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Full Length Research Paper

Sterol composition of caper (*Capparis spinosa*) seeds

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Caper is a perennial shrub of the Mediterranean Basin. The most important economical species is *Capparis spinosa.* Sterols of *C. spinosa* seed oil isolated from seven Tunisian stands were identified and quantified. *C. spinosa* contained high levels of phytosterols (2240.4 mg/kg of total extracted lipids), of which β-sitosterol, with 1390 mg/kg, was the most abundant (57.53%). Campesterol and stigmasterol accounted for 382 and 265 mg/kg, respectively (17.05 and 11.85% of the total sterols, respectively*). *C. spinosa* seed oil also contained a high level of 5-avenasterol (6%). We detect also brassicasterol (3.39 mg/kg). Cholesterol and campestanol are detected in much lower levels. These results bring attention to the richness of *C.spinosa* seed oil with sterols which are the most important class of the minor components.

Key words: Caper *(Capparis spinosa),* seed oil, unsaponifiables, sterols.

INTRODUCTION

Sterols are an important class of organic molecules. They are probably the most important class of the minor components and comprise a major portion of the unsaponifiable matter of most vegetable oils (Kiritsakis and Christie, 2000). Over 40 phytosterols have been identified; of these, campesterol, stigmasterol and sitosterol account for more than 95% of total phytosterol dietary intake (Calpe-Berdiel et al., 2009). These compounds have a considerable dietary and medicinal importance. It has been suggested that minor compounds protect against cardiovascular complications and could reduce the risk of heart attacks by 15 - 45% (Vanhanen et al., 1993; Law, 2000). Phytosterols have been shown to decrease the risk of certain types of cancer and enhance immune function (Awad and Fink, 2000; Bouic, 2001). Plant sterols and stanols (phytosterols/ phytostanols) are known to reduce serum low-density lipoprotein (LDL)-cholesterol level, and food products containing these plant compounds are widely used as a therapeutic dietary option to reduce plasma cholesterol and atherosclerotic risk (Calpe-Berdiel et al., 2009). Moreover, the National Cholesterol Education Program Adult Treatment Panel III guidelines recommend plant sterol-containing food as one of the lifestyle changes to lower cardiovascular risk (NCEP, 2002). There is increasing interest in isolating these biologically active components for nutraceutical applications and as ingredients for functional foods (Hendricks et al., 1999).

In the literature, little is known about these compounds in the caper. Caper is the common name of the genus Capparis, family of Capparaceae (Jacobs, 1965). As a spontaneous plant, caper has a large natural distribution in the Mediterranean Sea Basin; it grows from the Atlantic coasts of the Canary Islands and Morocco to the Black Sea to the Crimea and Armenia, and eastward to the Caspian Sea and into Iran (Romeo et al., 2007). *Capparis spinosa* is the most important species. Different parts of the caper plant can be used as a drug or a cosmetic (Afsharypuor et al., 1998; Akgul and Ozcan 1999; Tlili et al., 2009a).

This species become an interesting crop with an importance economic in the Mediterranean region over the last years (Romeo et al., 2007). The fresh aerial parts are stored in vinegar or brined and eaten pickled. Additionally, fruits with small, soft seeds are preferred for the production of pickles.

Previous chemical studies on *C.spinosa* have reported

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Abbreviations: TLC, Thin layer chromatography; **GC-FID,** gas chromatography- flame ionization detector.

Table 1. Location and total unsaponifiables and sterols of harvested *Capparis spinosa* seeds[a].

Code	Name	Location	Latitude	Longitude	Total unsapon-ifiables (%)	Sterol content mg/kg
D	Dahmani	North	35° 57' N	8° 48' E	1.99	2269.4
GM	Ghar el Melh	North	37° 10' N	10° 11' E	2.05	2170.1
KH	Ksar Hadada	South	33° 06' N	10° 19' E	2.53	2068
T	Chenini Tatouine	South	32° 55' N	10° 26 ' E	1.96	2470
M	Mateur	North	37° 02' N	9° 40' E	2.71*	2215.4
CH	Chouigui	North	36° 53' N	9° 47' E	1.67	2170.1
ST	Sidi Thabet	North	36° 55' N	10° 03' E	2	2323.4
Mean ± SD					2.13 ± 0.34	2240.4 ± 102.2

[a] values are mean of two repetitions; *values are significantly higher than the mean at p = 0.05.

the richness with tocopherols, carotenoids, flavonoids and glucosinolates in different parts of this plant (Germano et al., 2002; Matthaus et al., 2005; Tlili et al., 2009a,b). Commercial caper is also rich with these compounds (Tlili et al., 2009c). Moreover, *C. spinosa* seeds are rich in lipids, containing mainly unsaturated fatty acids (Matthaus et al., 2005; Tlili et al., 2009b).

