Food Bioprocess Technol (2012) 5:2978–2989
DOI 10.1007/s11947-011-0661-0

ORIGINAL PAPER

Drying Sage (*Salvia officinalis* L.) Plants and Its Effects on Content, Chemical Composition, and Radical Scavenging Activity of the Essential Oil

Ibtissem Hamrouni Sellami · Iness Bettaieb Rebey · Jazia Sriti · Fatma Zohra Rahali · Ferid Limam · Brahim Marzouk

Received: 9 March 2011 / Accepted: 21 July 2011 / Published online: 6 August 2011

Abstract Chemical composition and antioxidant activity of *Salvia officinalis* essential oil (EO) were studied under different drying methods of plant material. Results obtained showed that dried plant material yielded more essential oils than did the fresh one. The highest EO yields were obtained by infrared drying at 45 °C (0.39%) followed by air drying (0.30%) and oven drying at 45 °C (0.26%). The analysis of EOs by gas chromatography–mass spectrometry, showed in each of 55 identified compounds belonging mostly to oxygenated monoterpenes. This class of compounds was significantly affected by following drying methods: oven at 65 °C, microwave (500 W), and infrared at 45 °C. The main components of sage EO, 1,8-cineole, α and β-thujone, camphor, viridiflorol, and manool showed significant variation ($p<0.05$) with drying methods. Their concentrations increased significantly, particularly when drying sage at ambient air. Finally, the screening of antioxidant activity of the different sage EOs using the di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium radical (DPPH) assay showed an appreciable reduction of the stable radical DPPH, although microwave drying was the most efficient method with an IC50 of 1.60% as compared with fresh plant (IC50=5.32).

Keywords *Salvia officinalis* L. · Essential oil · Oven drying · Infrared drying · Microwave drying · Antioxidant activity

I. H. Sellami (✉) · I. B. Rebey · J. Sriti · F. Z. Rahali · F. Limam · B. Marzouk
Laboratory of Bioactive Substances,
Center of Biotechnology of the Techno pole Borj-Cedria,
BP. 901, 2050 Hammam-Lif, Tunisia
e-mail: ibtissem_hfr@yahoo.fr

Introduction

The genus *Salvia* (Lamiaceae) includes about 900 species spread throughout the world, some of which are economically important since they have been used as spices and flavoring agents (Longaray Delamare et al. 2007). The species *Salvia officinalis* L. is cultivated as a culinary herb and as a plant of great medicinal importance. Curative properties of sage are particularly recognized since earliest times, and its uses as a tonic, stimulant, carminative, antiseptic, and antihydrotic were reported (Kintzios 2000). Nowadays, many Mediterranean countries where sage grows have substantial gains from its production and export (Ben Taarit et al. 2009). Sage is an aromatic herb, and thus was previously considered mainly for its essential oil content (Santos-Gomes and Fernandes-Ferreira 2003). In addition to flavoring food, sage volatile oil can also act as antioxidant and preservative against food spoilage, while a broad range of applications in aromatherapy and health care are in extent (Hay and Waterman 1993).

The chemical composition of sage oil from different geographical origins has been the subject of many studies. The major constituents of different sage oils include α-thujone, β-thujone, camphor, and 1,8-cineole, which determine also their chemotypes (Länger et al. 1993; Gomes and Ferreira 2001; Radulescu et al. 2004).

The post-harvesting process of medicinal plants has great importance in the production chain because of its direct influence on the quality and quantity of the active principles in the product sold (Khorshidi et al. 2009). Aromatic plants are often dried before extraction to reduce moisture content. Drying is fundamental to the achievement of a high-quality product and, due to high investment and

energy costs, drying is also a large expense in medicinal plant production (Müller and Heindl 2006). Also, increasing usage of drying procedures within various technological lines in the food industry and biotechnology has made studies of the drying process of important practical interest. However, factors such as scale of production, availability of new technologies, and pharmaceutical quality standards must be considered for medicinal plant drying in modern times (Müller and Heindl 2006).

According to bibliographic data, it has been shown that drying method had a significant effect on oil content and composition of aromatic plants (Díaz-Maroto et al. 2003; Omidbaigi et al. 2004). Oil components are, however, lost during the drying process, therefore, the method of drying is very important (Asekun et al. 2007a). According to Venskutonis (1997), changes in concentrations of the volatile compounds during drying depend on several factors, such as the drying method and parameters that are characteristic of the product subjected to drying. On the other hand, energy requirements of drying are considerable and represent a major expense in the drying procedure, which is already the greatest cost in the processing of medicinal plants (Müller and Heindl 2006). Moreover, according to the same authors, drying performance takes authoritative influence on the quality of the product and, therefore, on its value.

Only one study has been performed on the effects of drying methods on the essential oil composition of both *S. officinalis* and *Thymus vulgaris* in which Venskutonis (1997) proceeded to the drying of sage and thyme leaves by two methods and various temperatures: freeze-drying and oven drying at 30 °C and 60 °C. So, according to bibliographic data, it seems that drying of sage material by air drying and both microwave and infrared drying methods are missing. Thermal drying technologies have attracted significant research and development efforts owing to the rising demand for improved product quality and reduced operating cost, as well as diminished environmental impact (Mujumdar and Law 2010). In recent years, microwave drying has gained popularity as an alternative drying method for a variety of food products such as fruits, vegetables, snack foods, and dairy products (Wang and Sheng 2006). In fact, air drying is one of the more energy-consuming processes, and thereby, industries using energy-intensive processes are being forced to explore ways for reducing their energy consumption (Ould Ahmedou et al. 2008). On the other hand, the use of infrared (IR) radiation technology in dehydrating foods has several advantages. These may include decreased drying time, high energy efficiency, high-quality finished products, uniform temperature in the product while drying, and a reduced necessity for air flow across the product (Mongpreneet et al. 2002). Finally, to the best of our knowledge, no reports were available on the effect of drying procedures on the antioxidant activity of sage essential oil.

The aim of this work is to study the effects of six drying methods on the chemical composition and antioxidant activity of *S. officinalis* essential oil. Another goal of the work is to determine the best drying method allowing production of the maximum levels of bioactive compounds such as 1,8-cineole, α-thujone, and camphor, and to evaluate the effect of these variations on the scavenging ability of sage essential oil against the di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium radical (DPPH).

