## Physicochemical and spectroscopical investigation of Pequi (*Caryocar coriaceum* Wittm.) pulp oil

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#### RESUMEN

# Investigación físico-química y espectroscópica del aceite de la pulpa de Pequi (Caryocar coriaceum Wittm.)

El presente trabajo describe la caracterización físico-química del aceite de la pulpa de Caryocar coriaceum (Wittm), "pequi". La composición química fue evaluada por GLC, que mostró el siguiente contenido de ácido graso: oleico (55,79%), palmítico (34,18%), heptadecenoico (5,86%), linoleico (1,80%), esteárico (1,73%), eicosenoico (0,37%) y palmitoleico (0,27%). Los resultados de la espectroscopia vibracional fueron típicos de un aceite comestible, y están en concordancia con el alto contenido de ácido graso insaturado. Las propiedades físicas tales como el contenido de agua, la acidez, índice de peróxido, índice de saponificación, densidad relativa, viscosidad e índice de refracción se presentan por primera vez para esta especie. Los frutos de pequi tienen un alto valor nutricional, y son ricos en proteínas y vitaminas; además su composición incluye ácidos grasos esenciales. El aceite de la fruta tiene un uso popular debido a su actividad antioxidante y antiinflamatoria. Hay un gran interés en extender el uso de este producto natural aprovechando los valores sociales, económicos y terapéuticos de sus derivados.

PALABRAS CLAVE: Análisis vibracional – Caracterización fisicoquímica – Caryocar coriaceum – Composición del aceite – Pequi.

#### SUMMARY

# Physicochemical and spectroscopical investigation of Pequi (*Caryocar coriaceum* Wittm.) pulp oil.

This work describes the physicochemical characterization of the fruit pulp oil of *Caryocar coriaceum* (Wittm), "pequi." The chemical composition was assessed by GLC, which demonstrated the following fatty acid contents: oleic (55.79%), palmitic (34.18%), heptadecenoic (5.86%), linoleic (1.80%), stearic (1.73%), eicosenoic (0.37%) and palmitoleic (0.27%). The vibrational spectroscopy results were typical of an edible oil, and are in accordance with the high unsaturated fatty acid content. Physical properties such as water content, acidity, peroxide index, saponification index, relative density, viscosity and refraction index are reported for the first time for this species. Pequi fruits have a high nutritional value, and are rich in protein and vitamins. In addition, their composition includes essential fatty acids, which accounts for the popular use of the fruit oil for antioxidant and antiinflammatory activity. There is great interest in extending the use of this natural product by exploiting the combined social, economic and therapeutic values of its derivatives.

KEY-WORDS: Caryocar coriaceum – Oil composition – Pequi – Physicochemical characterization – Vibrational analysis.

#### **1. INTRODUCTION**

The *Caryocar* genus is widespread in South America and comprises more than twenty species. In Brazil, considering an area including the states of Amazonas, Pará, Maranhão, Piauí, Goiás, Bahia, Ceará, São Paulo and Minas Gerais, the main species are *Caryocar brasiliense* (Cambess.) and *Caryocar coriaceum* (Wittm.) (Peixoto, 1973; Silva, 1939).

*Caryocar coriaceum* (Wittm.), popularly known as "piqui" or "pequi," is the only representative of the family Caryocaraceae in Ceará State, and is one of the most noticeable agrestal plants around Araripe (Ducke, 1959). It has drupaceous fruits that are orange-sized, globe-shaped, with greenyellowish peel and a large and fleshy nut; a whitish, butyraceous mesocarp is protected by a ligneous endocarp possessing thin and sharp erect spines (Almeida, 1998; Almeida *et al.*, 1998).

The fruit pulp is an excellent nutritional source, and is largely used by the population of the Cariri region, in Ceará State, and of neighboring cities of Pernambuco and Piauí states (Almeida, 1998; Braga, 1960). Besides being a nutritional source, the handmade oil extracted from the pulp or the nut is widely employed to treat respiratory tract infections, muscle pain, and chronic arthritis (Souza, 1997). It also plays a part in popular medicine preparations used by farmers for wounds, contusions, peelings and swelling in animals (Braga, 1960).

The diet of the Brazilian population is extremely lacking in lipids, especially in the North and Northeast regions of the country. Such low calorie diets, together with the lack of the main groups of nutrients, result in chronic conditions that worsen health (Castro *et al.*, 2009). The abundance of native plants of the species *C. coriaceum* (which is a rich source of essential fatty acids) on the Araripe plateau, and its observed therapeutic potential, suggest that it is a promising species for food and pharmaceutical industries (Bauer *et al.*, 1966; Cavalcante *et al.*, 2000). However, the scarcity of studies regarding this species is evident, and a thorough study of the physicochemical and microbiological properties of the pequi fruit pulp is justified.

