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Comparative analysis of supercritical CO₂ extracts and essential oils from an *Ocimum basilicum* chemotype particularly rich in T-cadinol

Andrea Occhipinti^a, Andrea Capuzzo^a, Simone Bossi^a, Chiara Milanese^b & Massimo E. Maffei^{a*}

Abstract

Ocimum basilicum (sweet basil) is an important essential oil plant used for different purposes (from food flavoring to pharmaceutical applications) characterized by the presence of several chemotypes. Here we show a comparative analysis between hydrodistillation of essential oils (EO) and supercritical CO₂ extraction (SFE) of an *O. basilicum* chemotype particularly rich in T-cadinol. SFE yielded a higher percentage of 1,8-cineole (10%; 4-fold), linalool (23.2%; 5.8-fold), eugenol (13.3%; 1.2-fold) and germacrene D (5.6%; 28-fold) with respect to EO. On the other hand, EO composition was characterized by higher percentages of T-cadinol (27.5%; 3-fold) and some other sesquiterpenes with respect to SFE. The presence of high percentages of T-cadinol in EO is of great importance owing to the biological activity of this compound in cancer therapy and as an antibiotic.

Keywords

- *Ocimum basilicum*,
- Lamiaceae,
- supercritical fluid extraction,
- essential oil,
- T-cadinol,
- GC-MS

Introduction

Basil (*Ocimum basilicum* L.) is an important aromatic as well as ornamental plant used since ancient times for its phytochemical properties, because of the presence of several bioactive compounds accumulated in leaf and flower secretory tissues (1–4). The essential oils of basil are used to flavor foods, whereas in pharmaceutical industry they find applications because of their insecticidal, nematicidal, fungistatic and antimicrobial properties (5–7). Basil cultivars have been classified according to their morphology (8) and essential oil chemical composition (9–13). However, the grouping of *Ocimum* species based on morphological characteristics does not correspond to the grouping based on volatile oil constituents (13). Many chemotypes are present with a variety of volatile oil compounds, including linalool, methyl chavicol, eugenol, geraniol and methyl cinnamate.

Supercritical fluid extraction (SFE) has been widely used for processing natural products because it produces extracts without solvent residues (14–17). Since SFE uses lower temperatures during extraction, it preserves thermo-sensitive compounds. This is the major reason why this technique is ideal for extracting plant volatiles, oleoresins and other active principles where the final product quality is of great importance (18,19). Recently, SFE technology was used to obtain extracts from *O. basilicum* with CO₂. It has been reported that through hydrodistillation the essential oil (EO)

yield ranged from 0.3% to 1.2% (w/w), while yields obtained using liquid and supercritical CO₂ ranged from 0.42% to 1.90% (w/w) (20).

As a part of a breeding program aimed to select methyl chavicol-free chemotypes, we isolated by massive selection and cultivation a chemotype particularly rich in T-cadinol (also known as 10-epi- α -muurolol). α -Cadinol (the isomer of T-cadinol) is found in some basil cultivars (20–23); however, to our knowledge this is the first report on a basil chemotype particularly rich in T-cadinol. Here we show a comparative analysis of essential oil and SFE extract of this basil chemotype.

Experimental

Plant material

Plants of *O. basilicum* L. were cultivated in the experimental fields of the Consorzio Sativa Soc. Coop. Agricola in Cesena, Italy. Plants were watered every three days and fertilized using a mixture of Osmocote® and Triabon®. When in full bloom, plants were harvested and air dried.

Essential oil distillation and CO₂ supercritical fluid extraction (SFE)

Basil was hydrodistilled by using a modified Clevenger apparatus as described previously (24). The SFE of dried basil aerial parts was carried out with a Waters SFE-2000F unit (Waters SFC Division, Pittsburg, PA, US) in a 2-L extractor. Food-grade (99.9% v/v purity) CO₂ (Air Liquide, Italy) was delivered for 30 minutes at a flow rate of 4.8 kg/hour and was compressed to the extraction pressure of 15 MPa by an air-driven liquid pump after cooling. The CO₂ was delivered to the extractor via a heat exchanger to maintain the extraction temperature at 50°C. Afterwards, 95% (w/w) ethanol (Carlo Erba, Italy) was added through the use of a co-solvent pump at the initial concentration of 2% (w/w) for 45 minutes and was increased to 20% (w/w) and maintained for 20 minutes. A final step with CO₂ alone was operated for 15 minutes. Two cyclonic separators (500 mL each) were used in order to obtain two different extracts: in the first separator (7.5 MPa, 25°C) waxes and other heavy components, in the second (2.5 MPa, 0°C) the terpenic fraction.