Materials and Methods

Chemicals

Solvents used in the experiments were purchased from Merck (Darmstadt, Germany). Anhydrous sodium sulphate (Na_2SO_4), 6-methyl-5-hepten-2-one used as internal standard, homologous series of C_8-C_{22} *n*-alkanes used for identification of aroma compounds (by calculation of their retention indexes), and commercially pure standards of aroma compounds were purchased from Fluka (Ridel-de Haën, Switzerland); butylated hydroxytoluene (BHT=2,6-bis(1,1-dimethylethyl)-4-methylphenol) and 1,1-diphenyl-2-picrylhydrazyl stable radical (DPPH) were obtained from Sigma-Aldrich (Stein-Heim, Germany). All other chemicals were of analytical grade.

Plant Material and Drying Process

Our study was carried out on aerial parts of sage (*S. officinalis* L.) which were collected in April 2009 from a house garden in the region of Hammam-Chatt in the southern suburbs of Tunis (Tunisia)—36°43'49"N and 10°22'98"E, 2 m above sea level (average annual minimum temperature=13.2 °C; average annual maximum temperature=23.5 °C; average annual precipitations=38.5 mm). The identity of the plant was confirmed by Prof. Abderrazzak Smaoui, taxonomist, and a voucher specimen was deposited in the herbarium at the Biotechnological Centre of Borj-Cedria under the reference number LN 08002. In order to preserve their original quality, fresh plant material was stored in a refrigerator at 4 °C until drying experiments. Samples were divided into seven batches containing three sets of 30 g each. One was used for fresh analysis, and the remaining batches were dried by using one of the following methods: (a) air drying at shade and ambient temperature (22 °C); (b) drying in a hot-air

oven at 45 °C; (c) drying in a hot-air oven at 65 °C; (d) drying in a microwave oven at 500 W; (e) drying in an IR moisture analyzer at 45 °C; and (f) drying in an IR moisture analyzer at 65 °C.

The drying conditions employed in each of these methods were selected after conducting trials to achieve a percentage moisture content of <10% (7% for air drying, 6% for oven drying at 45 °C, 5% for oven drying at 65 °C, 5% for microwave drying, 5% for both IR drying temperatures). The moisture content of the dried samples was determined in triplicate using a laboratory oven at 105 °C. The times needed for reaching the final drying point in each of the assayed drying methods were as follows—13 days for air drying, 8 days for oven drying at 45 °C, 5 days for oven drying at 65 °C, 5 min for microwave drying at 500 W, 14 h for IR drying at 45 °C, and 9 h for IR drying at 65 °C.

Drying Methods and Equipment

Air drying was carried out in the shade under normal air at daylight and ambient temperature (mean temperature=22 °C). Oven drying was conducted in a ventilated oven (Nüve FN 400) at two different temperatures (45 °C and 65 °C). Microwave drying was performed in a domestic digital microwave oven (Samsung M1719N) with technical features of ~230 V, 50 Hz, 800 W. Time adjustment is done with the aid of a digital clock located on the microwave oven. Drying was carried out using an intermediate power of 500 W. Infrared drying was realized by means of an IR moisture analyser (Sartorius MA 40) equipped with infrared source. The plant material was placed inside an aluminium cylindrical pan. The continuous weighing of the sample allowed reading of the drying kinetics. IR drying was carried out at 45 °C and 65 °C.

Essential Oil Extraction

Thirty grams of each sample (fresh and dried aerial parts) were subjected to hydrodistillation over 180 min using 0.5 L of distilled water. The extraction time was fixed after a kinetic survey during 30, 60, 90, 120, 150, 180, and 210 min. The distillate obtained was extracted with diethyl ether and dried over anhydrous sodium sulphate. The organic layer was then concentrated at 30 °C using a Vigreux column, and the resulting essential oil was subsequently analyzed. 6-Methyl-5-hepten-2-one was used as internal standard and was added to the organic layer before the concentration step.

All experiments were conducted in triplicate, and results were expressed on the basis of dry matter weight (even for fresh material). In this way, we should point out that we used three samples of 10 g of fresh material for the evaluation of their moisture content by drying them in an oven at 105 °C until constant weight was reached.

Gas Chromatography

Essential oils were analyzed by gas chromatography (GC) using a Hewlett-Packard 6890 apparatus (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and an electronic pressure control injector. A HP-Innowax capillary column (polyethylene glycol, 30 m×0.25 mm i.d.×0.25 mm film thickness; Agilent Technologies, Hewlett-Packard, CA, USA) was used; the flow of the carrier gas (N_2, U) was 1.6 ml/min. Analyses were performed using the following temperature program: oven isotherm at 35 °C for a duration of 10 min, from 35 °C to 205 °C at the rate of 3 °C/min, and isotherm at 205 °C for a duration of 10 min. Injector and detector temperatures were held, respectively, at 250 °C and 300 °C. Diluted samples of 2.0 μL were injected in the split/splitless mode (60:1 split). Quantitative data were obtained electronically from FID area percent data without using any correction factors.

Gas Chromatography–Mass Spectrometry

The gas chromatography–mass spectrometry (GC-MS) analyses were performed on a gas chromatograph HP 6890 interfaced with a HP 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) with electron impact ionization (70 eV). A HP-5MS capillary column (60 m× 0.25 mm i.d.×0.25 mm film thickness) was used. The column temperature was programmed to rise from 40 °C to 280 °C at a rate of 5 °C/min. The carrier gas was helium with a flow rate of 1.2 ml/min. Scan time and mass range were 1 s and 50–550 *m/z*, respectively. The injected volume was 1 μl, and the total run time was approximately 63 min.

Identification of Aroma Compounds

Identification of aroma compounds was based on the calculation of their retention indexes relative to (C_8–C_{22}) *n*-alkanes and their comparison with those mentioned in bibliographic data. Also, identification was made by comparison of retention times of sage compounds with those of commercially authentic standards analyzed in the same conditions. Further identification was made by matching the mass spectral fragmentation patterns of different compounds with corresponding data (Wiley 275. L library) and other published mass spectra (Adams 2001) as well as by comparison of their retention indexes with data from the Mass Spectral Library "Terpenoids and Related Constituents of Essential Oils" (Dr. Detley Hochmuth,

Scientific Consulting, Hamburg, Germany) using the Mass Finder 3 Software (http://www.massfinder.com). Relative percentage amounts of the identified compounds were obtained from the electronic integration of the FID peak areas.