To our knowledge, very few studies have been carried out on *C. spinosa* sterols. Matthaus and Özcan (2005) reported that in Turkish caper the level of phytosterols is about 6000 mg/kg. The goal of this work is to identify and quantify the sterol content in Tunisian *C.spinosa*.

The results offer an indication of the potential dietary, pharmaceutic and economic utility of caper seeds as a good source of phytosterols. Therefore, accurate data on the naturally occurring phytosterol content of seeds are essential to facilitate formulation of research diets, and development of dietary and medicinal recommendations related to the health impact of phytosterols.

MATERIALS AND METHODS

Plant Material

Sampling was performed from seven Tunisian regions in May 2008: Dahmani (D) Ghar el Melh (GM), Ksar Hadada (KH), Tatouine (T), Mateur (M), Chwigui (CH) and Sidi Thabet (ST) (Table 1). From each region, seeds were collected from 8 to 13 plants, mixed and then a representative sample was taken for further analysis. Seed oil was immediately extracted in the laboratory upon arrival as indicated below.

Oil extraction

The oil content was determined according to AOCS (1989) method Ce-66:1989 and ISO (1999) method 659:1998. About 5 g of the seeds were ground in a mortar until dough and extracted with petroleum ether in a soxhlet apparatus for 6 h. The solvent was concentrated using a rotary evaporator, under reduced pressure at 45°C. The oil was dried by using a stream of nitrogen and stored at -20°C until use.

Saponification of the lipids

To separate the unsaponifiable fraction, oil from *C. spinosa* seeds was treated with a potassium hydroxide solution to transform the fatty acyl esters into potassium salts that are soluble in water. Total extracted lipids were treated with 50 ml of 2M KOH-ethanol solution, and the mixture was refluxed, with constant stirring, for 1 h. Then, 50 ml of water were added. The unsaponifiable fraction was extracted with 3 × 40 ml of diethyl ether. The organic extract was separated and washed with 3 × 40 ml of distilled water and then dried over anhydrous sodium sulfate, filtered and concentrated using a rotary evaporator under reduced pressure at 60°C.

Separation of the sterolic fractions from unsaponifiables by thin-layer chromatography (TLC)

The unsaponifiable matter (5% in chloroform) was separated on TLC plates (20 × 20 cm) coated with KOH-methanol (2 N) impregnated silica gel (0.25 mm), previously activated by heating at 100°C for 1 h. The unsaponifiable fraction (250 µl) and internal standard 5α-cholestane-3β-ol (0.2%, w/v) were spotted on the plates. Elution was performed using hexane/ diethyl ether 65:35 (v/v) as the mobile phase. The plates were then sprayed with a 0.2% solution of 2', 7'-dichlorofluorescein in ethanol and the sterol pink bands appeared under UV light together with the spots of 5α-cholestane-3β-ol used as internal standard. Sterol bands were scraped off separately and dissolved into warm chloroform (5 ml). The obtained solutions were dried over anhydrous sodium sulfate and filtered through Whatman filter paper. The chloroform was evaporated by nitrogen stream and sterolic fractions were dried in an oven at 103°C.