Essential oil and SFE extract analyses

SFE extracts and EO were analyzed by gas chromatography (GC) on a Agilent Technologies 6890N gas chromatographer equipped with a Phenomenex capillary column ZB5-MS (model 7HG-G010-11, 30 m \times 0.25 mm ID, film thickness 0.25 μ m) coupled with an Agilent EI-quadrupole mass spectrometer detector (MSD) model 5973. Injection port was in split mode (split ratio 1:20) kept at 220°C. Carrier gas was helium with a constant flow of 0.9 mL/minute; oven program was as follows: initial temperature 60°C, then a 3°C/minute ramp was programmed up to a final temperature of 290°C. MSD conditions were: ionization energy 70 eV, electro multiplier 1859 V, ion source at 280°C, quadrupole at 150°C; the acquisition mode was in scan mode with a mass range 50–450 amu. Compounds were identified by GC–mass spectrometry (GC–MS) as previously reported (25). The column and the program parameters were the same as above. Further identification of compounds was made by comparison of their mass spectra with those stored in NIST 98 and other custom-made libraries or by co-injection with pure standards. Literature retention index values were taken from ref. (26). Essential oil and terpenic SFE extract content were calculated on a dry weight basis and based on pulegone as internal standard. The data reported are the mean of at least three replicates.

Results and discussion

During a breeding program aimed to select basil lacking methyl chavicol, we isolated several chemotypes. One of them was particularly rich in T-cadinol. In order to assess the better technique to extract the terpene fraction from this chemotype, we compared EO from hydrodistillation with supercritical CO₂ extracts. After extraction, SFE yields ranged from 1.10 to 1.30 g/kg fr. wt, whereas EO yields ranged from 0.90 to 0.98 g/kg fr. wt.

The chemical composition of basil EO and SFE was characterized by the presence of 1,8-cineole, linalool, eugenol, *trans*- α -bergamotene and T-cadinol (Table 1, Figures 1 and 2). A direct comparison revealed a higher percentage of 1,8-cineole (4-fold), linalool (5.8-fold), eugenol (1.2-fold) and germacrene D (28-fold) in SFE with respect to EO. On the other hand, EO composition was characterized by higher percentages of cubebol (3.5-fold), T-cadinol (3-fold) and α -cadinol (3.1-fold), with respect to SFE.

Table 1. CO₂ supercritical extract (SFE) and hydrodistillation of essential oil (EO) of *Ocimum basilicum*.

CSVPDFDisplay Table

Figure 1 Gas chromatography (GC) profile of *Ocimum basilicum* essential oil. **1:** α -pinene, **2:** sabinene, **3:** β -pinene, **4:** myrcene, **5:** limonene, **6:** 1,8-cineole, **7:** *cis*- β -ocimene, **8:** terpinolene, **9:** linalool, **10:** δ -terpineol, **11:** terpinen-4-ol, **12:** α -terpineol, **13:** bornyl acetate, **14:** α -terpinyl acetate, **15:** eugenol, **16:** α -copaene, **17:** β -elemene, **18:** unknown sesquiterpene hydrocarbon, **19:** methyl eugenol, **20:** *cis*- α -bergamotene, **21:** β -caryophyllene, **22:** *trans*- α -bergamotene, **23:** cadina-3,5-diene, **24:** α -humulene, **25:** *cis*-muurola-4(15),5-diene, **26:** β -acoradiene, **27:** germacrene D, **28:** unknown sesquiterpene hydrocarbon, **29:** bicyclogermacrene, **30:** δ -guaiene, **31:** germacrene A, **32:** γ -cadinene, **33:** δ -cadinene, **34:** β -sesquiphellandrene, **35:** unknown sesquiterpene hydrocarbon, **36:** *trans*-nerolidol, **37:** spathulenol, **38:** humulene epoxyde II, **39:** 1,10-di-epi-cubenol, **40:** T-cadinol (10-epi- α -muurolol), **41:** α -cadinol, **42:** intermedeol, **43:** β -bisabolol, **44:** α -bisabolol, **45:** farnesyl acetate.

