

## Article

# Optimization Studies and Compositional Oil Analysis of Pequi (*Caryocar brasiliense* Cambess) Almonds by Supercritical CO<sub>2</sub> Extraction

Livia Silva Mateus<sup>1</sup>, Juliete Martins Dutra<sup>1</sup>, Rogério Favareto<sup>1</sup> , Edson Antônio da Silva<sup>2</sup>, Leandro Ferreira Pinto<sup>3</sup> , Camila da Silva<sup>4</sup> and Lucio Cardozo-Filho<sup>4,\*</sup> 

<sup>1</sup> Instituto Federal de Educação, Ciência e Tecnologia Goiano, Campus Rio Verde, Rio Verde 75901-970, GO, Brazil

<sup>2</sup> Centro de Engenharias e Ciências Exatas, Universidade Estadual do Oeste do Paraná, Toledo 85903-000, PR, Brazil

<sup>3</sup> Campus of Rosana, Sao Paulo State University (UNESP), Rosana 19274-000, SP, Brazil

<sup>4</sup> Programa de Pós-graduação em Engenharia Química, Universidade Estadual de Maringá, Maringá 87020-900, PR, Brazil

\* Correspondence: lcfilho@uem.br; Tel.: +55-(44)-98840-9119

**Abstract:** *Caryocar brasiliense* Cambess (pequi) is the fruit of the *pequizeiro* tree found in the Brazilian Cerrado (savanna). Supercritical fluids have been used to effectively extract bioactive chemicals. In light of the paucity of research on the supercritical extraction of pequi, in this study, experimental tests were conducted on the extraction of pequi almond oil using supercritical CO<sub>2</sub>; the optimal extraction conditions were determined, and the fatty acids and active compounds in the oil were characterized. The experiments were conducted using the Box–Behnken experimental design of a three-variable system: pressure (15, 20, and 25 MPa), temperature (303.15, 318.15, and 333.15 K), and flow rate (2, 3, and 5 g·min<sup>-1</sup>). The optimal extraction conditions were 318.15 K, 25 MPa, and 5.0 g·min<sup>-1</sup>, which yielded 27.6 wt% of oil. The experimental kinetic curves were described using a second-order quadratic model (based on the Sovová model), which demonstrated a satisfactory correspondence with the kinetic curves. Significant amounts of squalene, stigmaterol, oleic fatty acids, and palmitic fatty acids were detected in pequi almond oil.

**Keywords:** pequi; supercritical extraction; fatty acids; bioactive compounds; squalene



**Citation:** Mateus, L.S.; Dutra, J.M.; Favareto, R.; da Silva, E.A.; Ferreira Pinto, L.; da Silva, C.; Cardozo-Filho, L. Optimization Studies and Compositional Oil Analysis of Pequi (*Caryocar brasiliense* Cambess) Almonds by Supercritical CO<sub>2</sub> Extraction. *Molecules* **2023**, *28*, 1030. <https://doi.org/10.3390/molecules28031030>

Academic Editors: Andrei Mocan and Mark P. Heitz

Received: 28 November 2022

Revised: 10 January 2023

Accepted: 13 January 2023

Published: 19 January 2023



**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

*Caryocar brasiliense* Cambess, commonly known as pequi, is a fruit of the *pequizeiro* tree that grows abundantly in Brazil's Cerrado (savanna) [1,2]. *Pequizeiro* is a leafy tree that can reach a height of 10 m, with twisted medium-sized stems and wide leaves [3]. Its fruits have a greenish epicarp (peel), yellowish mesocarp (pulp), brownish endocarp (thorny), and creamy chestnut hue [3]. Pequi is an excellent source of nutrients because it contains proteins, lipids, dietary fibers, carbohydrates, vitamins, carotenoids, and phenolic compounds [4–6]. Pequi almonds contain approximately 50% oil, which may be utilized to produce food, cosmetics, chemicals, and biofuels [1,2,7,8]. Rabbers et al. [9] reported that pequi oil is high in unsaturated fatty acids and possesses anti-inflammatory, antibacterial, antifungal, curative, antioxidant, and antitumor properties [10].

Studies have shown that pequi oil can be used in conjunction with physiotherapy to treat inflammation, lung infections, muscular discomfort, rheumatoid arthritis, and bruises [11]. Thus, research on the extraction of pequi oil is important because of its significant health benefits. Pequi oil is also a good source of squalene for human consumption, as well as for use in medicines and therapy. In addition, pequi oil contains active chemicals, such as stigmaterol,  $\gamma$ -tocopherol,  $\alpha$ -tocopherol, and  $\beta$ -sistosterol [12].

According to the literature, the most frequently used methods for extracting oil from pequi almonds include solvent extraction [13], mechanical processing [14], and enzymatic extraction [10]. Pressurized/supercritical gas technology can also be used to extract

oil [15,16]. This technique yields more oil compared to other techniques because of the intrinsic characteristics of supercritical fluid extraction, such as enhanced transport and diffusion of supercritical or near-supercritical gases into the matrix during the extraction process. Temperature and pressure control can also be used to extract specific bioactive components from the oleaginous matrix. Apart from the substantially higher yield, another advantage of supercritical extraction is the absence of residual organic solvents.

The use of carbon dioxide (CO<sub>2</sub>) eliminates the need for extensive heating while extracting thermolabile chemicals. Numerous studies have reported the extraction of oils and their active components using high-pressure gases (CO<sub>2</sub> and propane) [17,18].

To address the lack of comparative research in the literature, this study investigates the influence of temperature and pressure on oil extraction from pequi almonds. Pressurized CO<sub>2</sub> is used, focusing on improving the total yield and fatty acid profile and increasing the bioactive compound content. A second-order quadratic model is used to compute the mass transfers and characterize the observed kinetic curves.

## 2. Results and Discussion

The experimental conditions and total yields of pequi almond oil extracted using supercritical CO<sub>2</sub> are listed in Table 1. The highest extraction yield of 27.6 wt% was obtained at 318.15 K, 25 MPa, and 5.0 g.min<sup>-1</sup>.

**Table 1.** Experimental conditions and extraction yield results for pequi oil extraction using supercritical CO<sub>2</sub>.

Run	Temperature	Pressure	Flow Rate	Yield
	(K)	(MPa)	(g.min <sup>-1</sup> )	(wt%)
1	303.15	15	3.5	7.6
2	333.15	15	3.5	1.1
3	303.15	25	3.5	20.3
4	333.15	25	3.5	17.4
5	303.15	20	2.0	8.1
6	333.15	20	2.0	4.5
7	303.15	20	5.0	20.9
8	333.15	20	5.0	10.6
9	318.15	15	2.0	2.5
10	318.15	25	2.0	10.9
11	318.15	15	5.0	6.3
12	318.15	25	5.0	27.6
13	318.15	20	3.5	11.6
14	318.15	20	3.5	12.1
15	318.15	20	3.5	11.7

The yield in supercritical extraction may be associated with the duration of the experimental tests, as the kinetic curves did not present a maximum plateau. Figure 1 shows that there was no change in the inclination of the extraction kinetic curves. The changes in inclination were attributed to variations in the convective mass transfer mechanism. The mass transfer velocity was significantly affected by the convection mechanism in the fluid phase and relatively less by the diffusive mechanism. The gradual removal of lipid materials resulted in a discontinuity in the surface layer. At the beginning of this discontinuity, the extraction rate, governed by the diffusive mechanism, decreased. However, for pequi oil extraction, the extraction rate did not decrease despite variations in the CO<sub>2</sub> flow rate. Thus, it appears that the duration of the experimental extraction was insufficient to reach the extractive equilibrium region. The characteristics of the extraction kinetics were similar for all the studied flows.

