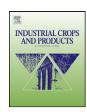
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Lipid, fatty acid and tocol distribution of coriander fruit's different parts

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ABSTRACT

High amounts of neutral lipids (NL) were found (95.65% of total lipids) in whole fruit oil, while glycolipids (GLs) occurred at high levels in pericarp oil (73.21% of total lipids). Triacylglycerol was found to be the principal lipid fraction of NL which formed 93% in seed and whole fruit. However, monoacylglycerol constituted the major fraction of NL in pericarp (34.5%). At least two GLs and five phospholipids (PL) were identified. The GL composition of the pericarp is characterized by monogalactosylacylglycerol as the major fraction with a percentage of 53.39% of total lipids. The major individual PL subclasses were phosphatidylcholine followed by phosphatidylethanolamine in seed and whole fruit. However, the PL were not detected in pericarp oil. The principal fatty acids identified in most lipid classes were petroselinic acid (C18:1n – 12), linoleic acid (C18:2n – 6), palmitic (C16:0) and oleic acid (C18:1n – 9). The total tocopherol and tocotrienol (tocol) contents were 27.78 mg/100 g oil in whole fruit, 26.42 mg/100 g oil in seed and 5.36 mg/100 g oil in pericarp. Fruit and seed oils were characterized by a high amount of γ -tocotrienol with 19.56 mg/100 g oil. However, α -tocopherol (1.82 mg/100 g oil) was found to be the tocol marker in pericarp oils.

The results are important as an indication of the potentially economical utility of *Coriandrum sativum* L. seed oil as a new source of PL.

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1. Introduction

The oleochemical industry is increasingly interested in custommade and novel oils with specific fatty acid compositions for applications in the oil and pharmaceutical industries (Murphy, 1999). Such oils can be used for the synthesis of high-quality products without expensive purification of raw materials. In addition, oilseed breeders are searching for species to produce beneficial new genotypes (Murphy, 1999).

In the development of new oil seed crops interest has turned to the members of the Apiaceae family. The Apiaceae represent one of the best-known plant families, widely distributed in temperate climate regions where they are often used as spices, vegetables or drugs owing to the presence of useful secondary metabolites. Some of the genera are known for their high level of petroselinic acid in seed oils which represent an interesting oleochemical for the food, cosmetics, and pharmaceutical industries. Petroselinic acid can be oxidatively cleaved to produce a mixture of lauric acid, a compound useful in the production of detergents, and adipic acid, a C6 dicarboxylic acid which can be utilized in the synthesis of nylon

polymer (Murphy, 1991). Species of interest include the coriander (*Coriandrum sativum* L.), which is widely known for its wide range of healing properties. It is generally used in gastrointestinal complaints such as anorexia, dyspepsia, flatulence, diarrhea, griping pain and vomiting (Usmanghani et al., 1997). Coriander is used as antiedemic, anti-inflammatory, antiseptic, emmenagogue, antidiabetic, antihypertensive, lipolytic and myorelaxant, and possess nerve-soothing property (Duke et al., 2002).

Different studies have proven its efficacy as antidiabetic (Swanston-Flatt et al., 1990), hypolipidemic, antioxidant (Chithra and Leelamma, 1997, 1999) and larvicidal (Consoli et al., 1988). Coriander fruit is also reputed as refrigerant, tonic, diuretic and aphrodisiac, while, the oil is considered useful in flatulent colic, rheumatism, neuralgia, etc. (Nadkarni, 1976).

The fatty acids profile is a main determinant of the oil quality in coriander fruit mainly with percentage of oleic, linoleic and petroselinic acids. Lipid components in fruits, though occurring in minor amounts, are presumed to contribute to the development of characteristic aromas and flavours during ripening as they are considered as precursors for various volatile odorous principles of fruits (Gholap and Bandyopadhyay, 1980). Supran (1978) reported that lipids contribute to the industrial and nutritional value as well as characteristic aromas and flavours. Recent studies on the compositional analysis of *C. sativum* L. fruits have described essential oil

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and fatty acid composition of fruit's different parts (Sriti et al., 2009) and the changes of fatty acid composition of coriander ripening (Msaada et al., 2009a). Msaada et al. (2009b) had also determined the influence of maturity stages and growing region on oil content and fatty acid composition.

Nevertheless, no work has been undertaken concerning the glycerolipid content of the Tunisian variety. Moreover, no published study has reported the lipid class and tocohperol contents of the various parts of the coriander fruit.

The main objective of the work presented here was to determine the lipid class composition, fatty acid distribution in the lipid pool and tocol contents of *C. sativum* in seed, pericarp and whole fruit.

2. Materials and methods

2.1. Plant material

Coriander fruits were collected from cultivated plants in June 2008 in the region of Korba (Northwestern Tunisia; latitude 36°34″38.22″(N); longitude 10°51″29.63″(E); altitude 637 m).

After sampling, 5 g of seeds were dried at 60 °C until constant weight was reached in order to determine their dry matter weight and moisture level.

Seeds were separated from the fruits and then manually divided into two parts, splitting the pericarp and the seed. It is interesting to note that coriander seed constitutes about 65.7% of fruit dry matter weight.

