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FATTY ACID COMPOSITION OF FRUITS OF TWO FORMS OF *Serenoa repens*

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*Extracts of saw palmetto (*Serenoa repens*) fruits are used widely in the treatment of benign prostatic hyperplasia (BPH). This study presents an analysis of the fatty acid content of saw palmetto fruits from both the yellow-green and the less-distributed waxy blue-green forms growing in the Rockdale Pineland Preserve in Miami-Dade County, Florida. Extracts of the fruits were analyzed by GC and GC/MS both before and after treatment with diazomethane. All the samples had a similar composition dominated by oleic acid (40%) and lauric acid (22%), with minor amounts of C_6-C_{18} free fatty acids and their naturally occurring methyl and ethyl esters. Fatty acid methyl and ethyl esters were quantified for the first time. Neither qualitatively nor quantitatively significant differences could be found in the fatty acid composition of the fruits from yellow-green and blue-green plants.*

Keywords: saw palmetto, *Serenoa repens*, *Sabal serrulata*, Arecaceae, benign prostatic hyperplasia, free fatty acids, fatty acid methyl esters, fatty acid ethyl esters.

Serenoa repens (W. Bartram) Small [synonym: *Sabal serrulata* (Michx.) Nutt. ex. Schult. & Schult. f.; Arecaceae], commonly called saw palmetto, is a dwarf palm that grows in the southern regions of North America [1]. Two forms of the plant occur in Florida, the more common yellow-green leaf form found throughout the state, and the blue-green leaf form restricted to coastal communities along Florida′s Atlantic coast [2]. *In vitro* studies have shown that the extracts contain noncompetitive inhibitors of the enzyme testosterone 5α-reductase and that free fatty acids, particularly lauric, oleic, myristic, and palmitic acids, may be the active agents of saw palmetto fruits [3].

Previous studies showed that the extracts of saw palmetto fruits are composed primarily of free fatty acids (FFAs). The major components are usually oleic acid and lauric acid; however, the composition of the extracts varies according to the site where the plants grow, the harvest season, and the extraction method employed [3–5]. This paper reports the fatty acid composition of fruit extracts of both the yellow-green and blue-green forms of saw palmetto growing in the Rockdale Pineland Preserve in Miami-Dade County, Florida. Ripe fruits of both leaf types were harvested from the same site and at the same time in order to minimize the influence of different soil composition or seasonal variation on the composition of the fruits.

Typically, fatty acids are analyzed after being converted into the methyl esters prior to analysis by GC or GC/MS [6]. Occasionally, free fatty acids have also been analyzed successfully [7]. The advantage of the derivatization is that the esters afford sharp peaks and better resolution, as opposed to the free fatty acids which may show tailing peaks due to the interaction of the free acids with the stationary phase [7]. However, derivatization has some drawbacks such as the possibility of incomplete esterification, the possible generation of artifacts due to side-reactions, and contamination of the sample with impurities in the reagent [6]. The most crucial limitation of the derivatization is that it prevents the study of naturally occurring methyl esters of the fatty acids (FAMEs). Moreover, the fatty acid ethyl esters (FAEEs) in the extracts can be lost by using common derivatization techniques (e.g., 5% H_2SO_4 in methanol, BF_3 in methanol) because the FAEEs are converted to FAMEs by transesterification [5]. In this study, we found that underivatized extracts can afford acceptable chromatograms (because of moderate broadening of the FFA peaks) when using a new nonpolar column (DB-5MS) and low amount of sample injected into the column.

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TABLE 1. Amounts of Free Fatty Acids, Naturally Occurring Fatty Acid Methyl Esters, and Fatty Acid Ethyl Esters in Saw Palmetto Fruits

