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Fatty acid composition, cardiovascular functionality, thermogravimetric-differential, calorimetric and spectroscopic behavior of pequi oil (*Caryocar villosum* (Alb.) Pers.)

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Abstract

The objective of this investigation was to determine the composition and fatty acids profile of pequi (*Caryocar villosum* (Alb.) Pers.) oil, as well as its functionality in cardiovascular health, thermogravimetric-differential, calorimetric and spectroscopic behavior. The methodology used followed the standards established by the American Oil Chemists Society (AOCS). The results show oil with high nutritional value with predominance of unsaturated fatty acids (70%), especially oleic acid (52.67%) and linoleic acid (15.20%). The oil functionality indexes were expressed as cardiovascular protectors with low atherogenic and thrombogenic indexes (0.38 and 0.75) with a high hypocholesterolemic ratio (2.58). The oil pattern in the spectral bands 2980 to 2870cm^{-1} confirmed the predominance of unsaturated fatty acids with prominent peaks corresponding to double bonded chemical groups bands. The oil behavior under progressive temperature rises in an air atmosphere, supports the potential applications of this fruit by-product, that exhibits high thermal and oxidative stability with reduced mass loss at temperatures (267 °C and 376 °C) above those habitually used in food and industrial uses. These data show that unconventional fruits can increase and diversify the number of ingredients that can be used in food preparations and in the food, pharmaceutical, dermocosmetic, biofuel and other industries.

Keywords: potential; oil; industry.

Practical Application: The Pequi has high lipid content, with potential for application in several industrial segments.

1 Introduction

The great variety of Amazonian fruit species with high macro and micronutrients content potential makes this region a considerable source of food resources and of several additions of raw materials applicable to different industrial sectors. Among them, those with the highest energy rate derived from their lipid constitution, the oil seeds, stand out (Pereira et al., 2019; Santos et al., 2019a, 2019b).

A number of investigations with fruits that stand out in the Brazilian trade balance have been carried out (Costa et al., 2016; Santos et al., 2018a, 2018b, Santos et al., 2019a, 2019b, 2020; Matos et al., 2019; Serra et al., 2019; Teixeira et al., 2019). However, with changes in eating habits, climate change and the search for innovations, spur the need for research with little explored fruits, among which the pequi (*Caryocar villosum* (Alb.) Pers.).

This fruit belongs to the *Caryocaracea* family with 25 species, with three of these found in the Brazilian cerrado. It is popularly known as "pequi", "piqui", "pequia", "almond of thorn", "horse grain" or "Brazil almond". The main use of the fruit is in typical regional dishes and in folk medicine. It has a nutritional and functional composition that shows relevant contents of lipids, proteins, vitamins, minerals and carotenoids. One of the most prominent highlights of this fruit is the extraction of its lipid content, that can be used in food, in the pharmaceutical industry, dermocosmetics, as fuel and lubricant, in addition to applications in folk medicine, in the indication of healing effect, in the treatment of wounds, suggesting a high anti-inflammatory activity (Bertolino et al., 2019; Colombo et al., 2015; Landen et al., 2016; Lorenzo et al., 2018).

Among the constituents of the proximate composition of the pequi, the lipid content stands out; it accounts for most of the high energy value present in this fruit. The fatty acid profile of the pequi oil shows a predominance of unsaturated fatty acids, with emphasis on oleic and palmitic acid (Bertolino et al., 2019; Colombo et al., 2015; Landen et al., 2016; Lorenzo, et al., 2018; Nascimento et al., 2020).

Only a few investigations carried out so far aim to indicate, with experimental and instrumental analyses, applications indications and the potential of products and by-products derived from fruits such as pequi, thus providing scientific data that evidence its action as an ingredient in the diversification of industrial raw materials. Thus, the objective of this investigation was to determine the fatty acid profile composition, cardiovascular health functionality, thermogravimetric-differential, calorimetric and spectroscopic behavior of pequi oil, with the purpose of evidencing the quality and behavior of this oil in the adverse conditions found in cooking and in the industrial practices.

