and solutes extraction[33]. Therefore, high-pressure values can increase extraction yield, but in some food matrices can concomitantly decrease selectivity, especially in the case of non-polar compounds[34]. Similarly, the addition of polar cosolvents can change the extraction of solutes in SC-$CO_2$ by increasing solubility, selectively or non-selectively, by altering fluid density and by polar interactions between the cosolvent and solutes, respectively[35].

Ethanol is freely miscible in SC-$CO_2$ under the conditions used, thereby increasing SC-$CO_2$ density and non-selectively improving solubility[36], favoring extraction beyond triacylglycerols and increasing oil yield[10]. Conversely, $H_2O$ tends to be immiscible with SC-$CO_2$ resulting in phase separation, especially when its concentration increases and temperature and pressure decrease[36]. Herein, extractions using SC-$CO_2$ plus $H_2O$ probably promoted a two-phase system, composed of supercritical fluid and liquid[37, 38]. Liquid water may facilitate the recovery of polar compounds by forming dipole-dipole interactions and H-bonding. Consequently, extraction selectivity is improved but not necessarily BO yield[36, 37].

γ-Tocopherol and α-tocopherol were the predominant tocols in BO, contributing 50% to 95% and 2% to 48%, respectively, to the total tocopherol contents, depending on the SC-$CO_2$ extraction conditions used (Table 1). Only small amounts (0.4% to 2%) of δ-tocopherol were detected in oils (data not shown). BO extracted by pure SC-$CO_2$ showed α- and γ-tocopherol contents that were 1.2 to 6.5 times as high as those in BO extracted by mechanical pressing, depending on the temperature and pressure used for supercritical fluid extraction. However, using alcohol as cosolvent impaired α-tocopherol extraction (especially at higher pressure), whereas $H_2O$ improved extraction of both tocols, but more so the gamma (Table 1). Possibly the use of lower concentration of $H_2O$, which is in the liquid state, increased the moisture of the baru almond and thus allowed for greater extraction of tocopherols by the system formed by the two phases ($CO_2$ and $H_2O$), moreover making the extraction solvent more polar and increasing the extraction of tocopherols that are moderately polar. In addition to parameters such solubility of bioactive compounds