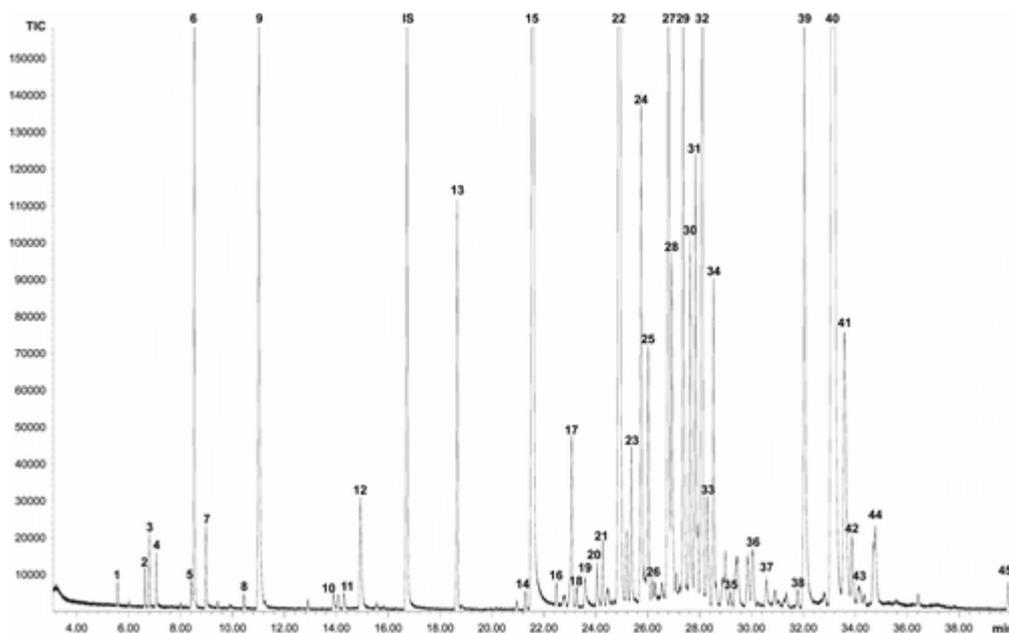
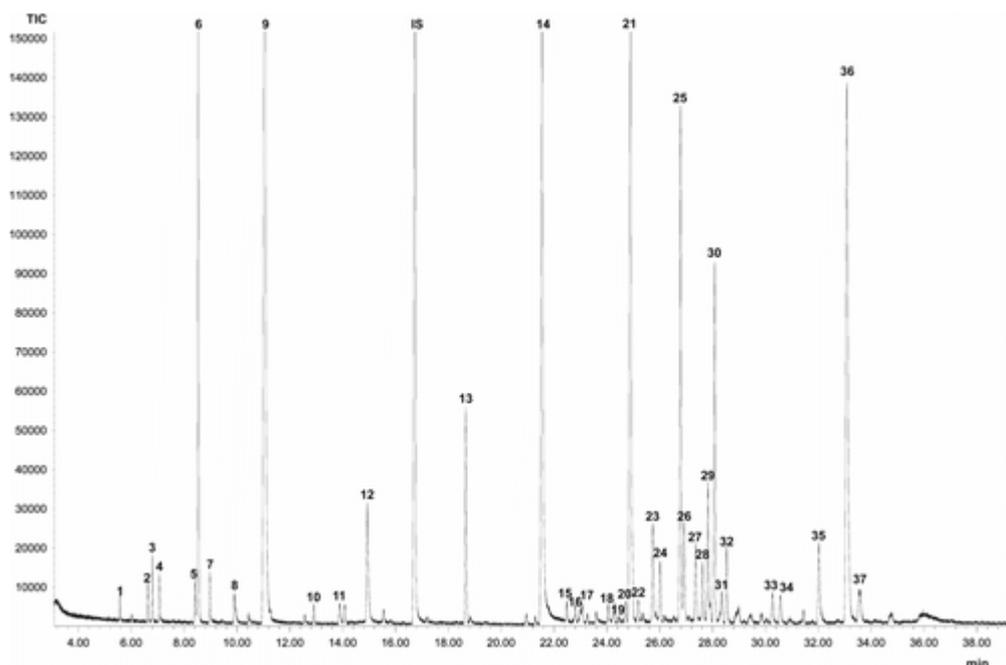