GC-FID Analysis

Sterolic fractions were treated with a derivatizing reagent obtained from Sigma-Aldrich France Ltd. (pyridine/hexamethyldisilazane/ trimethylchlorosilane, 9:3:1, v/v/v). A volume of 0.05 ml of reagent for each milligram of sterol was added. One microliter of this solution was injected into the gas chromatograph. TMS derivatives were analyzed in duplicate by GC in a Hewlett-Packard HP-4890D chromatograph equipped with a HP-5 (5% diphenyl-95% methylpolysiloxane) fused silica capillary column (30 m × 0.32 mm × 0.25 µm film thickness), operated isothermally at 250°C with an inlet carrier gas (nitrogen) pressure of 100 kPa. The injector with a split ratio of

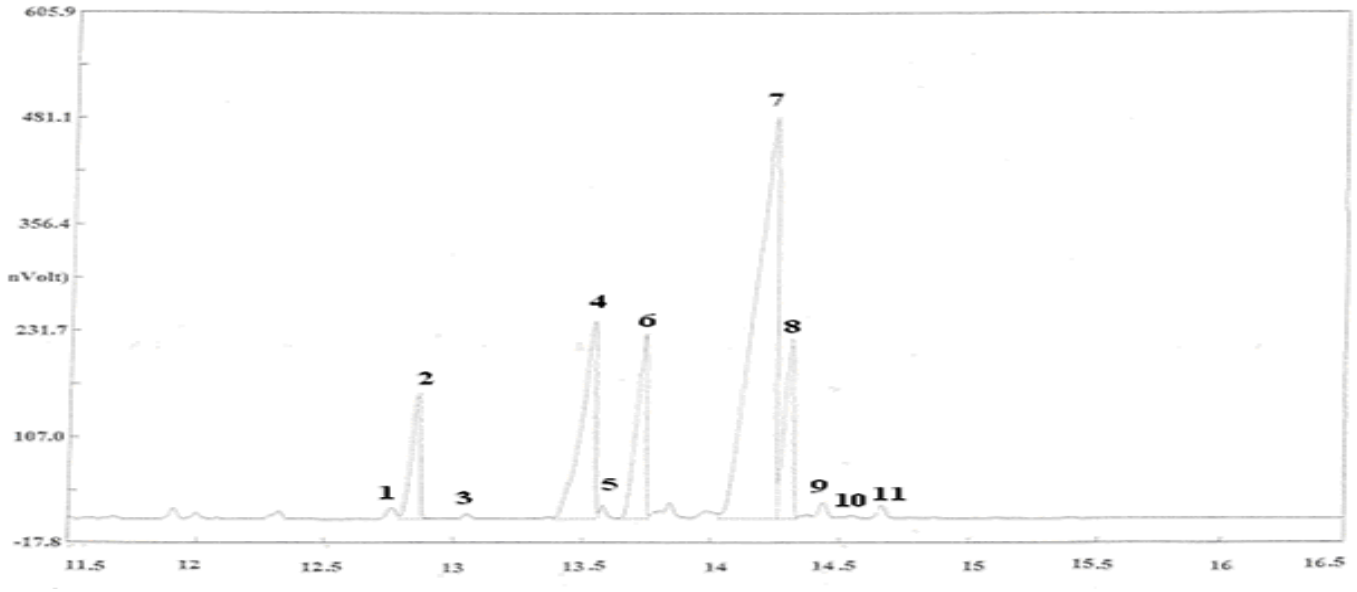

Figure 1. Typical chromatogram of sterols from unsaponifiable matter extracted from *C. spinosa* seeds. Analysis was performed on a HP-5 fused-silica capillary column 30 m (0.25 mm i.d., 0.25 µm film thickness) coated with a stationary phase of 5% cross-linked phenylmethylsilicone. Peaks : 1 Cholesterol, 2 Cholestanol (internal standard), 3 brassicasterol, 4 Campesterol, 5 Campestanol, 6 Stigmasterol, 7 β- Sitosterol, 8 Δ^{5} Avenasterol, 9 $\Delta^{5,24}$ Stigmastadienol, 10 Δ^{7} Stigmastenol , 11 Δ^{7} Avenasterol.

1:15 was maintained at 230°C and the flame ionization detector (FID) at 250°C. Sterols were identified by using a known mix of sterols chromatographed under the same conditions. Sterols were expressed as milligrams per kilogram of total extracted lipids by using 5α-cholestane-3β-ol as internal standard.

Statistical analysis

The experimental data were analyzed using the analysis of variance (ANOVA) and the statistical analysis system (XLSTAT 2008). Differences at $p \leq 0.05$ were considered statistically significant by Duncan's new multiple range test. All values were expressed as means ± standard deviation of at least duplicate repetitions.

