Composition of Lipids of Piqui (*Caryocar coriaceum* Wittm.) Seed and Pulp Oil

Heidrun Dresen*, R. B. N. Prasad, and Paul-Gerhard Gülz

Botanisches Institut der Universität zu Köln, Gyrhofstraße 15, D-5000 Köln 41, Bundesrepublik Deutschland

* Botanisches Institut der Universität/Gesamthochschule Essen, Fachbereich 9, Postfach 103764, D-4300 Essen 1, Bundesrepublik Deutschland

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The lipid composition of Piqui *(Caryocar coriaceum)* seed oil and pulp oil was analyzed and found to contain triacylglycerols (95.1/95.3%) as major components followed by free fatty acids (1.7/1.6%), diacylglycerols (1.6/1.5%), squalene (0.3/0.3%) and monoacylglycerols (0.1/0.1%). Phospholipids were found only in seed oil (0.2%). They were identified as phosphatidylethanolamine and phosphatidylinositol. The sterol fraction (0.1/0.1%) contained stigmasterol and β-sitosterol. In seed oil triacylglycerols the C-53 molecular species were dominated (52.8%) followed by C-55 (37.7%), C-57 (6.9%) and C-51 (2.6%) in minor quantities. In pulp oil triacylglycerols C-55 (51.7%) was predominant followed by C-53 (30.6%) and C-57 (17.7%). Palmitic (16:0) and oleic (18:1) acids were always the major fatty acids in both oils. In seed oil their quantities were nearly the same, whereas in pulp oil oleic acid was predominant.

Introduction

Caryocar coriaceum Wittm. (Caryocaraceae), popularly known as Piqui, is a typical tree of the Cerrado vegetation in North-East Brazil [1]. The tree grows up to 8 to 12 m and yields fruits with a size of 7 to 8 cm in diameter [2]. The fruit contains two types of oil, one from the pulp, the other from the seed. The pulp (mesocarp) surrounds the woody endocarp kernel as a 2 to 3 mm mighty fleshy layer which contains 70% of oil [2]. The hard endocarp kernel includes about 2 cm long kidney-shaped seeds. In the rural households of North-East Brazil, the oil from the Piqui fruit is well known for its medicinal properties [3]. The local industry produces edible oil from the Piqui fruit. However, little information is known about lipid composition of the *Caryocar coriaceum* seed and pulp oils. Therefore the present study describes the composition of the individual lipid classes namely triacylglycerols (TG), diacylglycerols (DG), monoacylglycerols (MG), free fatty acids (FFA), squalene (SQ), phospholipids (PL), and sterols (SL), including the fatty acid composition of acyl lipids.

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Materials and Methods

Piqui fruits were collected in the Cerrado vegetation of Piaui (Brazil) in 1988. Seeds were removed manually from the drupes. The pulp oil was obtained from fruits by traditional extraction methods of the region. Dry weight of seeds has been determined by heating them in an oven at 110 °C for 3 h. The seeds (50 g) were homogenized with an Ultra-Turrax and extracted with chloroform/methanol (2:1). The lipid extracts from seed and pulp oil were separated into different fractions by silica gel column chromatography. Elution was done with pentane, chloroform, acetone and methanol. Pentane eluted SQ, chloroform eluted TG, the acetone fraction contained DG, MG, FFA and SL, and phospholipids were obtained in the methanol fraction. The lipid classes were identified by TLC with solvent system I (hexane/diethyl ether/acetic acid 60:40:1) and compared with authentic samples (Sigma, Deisenhofen). All the lipid classes were visualized with iodine vapour. The presence of SL and SQ was confirmed by carbazole spray [4] after TLC in solvent II (dichloromethane/ethyl acetate 24:1). MG, DG and FFA were separated by preparative TLC from acetone eluate with solvent I. They were estimated quantitatively using methyl heptadecanoate (17:0) as an internal standard [5]. The phospholipids from methanol fraction were identified and separated as described for *Fagus* seed oil [6].

Methyl esters of fatty acids were obtained by refluxing the sample with 2 N HCl/methanol for 3 h.