In this work, we report on the physicochemical and vibrational spectroscopy studies of the pulp oil of *C. coriaceum*, in order to present an outlook of the potential of this fruit as a source of unsaturated fat. Its oil composition is compared to previous reports and to other species, namely *Caryocar brasiliensis*.

### 2. MATERIALS AND METHODS

#### 2.1. Plant material and oil extraction

Ripe fruits of *C. coriaceum* were collected on the Araripe plateau (line D of the National Araripe Forest) in the city of Crato, Ceará State, Brazil, and the exocarp (peel) was separated from the inner mesocarp plus endocarp (oily pulp with drupe). The oil was extracted from 752g of pequi pulp, carried out at 60 °C with hexane by the continuous technique in a Soxhlet extractor for 8 h. After that, the hexane fraction was dried with Na<sub>2</sub>SO<sub>4</sub> and the solvent was concentrated in a rotary evaporator under reduced pressure.

#### 2.2. Fatty acid analysis

Fatty acids were determined indirectly using their corresponding methyl esters. The oil (0.2 g) was saponified for 30 min under reflux with potassium hydroxide solution in methanol, following the method described by Hartman and Lago (1973). After adequate treatment and pH adjustment, the free fatty acids were methylated with methanol by acid catalysis in order to yield the respective methyl esters. The analysis of volatile constituents was carried out in a Hewlett-Packard GC/MS, model 5971, using the non-polar fused silica column DB-1 (30 m  $\times$  0.25 mm i.d., 0.25  $\mu$ m film), eluted with helium gas at 8 mL/min and with split mode. Injector and detector temperatures were set to 250 °C and 200 °C, respectively. The column temperature was programmed from 35 °C to 180 °C at 4 °C/ min, and then from 180 °C to 250 °C at 10 °C/ min. Mass spectra were recorded from 30 to 450 m/z, with an electron beam energy of 70 eV. The

individual components were identified by computer MS library searches, using retention indexes as a pre-selection routine, and visual inspection of the mass spectra from the literature for confirmation (Alencar *et al.*, 1984, 1990), as well as by visually comparing standard fragmentation to that reported in the literature (Adams, 2001; Stenhagen *et al.*, 1974).

#### 2.3. Physicochemical characterization

Physicochemical analysis was performed on the raw oil with regard to the following parameters: water content, acidity (as oleic acid), relative density, refraction index at 40 °C, peroxide index, and saponification index (AOCS, 1990; Lutz, 1985). Kinematic viscosity was measured at 40 °C in a Schott AVS 350 viscosimeter, with an AVSIS support coupled to a Schott CT-52 water-bath, capillary no. 520 with a 23-mm diameter, using Lawal methodology adapted to this equipment and sample material (Qian and Lawal, 2006). Viscosity was expressed in mm<sup>2</sup>/s according to the corresponding equation for this capillary.

#### 2.4. Spectroscopic analysis

Vibrational spectra of the lipid fraction of pequi pulp were measured using FT-Raman and FT-IR spectroscopies. Raman spectrum in the region 20-4000 cm<sup>-1</sup> was measured on a Bruker RFS/100, pumped by a Nd:YAG laser emitting at 1064 nm with output power of 150 mW. Spectral resolution was 4 cm<sup>-1</sup>, and the signal/noise ratio was enhanced by averaging 60 scans. FT-IR spectrum was obtained on a Bruker EQUINOX 55, using a spectral resolution of 4 cm<sup>-1</sup>. The signal/ noise ratio was enhanced by averaging 60 scans from the oil spread on a KBr pellet, and 30 scans for background signal, in the range of 3500 cm<sup>-1</sup> to 400 cm<sup>-1</sup>.

### 3. RESULTS AND DISCUSSION

On average, 40 mL of oil were obtained from the pulp (752g). After the identification of the methyl esters, it was possible to assess the percent chemical composition of major fatty acids from the lipid fraction of *C. coriaceum* pulp. The unsaturated fatty acids predominate over the saturated ones, with 64.09% of the former and 35.91% of the latter. This species, as well as others from the same genus which are spread over other regions (e.g., *C. brasiliense*), shows a variability in fatty acid content (see Table 1), which has been attributed mainly to geographic location and climate conditions.