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2 Materials and methods

2.1 Fruits harvest

The fruits of pequi (*Caryocar villosum* (Alb.) Pers.) used in this study were collected from adult trees located in the city of Bragança, PA, Brazil, in 2018, and, as a requirement for the largest number of fruits, after natural falling from the tree. In the "João Murça Pires" herbarium (MG), called Herbarium Amazonicum Musei Paraensis, the trees received the code MG n°. 205226, latitude 01°22'55.8 "S and longitude 46° 43'50.3" W. The fruits were selected according to the stage of maturity in which they are consumed (yellowish-orange color). At harvest, the integrity of the samples was considered satisfactory according to the fruit state of preservation.

2.2 Sample preparation

The samples were transported in low density polyethylene (LDPE) plastic bags and forwarded to the Laboratory of Operations and Separation of the Federal University of Pará, Belém, Brazil, where the fruits were washed in running water, immersed in a solution of chlorinated water at 150 ppm for 5 minutes. The samples were then shipped to the Food Technology Laboratory of the University of São Paulo (USP), Faculty of Pharmaceutical Sciences.

The pulp was mashed in a food processor (ARNO, Brasil where it was stored in plates and frozen at -86 °C in an ultra-freezer (TectalMaq, Brazil), and later lyophilized in the freeze drying equipment (Edwars vacuum, Brazil) for 3 days under 40 mbar pressure, and further packed in LDPE bags, sealed under vacuum to maintain the integrity of the material.

2.3 Oil extraction

The oil was Soxhlet extracted for 8 hours, with the heating mantle temperature varying between 50-55 °C using hexane as a solvent, following AOAC standards (Association of Official Analytical Chemists, 2016).

Infrared spectroscopy analysis

A drop of oil was placed in contact with the germanium crystal for ATT (Attenuance Total Reflectance) reading in the infrared spectrum (model Alpha Bruker, Germany), in the range of 500 to 4000 cm^{-1}.

Differential scanning calorimetry (DSC)

Performed by specialists in DSC-differential scanning instruments, series I (USA) in nitrogen atmosphere, with alumina crucible, at a temperature ranging from 25 to 200 °C.

Thermogravimetric and differential analyses (TG-DTA)

They were carried out in a TA Instrument thermobalance, model Q-500 (New Castle, DE, USA), using an air atmosphere, with a flow rate of 50 mL/min, heating ramp: 10 °C in the temperature range of 600 °C, alumina crucible 5mg ± 0,5.

2.4 Gas chromatography of pequi mesocarp oil

The preparation of fatty acid methyl esters followed the methodology of the international standardization organization ISO 5509 (International Organization for Standardization, 1978). Gas chromatography analysis (Varian 430-GC, Netherlands) with an injection temperature of 250 °C with a ramp of 140 °C for 5 minutes up to 240 °C at a rate of 4 °C/min, with detector at 280 °C using the column (SUPELCO, SPTM - 2560), with fused silica capillary 100 meters long, 0.25 mm in diameter and fused silica with 0.2 μm thick filament. The quantitative composition by area normalization, expressed in percentage by mass, followed the AOCS official method (American Oil Chemists' Society, 2005).

Indices of pequi oil functional quality

The functionality of the lipid fractions of the material analyzed in this investigation was determined by the proportions of fatty acids determined in their relevant lipid profiles, assessed by three composition indexes - Atherogenicity Index (IA), Thrombogenicity Index (TI) defined according to Ulbricht & Southgate (1991), and the hypocholesterolemic and hypercholesterolemic (HH) ratio defined by Santos-Silva et al. (2002) using the following calculations (Equation 1, 2 and 3):

$$I.A = \frac{\left[(C12:0) + (4XC14) + (C16)\right]}{(\Sigma AGMI + \Sigma \omega 6 + \Sigma \omega 3)} \quad (1)$$

$$I.T = \frac{(C14:0 + C16:0 + C18:0)}{\left[(0.5X \Sigma AGMI) + (0,5X \Sigma \omega 6) + ((3X \Sigma \omega 3)) + (\Sigma \omega 3 / (\Sigma \omega 6))\right]} \quad (2)$$

$$H.H = \frac{(C18:1\omega 9 + C18:2\omega 6 + C20:4\omega 6 + C18:3\omega 3 + C20:5\omega 3 + C22:5\omega 3 + C22:6\omega 3)}{(C14:0 + C16:0)} \quad (3)$$

2.4 Statistical analysis

The analyses were performed in triplicate, and the means and standard deviation were calculated with the software Statistica version 11 (StatSoft, 1985) and Microsoft Excel (Microsoft, USA).