Fatty acid	HP-InnoWax	DB-5MS	A	B	C	D
	RT		fruit, mg/g	hexane extract, mg/g	% composition (mean ± S.D.)	
6:0		6.01	0.8	5.1		
8:0		9.69	2.4	14.7		
8:0 methyl ester	8.77	8.75			0.67 ± 0.17	0.73 ± .03
8:0 ethyl ester	9.46	10.59	0.1	0.7	0.03 ± 0.03	0.04 ± 0.03
10:0		15.81	2.9	17.9		
10:0 methyl ester	11.94	14.59			1.62 ± 0.10	1.60 ± 0.18
10:0 ethyl ester	12.55	17.04	Tr.	0.3	0.03 ± 0.03	0.03 ± 0.02
11:0 methyl ester	13.40	18.11			Tr.	Tr.
12:0		23.15	35.7	222.8		
12:0 methyl ester	14.89	21.81	0.4	2.2	22.89 ± 0.73	21.60 ± 0.53
12:0 ethyl ester	15.42	24.31	0.7	4.5	0.78 ± 0.30	0.69 ± 0.12
13:0		26.71	0.1	0.4		
13:0 methyl ester	16.26	25.40			0.05 ± 0.03	0.08 ± 0.01
14:0		30.16	15.4	96.4		
14:0 methyl ester	17.58	28.99	0.1	0.4	11.19 ± 0.34	10.59 ± 0.13
14:0 ethyl ester	18.04	31.34	0.5	3.0	0.43 ± 0.13	0.37 ± 0.06
15:0 methyl ester	18.83	32.40			0.03 ± 0.03	0.03 ± 0.03
16:1 (9)		36.17	0.2	1.0		
16:1 (9) methyl ester	20.38	35.04			0.29 ± 0.02	0.27 ± 0.02
16:0		36.80	11.2	70.1		
16:0 methyl ester	20.04	35.76	0.1	0.5	11.32 ± 0.30	11.33 ± 0.20
16:0 ethyl ester	20.45	37.89	1.2	7.4	0.40 ± 0.14	0.37 ± 0.06
17:0 methyl ester	21.20	38.90			0.07 ± 0.05	0.09 ± 0.01
18:1 (9)		42.35	69.4	434.0		
18:1 (9) methyl ester	22.57	41.30	0.9	5.6	41.65 ± 1.29	44.12 ± 1.28
18:1 (9) ethyl ester	22.90	43.16	3.0	18.6	1.54 ± 0.50	1.57 ± 0.39
18:1 (11) methyl ester	22.64	41.39			0.75 ± 0.07	0.83 ± 0.04
18:2 (9,12) methyl ester	23.08	40.98			3.10 ± 0.39	2.94 ± 0.39
18:2 (9,12) ethyl ester		42.96			Tr.	Tr.
18:3 (9,12,15) methyl ester	23.79				0.71 ± 0.05	0.77 ± 0.10
18:0		42.95	0.9	6.0		
18:0 methyl ester	22.31	41.97			1.71 ± 0.09	1.61 ± 0.05
18:0 ethyl ester		43.92	Tr.	Tr.	Tr.	

A and B, composition of hexane extract #1 of fruits from yellow-green plants expressed as mg per gram of saw palmetto fruit (fresh weight) (A) and per gram of extract (B); C and D, average percentage composition of hexane extracts of saw palmetto fruits from yellow-green plants (C) and blue-green plants (D) treated with ethereal diazomethane; Tr.: trace.

This allowed the FFAs, FAMEs, and FAEEs to be simultaneously quantified. This method was unsuccessful in separating the unsaturated oleic, linoleic, and linolic acids from each other. Nevertheless, if that issue is set aside, the analysis of the fatty acids is simple and provides the opportunity to study the naturally occurring methyl and ethyl esters. The best resolution was achieved with the derivatized samples run in a polar column (HP-InnoWax); however, only the combined use of polar and nonpolar columns together with the analysis of the unesterified samples allowed the complete identification and quantification of the components of saw palmetto fruits as shown in Table 1.

Hexane extracts of the saw palmetto fruits from Rockdale Pineland State Park were analyzed by GC and GC/MS after treatment with diazomethane and also directly without previous derivatization. Hexane was the solvent of choice because esters are formed as artifact when alcoholic extracts are prepared (e.g., [5]). The analytical chromatographic procedure is described in the Experimental section. Analysis of the esterified samples was carried out in both polar (HP-InnoWax) and nonpolar (DB-5MS) columns. The naturally occurring FAMEs and FAEEs present in the saw palmetto fruits were quantified in underivatized samples by using the nonpolar column (DB-5MS). The combined use of both types of columns and extracts afforded the results shown in Table 1.

GC and GC/MS analyses of the hexane extracts obtained from different batches of saw palmetto fruits collected from Rockdale Pineland State Park plants showed almost identical chromatographs. More than 30 components could be detected in the extracts. Estimates of the free fatty acid and naturally occurring ester content in the fruits and extracts together are given in Table 1, along with the percentage composition based on extracts previously treated with diazomethane. Despite a number of papers dealing with the fatty acid composition of saw palmetto fruits, little attention has been paid to the FAMEs and FAEEs occurring in the extracts. Although ethyl laurate could be quantified [5] and an attempt was also made to determine the total FAMEs plus FAEEs content in different commercial brands of *S. repens* extract [3], a detailed study of the individual esters is lacking. A qualitative and quantitative estimate of the naturally occurring FAMEs and FAEEs detected in saw palmetto fruits is presented in Table 1. The total ethyl esters are more abundant than the methyl esters (3.5 g % and less than 1% in the extracts, respectively).