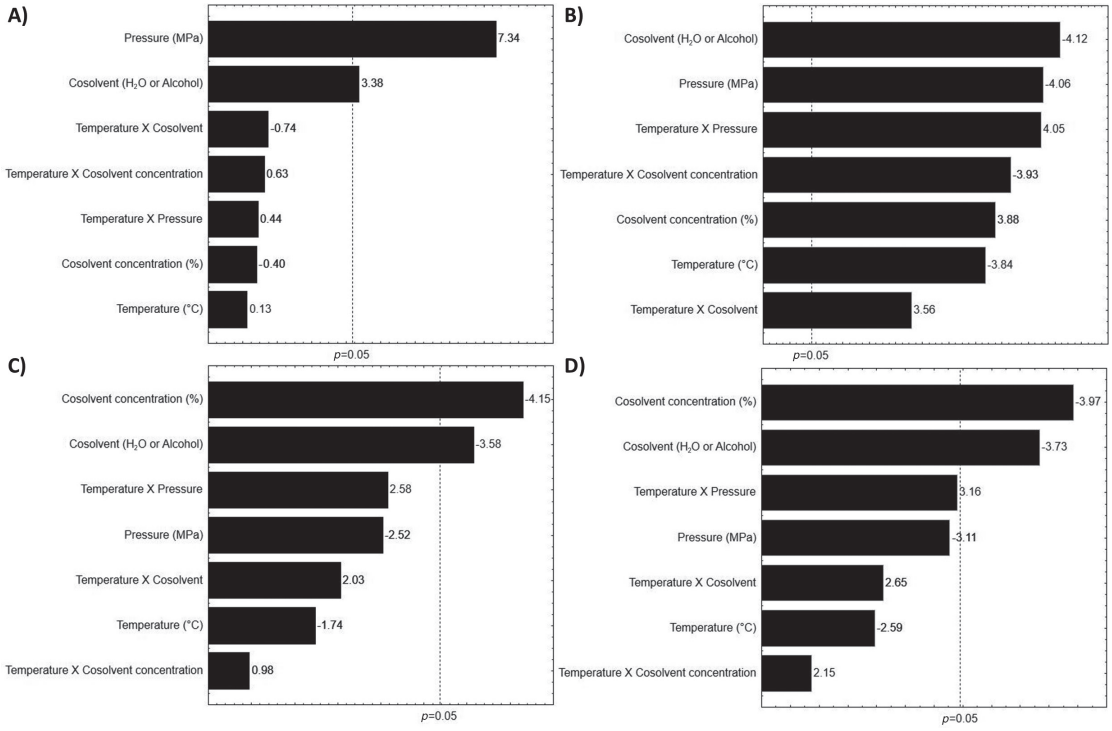


Fig. 1 Pareto charts of experimental design results, showing the extraction yield (g/ 100 g) (A); α-tocopherol content (mg/100 g oil) (B); γ-tocopherol content (mg/100 g oil) (C), and total tocopherols content (mg/ 100 g oil) (D), in baru almond (*Dipteryx alata* Vog.) oil obtained by SC-$CO_2$ extraction with polar cosolvents (alcohol; water; alcohol:water).

in the system formed by $CO_2$ and cosolvents, temperature and pressure, and particle size, moisture can also interfere in the extraction of bioactive compounds, such as tocopherols[39]. A similar behaviour was also observed by Silva *et al.*[20], who found that pomegranate seed oil extracted by SC-$CO_2$ was enriched in tocopherols when the cosolvent was composed of 90% water and 10% alcohol, compared to using pure alcohol.

All variables and interactions were statistically significant as determinants for α-tocopherol extractability (adjusted $R^2 = 0.91$), and their contribution was remarkably similar between experimental runs. Adding $H_2O$ to SC-$CO_2$ or reducing pressure improved vitamin E extraction. However, simultaneously increasing temperature and pressure changed the effect of pressure on α-tocopherol extraction (Fig. 1B).

Conversely, only the main effects of the type and concentration of cosolvent were determinants to γ-tocopherol (adjusted $R^2 = 0.82$) and total tocopherol content (adjusted $R^2 = 0.86$) (Figs. 1C, 1D and Table S2), suggesting a facilitated extraction of this tocol by SC-$CO_2$. Hence, adding small amounts of water as cosolvent enriched the oil in γ- and in total tocopherol, independently of the pressure and temperature used (Table 1).

Considering the use of a mixture of a 5% alcohol:water (50:50, v/v) as cosolvent to extract BO (runs *9*, *10* and *11*), contents of γ-tocopherol and total tocopherol were similar to the experiments using alcohol as a cosolvent at low temperatures (runs *5* and *7*). Conversely, in the experiments using small amounts of water as cosolvent higher contents of γ-tocopherol and total tocopherol were observed (runs *1* and *4*), showing that adding alcohol in the cosolvent mixture may have hampered the selectivity of liquid water towards the polar components in baru.

The solubility of tocopherols in SC-$CO_2$ is a matter of debate[37, 40]. Del Valle *et al.*[40] reported that the solubility of α-tocopherol in pure SC-$CO_2$ increased proportionally to pressure and temperature[40]. However, Antonie and Pereira[33] reported that α-tocopherol shows higher solubility in SC-$CO_2$ at low-pressure values (<28 MPa). In parallel, previous reports show that γ-tocopherol extraction can be easier than that of α-tocopherol, possibly due to its lower molecular weight that increases solubility[33, 41].

Therefore, even though using alcohol as a cosolvent contributed to the highest oil yield, $H_2O$ seems to have led to higher extraction selectivity. A major motivation to extract specialty oils by supercritical fluid techniques is to recover oils with valuable bioactive composition. Tocopherols are valuable components of edible oils due to their ability to protect biological (α-tocopherol) or food (γ-tocopherol) systems against lipid oxidation[22], which make them target compounds in the present study, providing a preliminary assessment of limiting conditions to SC-$CO_2$ extraction parameters. Therefore, conditions that extracted BO richer in α- and γ-tocopherols and total tocopherols (runs *1* and *4*) were selected for further characterization.