3 Results and discussion

3.1 Composition of the fatty material of pequi pulp oil

Table 1 shows the fatty acid composition of pequi pulp oil.

The results of pequi oil showed greater relevance of the following fatty acids: oleic, linoleic, α -linolenic, palmitoleic and gamma - linolenic, with conjugated linoleic acid (CLA), totaling 53.02% of monounsaturated fatty acids and 17.68% of polyunsaturated fatty acids.

In addition to oleic acid, another fatty acid of great prominence was palmitic acid, followed by stearic acid, which, when adding the amounts, in percentage, of the octanoic acids present, a total of 29.15% saturated fatty acids was obtained. The oleic acid content is smaller than in other oil species, as in the fruits of pracaxi, and cupuaçu (averages of 47.3% and 41.6% respectively) yet, close to the fruits of buriti (34.20%) and Brazil nuts (38.78%) (Bezerra et al., 2017; Forero-Doria et al., 2015; Serra et al., 2019).

Table 1. Fatty acid composition of pequi pulp oil.

Fatty acid	%
Octanoic (C8:0)	0.28 ± 0.00
Palmitic (C16:0)	26.50 ± 0.85
Palmitoleic (C16:1n7)	0.35 ± 0.04
Stearic (C18:0)	2.37 ± 0.30
Oleic (C18:1n9c)	52.67 ± 1.30
Linoleic (C18:2n6c)	15.20 ± 1.25
Gammalinolenic (C18:3n6)	0.25 ± 0.00
Alpha linolenic (C18:3n3)	0.46 ± 0.07
Conjugated Linoleic Acid - CLA c18,2 10 trans, 12 cis	1.77 ± 0.33
Arachidic (C20:0)	traces
Saturated (Σ)	29.15
Monounsaturated (Σ)	53.02
Polyunsaturated (Σ)	17.68
TOTAL	100

Data represent mean ± standard deviation.

Table 2. Functional quality index of pequi pulp oil.

Functional quality index	Results
Polyunsaturated/saturated	0.61
Atherogenicity Index	0.38
Thrombogenicity Index	0.75
Hypocholesterolemic and hypercholesterolemic ratio	2.58

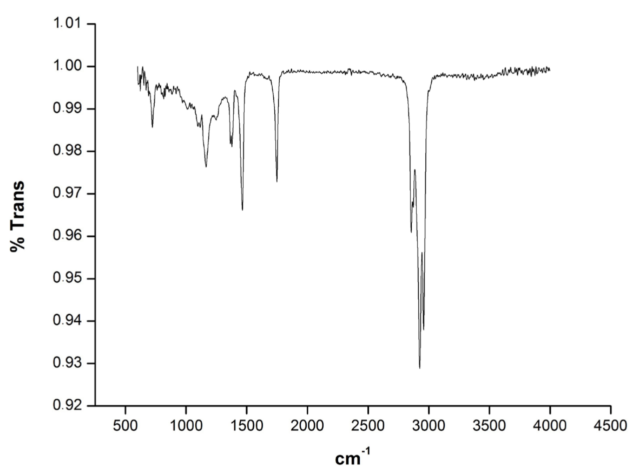

Figure 1. Region of the infrared spectrum of pequi pulp oil.

On the other hand, the predominance of polyunsaturated fatty acids (70.7%) content shows the functional potential of this raw material, that is active in the modulation of pathways that can influence cholesterolemia, with decreased hepatic production of VLDL, precursor of LDL, both by enhancing the catabolic effect of this fatty acid in the peroxisomes, and by interfering in the receptors and by increasing the hepatocytes membranes' liquid, altering the activity of LDL receptors and the quantity of liver receptors (Bezerra et al., 2017 ; Serra et al., 2019; Santos et al., 2020).

Table 2 shows the functional quality indices, calculated from the percentages of saturated, polyunsaturated and monounsaturated fatty acids of pequi oil, obtained from the quantitative data of its fatty acid profile.

