JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 5381–5385 5381
DOI:10.1021/jf900457p

Carotenoid and Tocopherol Composition of Leaves, Buds, and Flowers of *Capparis spinosa* Grown Wild in Tunisia

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High-performance liquid chromatography was used to determine carotenoids (β-carotene, lutein, neoxanthin, and violaxanthin) and tocopherols of leaves, buds, and flowers of Tunisian *Capparis spinosa*. This plant shows strong resistance to hard environmental conditions, and it is one of the most commonly found aromatics in the Mediterranean kitchen. In this study, the means of the total carotenoids were 3452.5 ± 1639.4, 1002 ± 518.5, and 342.7 ± 187.9 μg/g fresh weight (FW) in leaves, buds, and flowers, respectively. Lutein accounts for the high content. Violaxanthin provided the lowest portion of the total carotenoids. The principal form of tocopherol detected in leaves was α-tocopherol (20.19 ± 10 mg/100 g FW). In buds and flowers, there were both α- (49.12 ± 17.48 and 28.68 ± 9.13 mg/100 g FW, respectively) and γ-tocopherol (48.13 ± 15.08 and 27.8 ± 16.01 mg/100 g FW, respectively). The combined content of pro-vitamin A and vitamin E in capers encourages researchers to more explore and find developments for this plant.

KEYWORDS: ***Capparis spinosa*; tocopherols; carotenoids; leaves; buds; flowers**

INTRODUCTION

Mediterranean plants are exposed to a combination of environmental stress conditions, including low water availability, high irradiance, temperature fluctuations, and nutrient deprivation. Carotenoids and tocopherols are the most abundant groups of lipid antioxidants. Carotenoids are essential components of the photosynthetic apparatus in plants in which they protect against photo-oxidative damage and contribute to light harvesting for photosynthesis (*1*). They decrease the fluidity of biological membranes and play an important role by scavenging free radicals in both phototrophic and heterotrophic organisms (*2*). A few carotenoids, including β-carotene and lutein, have been found in humans (*3*). In mammals, β-carotene is the source of vitamin A (*1*). Tocopherols are known as vitamin E and are only synthesized by photosynthetic organisms. An increased tocopherol content has been correlated in the response of photosynthetic tissues to a variety of abiotic stresses (*4*). It has been reported that tocopherols protect chloroplast membranes from photo-oxidation (*5*). Also, it has been suggested that tocopherols are involved in the protection of pigments and proteins of the photosynthetic apparatus against oxidative degradation (*6*). In addition to antioxidant function, tocopherols also have nonantioxidant functions in membranes such as permeability and fluidity or protein kinase inhibition (*7*). Burton et al. (*8*) reported that vitamin E is an important antioxidant nutrient that can protect cell membranes from oxidative damage.

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In the literature, little is known about these compounds in the caper. Caper is the common name of the genus *Capparis*, family *Capparidaceae*. The plants show strong resistance to hard environmental conditions (*9*, *10*). Despite the adverse conditions of the Mediterranean summer, plants of *Capparis* did not seem to show any water stresses or any symptoms of photoinhibition, and it efficiently utilizes the high irradiance throughout the growth season (*9*). Different parts of the caper plant can be used as a drug or a cosmetic. The buds of the caper are also one of the most commonly found aromatics in the Mediterranean kitchen (*10*). Previous chemical studies on *Capparis spinosa* have reported the presence of alkaloids, lipid flavonoids, and glucosinolates (*11*, *12*). Moreover, it was reported that *C. spinosa* possesses some anti-inflammatory metabolites (*13*). This species has become an interesting and specialized crop of great economic importance in the Mediterranean area over the last years. Spain, Morocco, and Italy are the main producer countries (*10*). Because of its purposes, Tunisia has recently made considerable efforts to enhance production of this species.

To our knowledge, data on carotenoids and tocopherols in leaves, buds, and flowers of *C. spinosa* are very limited. The aim of this study is to determine the contents of some carotenoids and tocopherols in these parts of *C. spinosa* by an high-performance liquid chromatography (HPLC) analysis.