Antioxidant Activity of Essential Oils by DPPH Radical Scavenging Assay

Radical scavenging activity of sage essential oils against the stable DPPH radical was determined spectrophotometrically according to the method of Hanato et al. (1988). The antioxidant activities of essential oils were measured in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH. The colorimetric changes (from deep violet to light-yellow), when DPPH is reduced, were measured at 517 nm on a UV/visible light spectrophotometer. The 500 μL of various concentrations (2, 4, 6, 8, and 10 mg/ml) of the EOs in methanol as well as BHT (as standard antioxidant compound) were added to 0.5 ml of a 0.06 mM DPPH methanolic solution. The mixture was shaken vigorously and left standing in the dark at room temperature for 30 min. Absorbances of the resulting solutions were then measured at 517 nm. Methanol was used as a blank. The blank was prepared by adding 0.5 ml of the methanolic solution of DPPH to 500 μl of pure methanol. Special care was taken to minimize the loss of free radical activity of the DPPH radical stock solution. The antiradical activity (three replicates per treatment) was expressed as IC50 (milligrams per milliliter), the concentration required to causes a 50% DPPH inhibition. A lower IC50 value corresponds to a higher antioxidant activity (Patro et al. 2005). The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect}(\%) = \frac{(A_0 - A_1) \times 100}{A_0}$$

Where A_0 is the absorbance of the control at 30 min, and A_1 is the absorbance of the sample at 30 min. Samples were analyzed in triplicate.

Statistical Analysis

Statistical analyses were done using STATISTICA (Statsoft, 1998) program. Data were reported as means±standard deviation of three replicates. Differences were tested for significance by using the ANOVA procedure, using a significance level of $p \leq 0.05$. A principal component analysis was performed in order to discriminate between different drying methods on the basis of their volatiles contents.

Results and Discussion

Effect of Drying Methods on the Essential Oil Yield

The variation of sage essential oil yield as affected by drying method is illustrated in Fig. 1. Results showed that the EO yield of sage is significantly influenced by the drying method. Generally, the dried plant material yielded more essential oils than did the fresh one. As compared with fresh plants (EO yield=0.054%), three of the six drying methods resulted in an increase of the EO yields. These were the cases of IR drying at 45 °C (0.39%), air drying (0.30%), and oven drying at 45 °C (0.26%), whereas the other drying methods resulted in a decrease of the EO yields and did not differ significantly ($p<0.05$) from the fresh plant material. Also, our results showed that the lowest EO yield (0.02%) was obtained when drying sage plant material at oven (65 °C) whereas the highest yield (0.39%) was obtained when drying with IR at 45 °C.

On the other hand, our results showed that increasing drying temperature significantly decreased the EO content. This was the case of both drying at oven and IR. In fact, when changing drying temperature from 45 °C to 65 °C for mentioned techniques, the EO yield afforded a sharp decrease (from 0.26% to 0.02% in the case of oven drying and from 0.39% to 0.03% in the case of IR drying). These results suggested that, when increasing drying temperature by 20 °C, the losses in the essential oil yield could reach the 92.3%. The extensive release of EO from *S. officinalis* plants dried at 65 °C indicates that the biological structure of the oil glands of this species was strongly affected under such temperatures. In this way, studying anatomical characteristics of fresh and dried leaves of spearmint, Díaz-Maroto et al. (2003) found that epithelial cells in the dried samples can be observed to have collapsed and split open. Similar results have been also reported on oregano (Yusif et al. 2000) and basil (Yousif et al. 1999). These

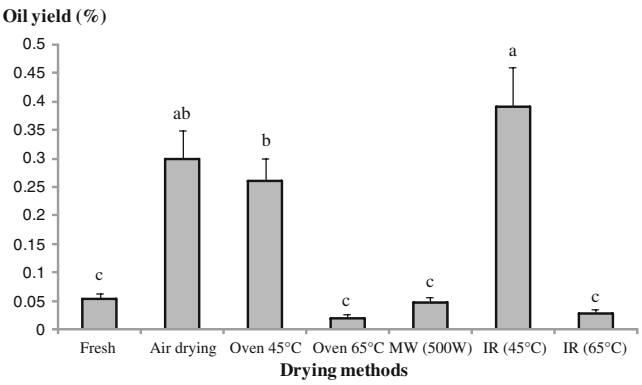

Fig. 1 Effects of drying methods on the yield of *S. officinalis* essential oil (percent). *Bars* with the *same small letter* did not share significant differences at $p<0.05$ (Duncan's multiple range test)

observations indicated that biomembrane structures were modified under sublethal temperatures that resulted in higher permeability.

The decrease in EO content with increasing drying temperature has been also reported by Venskutonis (1997) in sage and thyme when they were oven-dried at 60 °C. Similar results have been reported in other Lamiaceae species such as rosemary (*Rosmarinus officinalis*; Blanco et al. 2002), peppermint (*Mentha piperita*; Blanco et al. 2002), *Artemisia dracunculus* (Arabhosseini et al. 2006), and *Mentha longifolia* (Asekun et al. 2007b). According to Buggle et al. (1999), increasing temperature from 30 °C to 90 °C resulted in decreasing in EO oil content of *Cymbopogon citrates* (DC) Stapf. Similar results have been also reported by Braga et al. (2005) in *Piper hispidinervium* and by Khangholi and Rezaeinodehi (2008) in *Artemisia annua* plants as affected by drying method. According to these authors, under the operating conditions used, drying resulted in a higher EO content for temperatures up to 50 °C but for drying temperatures above 50 °C, this parameter was decreased. In contrast, other studies showed that increasing drying temperature could induce an increase in the EO yield of many plants such as *Lippia alba* (Castro et al. 2001) and *Satureja hortensis* (Sefidkon et al. 2006). These opposite results may be due to differences in plant species, secretory structures, and their localisation in the plant and chemical composition of essential oil (Khangholi and Rezaeinodehi 2008).