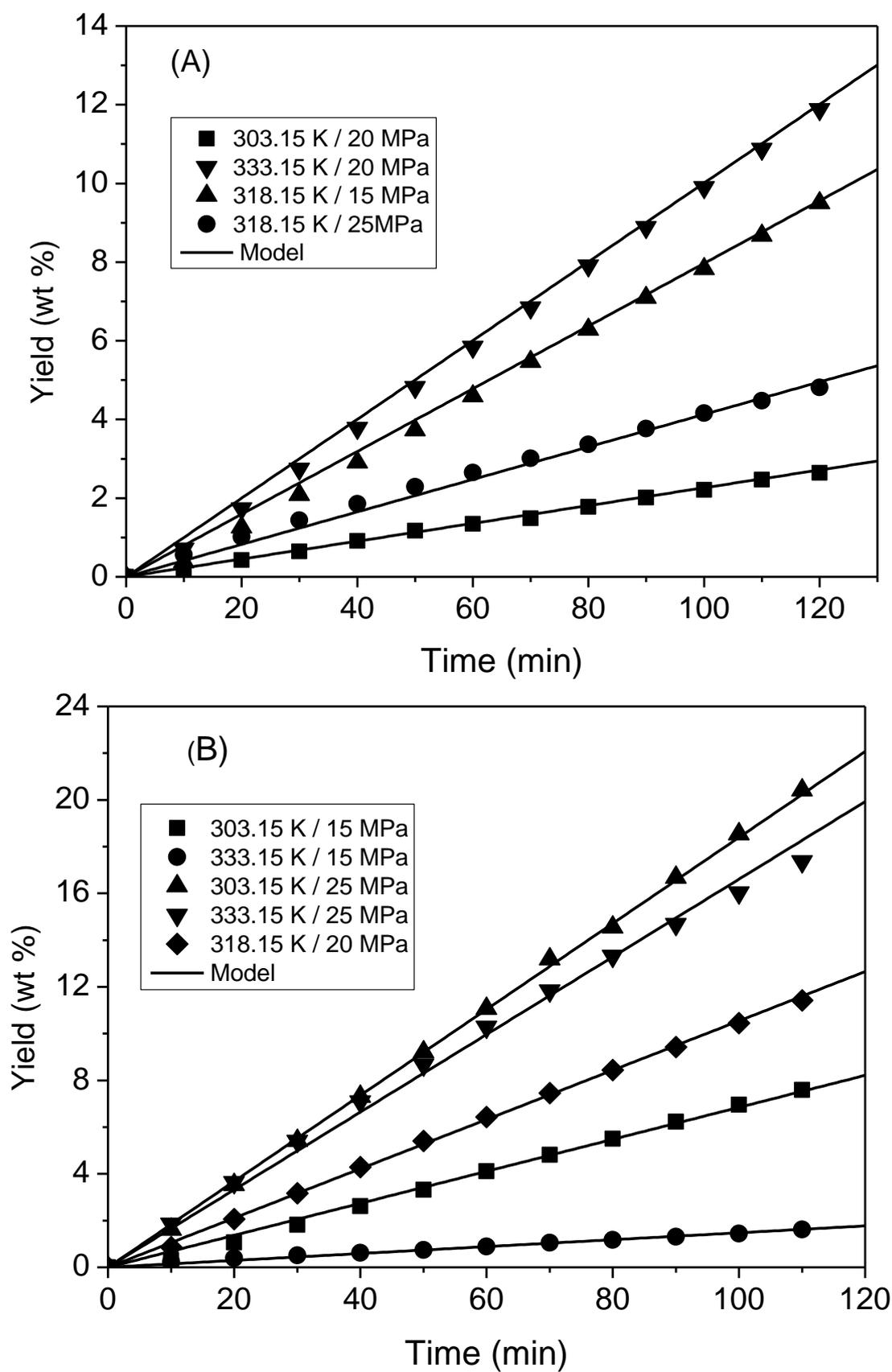
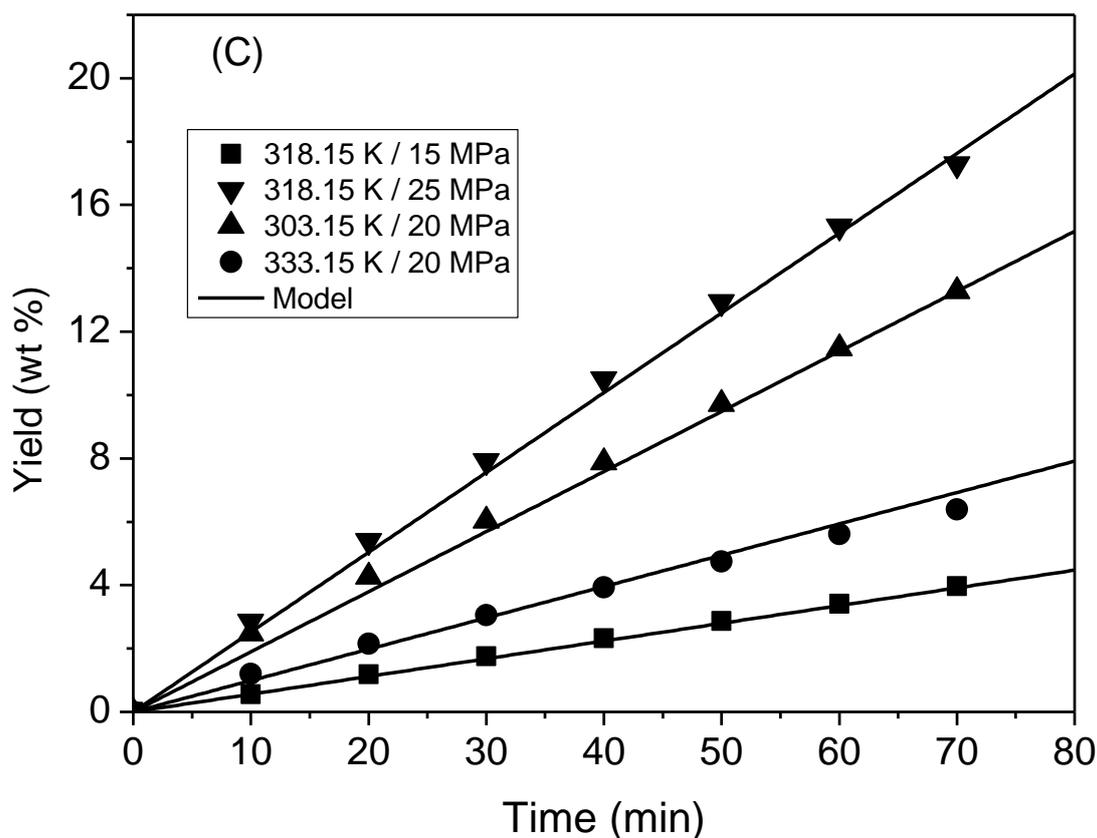


Figure 1. Cont.



**Figure 1.** Experimental kinetic curves for pequi almond oil extraction with supercritical CO<sub>2</sub> fitted using the second-order model. Flow rates of (A) 2.0 g.min<sup>-1</sup>, (B) 3.5 g.min<sup>-1</sup>, and (C) 5.0 g.min<sup>-1</sup>.

In general, increasing the CO<sub>2</sub> flow in the extractor bed facilitated oil extraction. The highest extraction yields were obtained at a flow rate of 5 g.min<sup>-1</sup>. However, being sensitive to changes in pressure and temperature, these yields fluctuated significantly. In contrast, at a flow rate of 3.5 g.min<sup>-1</sup>, the extraction yields exhibited smaller fluctuations with variations in pressure and temperature. Given that pressure is a key factor in obtaining high yields, high CO<sub>2</sub> densities help extract high oil yields.

Pressure also reduces the distance between molecules and enhances the interactions between CO<sub>2</sub> and the sample, which promotes convective mass transfer [19]. Shi et al. [20] and Wang et al. [19] found that increasing the temperature and pressure helped remove carotenoids from pumpkin seeds via supercritical CO<sub>2</sub> extraction.