MATERIALS AND METHODS

Chemicals. All solvents used in the experiments (acetone, methanol, dichloromethane, acetonitrile, and ethyl acetate) were purchased from Fluka (Ridel-de Haën, Switzerland). α-Tocopherol, γ-tocopherol, and β- tocopherol were purchased from Sigma-Aldrich (Steinheim, Germany).

Published on Web 05/27/2009

Table 1. Location and Names of Harvested Samples

samples			
code	location	latitude	longitude
GM	Ghar el Melh	37° 10′ N	10° 15′ E
ST	Sidi Thabeut	36° 55′ N	10° 05′ E
M	Mateur	37° 05′ N	9° 42′ E
CH	Chwigui	36° 53′ N	9° 47′ E
KH	Ksar Hdada	33° 02′ N	10° 26′ E

Plant Material. Leaves, buds, and flowers of wild *C. spinosa* were sampled in May 2007 from the following Tunisian regions: Ghar el Melh (GM), Sidi Thabet (ST), Mateur (M), Chwigui (CH), and Ksar Hdada (KH) (**Table 1**). After caper flower blossoming, other flower buds rapidly form, so flower buds and flowers exist in the same branch of wild *C. spinosa*. Samples were collected, immediately put in aluminum foil in the liquid nitrogen, and carried to the laboratory, where they were stored at −80 °C until analyzed and used within 1 week.

Carotenoids and Tocopherols Extraction. To extract carotenoids and tocopherols, we used the procedure described by Kimura and Rodriguez-Amaya (*14*) with some modifications. Samples were ground in a mortar under liquid nitrogen, and then, 1 g was subjected to extraction. Carotenoids and tocopherols were subsequently extracted with 5 mL of cold acetone and re-extracted with 10 mL of dichloromethane/methanol (2:1 v/v) until decoloration. The extraction was made in the ice with protection from light and within the shortest possible time to avoid degradation. The acetone−dichloromethane/methanol fraction was concentrated on a rotary evaporator under reduced pressure at 40 °C and brought to dryness under a stream of nitrogen. Before injection into the HPLC system, the samples were dissolved in 5 mL of mobile phase. All extracts were portioned and stored at −80 °C until the analyses, which were carried out within 2 weeks.

HPLC Analysis. *Carotenoids*. Carotenoids were determined using the HPLC gradient method described by Pfeifhofer (*15*), with a slight modification. Chlorophylls and carotenoids analysis was completed with a Spectra SYSTEM (ThermoFinnigan) equipped with a diode detector UV6000LP (350−550 nm) and separated using Column Zorbax C18 (250 mm × 4.6 mm). Two phases system were used as follows: phase A, acetonitrile/water 90:10 (v/v), and phase B, ethyl acetate. Carotenoid separation was a linear gradient from 100% A to 100% B in 35 min, followed by 5 min 100% B, return to 100% A in 5 min, followed by a 5 min 100% A. A flow rate of 1 mL min^{-1} was utilized. The detection wavelength was 440 nm. The injection volume was 10 μL.

Tocopherols. Tocopherol analysis was completed according to the method cited by Wildi and Lutz (*16*), which also was slightly modified. Tocopherol analysis was determined with a Spectra SYSTEM (ThermoFinnigan) equipped with a fluorescence detector FL3000 (excitation at 290 nm and emission at 330 nm). Samples were subjected to isocratic HPLC analysis (column Zorbax C_{18}, 250 mm × 4.6 mm) using acetonitrile/methanol/dichloromethane 65:35:5 (v/v/v) with a flow rate of 1 mL min^{-1}. Tocopherols were detected directly by fluorometry. The detection wavelength was 330 nm. The injection volume was 10 μL. Tocopherols were identified by coelution with authentic standards obtained from Sigma-Aldrich.

Statistical Analyses. The carotenoid and tocopherol concentrations of leaves, buds, and flowers of *C. spinosa* were calculated in μg/g fresh weight (FW) and mg/100 g FW, respectively. Statistical analyses were performed using SPSS (version 16.0). Data were expressed as means ± standard deviations (SDs) using analysis of variance. Differences at $p < 0.05$ were considered statistically significant by Duncan's new multiple range test.

RESULTS AND DISCUSSION

Carotenoid Contents. *C. spinosa* contains the carotenoid β-carotene and the xanthophylls neoxanthin, violaxanthin, and lutein. The content and percentage of carotenoid of *C. spinosa* from leaves, buds, and flowers of caper from the different samples can be seen in **Table 2**. The SD for the different part of *C. spinosa* is also included in the table.

