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Lipid Profiles of Tunisian Coriander (*Coriandrum sativum*) Seed

Jazia Sriti · Wissem Aidi Wannes · Thierry Talou · Baya Mhamdi · Muriel Cerny · Brahim Marzouk

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Abstract Coriander (*Coriandrum sativum* L.) seeds were harvested from the region of Korba (North-East Tunisia) in order to characterize their fatty acids, phytosterols, tocopherols and tocotrienols (tocols) profiles. Nine fatty acids, with petroselinic acid accounting for 76.6% of the total fatty acids, followed by linoleic, oleic and palmitic acids, accounting for 13.0, 5.4 and 3.4%, respectively, of the total fatty acids were identified. Neutral lipids (NLs) were mainly composed of triacylglycerols (98.4%). Polar lipids were mainly composed of phosphatidylcholine as the major phospholipid (PL) subclass, whereas digalactosyldiacylglycerol was the major galactolipid (GL). Total sterols content was estimated to be 36.93 mg/g oil. Stigmasterol accounted for 29.5% of the total sterols. Other representative sterols were *β*-sitosterol, Δ^7-stigmasterol and Δ^5, 24-stigmastadienol, which accounted for 24.8, 16.3 and 9.2%, respectively. Gamma-tocotrienol was the predominant tocol at 238.40 μg/g seed oil. This was equivalent to 72.8% of the total tocols followed by *γ*-tocopherol (8.06%) and *α*-tocopherol (7.6%).

Keywords *Coriandrum sativum* L. · Lipid classes · Phosphatidylcholine · Triacylglycerols · Sterol · Tocols

J. Sriti (✉) · W. A. Wannes · B. Mhamdi · B. Marzouk
Unité des Plantes Aromatiques et Médicinales,
Centre de Biotechnologie au Technopole de Borj-Cédria,
BP 901, 2050 Hammam-Lif, Tunisia
e-mail: sritijazia@yahoo.fr

T. Talou · M. Cerny
Laboratoire de Chimie Agro-industrielle, Unité Mixte de
Recherche INRA/INP. ENSIACET, 118 route de Narbonne,
31077 Toulouse Cedex 4, France

Introduction

During the last decades, in addition to oleaginous species, many new plant species have been investigated as potential sources of vegetable oils due to nutritional, industrial and pharmaceutical interests. Species of interest include the Apiaceae, which are widely distributed in temperate regions and used for their medicinal properties, mainly due to their high content of secondary metabolites such as coumarins. Recently, researchers have stated that seeds of some Apiaceae species may contain a significant amount of oil. In particular, coriander (*Coriandrum sativum* L.), an annual herb commonly used as a condiment or a spice in the Mediterranean area. In Tunisian, cultivation covers a surface of 284,000 ha and the yearly production is 2–2.5 t/ha [1].

Coriander oil is rich in petroselinic acid, which has many industrial applications. This acid can be used as a precursor of both lauric acid, which is a component of detergents and surfactants, and adipic acid, which is the monomeric component of nylon 66. Although, the fatty acid composition of coriander seed oil has been reported [2, 3], little is known about its oil composition.

Phospholipid (PL) composition also has potential as a multifunctional additive for food, pharmaceutical and industrial applications [4]. According to Rathjen and Steinhart [5], PLs are widely distributed in food, and possess pro-oxidant as well as antioxidant effects. Plant galactolipids are thought to be nutrients in the human diet, but little is known about their intestinal digestion and absorption in mammals [6].

Some other minor lipids, such as phytosterols, have attracted the interest of food researchers because of their peculiar functional properties [7]. Phytosterols account for the major proportion of the oil unsaponifiable fraction and

are one of the main components of membrane lipids. In plants, more than 200 different types of phytosterol have been reported, β-sitosterol, campesterol and stigmasterol being the most abundant [8]. Phytosterols play major roles in several areas, namely in pharmaceuticals (production of therapeutic steroids), nutrition (anti-cholesterol additives in functional foods, anti-cancer properties), and cosmetics (creams, lipstick) [9]. Epidemiologic and experimental investigations suggest that dietary sterols may offer protection against the most common cancers in Western societies, such as colon, breast and prostate cancers.

Another group of the oil minor fraction is represented by tocopherols, which are very interesting molecules due to their antioxidant properties [10]. These antioxidants are present in vegetable oils such as those from sunflower, maize, rape and olive. Their contents differ in refined and in virgin oils. The most common tocopherol is α-tocopherol whereas γ-tocopherol is the most abundant representative of this antioxidant group in other organs [11]. A major physiological role of these compounds is to prevent lipid peroxidation during seed dormancy, germination and early seedling development [12].