These yield variations were verified by statistical analysis (Table 2). The results showed a linear relationship between temperature and pressure (Equation (1)), where T, P, and F correspond to temperature, pressure, and solvent flow rate, respectively.

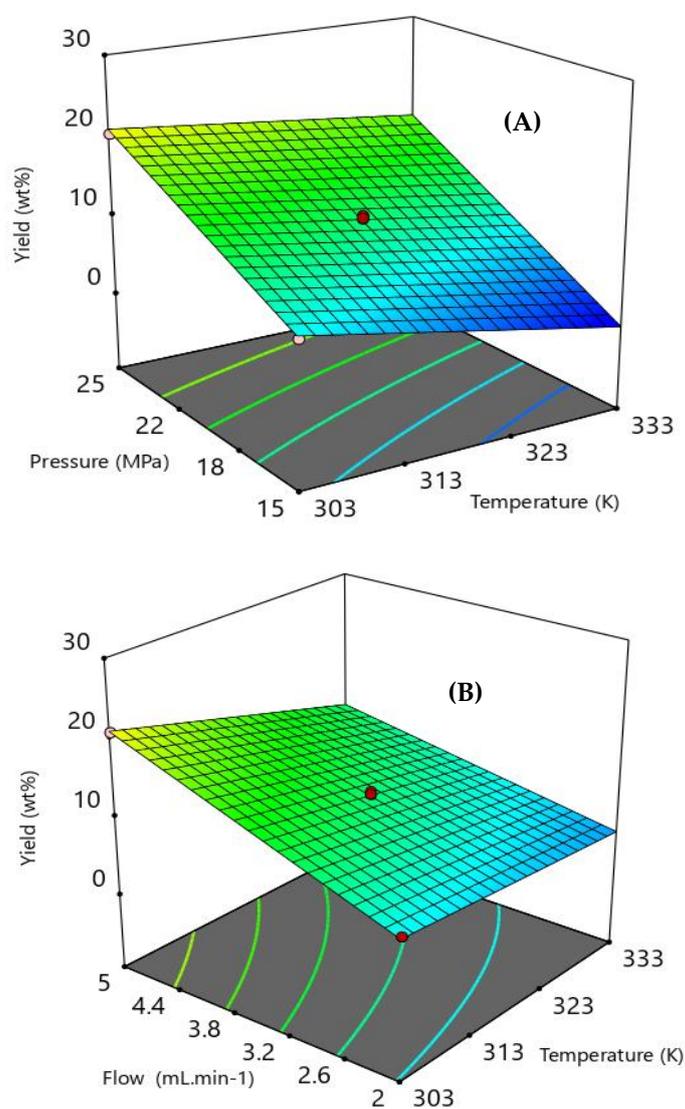
$$\text{Yield} = 11.53 - 2.91 T - 7.34 P + 4.94 F + 0.89 T.P - 1.66 T.F + 3.25 P.F \quad (1)$$

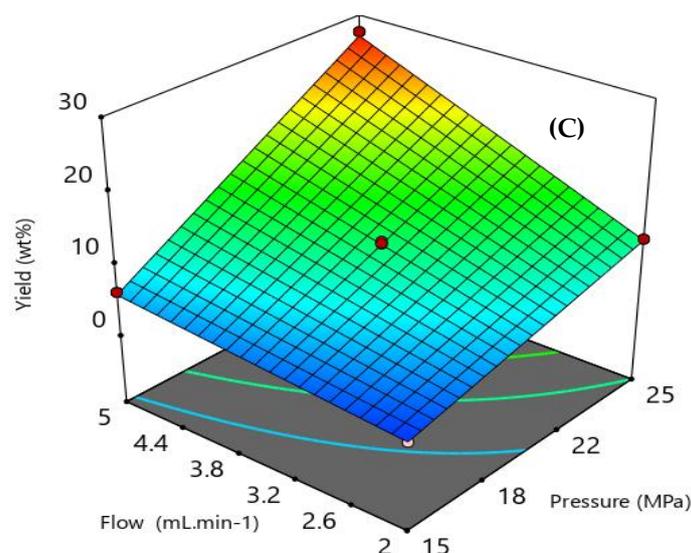
The coefficients of determination ( $R^2$ ) and adjusted  $R^2$  ( $R^2_{\text{adj}}$ ) for the model were 0.994 and 0.989, respectively. The F-value = 218 and  $p$ -value < 0.05 indicate the significance of the model. Thus, the temperature, pressure, and flow rate had synergistic effects, i.e., an increase in these parameters led to the highest oil yields (Figure 2).

**Table 2.** Data on ANOVA for oil obtained using Box–Behnken experimental design of a three-variable system for CO<sub>2</sub> extractions.

Terms	Sum of Squares	Degrees of Freedom	Mean Squares	F-Value	<i>p</i> -Value
Model	750.5	6	125.1	218	<0.0001
T	67.6	1	67.6	118	<0.0001
P	431.3	1	431.3	753	<0.0001
F	195.3	1	195.3	341	<0.0001
T.P	3.2	1	3.2	5.5	0.05
T.F	11.0	1	11.0	19.2	0.002
P.F	42.1	1	42.1	73.5	<0.0001
Residual	4.6	8	0.6		
Lack of Fit	4.5	6	0.7	13.6	0.07
Pure Error	0.1	2	0.06		
Cor Total	755.1	14			

T = Temperature; P = Pressure; and F = Flow rate.

**Figure 2.** Cont.



**Figure 2.** Response surface showing the behavior of the extraction yield as a function of (A) temperature and pressure with constant solvent flow rate of  $3.5 \text{ g}\cdot\text{min}^{-1}$ ; (B) temperature and solvent flow rate with constant pressure of 20 MPa; and (C) pressure and solvent flow rate with constant temperature of 318.15 K.

According to Table 3, the experimental parameters for the application of the mathematical model are as follows: bed density ( $\rho_{\text{bed}}$ ), porosity ( $\epsilon$ ), and apparent solubility ( $C_{\text{eq}}$ ). The model had only one adjustable parameter,  $k = 19.536 \text{ cm}^3\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ . An AARD of 7.3% indicates excellent agreement between the experimental data and model values. Klein et al. (2020) [21] employed a second-order kinetic model to describe the supercritical extraction of *Eugenia pyriformis* Cambess leaves. They obtained  $k$  values in the range of  $4\text{--}16 \text{ cm}^3\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ , which are of the same order of magnitude as the values estimated in this study. Parameter  $k$  was used to generate the calculated extraction kinetics curves, as shown in Figure 1.

**Table 3.** Experimental conditions, extraction yield results, and parameters of the mathematical model for pequi oil extraction using supercritical  $\text{CO}_2$ .

Run	Temperature (K)	Pressure (MPa)	Flow Rate ( $\text{g}\cdot\text{min}^{-1}$ )	$C_{\text{eq}} \times 1000^{\text{a}}$ ( $\text{goil}\cdot\text{cm}^{-3}$ )	Yield (wt%)
1	303.15	15	3.5	1.87	7.6
2	333.15	15	3.5	0.42	1.1
3	303.15	25	3.5	5.39	20.3
4	333.15	25	3.5	4.78	17.4
5	303.15	20	2.0	3.89	8.1
6	333.15	20	2.0	1.98	4.5
7	303.15	20	5.0	3.89	20.9
8	333.15	20	5.0	1.98	10.6
9	318.15	15	2.0	1.09	2.5
10	318.15	25	2.0	5.11	10.9
11	318.15	15	5.0	1.09	6.3
12	318.15	25	5.0	5.12	27.6
13–15 <sup>e</sup>	318.15	20	3.5	3.03	11.8

<sup>a</sup>  $C_{\text{eq}}$ , apparent solubility (g of oil per volume of solvent). <sup>e</sup> Mean  $\pm$  standard deviation ( $n = 3$ ).  $\epsilon$ , porosity: 0.8125.  $\rho_{\text{bed}}$ , density of the bed ( $\text{kg m}^{-3}$ ): 215.7.