In the literature, data on carotenoids of *C. spinosa* are limited. In this study (**Table 2**), the means of the total amount of carotenoids were 3452.5 ± 1639.4, 1002 ± 518.5, and 342.7 ± 187.9 μg/g FW in leaves, buds, and flowers, respectively. Previously, Özcan and Akgül (*10*) reported that the total carotenoids in buds of *C. spinosa* (size ≤ 8 mm) were between 5.61 and 6.96 μg/g dry weights; these differences may be due to cultivar variations, locations, and differing harvesting and extraction techniques, as cited by other authors (*17*, *18*). Levizon et al. (*9*) reported that *C. spinosa* is well-adapted to the adverse environment of the Mediterranean summer. Despite this adverse condition, plants of *Capparis* did not show any stress (*9*). The present study showed that different parts of caper plants contain an important level of carotenoids, which may be a response of these plants during the summer. A defining characteristic of these organisms is the use of readily available water as the reductant for photosynthesis (*19*). They are involved in photosystem assembly, light harvesting, and photoprotection (*1*). Indeed, it has been suggested that environmental stress could induce major changes in carotenoid profiles (*20*). The total carotenoid content found in this study makes *C. spinosa* a good natural source of these compounds, especially β-carotene and lutein, as compared with other natural sources such as pepper, tomato, and potato (**Table 3**).

According to the results shown in **Table 2**, leaves of sample CH contain the highest value of total carotenoid (6294.5 ± 19.32 μg/g FW). Apart from this sample, the characteristic level of total carotenoid in leaves was relatively stable (2191.7 ± 15.21−3331 ± 17.70 μg/g FW). It is interesting to show that buds of samples CH also contained the highest value (1762.3 ± 15.27 μg/g FW), whereas the total carotenoid content in the other samples varied from 411.3 ± 9.19 μg/g FW (GM) to 1231 ± 12.05 μg/g FW (M), whereas in flowers, the carotenoid content was from 142.5 ± 3.77 (KH) to 562.8 ± 5.85 μg/g FW (M). These differences may be due to the geographic location; in fact, samples were collected from the south (KH) and different regions in the north (GM, M, ST, and CH). Indeed, other research suggests that the geographic position affects the carotenoid content (*22*, *23*).

The content of lutein is 2346.3 ± 1292.9, 672.9 ± 402.7, and 214 ± 117.9 μg/g FW in leaves, buds, and flowers, respectively. The content of neoxanthin is 45.5 ± 21.3, 60.5 ± 40.1, and 20.4 ± 6.7 μg/g FW in leaves, buds, and flowers, respectively. Violaxanthin contents are 17.7 ± 8.8, 36.3 ± 24.4, and 8.5 ± 3.3 μg/g FW in leaves, buds, and flowers, respectively. Much research has suggested that the violaxanthin cycle protects plants from photooxidative damage (*24*). Moreover, it has been demonstrated that the high contents of these xanthophylls are the commune responses of plants during Mediterranean seasonal fluctuation (*25*).

Results show that β-carotene in *Capparis* was present at 1043 ± 376.9, 234 ± 97.8, and 103.5 ± 60.4 μg/g FW in leaves, buds, and flowers, respectively. It has been shown that this compound cooperates with tocopherol in limiting singlet oxygen-induced damage to photosystem II in some species (*26*).

Another study (*27*) suggested that depending on growth conditions and stress factors, the percentage of carotenoids can vary with ranges of lutein (40−60%), β-carotene (25−40%), violaxanthin (10−20%), and neoxanthin (5−13%). Also, Rodriguez-Amaya (*18*) reported that many factors such as variety, part of the plant utilized, and postharvest handling practices can modify the carotenoid composition.