The main objective of this study was to evaluate the neutral lipids, phospholipids, sterols and tocopherol contents of *C. sativum* in the seed. This result will be important in order to strengthen value-added use of coriander as a new source of edible oil with nutritional, industrial and pharmaceutical importance.

Materials and Methods

Materials

Overripe seeds of *Coriandrum sativum* var. *sativum* L. used in the present work were harvested in the year 2006 from the region of Korba in the northeast of Tunisia; latitude 36°34′38.22”(N); longitude 10°51′29.63”(E) and the altitude is 637 m. The precipitation average was 400–500 mm/year and the monthly average temperature was 17.7 °C. After harvesting, the seeds were stored at 4 °C until extraction.

Silica gel plates used for thin-layer chromatography (TLC) were from Merck (Darmstadt, Germany). Dihydrocholesterol (5α-cholestan-3β-ol) used as the internal standard for sterol quantification was obtained from Sigma-Aldrich Co. (St. Louis, MO). Fatty acid standards were purchased from Fluka (Riedel-de Haën, Switzerland) and Sigma-Aldrich (Steinheim, Germany). Sterol standards were purchased from Sigma (St. Louis, MO). Standards used for ST characterization, β-sitosterol, stigmasterol, campesterol, Δ^5 avenasterol and Δ^7 avenasterol were purchased from Supelco (Bellefonte, PA). Standards used for PL identification, phosphatidic acid (PA), phosphatidylethanolamine (PE), phosphatidylinositol (PI) from Bovine liver, phosphatidylcholine (PC) from soybean and phosphatidylglycerol (PG) from egg yolk were purchased from Sigma Chemical Co. (St. Louis, MO). α-, γ-, δ-tocopherol peaks from samples were identified by comparing their spectra with those of pure standards. All reagents and chemicals used in the study were of analytical grade.

Total Lipid Extraction

Samples of 200 seeds were extracted using the modified method of Bligh and Dyer [13]. Fruit samples were kept fixed in boiling water for 10 min to inactivate the phospholipases [14]. Then, one g of the fruit was finely ground in a china mortar into the chloroform–methanol–hexane (3:2:1, by volume) mixture [15]. Water (5 mL) was added and then the mixture was centrifuged at 2,000 rpm (3,000*g*) for 10 min. The organic phase containing total lipids was recovered, dried under a nitrogen stream and stored at −20 °C until analysis.

Fatty Acid Methylation

Fatty acids of glycerolipids were methylated using the sodium methoxide solution at 3% in methanol according to the method described by Cecchi et al. [16]. Methyl heptadecanoate (C17:0) was used as the internal standard.

Lipid Class Separation by Thin-Layer Chromatography

Lipid classes were separated by TLC using glass plates (20 × 20 cm) covered with silica gel (G60, Merck) at a thickness of 0.25 mm. For this, the plates were activated at 120 °C for 2 h immediately before use, and approximately 30 mg of total lipids per gram of adsorbent was fractioned. NLs were separated according to the method of Mangold [17] using a mobile phase of petroleum ether–ethanol–acetic acid (70:30:0.4, *v*/*v*/*v*). PLs were separated using a mixture of chloroform–acetone–methanol–acetic acid–water (50:20:10:10:5, *v*/*v*/*v*/*v*/*v*) as described by Lepage [18]. Lipid spots were detected after a brief exposure of the plates to iodine vapors saturating a tightly closed vat. The identification of lipid classes was made by comparing their Retention Factor (R_F) values with those of authentic standards chromatographed under the same conditions. After the detection of the lipid classes, the plates were submitted to a nitrogen stream in order to eliminate iodine, and individual bands were scraped from the plates and corresponding glycerolipids were recovered from the silica gel by elution with 5 mL of hexane.

Unsaponifiable and Sterols Extraction

Five mg of dihydrocholesterol (internal standard) was added to 140 mg of oil. Then, 3 mL of 1 M KOH in ethanol was added and the mixture was maintained at 75 °C for 30 min. After cooling at the ambient temperature, 1 mL of distilled water and 6 mL of isohexane were added to the mixtures. The isohexane phase was allowed to isolate unsaponifiable fraction which was analyzed by GC. Before GC analysis, samples were silylated by the addition of 1 mL *N*-methyl-*N*-trimethylethylsilyl-heptafluorobutyramide (MSHFBA) mixed with 50 µL of 1-imidazol methyl and heated for 5 min at 103 °C. All experiments were done in triplicate.