Analysis of fatty acids in pequi almond oil showed the following composition: oleic (~54%), palmitic (~37%), linoleic (~5%), and stearic (~1.5%) acids. Small amounts of myristic, palmitoleic, arachidic, linolenic, and gadoleic fatty acids were also observed (Table 4). The fatty acid profile obtained in this study was similar to that obtained by

Soxhlet extraction methods reported in the literature [22,23]. The predominance of oleic acid, with no significant differences across extractions, indicates that the supercritical CO<sub>2</sub> extraction process does not damage the primary fatty acids present in the oil.

**Table 4.** Fatty acid profile of pequi almond oils obtained by supercritical CO<sub>2</sub>.

Run	Fatty Acid (%) <sup>1</sup>								
	Myristic	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Arachidic	Linolenic	Gadoleic
1	0.35 ± 0.00 c	37.76 ± 0.04 d,e	0.41 ± 0.00 a,b	1.48 ± 0.04 d	53.66 ± 0.02 c	6.09 ± 0.01 b	0.08 ± 0.00 b,c	0.13 ± 0.00 d	0.05 ± 0.01 a
2	0.41 ± 0.00 a	35.18 ± 0.02 f	0.43 ± 0.01 a	1.56 ± 0.01 c,d	54.07 ± 0.03 b	6.035 ± 0.02 b	0.01 ± 0.00 a	0.15 ± 0.00 c	0.05 ± 0.00 a
3	0.31 ± 0.00 e	37.31 ± 0.03 f	0.36 ± 0.01 e,f	1.76 ± 0.00 a,b,c	54.23 ± 0.01 a,b	5.69 ± 0.02 c,d	0.08 ± 0.01 b	0.18 ± 0.00 b	0.06 ± 0.01 a
4	0.27 ± 0.00 <sup>f</sup>	37.35 ± 0.00 e,f	0.32 ± 0.01 g	1.92 ± 0.06 a,b	54.48 ± 0.04 a	5.32 ± 0.00 c,d	0.07 ± 0.00 c,d	0.21 ± 0.00 a	0.05 ± 0.01 a
5	0.33 ± 0.00 d	38.05 ± 0.12 d	0.39 ± 0.00 b,c,d	1.70 ± 0.01 a,b,c,d	53.58 ± 0.07 c	5.66 ± 0.05 c,d	0.08 ± 0.00 c	0.15 ± 0.00 c	0.04 ± 0.00 a
6	0.37 ± 0.00 b	40.56 ± 0.04 a	0.37 ± 0.00 c,d,e,f	1.95 ± 0.01 a	51.36 ± 0.02 g	5.10 ± 0.00 g	0.06 ± 0.00 d	0.17 ± 0.00 b	0.04 ± 0.00 a
7	0.31 ± 0.00 e	38.16 ± 0.03 c,d	0.37 ± 0.00 c,d,e,f	1.83 ± 0.00 a,b	53.24 ± 0.05 d,e	5.78 ± 0.01 c	0.07 ± 0.00 b,c,d	0.17 ± 0.00 b	0.05 ± 0.00 a
8	0.33 ± 0.01 c,d	38.80 ± 0.05 b	0.37 ± 0.00 c,d,e,f	1.69 ± 0.06 b,c,d	52.87 ± 0.01 f	5.64 ± 0.02 d,e	0.07 ± 0.00 c,d	0.15 ± 0.00 c	0.05 ± 0.00 a
9	0.32 ± 0.01 d,e	37.45 ± 0.01 e,f	0.37 ± 0.01 d,e,f	1.56 ± 0.04 c,d	54.26 ± 0.00 a,b	5.77 ± 0.03 c	0.07 ± 0.00 c,d	0.15 ± 0.00 c	0.04 ± 0.00 a
10	0.33 ± 0.00 c	0.39 ± 0.05 d	0.39 ± 0.00 b,c,d,e	1.18 ± 0.08 e	53.45 ± 0.04 c,d	6.25 ± 0.07 a	0.08 ± 0.00 b,c	0.15 ± 0.00 c	0.05 ± 0.00 a
11	0.35 ± 0.00 c	38.56 ± 0.00 b,c	0.40 ± 0.00 b,c	1.73 ± 0.06 a,b,c,d	52.99 ± 0.03 e,f	5.77 ± 0.02 c,d	0.07 ± 0.00 c,d	0.15 ± 0.00 c	0.04 ± 0.01 a
12	0.27 ± 0.01 <sup>f</sup>	37.13 ± 0.05 f	0.35 ± 0.00 f,g	1.92 ± 0.01 a,b	54.48 ± 0.04 a	5.52 ± 0.02 e	0.07 ± 0.00 c,d	0.21 ± 0.00 a	0.05 ± 0.00 a
13–15 <sup>a</sup>	0.32 ± 0.01 d,e	38.13 ± 0.35 d	0.41 ± 0.00 a,b	1.84 ± 0.18 a,b	53.29 ± 0.22 d	5.69 ± 0.04 c,d	0.08 ± 0.00 b,c	0.17 ± 0.01 b	0.04 ± 0.00 a

Mean ± standard deviation (n = 3)<sup>1</sup> in relative area. Means followed by the same letters (in each column) did not differ statistically ( $p > 0.05$ ).

The bioactive compounds detected in the extracts of pequi almonds were squalene, stigmasterol,  $\beta$ -sistosterol, octacosanol, triacontanol,  $\gamma$ -tocopherol, and  $\alpha$ -tocopherol. Among these, squalene had the highest concentrations of 3069–14,220 mg per 100 g of oil. The squalene content extracted with pressurized CO<sub>2</sub> is listed in Table 5.

The main fatty acids observed were oleic and palmitic acids. Under the optimal conditions, oleic acid in test 10 had the best extraction response, with a 6% statistical difference from that of the other tests. Palmitic acid exhibited the highest extraction in test 6 with 40% extraction. The differences between the experimental and quantitative extraction and identification results are attributed to the molecular mass of the fatty acids and to the temperature and pressure used in the supercritical extraction system [24,25].

Different extraction methods lead to variations in the fatty acid content. Although the supercritical extraction method is the most suitable, it is important to evaluate the extraction conditions, large-scale industrial processes, and industrial plants, which are influenced by the raw material and economic factors. In comparison, the Soxhlet extraction method remains the most widely applied process, although quantitative variations and degradation of molecules during heating limit further studies [26–28].

The highest solubility between CO<sub>2</sub> and squalene was obtained at approximately 15 MPa, 303.15 K, and 3.5 g·min<sup>-1</sup>. However, despite the mass of squalene/mass of oil obtained under these conditions, the amount of oil extracted under the same experimental conditions was significantly low (1.1 wt%). Thus, these conditions were not optimal when the mass of the oil and squalene binary system was considered.