In the present study, the relationship obtained meets the requirements of the Department of Health and Social Security (Her Majesty's Stationery Office, 1984) diets, in which a polyunsaturated/saturated ratio greater than 0.45 is considered beneficial and a ratio lower than 0.45, may lead to increased blood cholesterol levels. However, this index is limited when evaluated alone, as it does not take into account the metabolic effects of monounsaturated acids (Torres et al., 2017). Considering this proportion, and the value of monounsaturated fatty acids of 1.82%in this study, it is evidenced that pequi oil is a healthy food source for humans.

The results of the atherogenicity index and of the thrombogenicity index, found in reduced values in the composition of food and in the proportion of diets as a whole, are desirable factors, as they reveal a better nutritional and functional composition of the oil as adjuvant in the prevention of cardiovascular disease risks. In the present study, the two indices were lower than 0.8, positively influencing the reduction of cardiovascular impairment risk if added to usual diets (Pinto et al., 2018; Santos et al., 2020).

The results of the hypocholesterolemic/hypercholesterolemic relationship obtained (2.58) should be evaluated inversely to the atherogenicity and thrombogenicity index. When compared to another oilseed index, the indexes obtained in this investigation were higher than those of pupunha oil, with an index of 0.84. The high values exhibited in this relationship are directly related to cholesterol metabolism as they can promote the formation of high density lipoproteins (HDL). Thus, the higher its value, the more suitable the oil is for human consumption (Santos et al., 2020).

3.2 Infrared analysis of pequi pulp oil

Results of the pequi mesocarp infrared spectrum oil are shown in Figure 1.

In this spectrum, bands ranging from 2980 cm^{-1} to 2870 cm^{-1} were observed in the range of C-H bonds of the hydrocarbon skeleton, characteristic of strong alkanes. The absorption band (H - C and C = O) has a strong characteristic of the double bond functional groups, present in unsaturated fatty acids (Table 1), which can be found around 3000, 1600 and 750 cm^{-1}.

Bands around 1750 cm^{-1} have beem observed, being characteristic of the carbonyl group (C = O), methyl esters, ketones, aldehydes, which are frequent in long chain fatty acids. Bands around 1016 cm^{-1} and 1490 cm^{-1}, characteristic of saturated esters (C-O-C) and bands in the range of 1290 to 1050 cm^{-1}, characteristic of alcohols, esters, ethers, carboxylic acids and fatty acids were also observed. The lowest bands found are about 690 cm^{-1} and can be linked to alkenes (CH), which can be the sequence of aliphatic fatty acid chains (Rai et al., 2016; Santos et al., 2019a, b).

3.3 Thermal analysis of pequi pulp oil

The behavior of the thermogravimetric and differential curves (thermogravimetric and differential analysis - TG/DTA) of the fat material in an atmosphere of synthetic air is shown in Figure 2.

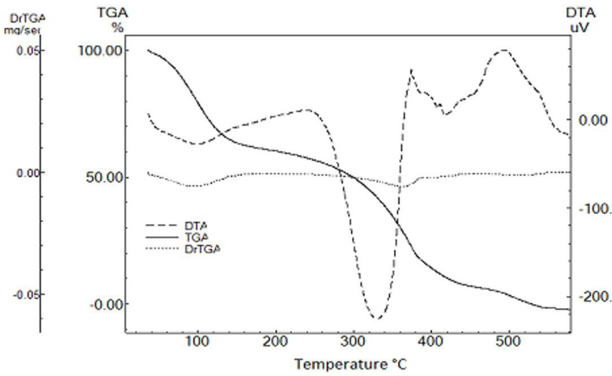

Figure 2. Derivative thermogravimetric (DTG) analysis of pequi pulp oil.

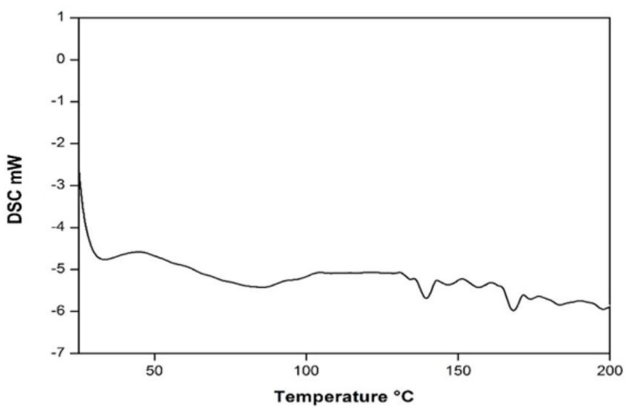

Figure 3. Differential scanning calorimetry (DSC) curve of pequi oil.