Gas Chromatography

The fatty acid methyl esters were analyzed by GC, using a Varian 3900 gas chromatography (Grenoble, FR) flame ionization gas chromatograph, with a fused silica capillary column, CP Select CB (50 m, 0.25 mm i.d., 0.25-µm film thickness; Grenoble, FR). The carrier gas was helium with a flow rate of 1.2 mL/min; split ratio was 1:100. The initial oven temperature was held at 185 °C for 40 min, increased at a rate of 15 °C/min to 250 °C and then held there for 10 min. The detector and injector temperatures were 250 °C. Analyses were done in triplicate.

Sterol samples were analyzed by GC using a FID-Perkin Elmer gas chromatograph (Courtaboeuf, FR) equipped with a CP-SIL 8CB capillary column (30 m; 0.25 mm i.d., 0.52-µm film thickness; Grenoble, FR). The carrier gas was hydrogen with a flow rate of 1 mL/min (split-splitless injection was used). Analyses were performed under the following temperature program: isotherm at 160 °C during 0.5 min, from 160 to 260 °C at a rate of 20 °C/min, 2 °C/min to 300 °C and 45 °C/min to 350 °C. Injector and detector temperatures were maintained respectively at 340 °C and 365 °C.

GC–MS

The GC–MS analyses were performed on a gas chromatograph HP 5890 (II) interfaced with a HP 5972 mass spectrometer (Agilent Technologies, Palo Alto, CA) with electron impact ionization (70 eV). A HP-5MS capillary column (60 m, 0.25 mm i.d., 0.25-µm film thickness; Agilent Technologies, Hewlett–Packard, CA) was used. The column temperature was programmed to rise from 40 to 280 °C at a rate of 5 °C/min. The carrier gas was He with a flow rate of 1.2 mL/min. Scan time and mass range were 1 s and 50–550 (*m*/*z*), respectively. Sterols were identified by comparing their mass spectra with those of authentic compounds.

Tocols Extraction

Fifteen grams of coriander seeds were ground in a china mortar using 150 mL of isooctane/isopropanol (3:2, *v*/*v*). The mixture obtained was centrifuged at 10,000*g* for 15 min. The organic layer was then recovered and filtered through a 0.45 µm filter. These steps were repeated twice. The extract was evaporated first, in a rotary evaporator and then under nitrogen, at ambient temperature.

High Performance Liquid Chromatography

Samples were analyzed by HPLC system consisting of a pump (P680, Bretonneux, FR) equipped with a KROMASIL Si-100-S column (Lapeyrouse- Fossat, FR) (5.0 µm, 4.0 × 250 mm) and a fluorescent detector (Dionex Model RF-2000 Fluorescence Detector, Bretonneux, FR) at 290 and 317 nm excitation and emission wavelengths, respectively. The mobile phase was isooctane/isopropanol (99.5:0.5, *v*/*v*) at a flow rate of 1 mL/min. Tocols identification was based on the comparison of their retention times with those of standard solutions (Supelco-Sigma).

Results and Discussion

Fatty Acids

The oil from the seeds of coriander extracted with *n*- the hexane constituted 22.6% on the basis of dry matter weight. Nine fatty acids were identified, where petroselinic was the major one accounting for 76.6%, followed by linoleic acids with 13% of total fatty acids (Table 1). In this context,

Table 1 Fatty acid composition of coriander seed

Fatty acid	RT of FAME	Total fatty acids (%)
14:0	4.639	0.08 ± 0.1
16:0	5.772	3.50 ± 0.05
16:1n-7	6.24	0.23 ± 0.00
18:0	7.726	0.78 ± 0.03
18:1n-12	8.364	76.65 ± 0.16
18:1n-9	8.411	5.47 ± 0.07
18:2n-6	9.592	13.05 ± 0.04
20:0	11.068	0.10 ± 0.01
18:3n-3	11.27	0.15 ± 0.01
SFA		4.46 ± 0.08
MUFA		82.35 ± 0.11
PUFA		13.2 ± 0.21
Oil yield (%)		22.65 ± 0.20

Values given are the means of three replicates ± standard deviation

RT retention time, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

Msaada et al. [19] showed that fruits collected in 2005 from the Charfine area (Northeastern Tunisia) had the same fatty acids, but, in addition, gadoleic, erucic and docosahexenoic acids were also present. Ramadan and Mörsel [20] reported similar results in seeds from Germany. The level of petroselinic acid detected in our samples was quite similar to that given by Kleiman and Spencer [21]. Petroselinic acid is metabolized and accumulated in the developing endosperm of some Umbelliferae species, including coriander and carrot [22]. This fatty acid is the product of the acyl carrier protein Δ^6 desaturase (ACP Δ^6 desaturase) activity. This polypeptide is highly expressed in the seed but is absent in tissues that do not biosynthesize petroselinic acid, including leaves and roots of coriander [23].