**Table 5.** Bioactive compounds of pequi almond oils obtained by supercritical CO<sub>2</sub>.

Run	Compound (mg per 100 g Oil)						
	Squalene	γ-Tocopherol	Octacosanol	α-Tocopherol	Stigmasterol	Triacantanol	β-Sitosterol
1	5032.05 ± 59.04 <sup>f</sup>	9.17 ± 0.12 <sup>e</sup>	27.59 ± 0.15 <sup>d</sup>	11.92 ± 0.14 <sup>d,e</sup>	133.92 ± 1.64 <sup>d</sup>	12.99 ± 0.12 <sup>c</sup>	35.96 ± 2.92 <sup>b,c</sup>
2	14219.82 ± 68.39 <sup>a</sup>	27.01 ± 1.11 <sup>a</sup>	34.06 ± 0.47 <sup>c</sup>	24.65 ± 0.77 <sup>a</sup>	324.88 ± 2.07 <sup>a</sup>	16.69 ± 0.86 <sup>b</sup>	70.61 ± 1.35 <sup>a</sup>
3	2972.47 ± 1.62 <sup>i</sup>	9.26 ± 0.05 <sup>e</sup>	9.89 ± 0.30 <sup>g</sup>	9.25 ± 0.87 <sup>f,g,h</sup>	90.48 ± 2.92 <sup>h</sup>	5.56 ± 0.03 <sup>f</sup>	25.68 ± 1.14 <sup>d,e,f</sup>
4	3069.31 ± 24.20 <sup>i</sup>	9.55 ± 0.11 <sup>e</sup>	9.59 ± 0.41 <sup>g</sup>	7.30 ± 1.39 <sup>h</sup>	93.54 ± 4.05 <sup>g,h</sup>	7.19 ± 0.21 <sup>e,f</sup>	23.73 ± 0.12 <sup>d,e,f</sup>
5	4225.7 ± 135.21 <sup>g</sup>	12.04 ± 0.78 <sup>d</sup>	20.73 ± 0.67 <sup>e</sup>	9.99 ± 0.19 <sup>e,f,g</sup>	119.50 ± 0.33 <sup>e</sup>	10.36 ± 0.19 <sup>d</sup>	24.05 ± 2.27 <sup>d,e,f</sup>
6	7076.92 ± 45.31 <sup>c</sup>	18.99 ± 0.01 <sup>b</sup>	40.65 ± 0.44 <sup>b</sup>	16.53 ± 0.39 <sup>b</sup>	185.65 ± 0.37 <sup>c</sup>	18.82 ± 1.03 <sup>b</sup>	39.18 ± 2.52 <sup>b</sup>
7	3057.3 ± 28.68 <sup>i</sup>	8.95 ± 0.38 <sup>e</sup>	14.08 ± 0.17 <sup>f</sup>	8.54 ± 0.70 <sup>g,h</sup>	88.60 ± 1.45 <sup>h</sup>	7.83 ± 0.43 <sup>e</sup>	20.80 ± 1.56 <sup>e,f</sup>
8	5507.90 ± 50.45 <sup>e</sup>	16.89 ± 0.35 <sup>c</sup>	22.52 ± 0.42 <sup>e</sup>	11.63 ± 0.22 <sup>d,e,f</sup>	143.42 ± 0.44 <sup>d</sup>	12.71 ± 0.54 <sup>c</sup>	30.20 ± 0.17 <sup>c,d</sup>
9	9021.06 ± 198.23 <sup>b</sup>	18.59 ± 1.04 <sup>b,c</sup>	36.91 ± 2.88 <sup>c</sup>	14.80 ± 0.57 <sup>b,c</sup>	197.97 ± 0.52 <sup>b</sup>	16.85 ± 1.13 <sup>b</sup>	38.18 ± 0.63 <sup>b</sup>
10	3756.2 ± 81.36 <sup>h</sup>	10.94 ± 0.26 <sup>d,e</sup>	10.99 ± 0.27 <sup>f,g</sup>	14.00 ± 0.79 <sup>c,d</sup>	103.97 ± 1.99 <sup>f,g</sup>	5.80 ± 0.21 <sup>e,f</sup>	20.60 ± 0.77 <sup>f</sup>
11	6671.90 ± 105.24 <sup>d</sup>	16.82 ± 0.12 <sup>c</sup>	22.48 ± 0.46 <sup>e</sup>	16.46 ± 0.13 <sup>b</sup>	181.58 ± 2.20 <sup>c</sup>	11.58 ± 0.19 <sup>c,d</sup>	38.36 ± 2.43 <sup>b</sup>
12	2826.37 ± 63.43 <sup>i</sup>	9.48 ± 0.30 <sup>e</sup>	3.08 ± 0.06 <sup>h</sup>	9.53 ± 0.49 <sup>e,f,g,h</sup>	83.55 ± 3.31 <sup>h</sup>	2.48 ± 0.46 <sup>g</sup>	19.76 ± 1.39 <sup>f</sup>
13–15 <sup>a</sup>	3848.6 ± 157.25 <sup>h</sup>	11.97 ± 0.29 <sup>d</sup>	48.77 ± 1.00 <sup>a</sup>	8.37 ± 0.07 <sup>g,h</sup>	113.37 ± 4.75 <sup>e,f</sup>	23.58 ± 0.54 <sup>a</sup>	27.22 ± 0.66 <sup>d,e</sup>

<sup>a</sup> Mean ± standard deviation (n = 3). Means followed by the same letters (in each column) did not differ statistically ( $p > 0.05$ ).

With regard to the quantitative variations between the tests, it should be noted that squalene, γ-tocopherol, α-tocopherol, stigmasterol, and β-sitosterol molecules promote numerous biological activities, such as hormone repositories, vitamin complexes, and cytotoxic agents in specific groups of cancer cell lines [29,30]. Quantitative analysis showed differences between the tests applied; however, in the test with high significance (run 2), there was a greater extraction per partition.

Among all extraction methods (e.g., hot or cold solvent extraction and crushing), the supercritical CO<sub>2</sub> extraction method has the highest yield. However, the extraction plants still have to deal with problems such as low laboratory-scale production [31,32]. Although the supercritical system remains a small-scale system, the use of residues, mainly those from the epicarp and mesocarp of pequi fruit, has gained increasing attention because of the economic potential of the bioactive compounds contained in this fruit [33,34].

### 3. Materials and Methods

#### 3.1. Sample Preparation

The pequi almonds were donated by farmers in Rio Verde-GO (17°47'52" S, 50°55'40" W) in Brazil. Prior to the experiments, the in natura samples were manually peeled and dried in a vented oven at 313.15 K for 24 h. The samples were shredded in a knife mill (Solab SL30, Brazil), sieved using a set of Tyler series (WS Tyler) sieves, and separated using a 20–42 mesh grain size. The true density ( $\rho_t$ ) of the particles was determined using helium gas pycnometry (Quantachrome Ultrapyc 1200 e, Germany). The samples were vacuum-packed in polyethylene bags and frozen.

#### 3.2. Supercritical CO<sub>2</sub> Extraction

The experiments were conducted using a CO<sub>2</sub> cylinder, two thermostatically regulated baths, an Isco 500D syringe pump, a jacketed extraction vessel (1.91 cm in diameter and 16.8 cm in height), and an absolute pressure transducer (Smar LD301, Brazil) (Supplementary Material, Figure S1). Additional information on the instruments and approaches used in the experiment is available in previous studies [15,16,18,35,36].

The extractor was supplied with 0.01 kg of dried pequi almonds, while the remaining space in the extraction cell was filled with glass spheres (inert bed). As a result, the CO<sub>2</sub> delivered to the extractor passed through the inert bed before reaching the ground seed. After reaching the appropriate extraction temperature, both the pump and extractor were simultaneously pressurized. After reaching the working pressure, the solution was allowed to cool for 30 min to achieve equilibrium and ensure that the solvent was saturated prior to the beginning of the extraction. The experiments were conducted using the Box–Behnken experimental design of a three-variable system: pressure (15, 20, and 25 MPa), temperature (303.15, 318.15, and 333.15 K), and flow rate (2, 3, and 5. and 5 g.min<sup>-1</sup>) (see Table 6). The

CO<sub>2</sub> (99.5% purity, Linde gas, Brazil) was compressed through the syringe pump at 293.15 K. For 110 min, the flow rate of the solvent was 3.5 g.min<sup>-1</sup>, regulated by a micrometric valve (Parker Autoclave Engineers, Erie, PA, USA), and maintained at 353.15 K. The total lipids extracted were collected in pre-weighed glass flasks at 10 min intervals (total of 110 min), and the extraction yield was measured gravimetrically using an analytical balance. The ultimate yield was determined by dividing the total mass extracted by the initial mass of leaves in the extractor (dry basis). The analyses were performed in a random order.