Notably, a fractional factorial design is a first-order model that was used as a screening model to give an overview on the phenomena under investigation, aiming at identifying the most relevant factors affecting the response variables[42]. Therefore, it would be of interest if future studies optimized BO extraction using pressure (10 to 30 MPa) and temperature (40 to 80℃) as factors and 0.3% $H_2O$ as a cosolvent in SC-$CO_2$.

### 3.2 Chemical profile of selected baru oil extracted by SC-$CO_2$+$H_2O$: Effect of pressure and temperature

Profiles of fatty acids, phytosterols, phenolic and volatile compounds, besides quality indices, were determined in BO extracted by SC-$CO_2$ in runs *1* and *4* (Table 1), using 0.3% $H_2O$ as cosolvent. In run *1* the lowest pressure (10 MPa) and temperature (50℃) values were applied and in run *4*, the highest ones (30 MPa and 70℃), allowing assessment of the effect of these parameters on SC-$CO_2$ extraction at a constant water concentration.

### 3.3 Baru oil quality indices

Peroxide value, acid value, and refractive index are com-

Table 2 Quality indices of fresh baru almond (*Dipteryx alata* Vog.) oils extracted by SC-$CO_2$ + 0.3% $H_2O$ (selected conditions; runs *1* and *4*).

| Quality Indices | Extraction by SC-$CO_2$ + 0.3% $H_2O$ | |
|---|---|---|
| | Experimental run *1* (50℃; 10 MPa) | Experimental run *4* (70℃; 30 MPa) |
| Peroxide value (mEq $O_2$/kg oil) | 0.45 ± 0.00b | 3.32 ± 0.20a |
| Acid value (mg KOH/g oil) | 0.47 ± 0.01a | 0.68 ± 0.03a |
| Refractive index | 1.48 ± 0.00a | 1.47 ± 0.00a |

Results are expressed as mean ± standard deviation of triplicate. Different letters on the same line indicate significant differences ($p < 0.05$; unpaired Student's *t*-test).

monly used in fresh oils to assess oxidative and hydrolytic transformations that can take place during extraction. Peroxide value in BO from run *1* was lower than in the oil from run *4*, probably because of the higher temperature used in the last experiment that might have accelerated oxidation. In contrast, acid value and the refractive index did not vary significantly between the oils (Table 2). Currently, there is no quality control recommendation for oils extracted via SC-$CO_2$ technologies. However, because in this case mild temperatures are used and a refining process is absent, it is fair to consider the limits adopted for cold-pressed oils, which must be below 15.0 mEq $O_2$/kg (peroxide value) and 4.0 mg KOH/g (acid value)[23].

### 3.4 Baru oil fatty acid composition

Fatty acid profile in BO extracted via SC-$CO_2$ + 0.3% $H_2O$ was slightly affected by pressure and temperature (Table 3 and Fig. S2). The content of behenic (22:0), eicosenoic (20:1*n*-9) and oleic (18:1*n*-9) acids was significantly affected by extraction conditions. However, these differences were only slight so that the profile in saturated, monounsaturated and polyunsaturated fatty acids was not affected. Monounsaturated fatty acids were the major ones with over 50 g/100 g, followed by polyunsaturated and saturated fatty acids. Oleic (18:1*n*-9) and linoleic (18:2*n*-6) acids were the most prevalent, agreeing with previous reports[10, 43]. The fatty acid composition varied slightly between runs *1* and *4* and between them and expeller-pressed BO, suggesting that it was mostly unaffected by the pressure and temperature used in SC-$CO_2$ + 0.3% $H_2O$ extractions. BO is a rich source of 18:1*n*-9, likewise peanut (35-69 g/100 g fatty acids), canola (61.9 g/100 g fatty acids), and olive (55-83 g/100 g fatty acids) oils[23]. Therefore, the positive health effects attributed to the intake of 18:1*n*-9 rich oils might be expected from the intake of BO[2].