The following fatty acids were identified: oleic acid, palmitic acid, heptadecenoic acid, linoleic acid, and stearic acid. The main fatty acid found in the pulp oil was oleic acid, followed by palmitic acid, as seen in *C. brasiliense* (Brasil, 1985; Facioli

Fatty Acid	Content / %			
	C.coriaceumª	C.coriaceum <sup>b</sup>	C.brasiliense°	C.brasiliense <sup>d</sup>
Palmitic (C16:0)	34.18	31.65	34.4	40.2
Palmitoleic (C16:1)	0.27	_	2.1	1.4
Estearic (C18:0)	1.73	1.83	1.8	2.3
Oleic (C18:1)	55.79	64.21	57.4	53.9
Linoleic (C18:2)	1.80	2.29	2.8	1.5
Linolenic (C18:3)	_	_	1.0	0.7
Araquidic (C20:0)	_	_	_	0.2
Heptadecenoic (C17:1)	5.86	_	_	_
Eicosenoic(C20:1)	0.37	_	_	_

 Table 1

 Methyl esters from C. coriaceum pulp compared to those from C. brasiliense

<sup>a</sup>Present research; <sup>b</sup>Figueiredo et al., 1989; <sup>a</sup>Brasil, 1985; <sup>d</sup>Facioli et al., 1998.

and Goncalves, 1998). Previous work reported a high oleic acid content (64.21%) and absence of heptadecenoic acid, which was found to be 5.86% in our investigation (Figueiredo *et al.*, 1989).

Fatty acids with an odd number of carbons, like heptadecenoic acid, are unusual in nature due to their synthesis making use of two acetyl units with two atoms. However, some researchers report these odd fatty acids in plants and bacteria. Heptadecenoic acid (17:1) has been recognized as a minor constituent of ruminant milk and intramuscular fat, mainly as the isomeric form 17:1 *cis*-9 (Alves *et al.*, 2006). However, the origin of this fatty acid is not clear and it has been suggested that it could be an endogenous product of  $\Delta^9$ -desaturation of heptadecanoic acid, forming the isomer cited before (Fievez *et al.*, 2003).

The protein content in pequi pulp is greater than that of rice and potato (Ribeiro, 1999; Ribeiro, 2000). Moreover, the oil has a high energy value of 3896 kJ/100 g (Peixoto, 1973). Mineral contents previously reported in the literature were: calcium, 122 mg/100 g; iron, 3.0 mg/100 g; and phosphorus, 49.05 mg/100 g (Lima, 1980). All these properties make pequi a fruit of considerable nutritional importance.

Physicochemical properties (means and standard deviations) of the oil from *C. coriaceum* pulp are presented in Table 2, and compared to results from *C. brasiliense*. The results of the physicochemical characterization demonstrate that the means differ little between the two species, and from the standards set by A.O.C.S. Yet, it should be noted that experimental precision, given by the standard variation, is within acceptable limits.

Water content from *C. coriaceum* is approximately 6% lower than that found in *C. brasiliense*, suggesting a slightly better chemical stability and quality (Cecchi, 2003). Stability against neutralization is also reflected in the low value obtained for the sample acidity. The peroxide index was also found to be lower for *C. coriaceum*, by about 11 %, indicating a higher resistance towards oxidation.

The saponification index lies above the limits set by Brazilian regulatory agencies, which ranges from 245 to 256 mg KOH/g of fat. The British standard, however, states that a first-class quality oil must have a saponification index between 177 and 187 mg KOH/g (Freire, 2001), but these limits refer to refined oils, which is not the case for the sample considered in this research.

Unfortunately, to the best of our knowledge, the density of the oil from *C. brasiliense* is not reported in the literature, but the differences in refraction indexes could be used to distinguish oils between these species. Viscosity values, besides being equal within experimental error, may also vary due to composition changes, as a result of varying geographic location, season, and climate conditions.

Vibrational spectroscopy techniques, such as FT-Raman and FT-IR, are valuable tools to characterize oils, mainly due to the ease of sample preparation and speed of results. They have been extensively used for quantitative and qualitative analyses of oils and fats (Baeten *et al.*, 1998; Guillen and Cabo, 1997; van de Voort *et al.*, 2001). Oils from different sources (vegetal and animal) may be distinguished and information on unsaturated bond structure may be obtained (Baeten *et al.*, 1998; Yang *et al.*, 2005).

The FT-Raman spectrum from the lipid fraction of pequi pulp is typical of an edible oil and is shown in Figure 1. Oil characterization is usually done considering five regions of the spectrum (Baeten *et al.*, 1998): A)  $3100 - 2805 \text{ cm}^{-1}$ ; B) 1770 - $1615 \text{ cm}^{-1}$ ; C)  $1500 - 1420 \text{ cm}^{-1}$ ; D)  $1345 - 1230 \text{ cm}^{-1}$ ; and, E) $1150 - 850 \text{ cm}^{-1}$ .