**Table 6.** Box–Behnken experimental design of a three-variable system.

Factors	Symbol	Unit	Levels		
			−1	0	+1
Temperature	T	K	303.15	318.15	333.15
Pressure	P	MPa	15	20	25
Flow rate	F	g.min <sup>-1</sup>	2	3.5	5

### 3.3. Kinetics of the Pequi Almond Oil Extraction

The second-order model, modified by De Sousa et al. [37], assumes that the extraction rate is proportional to both the residual capacity of the solvent for solute extraction and solute concentration in the solid matrix. The model is described by Equations (2) and (3), respectively.

$$\frac{\partial C}{\partial t} + \frac{\rho_{bed}}{\varepsilon} \frac{\partial q}{\partial t} + u \frac{\partial C}{\partial z} = 0 \quad (2)$$

$$\frac{\partial q}{\partial t} = -kq(C_{eq} - C) \quad (3)$$

where  $C$  is the solute concentration in the solvent (kg m<sup>-3</sup>),  $t$  is the extraction time (min),  $\rho_{bed}$  is the bed density (kg m<sup>-3</sup>),  $\varepsilon$  is the porosity,  $q$  is the solute concentration in the solid matrix (kg m<sup>-3</sup>),  $u$  is the interstitial velocity (m s<sup>-1</sup>),  $z$  is the axial coordinate of the bed,  $k$  is the kinetic constant (m<sup>3</sup> kg<sup>-1</sup> min<sup>-1</sup>), and  $C_{eq}$  is the equilibrium solute concentration of the solvent (kg m<sup>-3</sup>).

For the second-order model, the initial and boundary conditions are described by Equations (4)–(6).

$$C(0, z) = C_{eq} \quad (4)$$

$$q(0, z) = q_0 \quad (5)$$

$$C(t, 0) = \begin{cases} C_{eq} & t = 0 \\ 0 & t > 0 \end{cases} \quad (6)$$

where  $q_0$  is the initial solute concentration in the solid matrix (kg m<sup>-3</sup>).

Equation (7) describes the analytical solution of the second-order model.

$$\frac{C}{C_{eq}} = \begin{cases} 1 & t < t_r \\ 1 - \frac{1}{(e^A + e^{-B} - 1)e^B} & t > t_r \end{cases} \quad (7)$$

where  $A = (z/u)\beta$ ;  $B = [(-tu + z)\beta]/\alpha u$ ;  $\beta = kC_{eq}\alpha$ ;  $\alpha = (\rho_b q_0)/(\varepsilon C_{eq})$ , and  $t_r$  is the residence time of the solvent in the bed.

The total mass extracted is obtained by Equation (8).

$$m_{ext} = \int_0^{t_e} C_{z=H} w t = \begin{cases} C_{eq} w t & t < t_r \\ C_{eq} w t - \frac{C_{eq} w \alpha}{\beta} \ln \left( e^{\frac{(z\beta)}{u}} + e^{\frac{[-(tu+z)\beta]}{\alpha u}} - 1 \right) & t > t_r \end{cases} \quad (8)$$

where  $C_{z=H}$  is the solute concentration in the solvent at the extractor outlet (kg.m<sup>-3</sup>);  $H$  is the length of the extractor (m); and  $w$  is the volumetric flow rate of the solvent (m<sup>3</sup>.min<sup>-1</sup>). In the second-order kinetic model, only one adjustable parameter ( $k$ ) exists. A quadratic

interpolation algorithm was used to estimate the values of the parameter and the following objective function according to Equation (9):

$$F_{OBJ} = \sum_{j=1}^N \left( \frac{m_{extract,j}^{Exp} - m_{extract,j}^{Calc}}{m_{extract,j}^{Exp}} \right)^2 \quad (9)$$

The goodness of fit of the model was quantified using the average absolute relative deviation (AARD) according to Equation (10).

$$AARD(\%) = \frac{100}{N} \sum_{j=1}^N \left| \frac{m_{extract,j}^{Exp} - m_{extract,j}^{Calc}}{m_{extract,j}^{Exp}} \right| \quad (10)$$

where  $N$  is the number of experimental data points from all extraction curves, and  $m_{extract,j}^{Exp}$  and  $m_{extract,j}^{Calc}$  are the experimental and calculated mass of the extract, respectively.

### 3.4. Oil Characterization

To determine the fatty acid profile, the samples were derivatized using an adapted version of the methodology described by Santos Júnior et al. [38], in which the samples were derivatized in methanol with sulfuric acid and potassium hydroxide, followed by chromatographic analysis (Shimadzu, GCMS-QP2010 SE), as described by Rodrigues et al. [39].

Gas chromatography (Shimadzu, GCMS-QP2010 SE) linked to mass spectrometry and equipped with a Shimadzu Rtx-5MS capillary column (30 m × 0.25 mm × 0.25 mm) was used to assess the active substances using a modified version of the approach published by Du and Ahn [40]. The sample was derivatized using 20 µL N,O-bis(trimethylsilyl) trifluoroacetamide)/trimethylsilyl chloride (333.15 K for 30 min), followed by the addition of 5-cholestane. Data were collected using the GCMS Postrun Analysis program (Shimadzu), which includes the databases for the NIST14.lb and NIST14.lbs spectral libraries.

### 3.5. Statistical Analysis

The data were subjected to analysis of variance (ANOVA) at 5% significance, followed by Tukey's test. The main effects and interactions were computed using Design-Expert software version 12 [41]. Statistica software version 8.0 [42] was used to compute the main effects and interactions and to determine the influence of independent factors on the response.

## 4. Conclusions

The best pequi almond oil extraction conditions were pressure, temperature, and flow rate of 25 MPa, 318.15 K, and 5 g min<sup>-1</sup>, respectively, to obtain 27.6 wt% of oil. Oleic acid (>50 wt%) and palmitic acid (>35 wt%) were the most abundant fatty acids in the pequi almond oil. Fatty acid concentrations remained constant under all experimental conditions. The oil contained high amounts of squalene and stigmasterol. The second-order mathematical model effectively adjusted the extraction curves, contributing to the understanding of the extraction phases and mass transfer mechanisms.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules28031030/s1>. Figure S1: Schematic of the experimental supercritical extraction unit: 1-CO<sub>2</sub> cylinder; 2-Syringe pump; 3-Thermostatic bath; 4-Pressure indicator; 5-Temperature controller/indicator; 6-Extractor; 7-Valve; 8-Needle-type valve attached to an aluminum jacket for heating; 9-Thermostatic bath.

**Author Contributions:** Data curation, L.S.M.; investigation, J.M.D.; writing—original draft preparation, E.A.d.S.; writing—review and editing, L.F.P.; data curation, writing—review and editing, C.d.S.;

project administration, R.F.; supervision, L.C.-F. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Acknowledgments:** The authors would like to thank the Ministry of Science, Technology and Innovations (Ministério da Ciência, Tecnologia e Inovações—MCTIC), the Research Support Foundation of the State of Goiás (Fundação de Amparo à Pesquisa do Estado de Goiás—FAPEG), the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico—CNPq), the Coordination for the Improvement of Staff of Higher Education (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior -CAPES), the Financier of Studies and Projects (Financiadora de Estudos e Projetos—FINEP), and the PROPPI—Goiano Federal Institute (IF Goiano) for funding this research project. LF-P thanks for the financial support of the Fundação de Amparo à Pesquisa do Estado de São Paulo, FAPESP (Brazil), through the grant 2018/23063-1.