### 3.5 Tocopherol, phytosterol and phenolic composition

As shown in *3.1* above, BO extracted in runs *1* and *4* showed the highest contents of tocopherols, especially γ-tocopherol (Table 1 and Fig. S3). The lowest pressure and temperature clearly improved the extractability of α-tocopherol (Fig. 2A). The γ-to-α-tocopherol ratio was 10-fold lower in BO obtained in run *1* (1.04) than in run *4* (10.4), pointing to a more balanced extraction of both tocols under the experimental conditions in the first extraction.

Similarly, run *1* was most effective for extracting phytosterols, being 4-fold and 2-fold more effective than run *4* and expeller-pressed BO, respectively (Fig. 2B and Fig.

**Table 3** Fatty acid composition (g/100 g of total fatty acids) of baru almond (*Dipteryx alata* Vog.) oil extracted by SC-$CO_2$ + $H_2O$ (runs *1* and *4*; selected conditions by experimental design) and by mechanical pressing, used as a reference method.

| Fatty acid | Extraction by SC-$CO_2$ + 0.3% $H_2O$ | | Reference technique |
|---|---|---|---|
| | Experimental run *1* (50℃; 10 MPa) | Experimental run *4* (70℃; 30 MPa) | Expeller pressing |
| 16:0 | 6.45 ± 0.05a | 6.64 ± 0.00a | 6.17 ± 0.17a |
| 18:0 | 1.62 ± 0.18a | 1.83 ± 0.55a | 2.65 ± 0.69a |
| 20:0 | 1.20 ± 0.01a | 1.18 ± 0.02a | 1.30 ± 0.12a |
| 22:0 | 3.70 ± 0.02a | 3.30 ± 0.07b | 3.83 ± 0.02a |
| 24:0 | 4.30 ± 0.08a | 3.66 ± 0.03a | 4.27 ± 0.32a |
| 20:1*n*-9 | 2.66 ± 0.03b | 2.53 ± 0.02c | 2.81 ± 0.02a |
| 22:1*n*-9 | 0.30 ± 0.03a | 0.27 ± 0.02a | 0.25 ± 0.06a |
| 18:1*n*-9 | 50.76 ± 0.05a | 51.50 ± 0.68b | 50.75 ± 1.10a |
| 18:2*n*-6 | 28.87 ± 0.17a | 28.94 ± 0.10a | 27.84 ± 0.24b |
| 18:3*n*-3 | 0.14 ± 0.01a | 0.15 ± 0.02a | 0.13 ± 0.01a |
| *SFA*[a] | 17.27 ± 0.17a | 16.61 ± 0.63a | 18.22 ± 1.31a |
| *MUFA*[b] | 53.72 ± 0.01a | 54.30 ± 0.72a | 53.81 ± 1.06a |
| *PUFA*[c] | 29.01 ± 0.18a | 29.09 ± 0.09a | 27.97 ± 0.26b |

Results are expressed as mean ± standard deviation of triplicates. [a] SFA, saturated fatty acids; [b] MUFA, monounsaturated fatty acids; [c] PUFA, polyunsaturated fatty acids. Different letters on the same line indicate significant differences among samples ($p < 0.05$; ANOVA with Tukey's post-test).