Carotenoids also play an important role in human nutrition (*1*, *3*). Lutein, for example, has been reported to play a significant role in the health of eyes. β-Carotene has been reported as pro-vitamin A (*1*). Neoxanthin has also been reported to inhibit chemically induced carcinogenesis in the hamster buccal

Table 2. Content and Percent of Carotenoids of Leaves, Buds, and Flowers of *C. spinosa* (μg/g FW)[a]

		xanthophylls								
		noexanthin		violaxanthin		lutein		β-carotene		
samples		content	percent	content	percent	content	percent	content	percent	total carotenoids
leaves	GM	61.2 ± 2.14 b[b]	2.8	31.8 ± 1.28 a	1.4	1375.3 ± 14.56 e	62.7	723.5 ± 5.21 e	33	2191.7 ± 15.21 e
	KH	24.8 ± 1.07 e	0.7	20 ± 1.77 b	0.6	2015.2 ± 17.21 b	60.5	1271 ± 10.77 b	38.2	3331 ± 17.70 b
	ST	30.3 ± 1.14 d	1.1	9 ± 0.52 e	0.3	1749.5 ± 16.49 d	65.4	884.8 ± 6.41 c	33.1	2673.6 ± 13.35 d
	M	36.5 ± 1.11 c	1.3	12.4 ± 1.63 d	0.4	1977.9 ± 17.70 c	71.4	744.8 ± 4.19 d	26.8	2771.7 ± 15.29 c
	CH	74.5 ± 2.45 a	1.2	15.2 ± 0.10 c	0.2	4613.8 ± 22.43 a	73.3	1591 ± 15.13 a	25.3	6294.5 ± 19.32 a
	mean ± SD	45.5 ± 21.3	1.4 ± 0.7	17.7 ± 8.8	0.6 ± 0.4	2346.3 ± 1292.9	66.7 ± 4.9	1043 ± 376.9	31.26 ± 4.69	3452.5 ± 1639.4
buds	GM	60.7 ± 1.91 c	14.8	34.1 ± 1.28 c	8.3	223.5 ± 8.28 e	54.3	92.9 ± 1.77 e	22.6	411.3 ± 9.19 e
	KH	22.0 ± 0.78 e	3.1	12.4 ± 0.14 e	1.7	485.5 ± 5.47 d	68.2	191.7 ± 9.91 d	26.9	711.7 ± 9.46 d
	ST	24.3 ± 0.61 d	2.7	13.6 ± 0.42 d	1.5	596.4 ± 4.13 c	66.7	259.3 ± 2.63 c	29	893.6 ± 8.13 c
	M	77.2 ± 1.51 b	6.3	53.8 ± 0.56 b	4.4	754.5 ± 6.28 b	61.3	354.5 ± 5.47 a	28.1	1231 ± 12.05 b
	CH	118.5 ± 2.35 a	6.7	67.7 ± 0.49 a	3.8	1304.6 ± 9.11 a	74	271.5 ± 5.24 b	15.4	1762.3 ± 15.27 a
	mean ± SD	60.5 ± 40.1	6.7 ± 4.3	36.3 ± 24.4	3.9 ± 2.4	672.9 ± 402.7	64.9 ± 6.7	234 ± 97.8	24.4 ± 5	1002 ± 518.5
flowers	GM	18.6 ± 0.45 c	9.5	12.4 ± 0.29 a	6.3	118.6 ± 4.92 d	60.3	46.9 ± 0.84 e	23.9	196.5 ± 6.96 d
	KH	12.5 ± 0.36 e	8.8	7.5 ± 0.35 c	5.3	86.7 ± 1.9 e	60.8	53.8 ± 1.07 d	25.1	142.5 ± 3.77 e
	ST	16.5 ± 0.35 d	5.5	4.8 ± 0.14 e	1.6	195.9 ± 2.13 c	65.7	80.7 ± 0.9 c	27.1	297.9 ± 2.18 c
	M	29.5 ± 0.22 a	5.2	11.4 ± 0.21 b	2	357.1 ± 3.27 a	63.4	164.8 ± 2.08 b	29.7	562.8 ± 5.85 a
	CH	24.6 ± 0.45 b	4.8	6.1 ± 0.16 d	1.2	311.5 ± 4.06 b	60.6	171.5 ± 2.85 a	33.4	513.8 ± 4.05 b
	mean ± SD	20.4 ± 6.7	6.8 ± 1.9	8.5 ± 3.3	3.3 ± 2.1	214 ± 117.9	62.2 ± 2.1	103.5 ± 60.4	27.8 ± 3.4	342.7 ± 187.9

[a] Each value in the table is represented as a mean ± SD. [b] In each column, different letters mean significant differences ($p \leq 0.05$).