**Conflicts of Interest:** The authors declare that they have no conflict of interest.

## References

1. de Lima, A.; de Silva, A.M.O.e.; Trindade, R.A.; Torres, R.P.; Mancini-Filho, J. Composição Química e Compostos Bioativos Presentes Na Polpa e Na Amêndoa Do Pequi (Caryocar Brasiliense, Camb.). *Rev. Bras. Frutic.* **2007**, *29*, 695–698. [\[CrossRef\]](#)
2. Silva, T.A.; de Assunção, R.M.N.; Vieira, A.T.; de Oliveira, M.F.; Batista, A.C.F. Methylic and Ethylic Biodiesels from Pequi Oil (Caryocar Brasiliense Camb.): Production and Thermogravimetric Studies. *Fuel* **2014**, *136*, 10–18. [\[CrossRef\]](#)
3. Alves, A.M.; Fernandes, D.C.; de Sousa, A.G.O.; Naves, R.V.; Naves, M.M.V. Características Físicas e Nutricionais de Pequis Oriundos Dos Estados de Tocantins, Goiás e Minas Gerais. *Braz. J. Food Technol.* **2014**, *17*, 198–203. [\[CrossRef\]](#)
4. de Santana Magalhães, F.; de Souza Martins Sá, M.; Luiz Cardoso, V.; Hespagnol Miranda Reis, M. Recovery of Phenolic Compounds from Pequi (Caryocar Brasiliense Camb.) Fruit Extract by Membrane Filtrations: Comparison of Direct and Sequential Processes. *J. Food Eng.* **2019**, *257*, 26–33. [\[CrossRef\]](#)
5. do Nascimento-Silva, N.R.R.; Naves, M.M.V. Potential of Whole Pequi (Caryocar Spp.) Fruit—Pulp, Almond, Oil, and Shell—As a Medicinal Food. *J. Med. Food* **2019**, *22*, 952–962. [\[CrossRef\]](#)
6. Ferreira, M.A.; de Oliveira, M.E.S.; Silva, G.A.; Mathioni, S.M.; Mafia, R.G. Capillaureum Caryovora Gen. Sp. Nov. (Cryphonectriaceae) Pathogenic to Pequi (Caryocar Brasiliense) in Brazil. *Mycol. Prog.* **2019**, *18*, 385–403. [\[CrossRef\]](#)
7. Borges, K.A.; Batista, A.C.F.; de Souza Rodrigues, H.; Hernandes Terrones, M.; Tironi Vieira, A.; de Oliveira, M.F. Production of Methyl and Ethyl Biodiesel Fuel from Pequi Oil (Caryocar Brasiliensis Camb.). *Chem. Technol. Fuels Oils* **2012**, *48*, 83–89. [\[CrossRef\]](#)
8. Marques Cardoso, C.M.; Zavarize, D.G.; Gama Vieira, G.E. Transesterification of Pequi (Caryocar Brasiliensis Camb.) Bio-Oil via Heterogeneous Acid Catalysis: Catalyst Preparation, Process Optimization and Kinetics. *Ind. Crop. Prod.* **2019**, *139*, 111485. [\[CrossRef\]](#)
9. Rabbers, A.S.; Rabelo, R.E.; Oliveira, L.P.; Ribeiro, M.; Martins, V.C.A.; Plepis, A.M.G.; Vulcani, V.A.S. Additive Effect of Pulp Pequi Oil (Caryocar Brasiliense Camb.) on the Biocompatibility of Collagen and Gelatin Membranes in Subcutaneous Implants. *Arq. Bras. Med. Veterinária Zootec.* **2019**, *71*, 811–818. [\[CrossRef\]](#)
10. Malacrida, C.R.; Moraes, I.C.F.; de Rosso, V.V.; da Rodrigues, C.E.C.; de Souza, A.C. Effect of the Application of an Enzymatic Pretreatment on Bioactive Compounds of Caryocar Brasiliense Camb Pulp Oil. *J. Food Process. Preserv.* **2018**, *42*, e13828. [\[CrossRef\]](#)
11. da Silva, L.F.B.P.; Cerqueira Poty, J.A.; Martins, M.; de Machado Freitas Coelho, N.P.; Martins Maia Filho, A.L.; da Sena Costa, C.L.A. Anti-inflammatory action of pequi oil associated to ultrasound in tendinitis in rats: Macroscopic and histological analysis. *Man. Ther. Posturol. Rehabil. J.* **2016**, *14*, 347. [\[CrossRef\]](#)
12. Girardi, J.S. *Avaliação Da Influência D Ação Anti-Inflamatória Do Óleo de Pequi Associado Ao Ultrassom Na Tendinite Em Ratos: Análise Macroscópica e Histológicas Condições de Cultivo Sobre Os Teores de Compostos de Interesse Presentes Nos Extratos de Erva-Mate (Ilex Paraguariensis) Obtidos Por CO2 a Altas Pressões*; Universidade Federal de Santa Catarina: Florianópolis, Brazil, 2010.
13. Araújo, A.C.M.A.; de Resende Oliveira, É.; Menezes, E.G.T.; Dias, B.O.; Terra, A.W.C.; Queiroz, F. Solvent Effect on the Extraction of Soluble Solids from Murici and Pequi Seeds. *J. Food Process. Eng.* **2018**, *41*, e12813. [\[CrossRef\]](#)
14. Roll, M.M.; Miranda-Vilela, A.L.; Longo, J.P.F.; da Agostini-Costa, T.S.; Grisolia, C.K. The Pequi Pulp Oil (Caryocar Brasiliense Camb.) Provides Protection against Aging-Related Anemia, Inflammation and Oxidative Stress in Swiss Mice, Especially in Females. *Genet. Mol. Biol.* **2018**, *41*, 858–869. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Lemos, C.O.T.; Garcia, V.A.D.S.; Gonçalves, R.M.; Leal, I.C.R.; Siqueira, V.L.D.; Filho, L.C.; Cabral, V.F. Supercritical Extraction of Neolignans from Piper Regnellii Var. Pallescens. *J. Supercrit. Fluids* **2012**, *71*, 64–70. [\[CrossRef\]](#)