GC was carried out with a Hewlett-Packard 5710 unit fitted with a FID and an integrator 3380 S. Acylglycerols and sterols were analyzed on a capillary column OV 1 (10 m) and fatty acid methyl esters on FFAP (10 m) column. The column temperature was programmed between 150–340 °C as required. GC-MS detection of sterols was done by Finnigan MAT 4510, 70 eV, EI, with fused silica capillary column 15 m DB-1.

Results and Discussion

a) Piqui seed oil

The oil from the seeds of *Caryocar coriaceum* was colourless and without odour. The content of total lipids was 64.4% of the Piqui seed based on dry weight. The major component of the oil was TG with 95.1%, followed by FFA (1.7%), DG (1.6%), SQ (0.3%), PL (0.2%), SL (0.1%), and MG (0.1%) (Table I).

Triacylglycerols (95.1%) were obtained in pure form in the chloroform fraction. The analysis of fatty acid composition revealed oleic (18:1) and palmitic (16:0) acids as predominant components with 48.4% and 45.2%, respectively. Linoleic (18:2), stearic (18:0) and palmitoleic (16:1) acids followed in smaller quantities (Table II).

The molecular species of TG were analyzed by GC on the basis of chain length. The retention times of the molecular species of different TG were determined using authentic samples. The major molecular species of the Piqui seed oil was C-53 (52.8%) with di- C-16 + mono- C-18. Di- C-18 + mono- C-16 (C-55) was found in amounts of 37.7%. Tri- C-18 (C-57) and tri- C-16 (C-51) were also present in minor quantities of 6.9 and 2.6%, respectively (Table III). Analyses of hydrogenation products of the TG resulted in a similar composition of molecular species confirming the above values.

Table II. Fatty acid composition (peak area %) of the acyl lipids of *Caryocar coriaceum* seed oil.

Carbon No.	TG	DG	MG	FFA	PE	PI
16:0	45.2	28.7	43.9	28.3	34.0	50.2
16:1	1.0	0.6	+	0.7	–	–
18:0	1.5	1.3	10.0	1.5	13.6	13.0
18:1	48.4	62.4	43.5	63.4	47.9	33.0
18:2	3.9	7.0	2.6	6.1	4.5	3.8

Table III. Composition of triacylglycerol molecular species of *Caryocar coriaceum* seed and pulp oil (peak area %).

Carbon No.	Molecular species	% of TG Seed	% of TG Pulp
51	16, 16, 16	2.6	+
53	18, 16, 16 / 16, 18, 16	52.8	30.6
55	16, 18, 18 / 18, 16, 18	37.7	51.7
57	18, 18, 18	6.9	17.7

+ = Traces < 0.1.

Table I. Composition and yield of lipids of *Caryocar coriaceum* seed and pulp oil.

	Seed [mg]	Seed % of oil	Pulp [mg]	Pulp % of oil
Triacylglycerols (TG)	9305	95.1	5482	95.3
Free fatty acids (FFA)	166	1.7	92	1.6
Diacylglycerols (DG)	157	1.6	86	1.5
Squalene (SQ)	29	0.3	17	0.3
Phospholipids (PL)	20	0.2	–	–
Sterols (SL)	10	0.1	6	0.1
Monoacylglycerols (MG)	10	0.1	6	0.1
Unidentified and lost on column	88	0.9	64	1.1
	9785		5753	

Squalene was separated by silica gel column chromatography by eluting with pentane and found to be 0.3% of seed oil. The identification was done by TLC in solvent I (R_f1, 0.7), GC and GC-MS by comparison with authentic samples. Squalene is also reported in some plant oils like olive oil [7].

The acetone eluate was found to consist of MG, DG, FFA and SL which were identified by TLC using solvent I. MG, DG, and FFA were separated into individual classes by preparative TLC from acetone eluate using solvent I and estimated quantitatively with methyl heptadecanoate as an internal standard. They represented 0.1, 1.6 and 1.7%, respectively. Palmitic (16:0) and oleic (18:1) acids were predominant in MG, followed by stearic (18:0) and linoleic (18:2) acids. Apart from an increased amount of stearic acid (10%) this composition was similar to TG. DG and FFA also revealed palmitic and oleic acids as major components but in different quantities than in MG and TG (Table II).