RESULTS AND DISCUSSION

Unsaponifiable oil content

The unsaponifiable fraction of vegetable oils has applications in cosmetics and pharmacology to its biological properties. Results show that values of unsaponifiable oil content were between 1.67 (CH) and 2.71% (M) with an averaging of 2.13% (Table 1). Karleskind and Wolf (1996) reported that in sunflower, soybean or rapeseed the values are between 0.5 and 1.8%, while in shea nut the value is 10%. Nasri et al. (2007) reported that in pinus the value are between 1.32 and 2.09%.

Sterols contents

Sterols comprise a major portion of the unsaponifiable matter of most vegetable oils (Kiritsakis and Christie, 2000). Figure 1 shows a typical chromatogram corresponding to the GC analysis of sterol fraction. Ten sterols were identified. Seeds of *C. spinosa* are rich in sterols (Table 1). The levels ranged from 2068 mg/kg (KH) to 2470 mg/ kg (T), with an average of 2240.4 mg/kg. These values are lower than those published by Matthaus and Ozcan (2005) who reported for the same species an average of 6033 mg/kg. This difference could be due to the geographical effect.

Seeds of Tunisian C. spinosa contains levels of phytosterolsslightly higher than some oilseeds commonlyconsumed (Table 2), such as soybean (1610 mg/kg),almond (1430 mg/kg), olive oil (2210 mg/kg) or peanut (2200 mg/kg) (Abidi, 2001). Whereas, these values are stillsubstantially less than pine (4298 mg/kg) (Nasri et al.,2007), sesame oil (8650 mg/kg) or corn oil (9680 mg/kg)(Abidi, 2001). These values confirm the high nutritional and medicinal value of *C. spinosa* and its potential role in lowering serum cholesterol levels in humans (Westrate and Meijer, 1998;Law, 2000).

The sterol composition of the caper seeds analyzed is summarized in Table 3. β-sitosterol was the most abundant sterol with an average of 1289.54 mg/kg (57.53%), followed by campesterol with 382.37 mg/kg (17.05%) and stigmasterol with 265.31 mg/kg (11.85%). Cholesterol and campestanol are present in much lower proportions. These percentages are in agreement with those reported by Matthaus and Ozcan (2005). Grunwald (1975) reported that β-sitosterol, campesterol and stigmasterol predominate in higher plants. β-sitosterol is the major sterol in olive oil with 80% (Sanchez et al., 2004) in sesame with 50% (Mohamed and Awatif, 1998) or in coffee beans with 50% (Carrera et al., 1998).

Table 2. Sterol content in caper seeds and some selected foods oils (mg/kg).

Samples	Sterols	References
Potato	50	Abidi, 2001
Tomato	70	Abidi, 2001
Soybean	1610	Abidi, 2001
Almond	1430	Abidi, 2001
Chocolate	1670	Phillips et al., 2005
Olive	2210	Abidi, 2001
Peanut	2200	Abidi, 2001
Caper seeds	2240	This study
Pistachio	2790	Phillips et al., 2005
Pine	4298	Nasri et al., 2007
Caper seeds	6033	Matthaus and Ozcan, 2005
Sesame	8650	Abidi, 2001
Corn	9680	Abidi, 2001

Table 3. Content and percent of sterols of *Capparis spinosa* seed oil (mg/kg).