2.2. Reagents and standards

All solvents used in the experiments (diethyl ether, chloroform, hexane, toluene, ethanol, acetone and methanol) were purchased from Merck (Darmstadt, Germany). Sodium methylate, sodium chloride, sulphuric acid, acetic acid and anhydrous sodium sulphate (Na₂SO₄) were purchased from Sigma–Aldrich (Steinheim, Germany). Fatty acid and glycerolipid standards were purchased from Fluka (Ridel-de Haën, Switzerland) and Sigma–Aldrich (Steinheim, Germany). Tocopherol peaks from samples were identified by comparing their spectra with those of pure standards (Sigma–Aldrich). All reagents and chemicals used in the study were of analytical grade.

2.3. Total lipid extraction

Triplicate sub-samples of 1 g were extracted using the modified method of Bligh and Dyer (1959). Thus, fruit samples were fixed in boiling water for 5 min and then ground manually in a China mortar using a mixture of chloroform/methanol/hexane (3:2:1, v/v/v). After washing with water of fixation and decantation during 24 h at +4 °C, the organic phase containing total glycerolipids was recovered and dried under a nitrogen stream. Finally, the residue was dissolved in a known volume of toluene–ethanol (4:1, v/v) and stored at -20 °C for further analyses.

2.4. Lipid class separation by thin-layer chromatography (TLC)

Lipid classes were separated by TLC using $20\,\mathrm{cm} \times 20\,\mathrm{cm} \times 0.25\,\mathrm{mm}$ silica gel plates (G60, Merck, Darmstadt, Germany). Neutral lipid separation by the method described by Manglod (1964) using a development system composed of petroleum ether-diethyl ether-acetic acid (70:30:0.4; v/v/v). Polar lipids were separated using a mobile phase mixture of chloroform-acetone-methanol-acetic acid-water (50:20:10:10:5; v/v/v/v) as described by Lepage (1967). Glycerolipid spots were detected after a brief exposure to iodine and

each spot was identified by co-chromatography of pure lipid standards.

2.5. Fatty acid methylation and analysis

The fatty acids of each lipid fraction were converted to methyl esters as described by Cecchi et al. (1985). Transmethylation was made by the addition of 2 mL of hexane, 0.5 mL of 3% sodium methylate, a known amount of heptadecanoic acid methyl ester (C17:0) used as the internal standard, 0.2 mL of 1N $\rm H_2SO_4$ and 1.5 mL of 10% sodium chloride. The hexanic phase that contains fatty acid methyl esters (FAMEs) was recovered and its volume reduced using a stream of nitrogen, prior to analysis.

FAMEs were analyzed on a HP 6890 gas chromatograph (Agilent Palo Alto, CA, USA) equipped with a flame ionization detector (FID). The esters were separated on a RT-2560 capillary column (100 m length, 0.25 mm i.d., 0.20 mm film thickness). The oven temperature was kept at 170 °C for 2 min, followed by a 3 °C/min ramp to 240 °C and finally held there for an additional 15 min period. Nitrogen was used as carrier gas at a flow rate of 1.2 mL/min. The injector and detector temperatures were maintained at 225 °C. FAMEs were identified by comparison of their retention times with those of pure reference standards. Gas chromatograph was connected to HP Chemstation (Rev.A.0401) software for peak area and fatty acid percentage calculation.

2.6. Dry matter, protein and mineral content

Dry matter was determined gravimetrically after drying at $105\,^{\circ}\text{C}$ for $24\,\text{h}$ (NF V03-908).

Protein content was determined by Kjedhal method according to the French Standard NF V18-100 consisting of mineralization of organic nitrogen content in the sample to mineral nitrogen. By convention, the protein content of the sample was then obtained by multiplying the total nitrogen content by a conversion factor empirically (6.25).

The mineral content was determined according to standard French NF V03-322. The sample underwent a calcination in an oven at $550\,^{\circ}\text{C}$ until constant weight. All experiments were done in triplicates.

2.7. Tocol extraction and analysis

Five grams of fruits (pericarp) and seeds of coriander were first ground into a fine powder and combined with $50\,\mathrm{mL}$ of hexane. The mixture obtained was centrifuged at $10,000\times g$ for $15\,\mathrm{min}$. The organic layer was then recovered and filtered. These steps were repeated twice. The extract was evaporated first, in a rotary evaporator and then under nitrogen, at room temperature. For the determination of tocopherols a solution of $10\,\mathrm{mg}$ oil in $1\,\mathrm{mL}$ hexane was directly used for the high-performance liquid chromatography (HPLC) analysis.

Samples were analyzed by high-performance liquid chromatography (HPLC) consisting a pump P680 equipped with a KROMASIL Si-100-S column (Lapeyrouse-Fossat, FR) (5.0 μ m, 4.0 mm \times 250 mm) with fluorometrical detection (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. Tocopherols identification was based on the comparison of their retention times with those of standard solutions (Supelco-Sigma).

2.8. Statistical analysis

Data were subjected to statistical analysis using statistical program package STATISTICA (Stasoft, 1998). Total volatile compounds

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