Figure 2 Gas chromatography (GC) profile of *Ocimum basilicum* supercritical CO₂ extract. **1:** α -pinene, **2:** sabinene, **3:** β -pinene, **4:** myrcene, **5:** limonene, **6:** 1,8-cineole, **7:** *cis*- β -ocimene, **8:** *cis*-sabinene hydrate, **9:** linalool, **10:** camphor, **11:** δ -terpineol, **12:** α -terpineol, **13:** bornyl acetate, **14:**

eugenol, **15**: α -copaene, **16**: β -bourbonene, **17**: β -elemene, **18**: *cis*- α -bergamotene, **19**: β -caryophyllene, **20**: calarene, **21**: *trans*- α -bergamotene, **22**: *trans*- β -farnesene, **23**: α -humulene, **24**: *cis*-muurola-4(**15**),5-diene, **25**: germacrene D, **26**: unknown sesquiterpene hydrocarbon, **27**: bicyclogermacrene, **28**: δ -guaiane, **29**: germacrene A, **30**: γ -cadinene, **31**: δ -cadinene, **32**: β -sesquiphellandrene, **33**: unknown sesquiterpene alcohol, **34**: spathulenol, **35**: 1,10-di-epi-cubenol, **36**: T-cadinol (10-epi- α -muurolol), **37**: α -cadinol.



With reference to minor compounds, both extraction methods revealed the presence of equal percentages of several monoterpenes and sesquiterpenes (Table 1). However, EO analysis revealed a higher presence of terpinolene, terpenen-4-ol, α -terpinyl acetate, methyl eugenol, cadina-3,5-diene, β -acoradiene, *trans*-nerolidol, humulene epoxyde II, intermedeol, β -bisabolol, α -bisabolol and farnesyl acetate. On the other hand, SFE allowed a better characterization of *cis*-sabinene hydrate, camphor, β -bourbonene, calarene and *trans*- β -farnesene, which were either not detectable or detectable in trace amounts in EO GC-MS analyses (Table 1, Figures 1 and 2).

The terpene composition of basil chemotypes is highly variable, depending on environmental conditions (phenotypic plasticity) and genotype. Several studies have identified tens of chemotypes and an effort has been made to group chemotypes. The SFE and EO analyses of the basil chemotype here analyzed indicate the major presence of linalool, eugenol and T-cadinol. However, the different extraction techniques may lead to different grouping, particularly if linalool is considered. By comparing thyme SFE and EO, Grosso and co-workers showed that the main difference was found to be the relative percentage of thymoquinone (not found in the essential oil) and carvacryl methyl ether (1.0–1.2% for HD versus 0.4% for SFE), and this difference was correlated to the higher antioxidant activity of the SFE volatiles when compared with EO (27). Thus, SFE extract may lead to different chemical composition that might enhance the biological activity of the terpene fractions. From a chemotaxonomic point of view, classification based only on the quantitative parameter may lead to different interpretation when different extraction techniques are used. This is the main reason why other methods, such as DNA fingerprinting, should be used to support classification of aromatic plants (28).

The use of SFE for terpene characterization has been successfully used in several aromatic plants (14, 18, 19, 29, 30), with particular reference to the *Lamiaceae* (31) and the genus *Ocimum* (20, 32, 33). Our results are in line with previous investigations by showing a higher SFE extraction of low molecular weight terpenes (e.g. 1,8-cineole, linalool and eugenol, in our case) and a reduced

extraction of high-boiling point sesquiterpenes (e.g. T-cadinol, cubebol and α -cadinol), when compared with EO hydrodistillation. Recently Kamali and co-workers showed that SFE extracts from lavandin flowers increased up to 80% (w/w) extractions of low-boiling point terpenes such as 1,8-cineole (8.1%), linalool (34.1%) and linalyl acetate (30.5%) (34). Eugenol is present in sweet basil extracts, but usually not as a major compound (11, 12, 35, 36). Leal et al. (32) reported data on the obtainment of extracts of clove basil (*Ocimum gratissimum*) by SFE where eugenol was the major compound (41.5–46.6%, area). With regards T-cadinol, this sesquiterpene alcohol is known for its biological activity. T-Cadinol activity on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated RAW 264.7 macrophages was investigated (37), whereas its antibacterial activity has been determined against a number of *Staphylococcus aureus* strains with minimum inhibitory concentration (MIC) values in the range of 4–256 $\mu\text{g/mL}$ (38, 39). Furthermore, T-cadinol may be used in dendritic cells-based immunotherapy for cancer (40) and represent a new chemical class of calcium antagonists, which interact with dihydropyridine binding sites on the voltage-operated calcium channels (41, 42).

In conclusion, we described the SFE potential for low molecular weight terpenes in sweet basil and we also showed its limit when sesquiterpene recoveries are compared with EO hydrodistillation. The finding of an *O. basilicum* T-cadinol chemotype has important implications and pharmacological interest owing to the biological properties of this sesquiterpene alcohol.

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