Sterols (R_f2, 0.25) were detected by positive color reaction with carbazole after developing with solvent II. They were present in 0.1% of the oil. Identification of stigmasterol and β-sitosterol was done by GC and GC-MS. They showed identical behaviour to those of sterols found in *Citrus halimii* [8] and also in beech seed oil [6]. The contents were found to be 71% of stigmasterol and 29% of β-sitosterol based on peak area %.

The methanol fraction was found to contain PL (0.2% of seed oil). The presence of phosphatidylethanolamine (PE) and phosphatidylinositol (PI) was clearly identified by TLC with two different solvent systems [6] along with authentic samples and typical response to ninhydrin [9] and sodium metaperiodate-benzidine [10] spray reagents. Phosphatidylcholine (PC) was found only in traces with undetectable amounts. The composition of PE and PI was found to be 28% and 72% respectively, based on a colorimetric estimation of phosphorus according to Bartlett [11]. The content of PL in Piqui seed is similar to that of *Simmondsia chinensis* seed oil [12], although PL are present in higher amounts in most of the seed oils [13, 14].

Oleic and palmitic acids were found to be the major fatty acids of PE, whereas palmitic acid was significantly predominant in PI followed by oleic and stearic acids (Table II).

PC is present in most seed oils [15, 16] but could not be isolated from Piqui seed oil. PC has been reported in traces by Prasad and Gülz [6] in beech *(Fagus sylvatica)* seed oil, too.

b) Piqui pulp oil

The oil from Piqui pulp was analyzed following the same methods as described for seed oil. Major component of pulp oil was TG with 95.3% (Table I). Analyses of fatty acid composition revealed oleic acid (65.6%) as predominant, followed by palmitic acid (32.2%). Linoleic and palmitoleic acids appeared only in small quantities, stearic acid was found in traces (Table IV).

Table IV. Fatty acid composition (peak area %) of the acyl lipids of *Caryocar coriaceum* pulp oil.

Carbon No.	TG	DG	MG	FFA
16:0	32.2	24.3	28.3	20.1
16:1	0.4	+	+	+
18:0	+	1.2	6.2	1.1
18:1	65.6	73.3	61.6	77.7
18:2	1.8	1.2	3.9	1.1

Molecular species of the type di- C-18 + mono-C-16 (C-55) was predominant (51.7%) in pulp oil TG, whereas di- C-16 + mono- C-18 (C-53) was the major species in seed oil TG. The species consisting of tri- C-18 (C-57) were found also in higher concentrations than in seed oil TG. The more content of C-55 and C-57 in pulp oil than in seed oil is because of the higher concentration of oleic acid in the former oil. Tri- C-16 appeared only in traces (Table III).

As in seed oil, the pentane fraction of pulp oil resulted in the isolation of squalene (0.3%).

The acetone fraction was found to contain DG, MG, FFA and SL in quantities of 1.5, 0.1, 1.6 and 0.1%, respectively (Table I). Fatty acid compositions of DG, MG and FFA were similar to TG with oleic acid as predominant, followed by palmitic acid (Table IV).

The sterol fraction of the pulp oil was analyzed as described in seed oil and found to contain stigmasterol (62.9%) and β-sitosterol (37.1%), which is similar to that of seed oil.

In the methanol fraction of the pulp oil phospholipids were not detectable.

The lipid composition of Piqui seed and pulp oil is almost similar. Beside TG, DG, MG and FFA, both types of oil from the Piqui fruit contain squalene and

the sterols (stigmasterol and β-sitosterol) in comparable quantities. However, phospholipids were found only in seed oil.

The fatty acids of the TG showed a specific composition of the two Piqui oils. Palmitic and oleic acids were the predominant fatty acids in Piqui seed oil. Their quantities were found to be nearly equal (45.2 and 48.4%). This fatty acid composition is similar to that of palm oil [17]. In pulp oil TG, oleic acid was dominating with more than 65%. Similar high amounts of oleic acid were also found for DG, MG and FFA of the pulp oil.

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