Hexane extracts contained more than 80 g of free fatty acids per 100 g of extract (Table 1). The content of the major components, oleic and lauric acids (ca. 0.4 and 0.22 g per g of extract, respectively), the total amount of free fatty acids (0.87 g per gram of extract), and the total amount of free unsaturated acids (0.44 g per gram of extract) are consistent with the fatty acid contents previously reported for hexane extracts [4, 8]. This study shows that the analyses of derivatized samples with polar and nonpolar GC columns complemented with the analysis of underivatized samples in nonpolar columns could provide detailed information about the FFA, FAME, and FAEE composition of the saw palmetto fruits.

The analyses of fruit extracts from yellow-green and blue-green saw palmetto plants indicated no significant differences in fatty acid composition. By extraction with *n*-hexane, both types of fruits afforded the same amount of lipophilic extract (ca. 3 g per 100 g of fruit, fresh wt.) and the same percentage composition (Table 1). Small differences in percentages between either forms are not significant due to the scarce number of samples analyzed. This study shows that the morphological characteristics described for yellow-green and blue-green saw palmetto plants are not associated with qualitative or quantitative changes in the fatty acid composition of the fruits. Since the clinical benefits of saw palmetto extracts are largely attributed to their free fatty acid content [3], the fruits from both types of plants should have the same therapeutic value for the treatment of benign prostatic hyperplasia.

EXPERIMENTAL

Plant Materials. Four batches (# 1–4) of ripe fruits from yellow-green saw palmetto plants and five batches (# 5–9) from blue-green plants were collected in Rockdale Pineland Preserve on August 15th–25th, 2003 and stored at –80°C until analyzed. Ten representative fruits of each batch were separated (weight of each group of drupes ca. 42 g; average weight 4.18 g per fruit, fresh wt.) and dried in an oven for 72 h at 55°C. The dry fruits (aprox. 16.3 g per batch; average weight 1.63 g per fruit, dry wt.) were ground to a fine powder using a Mr. Coffee Model IDS 55 coffee grinder.

Preparation of the Lipophilic Extracts. Ground dried plant material of each batch was macerated at room temperature with 100 mL of *n*-hexane in an orbital shaker overnight. The suspension was filtered and washed with *n*-hexane (50 mL). The plant material was extracted once more under the same conditions. Filtrates and washes were combined (250 mL) and evaporated to dryness under reduced pressure, yielding a viscous oil (yellow-green plant fruits: mean ± S.D. = 1.26 ± 0.16 g; blue-green plant fruits: mean ± S.D. = 1.29 ± 0.24 g). The lipophilic extracts were directly analyzed by GC and GC/MS or, alternatively, an aliquot of the extract was treated with excess ethereal diazomethane and the fatty acids converted to methyl esters prior to GC analysis. Typically, ca. 10 mg of exactly weighed oil was reconstituted in 1 mL *n*-hexane, and 2 μL of the sample solution was injected.

Conditions of GC/MS and GC/FID Analysis. GC/MS determinations were carried out in a Hewlett Packard model 6890 instrument coupled to a Q-Mass 910 quadrupole selective detector at 70 eV. Two fused capillary columns of different polarity were used. Polar column: HP-InnoWax polyethylene glycol (30 m × 0.25 mm; nominal film thickness; 0.25 μm; J & W Scientific); injection port temperature, 230°C, split ratio 1/20; detector temperature, 280°C; carrier gas, helium at 0.7 mL/min; temperature program: 60°C for 1 min, then ramped at 8°C/min to 240°C, 240°C held for 20 min. Nonpolar column: DB-5MS (30 m × 0.25 mm i.d.; film thickness 0.25 μm; J & W Scientific); injection port temperature, 230°C; split ratio 1/20; detector temperature, 300°C; carrier gas, helium at 0.7 mL/min; temperature program: 50°C to 300°C linear increase at 3°C/min.

GC/FID Analysis. Determinations were performed on a Trace GC Ultra apparatus (Thermo Electron Corporation) equipped with a flame ionization detector. The output was recorded using a ChromQuest version 4.1 data system. Capillary columns: HP-InnoWax (conditions as above) and DB-5MS, injector temperature, 200°C, split ratio 1/50; detector temperature, 270°C; carrier gas helium, 1 mL/min; temperature program: 105°C to 240° linear increase at 3°C/min, 240°C maintained for 20 min.

Identification. Fatty acids, free or derivatized as methyl esters, were identified by the use of standards and comparison of their mass spectra with reference mass spectra in NIST G 1701 BA Version B.01.00 database (Hewlett Packard 1989–1998). Fatty acids were quantified by FID area values against calibration curves determined with standards of the main acids and methyl esters.

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