Other representative fatty acids were oleic (5.4%), palmitic (3.5%) and stearic (0.7%) acids. In addition, palmitoleic, α-linolenic, arachidic and myristic acids were minor fatty acids constituting 0.2, 0.15, 0.10 and 0.08%, respectively (Table 1). In seeds, saturated fatty acids represented 4.4% of total fatty acids, while monounsaturated fatty acids accounted for 82.3%. However, the fatty acid profile estimated in the present investigation was slightly different from those in literature that varied considerably. For example, in this study, lauric acid was not found as reported by Subbaram and Young [3].

Although the substantial data on chemical composition of coriander, there are no previous phytochemical on coriander cultivated in Tunisia.

Table 2 shows the proportions of NL classes in coriander seed oil, the data showed that NLs, which represent 94.88% of the total lipids, were mainly composed of TAG (95.5%), FFA (2.05%), DAG (1.8%) of NLs and 0.5% MAG in the NLs.

PLs from coriander seed oil were separated into five classes by TLC and were identified as PI, PC, PG, PE and PA by comparing their retention times with those of authentic standards analyzed under the same conditions. PC was the most abundant class with 35.98% of the total PL content followed by PE and PI. As for PA and PG, these were detected in lower levels with approximately 8.11 and 6.68%, respectively. According to Ramadan and Mörsel [20], PC and PE together make up to 70.5% of the total PLs from the seeds of *C. sativum*, which originated from Germany. On the other hand, these authors have shown that the solvent (or mixtures) used plays an important role in the amount and composition of the recovered lipids.

GLs were separated by TLC according to Lepage [18] into two subclasses: monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG). The most abundant subclass was DGDG, representing 62.32% of the total GL content followed by MGDG with 37.68%.

Table 2 Glycerolipid composition of *Coriandrum sativum* L. seed oil

	NLs (g/100 g)	PhLs (g/100 g)	GLs (g/100 g)
MAGs	0.57 ± 0.04	–	–
DAGs	1.88 ± 0.02	–	–
TAGs	95.50 ± 0.45	–	–
FFAs	2.05 ± 0.15	–	–
PI	–	15.40 ± 0.12	–
PC	–	35.98 ± 0.31	–
PG	–	6.68 ± 0.17	–
PE	–	33.83 ± 0.23	–
PA	–	8.11 ± 0.14	–
MGDG	–	–	37.68 ± 0.65
DGDG	–	–	62.32 ± 0.34

Values are given as means of three replicates ± SD

NLs neutral lipids, *PhLs* phospholipids, *GLs* galactolipids, *MAGs* monoacylglycerols, *DAGs* diacylglycerols, *FFAs* free fatty acids, *TAGs* triacylglycerols, *PI* phosphatidylinositol, *PC* phosphatidylcholin, *PG* phosphatidylglycerol, *PE* phosphatidyl ethanolamine, *PA* phosphatidic acid, *MGDG* monogalactosyldiacylglycerol, *DGDG* digalactosyldiacylglycerol

Sterol Composition

Sterols constitute the major fraction of the unsaponifiable matter in many oils. They are of interest due to their antioxidant activity and beneficial impact on human health [24, 25]. Moreover, analysis of sterols provides a powerful tool for oil authenticity and quality control otherwise not recognized by the fatty acids profile. Thus, the sterol profile constitutes a perfect finger print of vegetable oils.

The content and composition of sterol in coriander seed oil are presented in Table 3. High level of total sterols was estimated, which make up 36.93 mg/g oil (3.69%). Compared with others vegetables oils such as corn (23 mg/g of oil), soybean (9 mg/g of oil), rapeseed (0.05 mg/g of oil) and coconut (0.008 mg/g of oil) oils [26], coriander seed

Table 3 Sterol composition of coriander seed oil

Sterols	RT	Total sterols (%)
Cholesterol	20.725	1.02 ± 0.23
Campesterol	22.674	8.82 ± 0.56
Stigmasterol	23.16	29.54 ± 1.49
β-Sitosterol	24.385	24.82 ± 1.06
Δ^5 Avenasterol	42.713	4.81 ± 0.33
Δ^5 24 Stigmastadienol	24.917	9.24 ± 0.28
Δ^7 Stigmasterol	25.702	16.30 ± 0.37
Δ^7 Avenasterol	26.035	5.44 ± 0.56
Total sterols (%)		3.96 ± 0.25