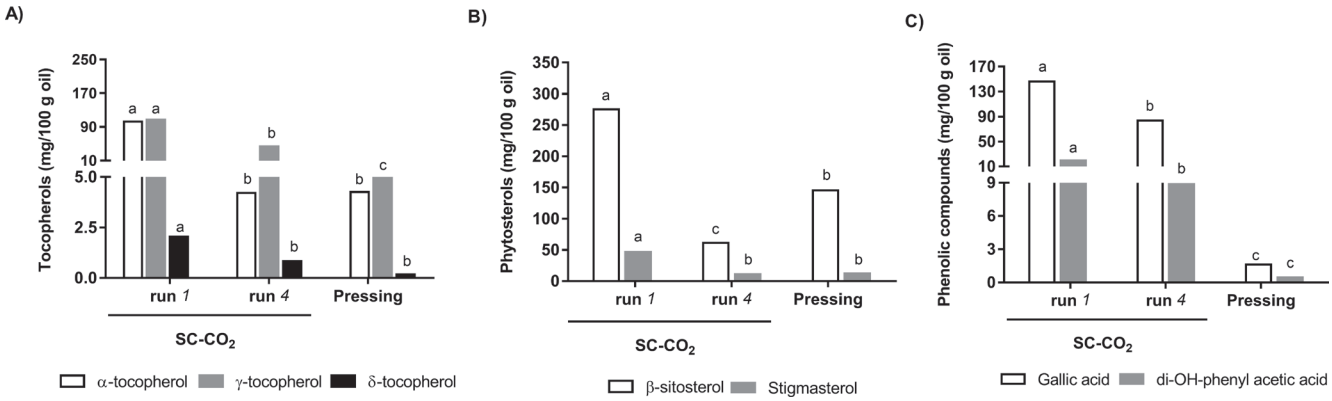


Fig. 2 Minor components in baru almond (*Dipteryx alata* Vog.) oil obtained by SC-$CO_2$ + 0.3% $H_2O$ (selected conditions; runs *1* and *4*); and expeller pressing. (A) Tocopherol contents (mg/100 g); (B) Phytosterol contents (mg/100 g); (C) Phenolic acid compounds (mg/100 g). Different letters in bars indicate significant differences between extraction conditions (one-way ANOVA with Tukey's post-test, $p < 0.05$).

S4). β-Sitosterol and stigmasterol were the major sterols in BO, and the first was the most concentrated one independently of the extraction conditions used. A similar sterol profile was previously reported in expeller-pressed BO[8]. Our data indicated that SC-$CO_2$ extraction might increase phytosterols' content in BO, which is an added value to this oil as plant sterols inhibit dietary cholesterol absorption.

The two principal phenolic acids extracted by SC-$CO_2$ + 0.3% $H_2O$, independently of the temperature and pressure used, were gallic acid and 3,4-di-hydroxyphenylacetic acid (Fig. S5). Both phenolic acids were more concentrated in run *1* compared to run *4* (2-fold) and to expeller-pressed BO (75-fold) (Fig. 2C). Our data confirmed the apparent suppressive effect of pressure and temperature for the extraction of phenolic compounds by SC-$CO_2$[10].

Taken together, our results showed that by using low values of pressure and temperature for SC-$CO_2$ + 0.3% $H_2O$ extraction, the composition of tocopherol, phytosterol, and phenolic compounds in BO was sensibly improved (Fig. 2). Solvating power is related to pressure and temperature due to their solvent density effects. However, other factors such as the molecular mass of solute, molecular structure complexity, the vapor pressure of solute, and if they are free or bound to cellular structural components can impact the extractability of bioactive compounds by SC-$CO_2$[33].

The solvation capacity of SC-$CO_2$ is usually directly proportional to pressure, but inversely to temperature[15]; thus, the extraction efficiency decreased by increasing temperature. Conversely, high temperatures increase the vapor pressure of solutes, increasing solubility in SC-$CO_2$. Therefore, the effect of temperature on extraction efficiency depends on the pressure used. Usually, the negative impact of high temperatures decreasing solvent density prevails at low-pressure values (< 18 MPa). In contrast, the beneficial effect of temperature on vapor pressure predominates at high pressures (> 18 MPa), and this phenomenon is called retrogradation[33]. It is reasonable to hypothesize that the solvents' mixture in run *1* was denser than in run *4*, increasing the solvation power of SC-$CO_2$ + 0.3% $H_2O$, and improving the extraction of high molecular weight compounds, such as tocopherols ($\alpha$ = 431 and $\gamma$ = 416 g/mol) and phytosterols (β-sitosterol = 415 and stigmasterol = 413 g/mol).

### 3.6 Volatile and semi-volatile fraction profile in baru oil

BO obtained from runs *1* and *4* showed seventeen volatile and semi-volatile compounds from eight chemical classes (Table 4 and Fig. S6). Most of them were furans, pyrazines, or pyrroles, heterocycles typically formed by toasting, roasting, and other heat processes[43].