Sterols	D		M		CH		ST		GM		KH		T		Mean
	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	Content	%	
Cholesterol	18.83*	0.83	8.64	0.39	12.81	0.59	6.74	0,29	8.46	0.39	4.54	0,22	18.52*	0.75	11.22
Brassicasterol	1.58	0.07	7.31*	0.33	1.95	0.09	5.34	0.23	2.6	0.12	1.03	0.05	3.95	0.16	3.39
Campesterol	439.81*	19.38	383.04	17.29	396.04	18.25	392.42	16.89	341.57	15,74	321.57	15,55	402.12	16.28	382.37
Campestanol	6.35	0.28	7.31	0.33	6.73	0,31	4.18	0,18	2.82	0,13	2.68	0,13	1.48	0,06	4.51
Stigmasterol	315.9*	13.92	220.87	9.97	288.19	13.28	262.08	11.28	266.48	12.28	232.23	11.23	271.45	10.99	265.31
β-sitosterol	1320.79	58.20	1381.3	62.35	1220.89	56.26	1180.29	50.80	1263.65	58.23	1172.14	56,68	1487.68*	60,23	1289.54
Δ5 Avenasterol	129.81	5.72	179.67*	8.11	129.34	5,96	160.78	6,92	116.53	5,37	140.42	6,79	134.86	5,46	141.63
$\Delta^{5,24}$Stigmastadienol	16.56	0.73	14.62	0.66	16.05	0,74	20.68*	0.89	16.71	0,77	6.82	0,33	20.5*	0,83	15.99
Δ^{7} Stigmastenol	2.95	0,13	1.55	0,07	6.94	0,32	2.32	0,10	2.6	0,12	3.51	0,17	7.41*	0,30	3.89
Δ^{7} Avenasterol	16.79*	0,74	11.74	0,53	9.76	0,45	11.85	0,51	3.47	0,16	7.44	0,36	7.41	0,30	9.78
Total	2269,4 ± 89		2215.4 ± 97.5		2170.1 ± 78.6		2323.4 ± 109.3		2170.1 ± 88.4		2068 ± 88.2		2470 ± 98.6		2240.4 ± 102.2

[a] values are mean of two repetitions; *values are significantly higher than the mean at p ≤0.05.

Recent evidence suggests that phytosterols/ phytostanols may regulate proteins implicated in cholesterol metabolism both in enterocytes and hepatocytes (Calpe-Berdiel et al., 2009). β-sitosterol plays a major role in treatment of many disease such as treatment to lower serum cholesterol for hypercholesterolemic patients (Pollak, 1953) or breast cancer (Awad et al., 2008). It has recently been reported that accumulations of some

phytosterols such as stigmasterol with an unsaturation within the side chain, disrupt cholesterol metabolism (Yang et al., 2004, 2006). Other authors suggested that campesterol, as one of the phtytosterols, can regulate lipoprotein metabolism in the intestine and regulate biliary secretion (Sudhop et al., 2002; Ho and Pal, 2005). The high amount of β-sitosterol, stigmasterol and campesterol in seed oil of *C. spinosa* gives to this species a nutritional and pharmaceutic value.

Results show also the presence of Δ5-avenasterol with an average of 141.63 mg/kg (ca. 6%). These values are less than those reported by Matthaus and Ozcan (2005) for the same species (ca. 336 mg/kg). Our values are slightly less than those found in other species such as sunflower oil (170 mg/kg) or rapeseed oil (250 mg/kg). This compound is known to act as an antioxidant (Savage et al., 1997). Moreover, this compound could protect the oil from oxidation during prolonged heating as is the case of sesame oil (Mohamed and Awatif, 1998).

Minor lipid compounds are often reliable species-specific biochemical indicators. Sterols have also been used to differentiate among populations and varieties (Carrera et al., 1998; Gauvin et al., 2004). Of the sterols identified in oils of seeds of *C. spinosa* was the presence of brassicasterol (3.39 mg/kg), which is a characteristic sterol of the family Brassicaceae (Appelquist et al., 1981). This level is low compared to those reported by Matthaus and Ozcan (2005) in seed oils of Turkish caper (13.4 mg/ kg) and much lower than the values found in oil seeds of Brassica napus (415 mg/kg) (Amar et al., 2008). Some authors suggested that phytosterols containing a double bond at C-22 in the side chain, such as brassicasterol, regulate cholesterol metabolism in intestinal cells (Fernandez et al., 2002).

It is concluded that *C. spinosa* seed oil contained 2.13% of unsaponifiable fraction. High amount of phytosterols, with dietary and medicinal importance, (ca. 2240 mg/kg) was quantified. β-sitosterol was the major sterol (ca. 58%) followed by campesterol and stigmasterol (ca.17 and 12%, respectively). An important contents of 5-avenasterol was also detected (ca. 141 mg/kg). Brassicasterol, characteristic sterol of the family Brassicaceae, was also quantified. Cholesterol and campestanol are present in much lower proportions. These compounds are known to have a wide range of beneficial biological activities and physical pro- perties. The oil unsaponifiable matter of *C. spinosa* seeds confirms their nutritional importance and pharmaceutic value.

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