Effect of Drying Methods on Essential Oil Composition

As shown in Table 1, in fresh *S. officinalis* aerial parts, 36 volatile components were identified representing 95.97% of total volatiles. The principal components of the oil were 1,8-cineole (29.41%), α-thujone (22.58%), camphor (20.22%), viridiflorol (5.81%), β-thujone (2.90%), and terpinen-4-ol (2.27%). According to these results, it seems that Tunisian *S. officinalis* cultivated in the region of Hammam-Chatt is of the 1,8-cineole chemotype. According to Tucker and Maciarello (1990), chemotypes of Dalmatian sage with α-thujone, β-thujone, camphor, or 1,8-cineole as the main component have been proposed. The volatile compounds from Tunisian fresh sage could be grouped in the following main chemical groups: oxygenated monoterpenes (83.24%), oxygenated sesquiterpenes (8.65%), monoterpene hydrocarbons (2.49%), and sesquiterpene hydrocarbons (0.06%).

In order to study the effects of drying method on the chemical composition of *S. officinalis* EO, percentages of volatile compounds of the dried plants were compared with those found in fresh leaves.

The effects of the different drying methods on the volatile composition of *S. officinalis* aerial parts have been shown in Table 1. In total, 42, 32, 21, 22, 32, and 24 constituents were identified in air, oven 45 °C, oven 65 °C, microwave (500 W), IR 45 °C, and IR 65 °C drying techniques. These identified compounds represented 98.96%, 98.01%, 96.48%, 94.83%, 97.68%, and 97.90% of total volatiles, respectively, for the different drying methods.

Following treatment by the different drying methods, significant changes ($p<0.05$) were observed in the chemical profile of the EOs. Nevertheless, oxygenated monoterpenes remained the major class (68.16–94.19%) followed by oxygenated sesquiterpenes (0–22.07%), sesquiterpene hydrocarbons (0–2.17%), and monoterpene hydrocarbons (0.22–1.83%). On the other hand, the relative percentages of the individual volatiles varied significantly ($p<0.05$) under various drying methods. The percentage of the main compound, 1,8-cineole, increased when drying plant material at ambient air (36.39%) whereas it decreased significantly when drying with oven 65 °C (17.25%), MW (22.30%), and IR 65 °C (25.16%). The second main compound, α-thujone, decreased under the different drying techniques; its lowest percentage is detected in the case of IR drying at 45 °C. The percentage of camphor increased significantly whatever the method of drying is and reached 46.33% when drying plant material under IR at 65 °C. As for β-thujone, it increased significantly when drying plant material and its highest percentage is obtained in the case of oven drying at 45 °C where it reached 5.30% of total volatiles. The rates of viridiflorol increased in the case of drying in oven at 65 °C, in MW oven at 500 W and in IR analyzer at 45 °C whereas, the percentages of terpinen-4-ol remained constant in the case of IR drying at 65 °C and decreased using the other drying techniques. Based on these results, it seems that oven drying at both 45 °C and 65 °C, and MW (500 W) and IR drying at both 45 °C and 65 °C resulted in a transformation of the sage EO chemotype from 1,8-cineole to camphor type.

Using other drying techniques (convective oven drying at 30 °C and 60 °C and freeze-drying), Venskutonis (1997) reported a decrease in the levels of 1,8-cineole and α-thujone whereas those of β-thujone and camphor increased in dried *S. officinalis* leaves as compared with fresh ones.

Drying of sage plants resulted in the losses of certain volatiles such as sabinene, α-terpinene, valencene, methyl eugenol, and others. These compounds seem to have more affinity to the water fraction contained in sage plants, and thereby, they were lost with water during drying process. Indeed, according to Moyler (1994), when drying plant material, many compounds which are dragged to the leaf surfaces by the evaporating water are lost. Between the different drying techniques

Table 1 Chemical composition (percent) of the essential oils of fresh and dried sage (*S. officinalis* L.) plants