16. Gonçalves, R.M.; Lemos, C.O.T.; Leal, I.C.R.; Nakamura, C.V.; Cortez, D.A.G.; da Silva, E.A.; Cabral, V.F.; Cardozo-Filho, L. Comparing Conventional and Supercritical Extraction of (-)-Mammea A/BB and the Antioxidant Activity of Calophyllum Brasiliense Extracts. *Molecules* **2013**, *18*, 6215–6229. [[CrossRef](#)]
17. Pederssetti, M.M.; Palú, F.; da Silva, E.A.; Rohling, J.H.; Cardozo-Filho, L.; Dariva, C. Extraction of Canola Seed (Brassica Napus) Oil Using Compressed Propane and Supercritical Carbon Dioxide. *J. Food Eng.* **2011**, *102*, 189–196. [[CrossRef](#)]
18. Silva, M.O.; Camacho, F.P.; Ferreira-Pinto, L.; Giufrida, W.M.; Vieira, A.M.S.; Visentaine, J.V.; Vedoy, D.R.L.; Cardozo-Filho, L. Extraction and Phase Behaviour of Moringa Oleifera Seed Oil Using Compressed Propane. *Can. J. Chem. Eng.* **2016**, *94*, 2195–2201. [[CrossRef](#)]
19. Wang, X.; Wang, C.; Zha, X.; Mei, Y.; Xia, J.; Jiao, Z. Supercritical Carbon Dioxide Extraction of  $\beta$ -Carotene and  $\alpha$ -Tocopherol from Pumpkin: A Box-Behnken Design for Extraction Variables. *Anal. Methods* **2017**, *9*, 294–303. [[CrossRef](#)]
20. Shi, J.; Yi, C.; Ye, X.; Xue, S.; Jiang, Y.; Ma, Y.; Liu, D. Effects of Supercritical CO<sub>2</sub> Fluid Parameters on Chemical Composition and Yield of Carotenoids Extracted from Pumpkin. *LWT-Food Sci. Technol.* **2010**, *43*, 39–44. [[CrossRef](#)]
21. Klein, E.J.; Johann, G.; da Silva, E.A.; Vieira, M.G.A. Mathematical Modeling of Supercritical CO<sub>2</sub> Extraction of Eugenia Pyriformis Cambess. Leaves. *Chem. Eng. Commun.* **2021**, *208*, 1543–1552. [[CrossRef](#)]
22. Tatiana Nogueira De Deus *Extração e Caracterização de Óleo Do Pequi (Caryocar Brasiliensis Camb.) Para o Uso Sustentável Em Formulações Cosméticas Óleo/Água (o/A)*; Universidade Católica de Goiás: Goiânia, Brazil, 2008.
23. Lopes, R.M.; da Silva, J.P.; Vieira, R.F.; da Silva, D.B.; da Gomes, I.S.; da Agostini-Costa, T.S. Composição de Ácidos Graxos Em Polpa de Frutas Nativas Do Cerrado. *Rev. Bras. Frutic.* **2012**, *34*, 635–640. [[CrossRef](#)]
24. Miranda-Vilela, A.L.; Grisolia, C.K.; Resck, I.S.; Mendonça, M.A. Characterization of the Major Nutritional Components of Caryocar Brasiliense Fruit Pulp by Nmr Spectroscopy. *Quim. Nova* **2009**, *32*, 2310–2313. [[CrossRef](#)]
25. Pessoa, A.S.; Podestá, R.; Block, J.M.; Franceschi, E.; Dariva, C.; Lanza, M. Extraction of Pequi (Caryocar Coriaceum) Pulp Oil Using Subcritical Propane: Determination of Process Yield and Fatty Acid Profile. *J. Supercrit. Fluids* **2015**, *101*, 95–103. [[CrossRef](#)]
26. Rajaei, A.; Barzegar, M.; Yamini, Y. Supercritical Fluid Extraction of Tea Seed Oil and Its Comparison with Solvent Extraction. *Eur. Food Res. Technol.* **2005**, *220*, 401–405. [[CrossRef](#)]
27. Sporning, S.; Bøwadt, S.; Svensmark, B.; Björklund, E. Comprehensive Comparison of Classic Soxhlet Extraction with Soxtec Extraction, Ultrasonication Extraction, Supercritical Fluid Extraction, Microwave Assisted Extraction and Accelerated Solvent Extraction for the Determination of Polychlorinated Biphenyls. *J. Chromatogr. A* **2005**, *1090*, 1–9. [[CrossRef](#)] [[PubMed](#)]
28. Ferrentino, G.; Giampiccolo, S.; Morozova, K.; Haman, N.; Spilimbergo, S.; Scampicchio, M. Supercritical Fluid Extraction of Oils from Apple Seeds: Process Optimization, Chemical Characterization and Comparison with a Conventional Solvent Extraction. *Innov. Food Sci. Emerg. Technol.* **2020**, *64*, 102428. [[CrossRef](#)]
29. Liu, S.; Hu, H.; Yu, Y.; Zhao, J.; Liu, L.; Zhao, S.; Xie, J.; Li, C.; Shen, M. Simultaneous Determination of Tocopherols, Phytosterols, and Squalene in Vegetable Oils by High Performance Liquid Chromatography-Tandem Mass Spectrometry. *Food Anal. Methods* **2021**, *14*, 1567–1576. [[CrossRef](#)]
30. Sipiencie, E.; Mišina, I.; Qian, Y.; Grygier, A.; Sobieszcańska, N.; Sahu, P.K.; Rudzińska, M.; Patel, K.S.; Górnaś, P. Fatty Acid Profile and Squalene, Tocopherol, Carotenoid, Sterol Content of Seven Selected Consumed Legumes. *Plant Foods Hum. Nutr.* **2021**, *76*, 53–59. [[CrossRef](#)]
31. Ahmadkelayeh, S.; Hawboldt, K. Extraction of Lipids and Astaxanthin from Crustacean By-Products: A Review on Supercritical CO<sub>2</sub> Extraction. *Trends Food Sci. Technol.* **2020**, *103*, 94–108. [[CrossRef](#)]
32. Geow, C.H.; Tan, M.C.; Yeap, S.P.; Chin, N.L. A Review on Extraction Techniques and Its Future Applications in Industry. *Eur. J. Lipid Sci. Technol.* **2021**, *123*, 2000302. [[CrossRef](#)]
33. Johner, J.C.F.; Hatami, T.; Zabot, G.L.; Meireles, M.A.A. Kinetic Behavior and Economic Evaluation of Supercritical Fluid Extraction of Oil from Pequi (Caryocar Brasiliense) for Various Grinding Times and Solvent Flow Rates. *J. Supercrit. Fluids* **2018**, *140*, 188–195. [[CrossRef](#)]
34. Johner, J.C.F.; Hatami, T.; Meireles, M.A.A. Developing a Supercritical Fluid Extraction Method Assisted by Cold Pressing for Extraction of Pequi (Caryocar Brasiliense). *J. Supercrit. Fluids* **2018**, *137*, 34–39. [[CrossRef](#)]
35. Garcia, V.A.D.S.; Cabral, V.F.; Zanoelo, É.F.; da Silva, C.; Filho, L.C. Extraction of Mucuna Seed Oil Using Supercritical Carbon Dioxide to Increase the Concentration of L-Dopa in the Defatted Meal. *J. Supercrit. Fluids* **2012**, *69*, 75–81. [[CrossRef](#)]
36. Rogério Favareto *Extração de Compostos Bioativos Utilizando CO<sub>2</sub> Supercrítico de Espécies Do Cerrado*; Instituto Federal de Educação, Ciência e Tecnologia Goiano-Campus Rio Verde: Goiânia, Brazil.
37. Souza, A.T.; Benazzi, T.L.; Grings, M.B.; Cabral, V.; Silva, E.A.; Cardozo-Filho, L.; Ceva Antunes, O.A. Supercritical Extraction Process and Phase Equilibrium of Candeia (Eremanthus Erythropappus) Oil Using Supercritical Carbon Dioxide. *J. Supercrit. Fluids* **2008**, *47*, 182–187. [[CrossRef](#)]
38. Santos Júnior, O.O.; Montanher, P.F.; Bonafé, E.G.; do Prado, I.N.; Maruyama, S.A.; Matsushita, M.; Visentaine, J.V. A Simple, Fast and Efficient Method for Transesterification of Fatty Acids in Foods Assisted by Ultrasound Energy. *J. Braz. Chem. Soc.* **2014**, *25*, 1712–1719. [[CrossRef](#)]
39. de Menezes Rodrigues, G.; Cardozo-Filho, L.; da Silva, C. Pressurized Liquid Extraction of Oil from Soybean Seeds. *Can. J. Chem. Eng.* **2017**, *95*, 2383–2389. [[CrossRef](#)]
40. Du, M.; Ahn, D.U. Simultaneous Analysis of Tocopherols, Cholesterol, and Phytosterols Using Gas Chromatography. *J. Food Sci.* **2002**, *67*, 1696–1700. [[CrossRef](#)]

41. Stat-Ease, I. Design Expert Software Version 7.1.3 2008. Available online: <https://design-expert-trial.updatestar.com/en> (accessed on 28 November 2022).
42. StatSoft, I. Statistica: Data Analysis Software System Version 8.0 2008. Available online: [https://www.scirp.org/\(S\(lz5mqp453edsnp55rrgjt55\)\)/reference/ReferencesPapers.aspx?ReferenceID=1924761](https://www.scirp.org/(S(lz5mqp453edsnp55rrgjt55))/reference/ReferencesPapers.aspx?ReferenceID=1924761) (accessed on 28 November 2022).

**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.