In the results presented by the thermogravimetric and differential curves (TG/DTG and DTA) for pequi oil (Figure 2), a mass loss event can be observed, starting at 37 °C and ending at 135 °C. This change is related to the boiling of the water present in this material. The mass loss follows in parallel with the progressive increase in temperature, with a more evident loss in the event occurring between the temperatures of 267 °C and 376 °C, with the loss of 40.7% of the oil mass; this behavior continues until the threshold of destruction of the sample is reached at around 529 °C.

The derived thermogravimetry (DTG) of the fat material was plotted to accentuate the most accurate point of mass loss, being more evident from its first mathematical derivative. The behavior was evident with projections of three peaks, the second one being of high intensity. The main peak corresponds to a temperature close to 279 °C and 363 °C, and the most evident peak point for mass loss is around 330 °C, presenting ΔH of -15 kJ/g, and therefore characterized as endothermic peak. The last event of lesser intensity was found at the temperature range of 500 °C, similar to that presented in the thermogravimetric curve.

Ramos et al. (2015) have shown in the results of the TG/DTG analysis that pequi oil is stable at 265 °C, the decomposition of the compound occurred in two consecutive stages of mass loss between 265-371 °C and 371-498 °C with mass loss of 84.36% and 15.63%, respectively.

When compared to the behavior of other oilseeds oils, such as Sapucaia and Pracaxi (Santos et al., 2019a, b), the TG/DrTGA and DTA curves in air flow in Sapucaia demonstrated a behavior of more intense mass loss after the temperature of 200 °C, continuing until the complete reduction of the sample close to the temperature of 650 °C. On the other hand, the curves of Pracaxi showed a behavior similar to those of the pequi oil, with mass loss close to 100 °C, continuing intensely until total reduction close to 400 °C.

These comparisons show a variation in terms of thermal stability, specific for each oil constitution of these fruits. These behaviors are related to the deteriorating action of oxidative elements with plenty oxygen in the analysis atmosphere and the progressive rise in temperature. However, material stability is observed at temperatures considered high, when compared to kitchen frying temperatures and industrial processing. Figure 3 shows the chart of the exploratory differential scanning calorimetry analysis (DSC) of pequi pulp oil.

The oil extracted from the fruit mesocarp was heated to a temperature of up to 200 °C, to assess its stability at high temperatures, depending on the applications in which it would be used; three endothermic curves were plotted, the first due to the remaining water evaporation, between 50 °C and 95 °C, with change in enthalpy ΔH = -7.88 J/g; the second presented ΔH = -34.85 J/g and the third peak with ΔH = -1.84 J/g corresponds to the volatilization or decomposition of compounds, showing that the oil is stable at temperatures up to 200 °C; thus it can be applied as frying and roasting material and with no risk of degradation of the lipid material.

The structuring and knowledge of the best parameter for the melting point, solidification, phase changes and other behaviors caused to the oil by the heat flow are essential data for the research and product development segment. These databases allow a reduction in testing and elaboration time, adjustment of parameters, savings in all aspects, as it occurs in different applications and design of industrial products, such as the production of edible oils, lubricants, biodiesel among others. These information bases can be expressed and ratified by the DSC curves (Santos et al., 2012, 2019b).

4 Conclusion

In the pequi oil, the main fatty acids included palmitic, oleic and linoleic fatty acids, which influenced the functional quality indexes of the oil, demonstrating that it has low atherogenic capacity as the polyunsaturated/saturated ratio is in the range indicated as beneficial to health, and the hypocholesterolemic/hypercholesterolemic index is also adequate.

In the spectroscopic profile, the most prominent bands indicate the presence of hydrocarbon bonds, characteristic of double bond functional groups, present in unsaturated fatty acids. Chemical groups frequently found in long chain fatty acids of saturated esters were observed. These findings also confirm the oil nutritional potential considering the fatty acid profile and functional indices.

The differential thermogravimetric curves indicated that the pequi oil maintains its thermal stability at temperatures above those commonly used, demonstrating that the pequi fruit pulp and its by-product, the oil, can be applied in culinary preparations and in the food industry, such as baking and confectionery among others, without major mass losses and relevant oxidative changes.

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