Table 3. β-Carotene and Lutein Contents (μg/g FW) in the Current Study and Published Studies

samples	β-carotene	lutein	study reference
leaves			
caper	2346.3	1043	this study
pepper	31.68	86.86	*17*
sesame	25.81	44.48	*17*
radish	15.7	19	*21*
edible portion			
caper (buds)	672.9	234	this study
pepper	2.24	4.89	*17*
cucumber	8.83	14.08	*17*
sweet potato	78.3	0.5	*21*
tomato, ordinary	7.78	195.9	*21*
tomato, high β-carotene	23.5	24.5	*21*

pouch (*28*). The identified lutein, β-carotene, and neoxanthin contents in *C. spinosa*, especially buds, could be used to enhance the intake of these compounds in foods.

Tocopherol Contents. The different parts of *C. spinosa* contain α- and γ-tocopherol. The absence of β-tocopherol was confirmed by using the same method described by ref (*29*). Tocopherol contents and the SDs of the different samples and parts of capers are given in **Table 4**.

As seen in **Table 4**, we notice that the total tocopherols varied within parts of plants; 20.19 ± 10, 98.5 ± 28.9, and 55.97 ± 23.8 mg/100 g FW in leaves, buds, and flowers, respectively. α-Tocopherol is the principal form in the leaves of *C. spinosa* (20.19 ± 10 mg/100 g FW). Indeed, a previous study indicates that this compound is commonly accumulated in leaves (*26*). In buds and flowers, there were two isomers, α-tocopherol (49.12 ± 17.48 and 28.68 ± 9.13 mg/100 g FW, respectively) and γ-tocopherol (48.13 ± 15.08 and 27.8 ± 16.01 mg/100 g FW, respectively). In general, γ-tocopherol was associated with nongreen plant tissues including reproductive tissues (*30*). A previous study suggested that the primary function of tocopherols is to protect photosynthetic membranes from oxidative stress by acting as a lipid-soluble antioxidant (*5*). Tocopherol synthesis is regulated in

Table 4. Tocopherol Content of Leaves, Buds, and Flowers of *C. spinosa* (mg/100 g FW)[a]

samples		α-tocopherol	γ-tocopherol	total tocopherols
leaves	GM	12.35 ± 0.21 d[b]	tr[c]	12.35 ± 0.21 d
	KH	32.40 ± 0.28 a	tr	32.40 ± 0.28 a
	ST	19.20 ± 0.28 c	tr	19.20 ± 0.28 c
	M	11.47 ± 0.60 d	tr	11.47 ± 0.60 d
	CH	30.54 ± 0.65 b	tr	30.54 ± 0.65 b
	mean ± SD	20.19 ± 10	tr	20.19 ± 10
buds	GM	32.55 ± 0.49 e	34.1 ± 0.14 e	66.40 ± 0.56 e
	KH	46.35 ± 0.21 c	50.15 ± 1.07 b	96.50 ± 0.42 c
	ST	35.1 ± 0.14 d	40.11 ± 0.93 d	75.58 ± 0.70 d
	M	64.03 ± 1.02 b	73.10 ± 1.15 a	137.55 ± 1.46 a
	CH	72.05 ± 1.21 a	43.55 ± 0.5 c	114.44 ± 1.51 b
	mean ± SD	49.12 ± 17.48	48.13 ± 15.08	98.50 ± 28.90
flowers	GM	28.25 ± 0.35 c	15.13 ± 0.09 d	43.1 ± 0.14 d
	KH	15.04 ± 0.06 a	13.45 ± 0.63 e	28.65 ± 0.35 e
	ST	28.35 ± 0.49 c	34.35 ± 1.21 b	62.5 ± 0.42 b
	M	40.55 ± 0.49 a	52.05 ± 1.07 a	92.04 ± 1.05 a
	CH	31.25 ± 0.35 b	22.47 ± 0.46 c	53.59 ± 0.57 c
	mean ± SD	28.68 ± 9.13	27.8 ± 16.01	55.97 ± 23.8

[a] Each value in the table is represented as a mean ± SD. [b] In each column, different letters mean significant differences ($p \leq 0.05$). [c] tr: trace.

plant responses to environmental stress and stress hormones such as low temperature (*31*), salinity (*4*), jasmonic, salicylic acid (*26*), Ni (*32*), UV radiation (*33*), and Zn and Cd (*34*). The extraordinary capacity of *C. spinosa* to exploit the limited water resources and to modulate the water potential (*9*) may be related to the important levels of tocopherols in this plant. Recently, another study (*35*) has also reported that the seeds of *C. spinosa* were rich in tocopherols.