Compound	RI_a	RI_p	Percent of total volatiles							Identification
			Fresh	Air drying	Oven 45 °C	Oven 65 °C	MWO (500 W)	IR 45 °C	IR 65 °C	
Methanol	–	–	0.02ab±0.01	–	–	0.07a±0.04	–	0.07a±0.02	–	GC/MS, CoI
Ethanol	–	–	0.05b±0.01	–	0.16a±0.08	–	–	–	–	GC/MS, CoI
trans-2-Hexenal	847	1,215	–	0.20a±0.05	–	–	–	–	–	GC/MS, CoI
cis-3-Hexenol	853	1,408	–	0.02a±0.00	–	–	–	–	–	GC/MS, CoI
Camphene	944	1,077	0.24b±0.09	0.12b±0.08	0.17b±0.09	1.25a±0.42	–	–	–	GC/MS, CoI
Sabinene	970	1,102	0.83a±0.10	–	0.34b±0.09	–	–	0.38b±0.08	–	GC/MS, CoI
β-Pinene	980	1,095	–	0.35b±0.62	–	0.59b±0.27	–	0.41b±0.09	1.24a±0.40	GC/MS, CoI
Myrcene	990	1,155	0.33a±0.08	0.36a±0.03	–	–	0.22ab±0.05	–	–	GC/MS, CoI
Octanal	1,005	1,287	–	0.03b±0.00	0.06a±0.01	0.08a±0.02	–	0.06a±0.01	–	GC/MS, CoI
Δ3-Carene	1,014	1,134	–	0.02a±0.00	–	–	–	–	–	GC/MS, RI
α-Terpinene	1,017	1,177	1.09a±0.09	–	–	–	–	0.13b±0.05	0.42b±0.09	GC/MS, CoI
1,8-Cineole	1,028	1,193	29.41b±0.15	36.39a±1.03	29.2b±1.12	17.25d±0.99	22.30cd±0.38	26.58bc±2.63	25.16bc±2.83	GC/MS, CoI
E-β-Ocimene	1,042	1,049	–	–	0.2a±0.05	–	–	–	–	GC/MS, RI
Butyl acetate	1,070	–	–	–	–	–	0.70a±0.21	–	–	GC/MS, RI
Octanol	1,073	1,562	–	0.05a±0.00	–	–	–	0.06a±0.01	–	GC/MS, CoI
trans-Linalool oxide	1,088	1,475	0.50a±0.01	0.36b±0.04	–	–	–	0.12c±0.04	–	GC/MS, RI
α-Campholenal	1,092	1,374	0.22bc±0.06	0.86a±0.17	0.12bc±0.03	0.33b±0.08	0.09bc±0.01	0.31bc±0.10	–	GC/MS, RI
trans-Sabinene hydrate	1,094	1,548	–	–	–	–	0.23a±0.09	–	–	GC/MS, RI
Fenchol	1,098	1,646	0.10ab±0.01	0.12a±0.02	0.10ab±0.03	–	–	0.08b±0.03	–	GC/MS, CoI
Linalool	1,100	1,448	0.07bc±0.02	0.10ab±0.02	0.10ab±0.02	0.12a±0.02	–	0.04c±0.01	–	GC/MS, CoI
α-Thujone	1,102	1,321	22.58a±0.90	10.15bc±1.13	17.6ab±0.47	7.48bc±0.94	18.05ab±1.43	12.21bc±0.09	16.50ab±1.83	GC/MS, CoI
β-Thujone	1,115	1,336	2.90c±0.85	2.30c±0.08	5.30a±0.44	2.23c±0.24	3.89b±0.99	4.28b±0.12	3.76b±0.91	GC/MS, CoI
cis-Verbenol	1,141	–	0.21a±0.05	0.18a±0.08	0.20a±0.09	–	0.15a±0.05	0.12ab±0.02	–	GC/MS, RI
Camphor	1,143	1,398	20.22c±1.06	33.10b±1.04	34.26b±1.03	36.71ab±1.84	26.81bc±1.87	31.95b±2.30	46.33a±2.34	GC/MS, CoI
p-Cymen-8-ol	1,159	1,832	0.13b±0.01	0.20a±0.01	0.19a±0.03	–	0.22a±0.03	0.22a±0.05	–	GC/MS, RI
Borneol	1,168	1,690	2.55a±0.90	2.96a±0.24	2.92a±0.41	3.04a±0.70	3.1a±0.47	3.33a±0.10	–	GC/MS, CoI
Terpinen-4-ol	1,175	1,591	2.27a±0.90	0.06b±0.01	0.06b±0.00	–	0.06b±0.01	1.48ab±0.09	2.43a±0.73	GC/MS, CoI
Nerol	1,179	1,780	0.11c±0.05	0.30a±0.01	0.24ab±0.04	0.20abc±0.03	0.25ab±0.02	0.16bc±0.07	–	GC/MS, CoI
cis-Dihydrocarvone	1,191	1,645	0.67a±0.03	0.35b±0.08	0.14bc±0.08	–	0.15bc±0.02	0.12bc±0.02	–	GC/MS, RI
α-Terpineol	1,192	–	0.33bcd±0.07	1.04ab±0.46	0.65abcd±0.35	–	1.27a±0.21	0.89abc±0.12	–	GC/MS, CoI
Linalyl acetate	1,257	1,556	–	0.13a±0.02	–	–	–	0.06b±0.01	–	GC/MS, CoI
Bornyl acetate	1,285	1,570	–	–	–	–	0.14a±0.02	–	–	GC/MS, CoI
2-Undecanone	1,292	–	–	–	–	–	2.38a±0.35	–	–	GC/MS, CoI
Thymol	1,295	2,175	0.18b±0.03	0.07bc±0.02	0.16b±0.04	0.43a±0.05	–	0.07bc±0.03	–	GC/MS, CoI
Undecanal	1,307	–	0.11b±0.03	–	–	0.85a±0.27	–	–	0.95a±0.21	GC/MS, CoI
Eugenol	1,337	2,144	0.05ab±0.02	0.04ab±0.01	0.27ab±0.05	0.34a±0.04	–	0.07ab±0.02	–	GC/MS, CoI
α-Terpinyl acetate	1,350	1,693	0.98a±0.07	0.32b±0.04	0.24b±0.03	–	0.23b±0.05	0.23b±0.01	–	GC/MS, CoI
Neryl acetate	1,365	1,726	–	0.11a±0.02	–	–	–	–	–	GC/MS, CoI
Geranyl acetate	1,380	1,746	0.04b±0.01	0.06a±0.02	–	–	–	–	–	GC/MS, CoI
Cinnamyl alcohol	1,384	–	0.08c±0.02	0.12c±0.06	1.02b±0.46	2.23a±0.67	0.36c±0.16	0.21c±0.07	1.11b±0.34	GC/MS, CoI
β-Damascone	1,391	1,805	–	0.05b±0.01	0.09a±0.03	–	–	–	–	GC/MS, CoI
Methyl eugenol	1,404	2,005	0.68a±0.09	–	–	–	–	0.11b±0.04	–	GC/MS, RI
β-Caryophyllene	1,418	1,577	–	1.93a±0.40	0.99b±0.27	–	–	–	–	GC/MS, CoI
α-Humulene	1,453	1,647	–	0.15a±0.02	0.04b±0.02	–	0.13a±0.02	–	–	GC/MS, CoI
α-Selinene	1,480	1,703	–	0.05ab±0.00	0.04b±0.01	–	0.06a±0.02	–	–	GC/MS, RI
β-Elemene	1,481	1,390	–	0.03b±0.00	–	–	–	0.06a±0.00	–	GC/MS, RI
Eugenyl acetate	1,485	2,107	0.16c±0.01	0.24c±0.10	0.60b±0.17	0.75a±0.06	–	0.12cd±0.03	–	GC/MS, RI
Valencene	1,495	1,740	0.06a±0.02	–	–	–	–	–	–	GC/MS, CoI
Geranyl-2-methyl Butyrate	1,562	1,880	0.05b±0.01	0.08a±0.00	–	–	0.07ab±0.03	–	–	GC/MS, RI
caryophyllene oxide	1,585	2,007	0.44ab±0.09	0.37b±0.17	0.19bc±0.05	–	0.71a±0.24	0.42ab±0.10	–	GC/MS, CoI
Farnesol	1,724	2,351	0.17b±0.07	0.11b±0.07	0.24b±0.07	0.90a±0.11	0.30b±0.10	0.26b±0.08	–	GC/MS, CoI
Nonadecane	1,897	1,900	0.10c±0.03	0.92a±0.32	0.42b±0.03	0.47b±0.16	–	0.21bc±0.07	–	GC/MS, CoI
Viridiflorol	1,937	1,560	5.81bc±1.03	3.84cd±1.01	–	12.29a±1.44	10.24ab±1.03	9.32ab±1.03	–	GC/MS, RI
Manool	1,961	2,056	2.23b±0.91	0.72b±0.10	1.70b±0.41	8.87a±0.76	2.72b±0.80	3.42b±0.81	–	GC/MS, RI