In the first region, many bands are observed, as occurs with several organic materials. The spectral region corresponding to C – H stretching vibrations includes the most intense bands of the Raman spectrum (Almeida *et al.*, 2006, Lima *et al.*, 2005). The peak observed at 3007 cm<sup>-1</sup> was assigned to the C--H stretching vibration of the olefin moiety (=C – H). The peaks observed at 2964 cm<sup>-1</sup> and 2894 cm<sup>-1</sup> were assigned to C--H asymmetric stretching vibration and C--H symmetric stretching vibrations of CH<sub>2</sub> were observed as peaks at 2929 cm<sup>-1</sup> (asymmetric) and at 2851 cm<sup>-1</sup> (symmetric).

to C. brasiliense					
C. coriaceum	C. brasiliense <sup>a</sup>				
1.145 ± 0.01	$1.216 \pm 0.03$				
$0.68 \pm 0.02$	NA				
$2.65 \pm 0.09$	$2.98 \pm 0.70$				
266.83 ± 1.24	268.16 ± 0.92				
$0.9082 \pm 0.0014$	NA				
42.89 ± 0.51	$43.52 \pm 0.72$				
1.4619 ± 0.0002	$1.4640 \pm 0.0004$				
	C. coriaceum $1.145 \pm 0.01$ $0.68 \pm 0.02$ $2.65 \pm 0.09$ $266.83 \pm 1.24$ $0.9082 \pm 0.0014$ $42.89 \pm 0.51$				

 Table 2

 Physicochemical properties of the oil from C. coriaceum pulp compared to C. brasiliense

<sup>a</sup>Brandão *et al.*, 2006, NA-value not available.

In the B region, we observed a peak at 1747 cm<sup>-1</sup> which was assigned to an ester C=O stretching vibration, and a band at 1654 cm<sup>-1</sup> which was assigned to a non-conjugated *cis* C=C stretching vibration. In the C region, a band was observed at 1438 cm<sup>-1</sup>, and was regarded as a bending vibration of the CH<sub>2</sub> unit.

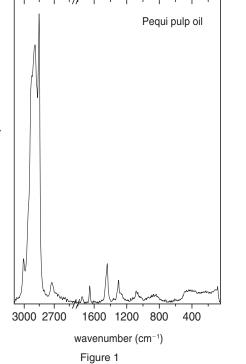
The fourth region considered shows a peak at  $1301 \text{ cm}^{-1}$ , corresponding to a  $\text{CH}_2$  in phase twisting vibration and another at  $1270 \text{ cm}^{-1}$  which was assigned to a *cis*, non-conjugated ==C-H bending vibration. There is also a weak peak at  $1256 \text{ cm}^{-1}$ , which is indicative of the presence of low-saturated fatty acids (or short-chain fatty acids). This is in accordance with the chromatography results.

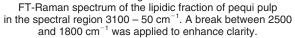
The peaks at 1654 cm<sup>-1</sup> and 1270 cm<sup>-1</sup> together indicate that the sample consists of unsaturated,

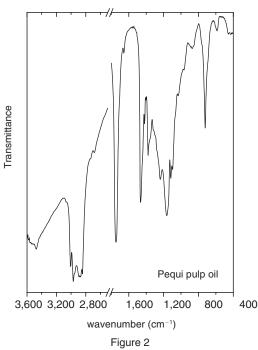
non-conjugated (mono-unsaturated) fatty acids found in the *cis* conformation. Indeed, our sample showed a low content of linoleic acid (doubly unsaturated), only 1.8%.

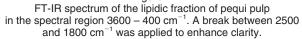
In the E region, we observed bands assigned to the carbon chain C - C and also C - O stretching vibrations. As these are common to all fatty acids, they were not considered in this study.

The FT-IR spectrum is complementary to the FT-Raman measurements, and is shown in Figure 2. A band associated with the O – H stretching vibration is observed at 3465 cm<sup>-1</sup>. The bands appearing between 2800 and 3000 cm<sup>-1</sup> are associated with C – H stretching vibrations, as in the FT-Raman scattering measurements. The strong peak near 1740 cm<sup>-1</sup> can be associated with a C=O stretching vibration. There is a strong peak observed at 1239 cm<sup>-1</sup>, which remained unassigned. It is interesting to note that









ntensity

some vibrations are observed only in the Raman spectrum; this is true for the =C–H bending vibration that is seen at 1270 cm<sup>-1</sup> and the CH<sub>2</sub> twisting vibration that appears as a band at 1301 cm<sup>-1</sup>.

#### 4. CONCLUSIONS

In conclusion, the oil from pequi pulp was studied by a variety of techniques and its composition and properties were determined. These results are unparalleled in the literature for the species *C. coriaceum* and are a valuable source of data for characterizing the oil. Its composition was determined by GLC, and spectroscopic data are in accordance with the analysis. The observed properties characterize pequi oil as a valuable nutritional source of unsaturated fatty acids, with a high energy content.

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