Values given are the means of three replicates ± standard deviation

RT retention time

oil represented an important source of sterols. Stigmasterol (29.5%) represents the main component followed by β-sitosterol (24.8%). These compounds characterize the Nigella seed oils constituting about 65% of total sterols [27]. On the other hand, Ramadan and Mörsel [20] showed that stigmasterol (29.8%) and β-sitosterol (28.2%) were the most predominant components for coriander seeds grown in Germany. Stigmasterol is related to various parameters of the quality of virgin olive oil. Indeed, most vegetable seeds such as *Ornithopus sativus* [26], millet, sunflower [28] and fenugreek [29] were characterized by the predominance of β-sitosterol. The latter is the sterol marker in extra virgin olive oil and ranges from 75 to 87% of total sterols [30]. In soybeans and peanuts, β-sitosterol and campesterol were detected as major sterols.

The next major components were Δ^7 stigmasterol (16.3% of total sterol) and Δ^5 24-stigmastadienol (9.2% of total sterol). Campesterol was present at a level of 8.8% of total sterol, whereas Δ^7 avenasterol and Δ^5 avenasterol were detected in lower amounts with approximately 5 and 4%, respectively. In this study, a small amount of cholesterol was detected at 1.02% of total sterol; it has also been detected in black cumin seeds from Egypt [31], where it represented 7.2% of total sterols in black cumin from Tunisia (2% of total sterols) [32]. However, this component was not detected in *C. sativum* L. seeds from Germany [33].

Tocols Composition

Tocotrienols are mostly associated with seeds, fruits and latex, rather than with mature leaves [34]. The compositions of tocols in coriander seed analyzed using HPLC are summarized in Table 4. Total tocols contents in coriander seed oils were 327.47 µg/g seed. The major tocopherol was γ-tocopherol (26.40 µg/g seed), followed by δ-tocopherol (13.50 µg/g seed) and α-tocopherol (11.70 µg/g seed). Coriander seed contained higher amounts of total tocotrienol where γ-tocotrienol was the main compound (238.40 µg/g seed), followed by α-tocotrienol (24.90 µg/g seed) and δ-tocotrienol (12.57 µg/g seed). The amounts of tocopherols and tocotrienols in coriander seed are seldom described in the literature. György et al. [33] who studied seed tocols of Belgian coriander found δ-tocopherol (36.5 µg/g seed) as the major compound followed by α-tocotrienol (6 µg/g seed). The composition of tocols can be affected not only by genetic factors (cultivars) but also by region and growing conditions (e.g. climate, soil). In particular, these resulted from the combined effects of climatic, genetic and abiotic conditions (e.g. climate, soil) under which the crop was grown [10]. Other studies showed the presence of tocotrienols in monocotyledonous seeds [34, 35] and dicotyledonous plants, including *Carum carvi* L [36].

Table 4 Tocol composition of coriander seed

Tocols	RT	Seed (µg/g)
α-Tocopherol	4.48	11.7 ± 0.68
α-Tocotrienol	4.9	24.90 ± 0.79
γ-Tocopherol	7.45	26.40 ± 1.47
γ-Tocotrienol	8.16	238.40 ± 2.72
δ-Tocopherol	11.42	13.50 ± 2.15
δ-Tocotrienol	12.66	12.57 ± 0.80
Total Tocols		327.47 ± 1.24

Values given are the means of three replicates ± standard deviation

RT HPLC retention time

Bonvehi et al. [37] proved that α- and γ-tocopherols were the major tocopherols in vegetable oils and fats. Wheat germ oil was found to be by far the best source of α-tocopherol, followed by sunflower and, again by quite a large margin, olive oils.

Conclusions

The present investigation provides evidence that coriander seed oil is a rich source of essential fatty acids such as linoleic acid and an unusual acid, petroselinic acid, characterizing Umbelliferae seed lipids. The PL fraction has an appreciable proportions of PC and PE , making this oil a good source of these components. As for GLs, their presence in appreciable amounts makes coriander seeds an excellent source of these compounds in the human diet.

On the others hand, it is clear that *Coriandrum sativum* L. seed oil constitutes a good source of sterols mainly of stigmasterol and β-sitosterol which exhibit an inhibitory effect on the absorption of dietary cholesterol. The tocols composition indicates that the coriander seeds are rich in tocotrienol.

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