Table 1 (continued)

Compound	RI_a	RI_p	Percent of total volatiles							Identification
			Fresh	Air drying	Oven 45°C	Oven 65°C	MWO (500W)	IR 45°C	IR 65°C	
Unknown			4.03a±0.58	1.03a±0.43	1.99a±0.37	3.52a±0.86	5.17a±1.04	3.01a±0.66	2.10a±0.69	
Monoterpene hydrocarbons			2.49a±0.86	0.86bcd±0.24	0.72cd±0.32	1.83ab±0.51	0.22d±0.05	0.93bcd±0.20	1.66abc±0.40	
Oxygenated monoterpenes			83.24ab±2.03	88.65a±2.25	91.52a±2.38	68.16c±1.35	76.61bc±2.39	82.22ab±2.77	94.19a±1.91	
Sesquiterpene hydrocarbons			0.06c±0.01	2.17a±0.5	1.08b±0.51	0c	0.19c±0.04	0.06c±0.00	0c	
Oxygenated sesquiterpenes			8.65bc±0.87	5.05bc±1.04	2.15c±0.68	22.07a±1.07	13.98ab±1.30	13.42ab±1.16	0c	
Others			1.61cd±0.50	2.37c±0.36	2.65bc±0.62	3.60ab±0.68	3.88a±1.14	1.05d±0.22	2.07cd±0.23	
Total identified			95.97	98.96	98.01	96.48	94.83	97.68	97.90	

Components are listed in order of elution in apolar column (HP-5); values followed by the same small letter did not share significant differences at $p<0.05$ (Duncan's test)

RI_p and RI_a retention indices calculated using respectively a polar column (HP-Innowax) and an apolar column (HP-5), *MWO* microwave oven, *IR* infra-red, *MS* mass spectrometry, *CoI* co-injection, *RI* retention indices

used in this work, it seems that most of the losses in sage volatiles occurred under oven (65 °C), MW (500 W), and IR (65 °C) drying procedures.

New volatile compounds which were absent from fresh material appeared under drying such as β-pinene, octanal, β-caryophyllene, α-humulene, α-selinene, and others. According to Huopalahti et al. (1985), the formation of new compounds have in some cases been observed after drying, probably as a consequence of oxidation reactions, hydrolysis of glycosylated forms, or the release of substances following the rupture of cell walls.

On the other hand, EO obtained under microwave drying (500 W) showed substantial losses in most of volatiles which may be due to expansion of the structure of the epidermis of plants. In fact, microwave drying of basil and carrots generates expansion effects of the epidermis structure (Lin et al. 1998; Yousif et al. 1999). This effect may cause volatiles to be released into the air (Díaz-Maroto et al. 2003). Emission into the atmosphere being one of the main ways of loss of volatile compounds in plants (Lerdau et al. 1997).

The variation of individual compounds under the effect of drying affects the composition of the different chemical classes. Indeed, the percentages of monoterpene hydrocarbons decreased significantly ($p<0.05$) and reached its lowest value (0.22%) in the case of MW (500 W) drying. The rates of oxygenated monoterpenes decreased under oven (65 °C) and MW (500 W) drying techniques (68.16% and 76.61%) whereas they increased under the other drying methods. The highest level of oxygenated monoterpenes was detected in the case of IR (65 °C) drying procedure. As for sesquiterpene hydrocarbons, this class increased only under air and oven (45 °C) drying (5.05% and 2.15%). Finally, oxygenated sesquiterpenes increased under all the drying techniques except for IR (45 °C) drying where their rates decreased significantly and reached 1.05%.

Effect of Drying Methods on the Amounts of the Main Essential Oil Components

The effects of the different drying methods on the concentration of the main sage EO components as expressed in micrograms per gram of dry weight are resumed in Table 2. Results showed that oxygenated monoterpenes constitute the main chemical class of the different EOs. Their contents increased significantly ($p<0.05$) in the cases of air drying, oven (45 °C), and IR (45 °C) and were respectively 5.9, 5.4, and 4.9 times higher than those of fresh plants. These rises were mainly due to increases in the contents of the main compounds of this class such as 1,8-cineole, camphor, and borneol which reached their highest concentrations in the case of air drying with 1107.49, 1028.53, and 92.32 μg/g of dry weight, respectively. As for α- and-β-thujone, their highest levels were detected in the case of oven drying at 45 °C under which they were respectively 4 and 9.2 times higher than those found in fresh plants.

An increase in the contents of both 1,8-cineole and linalool in the EOs of spearmint (Lamiaceae) have been previously reported by Díaz-Maroto et al. (2003) in the cases of both ambient air and oven drying at 45 °C.

Based on our results, it seems that the highest losses in volatiles occurred in samples dried by IR at 65 °C. This was particularly the case of sesquiterpenes which were completely absent from EOs. According to Díaz-Maroto et al. (2003), sometimes, substances of relatively low volatility, like sesquiterpenes, are released more readily than more volatile compounds. Some authors have suggested that there may be membranes selectively more permeable to certain volatiles or separate compartments for the synthesis of emitted volatiles and stored substances (Loughrin et al. 1994; Gershenzon et al. 2000). In general, rapid drying under high temperature causes complex and physical degradative changes and losses of volatile

Table 2 Concentrations of volatile components (micrograms per gram of dry weight) of fresh and dried sage (*S. officinalis* L.) plants