The volatile and semi-volatile fraction profile in BO was also sensibly affected by temperature and pressure. Extraction by SC-$CO_2$ + 0.3% $H_2O$ under 30 MPa and 70℃ (run *4*) provided BO with higher diversity and contents of volatile and semi-volatile compounds, and this was evident as a distinct flavor when sniffed. Moreover, this BO also showed an improved aroma profile compared to the expeller-pressed BO. The reason for a higher diversity and contents of volatile and semi-volatile compounds in the BO obtained in run *4* is that volatile compounds are small molecules with medium to high vapor pressure and are usually free in the food matrix. Consequently, these compounds tend to be easily extractable under high pressure, which was possibly favored in run *4* because of solutes' vapor pressure rather than due to the solvent's density in this case[33].

Taken from their peak areas, the major volatile compounds in BO from run *4* are typically related to the aroma of roasted almonds formed by the Maillard reaction, especially 2,5-dimethy-pyrazine and trimethylpyrazine[43–45]. Furthermore, 2,5-dimethyl-hydroxy-furanone was the

Table 4 Volatile and semi-volatile compounds profile[a,b] in baru almond oil extracted by SC-$CO_2$ + 0.3% $H_2O$ (selected conditions, runs *1* and runs *4*) and by expeller pressing, used as a reference method.

| Volatile compounds[b] | Similarity index (%) | Linear retention index ($Error_{LRI}$, %)[c] | Aroma notes | SC-$CO_2$ extraction | | Reference technique |
|---|---|---|---|---|---|---|
| | | | | run *1* (50℃; 10 MPa) | run *4* (70℃; 30 MPa) | Expeller pressing |
| *Alcohols* | | | | | | |
| Ethanol, 2-nitro- | 85 | 737 | Sweet [d] | n.d [i] | + + | n.d |
| Ethanol, 2-(vinyloxy) | 86 | 779 | Sweet [d] | n.d | + | n.d |
| *Acids* | | | | | | |
| OH-propanoic acid | 85 | 789 | − | n.d | + | n.d |
| Hexanoic acid | 82 | 974 (− 0.1) | − | n.d | + | n.d |
| Propanoic acid, 2-oxo-ethyl ester | 83 | 1041 | Pungent, rancid, soy [d] | n.d | + + | n.d |
| *Aldehydes* | | | | | | |
| Hexanal | 82 | 797 (− 0.5) | Grass, tallow, fat [d] | n.d | + | + + |
| *Ester* | | | | | | |
| Pantolactone | 89 | 1037 | Cotton candy [d] | n.d | + | n.d |
| *Furan type compounds* | | | | | | |
| 2,5-Dimethyl-hydroxy-furanone | 89 | 1060 (+ 0.5) | Sweet, caramel-like [e] | + | + + + | n.d |
| Furfural | 87 | 831 (+ 0.1) | Bread, almond, sweet [d] | n.d | + + | n.d |
| 2-Furanmethanol | 88 | 855 (+ 1.3) | Very mild, slightly caramel-like, warm-oily [f] | n.d | + | n.d |
| 5-Methyl, 2-furancarboxaldehyde | 73 | 964 (− 0.2) | Caramel-like, sweet, fruity, and nutty [f] | n.d | + | n.d |
| *Pyrazines* | | | | | | |
| 2,5-Dimethyl-pyrazine | 87 | 913 (+ 2.5) | Roasted; nutty [g] | n.d | + + + | + + + |
| Trimethyl-pyrazine | 83 | 1006 (+ 0.1) | Roasted; nutty; baked potato-like, grassy [f,g] | n.d | + + | + + |
| 3-Ethyl-2,5-Dimethylpyrazine | 76 | 1082 (0.0) | Roasted; nutty; earthy, baked potato-like [f,g] | n.d | + | n.d |
| *Pyrroles* | | | | | | |
| 1H-Pyrrole-2-carboxaldehyde | 79 | 1009 (0.0) | Powerful, smoky, woody [h] | n.d | + | n.d |
| 2-Pyrrolidinone | 84 | 1077 (+ 0.1) | − | + | + + | n.d |
| *Alkane* | | | | | | |
| 2,3-Dimethyl-oxirane | 95 | 1149 | − | + + | + + + | n.d |