The presence of a high content of γ-tocopherol in flower buds and flowers as compared to leaves suggests that maybe γ-tocopherol has specific functions that differ between the different parts of *C. spinosa*. In a previous paper (*31*), the authors suggested that γ-tocopherol can functionally replace α-tocopherol in some conditions. On the other hand, γ-tocopherol exerts a specific function in osmoprotection in vivo, providing evidence that α- and γ-tocopherol may not be functionally equivalent in

Table 5. Tocopherols Contents (mg/100 g FW) in the Current Study and Published Studies

samples	α-tocopherol	γ -tocopherol	study reference
leaves			
caper	20.19	tr[a]	this study
Citrus hystrix	40	NM[a]	*36*
Mentha arvensis	5	NM	*36*
Ilex aquifolium	19	NM	*37*
sesame	0.5	ND[a]	*17*
edible portion			
caper (buds)	49.12	48.13	this study
green *Capsicum annum*	8	NM	*36*
apple	0.5	ND	*38*
tomato	0.7	0.2	*39*
almond	26	0.8	*40*
flower			
caper	28.68	27.8	this study
Musa sapientum	3	NM	*36*

[a] tr, trace; NM, not mentioned; ND, not detected.

many respects (*30*). As compared with other natural sources such as sesame, tomato, and almond, the content of tocopherols found in this study makes *C. spinosa* a good natural source of these compounds (**Table 5**).

The content of total tocopherol varied between locations. The levels are between 11.47 ± 06 (M) and 32.4 ± 0.28 mg/100 g FW (KH) in leaves, from 66.4 ± 0.56 (GM) to 137.55 ± 1.46 mg/100 g FW (M) in buds, and between 28.65 ± 0.35 (KH) and 92.04 ± 1.05 mg/100 g FW (M) in flowers. These results indicate that the harvesting region is an important factor influencing the value of these compounds. Other authors reported that tocopherol content can be affected by geographic location (*40*).

Previous studies indicate that tocopherols and carotenoids cooperate in limiting stress condition damage (*26*). It is interesting to show that with high levels of total carotenoids [6294.5 ± 19.32 (CH) and 3331 ± 17.7 μg/g FW (KH) in leaves, 1762.3 ± 15.27 (CH) and 1231 ± 12.05 μg/g FW (M) in buds, and 562.8 ± 5.85 μg/g FW (M) in flowers], we detect the high values of tocopherols [30.54 ± 0.65 (CH) and 32.4 ± 0.28 mg/100 g FW (KH) in leaves, 114.44 ± 1.51 (CH) and 137.55 ± 1.46 mg/100 g FW (M) in buds, and 92.04 ± 1.05 mg/100 g FW (M) in flowers]. It seems that these compounds in different parts of *C. spinosa* cooperate to help this plant to resist to the environmental stress conditions.

Vitamin E is an important antioxidant in the diet. Our results show that *C. spinosa*, especially the buds, is a rich source of natural vitamin E. Moreover, the buds of *C. spinosa* present an important level of γ-tocopherol. This compound plays an important preventive role against many diseases like cancer and cardiovascular disease (*30*). From all of these facts, *C. spinosa* confirms its high nutritional value.

In summary, results show that the different parts of *C. spinosa* are rich in carotenoids and tocopherols. These compounds cope with oxidative stress. Qualitative and quantitative vitamin E and pro-vitamin A reported in this study should improve the nutritional value of caper (*C. spinosa*) and encourage the use of this plant for both food and pharmaceutical industries.

ACKNOWLEDGMENT

We are very grateful to the INRGREF for help in collecting samples.

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Received December 3, 2008. Revised manuscript received April 23, 2009. Accepted May 11, 2009.