Compound	RI_a	RI_p	Micrograms per gram of dry weight						
			Fresh	Air drying	Oven 45 °C	Oven 65 °C	MWO (500 W)	IR 45 °C	IR 65 °C
Main compound									
1,8-Cineole	1,028	1,193	160.54b±14.56	1107.49a±130.12	761.28a±80.35	157.84b±10.09	117.55b±14.64	670.33a±50.45	70.21b±5.55
α-Thujone	1,102	1,321	116.80b±3.62	321.44a±18.23	466.80a±42.03	60.95b±3.11	97.52b±13.46	450.81a±31.14	57.51b±4.58
β-Thujone	1,115	1,336	15.05b±0.95	70.83b±6.44	139.48b±27.39	18.64b±2.51	21.19b±5.93	116.06b±13.31	12.91b±2.94
Camphor	1,143	1,398	110.43c±9.21	1028.53a±126.61	910.70a±116.23	290.24bc±15.98	140.48c±13.52	815.09ab±107.71	127.48c±10.59
Borneol	1,168	1,690	14.28b±1.07	92.32a±4.86	78.50a±5.53	21.88b±1.97	16.02b±3.39	86.16a±4.23	–
Terpinen-4-ol	1,175	1,591	12.78b±0.82	2.10b±0.90	1.55b±0.42	–	0.36b±0.08	37.32a±2.10	9.73b±2.07
2-Undecanone	1,292	–	–	–	–	–	16.50a±0.98	–	–
Viridiflorol	1,937	1,560	32.94b±1.43	120.68ab±8.22	–	112.34ab±6.73	51.57ab±9.09	213.95a±12.03	–
Manool	1,961	2,056	12.83a±1.07	25.39a±2.09	48.28a±3.05	112.24a±5.82	14.97a±3.12	61.22a±9.02	–
Unknown			22.30b±2.54	100.71a±6.12	51.35ab±3.42	26.42b±1.88	24.49b±2.07	54.36ab±7.71	4.89b±0.97
Chemical classes									
Monoterpene hydrocarbons			14.07ab±1.66	27.46a±2.21	17.90ab±1.55	15.96ab±1.20	1.20b±0.43	26.84ab±6.14	3.49ab±0.83
Oxygenated monoterpenes			448.22b±12.24	2668.59a±162.28	2418.15a±229.12	534.34b±48.31	406.46b±37.87	2233.26a±361.12	277.87b±13.51
Sesquiterpene hydrocarbons			0.30b±0.08	70.49a±2.10	30.21b±3.03	–	1.04b±0.38	1.47b±0.19	–
Oxygenated sesquiterpenes			49.28a±3.12	161.16a±12.84	61.01a±5.21	242.81a±11.24	71.72a±18.28	289.43a±16.22	–
Others			9.00b±1.06	71.86a±1.99	70.04a±8.40	33.00ab±2.05	20.54b±5.78	25.57b±4.98	5.77b±0.99

Components are listed in order of elution in apolar column (HP-5); values followed by the same small letter did not share significant differences at $p<0.05$ (Duncan test)

RI_p and RI_a retention indices calculated using respectively a polar column (HP-Innowax) and an apolar column (HP-5), *MWO* microwave oven, *IR* infra-red, *MS* mass spectrometry, *CoI* co-injection, *RI* retention indice

compounds such as flavor and aroma in plant material (Fellows 1988).

In order to determine the relationship between the different drying methods, cluster analysis was carried out on the basis of the volatile compounds contents (Fig. 2). Results obtained showed the presence of one well-defined group including the fresh material together with MW- (500 W), IR- (65 °C), and oven-dried (65 °C) materials, suggesting similar compositions and another defined group composed of air drying, oven (45 °C), and IR (45 °C). The two groups were clearly distinguished both in quality and in quantity.

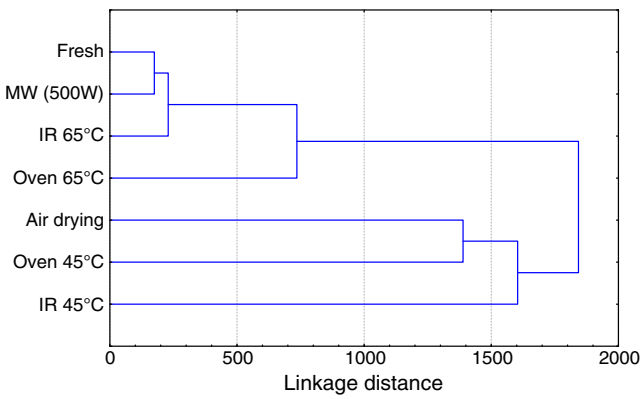

Fig. 2 Two-dimensional dendrogram obtained in the cluster analysis of the different extraction methods based on volatile compound contents of *S. officinalis* essential oils

Effect of Drying Methods on the DPPH Radical Scavenging Activity of EOs

Antioxidant activities of EOs from aromatic plants are mainly attributed to the active compounds present in them. This could be due to the high percentage of main constituents but also to the presence of other constituents in small quantities or to synergy among them (Romeilah et al. 2010). In the present investigation, the antioxidant activities of *S. officinalis* EOs obtained by the different drying methods as compared with BHT as reference antioxidant compound were determined, and the results are summarized in Table 3. Results indicated that the radical scavenging activity (percent inhibition) of the EOs obtained by oven drying (65 °C) and fresh material were the highest (88.56% and 85.75%, respectively) at the concentration of 10 mg/ml followed by IR (65 °C), MW(500 W), and air drying EOs (84.11%, 84.33%, and 84.64%, respectively). The lowest inhibition percentages were found in the EOs obtained by IR- (45 °C) and oven-drying (45 °C) techniques (61.07% and 59.87%, respectively). It was

Table 3 Antioxidant activities of the essential oils of fresh and dried sage (*S. officinalis* L.) plants measured by the DPPH method

	DPPH inhibition (%)				
	2 mg/ml	4 mg/ml	6 mg/ml	10 mg/ml	IC50
Fresh	12.15eC±1.29	48.71dB±8.77	58.12cB±5.14	85.75abA±2.09	5.32b±0.38
Air drying	29.19dC±1.61	58.26cB±1.10	81.63bA±2.38	84.64bA±8.65	3.33c±0.17
Oven 45 °C	10.12eD±1.57	27.45eC±1.90	36.96eB±1.88	59.87cA±1.39	8.23a±0.19
Oven 65 °C	19.73deD±2.41	32.97eC±3.30	51.83dB±1.63	88.56abA±7.03	5.70b±0.53
MW (500 W)	61.20bB±10.94	80.89bA±6.92	83.3bA±9.26	84.33bA±9.06	1.60de±0.49
IR 45 °C	18.26eC±2.43	33.43eB±3.19	51.01dA±1.52	61.07cA±9.12	7.79a±1.36
IR 65 °C	39.68cB±8.48	77.06bA±5.74	80.90bA±1.23	84.11bA±10.80	2.43d±0.33
BHT	96.48aC±0.39	97.05aB±0.09	97.32aAB±0.25	97.51aA±0.12	0.90e±0.00