[a] The number of crosses (+) indicates the proximate amounts of the compounds and was based on peak areas, as follows (arbitrary units): < 300,000; ≥ 300,000 and ≤ 600,000; > 600,000. [b] Compounds peak identity was signaled by two criteria: similarity index from NIST library (SI) > 70 % and $Error_{LRI}$ < 0.5%; or SI > 80 %. [c] $Error_{LRI}$ was the percent difference between experimental and published LRI values (NIST library; accessed on http://webbook.nist.gov/chemistry/), and wasn't reported in this Table when LRI data wasn't available. Aroma notes described in: [d] Acree and Arn[47]; [e] Ho *et al.*[48]; [f] Liu *et al.*[49]; [g] Agila and Barringer[44]; Vázquez-Araújo *et al.*[45]; Vázquez-Araújo *et al.*[50]; Wirthensohn *et al.*[51]; [h] Kendirci and Onoğur[52]. [i] n.d = Not detected.

second major compound in BO from run *1* and contributes to a strong sweet aroma of roasted almonds[44] (Table 4). These compounds possibly contribute to the nutty and toasted flavor in the toasted baru almond, and gave a distinct character to BO, especially the one extracted by SC-$CO_2$ + 0.3% $H_2O$ at 30 MPa and 70℃ (run *4*).

BO obtained by expeller pressing and by SC-$CO_2$ at the highest temperature (70℃) showed low hexanal contents (Table 4). High levels of hexanal in oils correlate with a rancid off-flavor, and this aldehyde is typically formed by oxidation of 18:2*n*-6. However, low levels of hexanal have been linked to a green aroma note in olive oil, which is a positive attribute in this case[46]. Future investigations relating the volatile and semi-volatile profile of BO with sensory and olfactometric analyses are welcome to establish the superior quality of one extraction method over the other. Although in the present study we are not able to ascertain the influence of edafoclimatic conditions on BO composition, we should not rule out that the chemical profile observed herein was impacted to some extent by the ENSO climate phenomenon (with warmer and drier weather in the Brazilian savannah). Future studies are welcome to determine the range of BO chemical profile in varying conditions of the weather.

## 4 Conclusions

Using alcohol as polar cosolvent increased BO extraction yield obtained by SC-$CO_2$, especially at high pressure, whereas using a small amount of $H_2O$ provided a value-added oil enhanced in tocopherols, mainly γ-tocopherol. Extraction of bioactive compounds in baru oil was improved by the use of $H_2O$ in SC-$CO_2$ and was modulated by temperature and pressure. Lower temperature and pressure values favored the extraction of a better γ-/α-tocopherol ratio, besides leading to higher contents of phytosterols and phenolic compounds. However, higher temperature and pressure values favored a distinctive volatile profile. Therefore, our study indicates that extraction by SC-$CO_2$ + $H_2O$ can be used to obtain a value-added BO and that the oil chemical profile might be tailored for food applications by tuning extraction temperature and pressure. Future efforts are welcome to determine if SC-$CO_2$ extraction can be optimized to enable extraction of BO simultaneously enriched in bioactive and aroma-active compounds besides the highest possible extraction yield, particularly further investigating the effects of pressure and temperature on BO extracted using water as cosolvent.

## Authors' Contributions

V.O.D.S.P: Methodology, Formal Analysis, Investigation, Writing - Original Draft. L.O.S: Formal Analysis, Methodology. V.N.C.B: Conceptualization, Writing - Review & Editing, Supervision. A.G.T: Conceptualization, Writing - Review & Editing, Supervision, Funding acquisition.

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## Conflicts of Interest

The authors declare there are no conflicts of interest.

## Supporting Information

This material is available free of charge via the Internet at doi: 10.5650/jos.ess21115

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