Values followed by the same capital letter within the same line did not share significant differences at $p<0.05$ according to Duncan's test. Values followed by the same small letter within the same column did not share significant differences at $p<0.05$ according to Duncan's test

IC50 concentration (milligrams per milliliter) for a 50% inhibition

noticed that the scavenging activities of the EOs increased with the increasing of the EOs concentrations. All the tested samples showed lower DPPH radical scavenging activity when compared with the standard BHT. On the other hand, the highest antioxidant activity was obtained with BHT with an IC50 value of 0.90 mg/ml. All the tested EOs were able to reduce the stable radical DPPH reaching 50% of reduction with IC50 values. The EO obtained by MW (500 W) showed the lowest IC50 value (1.60) that corresponds to the highest antioxidant activity. Also, EOs obtained by IR (65 °C) and air drying methods showed good antioxidant activities with IC50 values of 2.43 and 3.33, respectively. On the other hand, EOs obtained by IR-(45 °C) and oven-drying (45 °C) techniques together with fresh material showed the lowest antioxidant capacities with IC50 values of 7.79, 8.23, and 5.32, respectively. The difference in DPPH radical scavenging activity between the different EOs is attributable to the chemical composition of each EO. In general, antioxidant activity of various *Salvia* species extracts is described (Murat et al. 2009; Janicksak et al. 2010), but not so much is reported on their essential oils to this regard. According to Burits et al. (2001), the components including 1,8-cineole, camphor, borneol, and terpinen-4-ol were found not to possess strong reducing effects using the DPPH assay. Based on this, it seems that the antioxidant activity of sage EO as screened by the DPPH system was not attributable to its main components. According to Kim et al. (2004) and Agnaniet et al. (2005), minor compounds might play a significant role in the antioxidant activity of many EOs, and synergistic effects were reported. Finally, we should point out that the consequences of food processing and preservation procedures on the overall antioxidant activity of foods are generally the results of different, and sometimes opposite

Table 4 Correlation coefficients between major components and antioxidant activity of essential oils of fresh and dried sage (*S. officinalis* L.) plants

Variables	DPPH inhibition						
	Fresh	Air drying	Oven 45 °C	Oven 65 °C	MW (500 W)	IR 45 °C	IR 65 °C
α-Terpinene	0.26	–	–	–	–	0.47	
1,8-Cineole	−0.37	−0.42	−0.60	−0.38	−0.51	−0.39	−0.44
α-Thujone	0.96	0.88	0.97	0.73	0.97	0.94	0.95
β-Thujone	0.73	0.60	0.82	0.65	0.78	−0.71	−0.84
Camphor	−0.12	−0.10	−0.31	−0.20	−0.20	−0.29	−0.18
2-Undecanone	–	–	–	–	0.70	–	–
α-Terpineol	0.87	0.72	0.77	–	0.75	0.90	–
Borneol	−0.67	−0.49	−0.42	−0.44	−0.55	−0.62	–
Viridiflorol	0.18	0.28	–	0.17	0.42	0.37	–
Manool	0.72	0.75	0.61	0.65	0.71	0.72	–

Correlation is significant at the 0.05 level (two-tailed), $r<0.5$ at $p<0.05$

events which can take place consecutively or simultaneously (Tomaino et al. 2005). Processing methods may also improve the properties of naturally occurring antioxidants or induce the formation of new compounds having antioxidant properties, so that the overall antioxidant activity of plant raw material can remain unchanged or increased despite the eventual loss of active ingredients (Tomaino et al. 2005).

Correlation Between Main Sage EO Constituents as Obtained by Drying Methods and DPPH Radical Scavenging Activity

Table 4 shows correlation coefficients between main components and DPPH scavenging activity in the case of fresh and dried plants. Results showed that some volatile compound such as β-thujone, 2-undecanone, α-terpineol, and manool were positively correlated ($p<0.05$) with DPPH scavenging activity. According to Ruberto and Baratta (2000), alcohols could have good antioxidant activity which can explain the significant positive correlation between viridiflorol, manool, and antioxidant DPPH assay. As for the other main EO components such as 1,8-cineole, camphor, and borneol, they showed negative correlation with DPPH radical scavenging ability. These results are confirmed by the work of Kelen and Tepe (2008) who reported that some of these compounds (1,8-cineole, camphor, and borneol) have been tested separately for their antioxidant power using different model systems including DPPH assay, and not one of them exhibited strong antioxidative activity in all methods employed. On the other hand, a moderate positive correlation ($p<0.05$) was observed between DPPH activity and both monoterpene hydrocarbons and oxygenated sesquiterpenes, whereas a moderate to high negative correlation was found between DPPH scavenging ability and both oxygenated monoterpenes and sesquiterpene hydrocarbons.

Finally, the presence of 2-undecanone only in the EO of microwave dried samples and its high positive correlation with DPPH scavenging activity leads us to think that this compound contributes to the high antioxidant power of the EOs obtained from microwave dried plants as compared with other drying methods. This does not exclude the role of other EO components such as β-thujone, α-terpineol, and manool which may act in synergy together with 2-undecanone.

Based on these results, it seems that high antioxidant activities observed in the cases of microwave (500 W), air drying, and IR (65 °C) drying methods were probably attributable to the particular composition of these EOs and to the synergism between their main constituents.

Conclusion

In summary, our investigation showed that drying sage aerial parts at ambient air, by oven at 45 °C, and by infrared at 45 °C resulted in small losses of the volatile compounds as compared with fresh material. Also, in some cases, these methods may improve the yield of some bioactive compounds such as 1,8-cineole, α and β-thujone, camphor, and others. These compounds are well known for their antibacterial and antifungal activities, so, the above-mentioned drying methods could give EOs characterized by their important disinfectant properties which could be utilized in food industries as preservative agents. On the other hand, the antioxidant activity of the EOs as determined by the DPPH assay showed that oils obtained from MW (500 W), air drying, and IR (65 °C) drying techniques were the most efficient in the inhibition of the degradation of the DPPH stable radical. These results suggest that these drying methods could be retained if antioxidant activity of sage EO is in demand especially in food, cosmetic, and pharmaceutical industries.

Acknowledgments We thank Dr. Mihoubi Daoued (Energetic and Thermal Processing Laboratory, CRTE, B.P. 95, 2050 Hammam-Lif, Tunisia) for the technical support of infrared drying experiments and for his helpful recommendations.

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