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Seasonal Variation of Essential Oil Yield and Composition of Sage (*Salvia officinalis* L.) Grown in Castilla - La Mancha (Central Spain)

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Abstract

Links between phenology, yield and composition of the essential oil of common sage, *Salvia officinalis* L., grown in Guadalajara (Central Spain) were determined in the different phases of the biological cycle during one year. Data showed an average yield about 1.0%. The analysis of the oil components was carried out by GC-FID and GC/MS. The main oil constituent was alpha thujone (40.1-46.5%). Other identified compounds are beta pinene (2.6-4.5%), cineole (3.5-8.7%), beta thujone (4.1-5.6%), camphor (4.1-8.0%), borneol (1.3-3.7%), alpha humulene (3.8-7.3%), viridiflorol (3.4-12.6%) and manool (0.1-4.5%). The highest yield of oil was obtained in the period of full flowering and the highest concentration of alpha thujone in the period of initial flowering.

Keywords: alpha thujone, essential oil composition, *Salvia officinalis,* Spain

Introduction

Aromatic and medicinal plants make up an interesting group because of the active principles they content. Cultivation of these species has a long tradition in Central Spain (Gaviña, 1966; Torner, 1974) and is an alternative to farming surpluses and to exploitation of abandoned lands, as well as a handful tool for diversification.

Sage is the general name for the herbs of the *Salvia* species, all of which are native of southern Europe and the Western Mediterranean. Common sage, *Salvia officinalis* L., is a perennial herb of the *Labiatae* family and is now produced worldwide. Sage oil has a camphoraceous, thujone-like aroma and is used in the fragrance industry. The oil of *Salvia officinalis* is also known for its medicinal-biological activities, such as antimicrobial and fungicidal effects (Barlcevic *et al*., 2000; Carta *et al*., 1996; Edris *et al*., 2007).

ISO 9909 for medicinal uses regulates the amounts of several constituents in the sage essential oils: cis-thujone (18.0-43.0%), camphor (4.5-24.5%), 1,8 cineole ((5.5-13.0%), trans-thujone (3.0-8.5%), alpha-humulene (>12.0%), alpha-pinene (1.0-6.5%), camphene (1.5-7.0%), limonene (0.5-3.0%), bornyl acetate (>2.5%) and linalool + linalyl acetate (>1%).

The oil is obtained by steam distillation from fresh or dried leaves and flowering tops of the plant. Oil yield is between 1.4 and 2.0%. The composition of oil of *S. officinalis* from different countries has been studied by several authors (Fellah *et al*., 2006; Kanias *et al*., 1998; Pino *et al*., 1997; Radulescu *et al*., 2004; Tsankova *et al*., 1994; Velickovic *et al*., 2002). It is composed mainly of monoterpene and sesquiterpene hydrocarbons and their oxygen derivates. Seasonal variation of oil of *S. officinalis* has also been reported (Asllani, 2000; Mirjalili *et al*., 2006; Perry *et al*., 1999; Pitarevic *et al*., 1984; Santos-Gomez and Fernandez-Ferreira, 2001).

The aims of the present paper were (a) to study the variation of the yield of oil, (b) to characterize the chemical composition of the oil and (c) to assess the evolution of the volatile active principles present in sage oil during one year.

Material and methods

Plant material

Samples of *S. officinalis* were collected from the cultivations belonging to the Centro Agrícola of Marchamalo (dependent on the Delegation of Agriculture and Environment of Guadalajara), between November 2008 and August 2009. Collection took place once a month during all the phenological periods, except full flowering and after flowering, when two or three samples were taken. The upper 20 cm aerial parts were harvested manually and dried in a ventilated chamber to constant weight. Water content was determined by azeotropic distillation with xylene.

Distillation

The oil was obtained by hydrodistillation of the dried ground material (only leaves or leaves and flowers, depending on each phenological period, because farmers do not distill leaves and flowers separately). The distillation was carried out in a Clevenger-type apparatus, according to the French Pharmacopeaea (IX Ed.). The oil was recovered directly and was stored with anhydrous Na_2SO_4 in dark vials at 4°C.

Gas Chromatography/Mass Spectrometry

GC/MS Samples were analysed by means of gas chromatography/mass spectrometry using an HP 5890A gas chromatograph connected to an HP 5971A mass detector (EI, 70 eV) and equipped with a 30 m × 0.25 mm internal diameter, DB-5 column (0.25 mm film thickness), upon the following conditions: injector temperature 260°C, column temperature 60°C (2 min), 4°C (1 min), 260°C (5 min), carrier gas He 1 mL/min, split 1:20, scan time 1 s., acquisition mass range 40-650 m/z.

The composition of the oil of *S. officinalis* was determined by comparing the relative GC retention times and the mass spectra of oil components with those of authentic compounds and the mass spectral data obtained from libraries.

Gas Chromatography/Flame Ionization Detection (GC/FID)

A gas chromatograph Hewlett Packard HP 5890 Series II, equipped with flame ionization detector (FID), an auto sampler and a split-splitless injector was used. The capillary column and conditions were similar to GC/MS. The temperature of FID was 270°C. The quantitative data were expressed as relative percentage of the oil constituents calculated from the GC peak areas without using correction factors. Each determination was carried out in duplicate.

Chemicals

Alpha thujone, beta pinene, cineole, beta thujone, camphor, borneol and alpha humulene were obtained from Sigma-Aldrich. All compounds were analytical standard grade and anhydrous Na_2SO_4 was analytical reagent grade from Panreac.

Results and discussion

Tab. 1 shows the yield of oil obtained from every sample for each phenological period.

Tab. 1. Essential oil yield (% v/w) of *Salvia officinalis*

Sample	Yield (% v/w)
VS1	0.6
VS2	0.7
VS3	0.8
VS4	0.8
IF1	0.7
IF2	1.1
IF3	1.2
FF1	1.4
FF2	1.5
FF3	1.3
AF1	1.0
AF2	1.0
AF3	0.9
AF4	0.8

VS = Vegetative Stage; IF = Initial Flowering; FF = Full Flowering; AF = After Flowering

The oil yield was calculated referring volume of oil to weight of every dried sample. Data and calculations are referred to leaves, and also flowers during flowering periods. The average oil yield (v/w) was approximately 1.0 %. It is higher during initial and full flowering (1.1-1.5 %), being lower after flowering and in the vegetative stand period (less than 1.0 %).

Tab. 2 shows the qualitative and average quantitative composition of the main constituents in the oil samples of sage obtained during the period of full flowering.

Tab. 2. Average composition of *Salvia officinalis* during the vegetative cycle

Compound	Percentage (%)	Compound	Percentage (%)
Monoterpenes		borneol	2.5
alpha-pinene	4.7	bornyl acetate	0.3
camphene	0.8	*Sesquiterpenes*	
beta-pinene	1.9	caryophyllene	1.8
myrcene	1.2	alpha-humulene	4.6
p-cymene	1.0	gamma cadinene	0.5
1,8 cineole	6.7	viridiflorol	8.1
alpha-thujone	43.6	*Diterpenes*	
beta-thujone	5.1	manool	1.7
camphor	8.1		

Qualitative composition is similar to that described in literature (Perry *et al*., 1999; Pino *et al*., 1997; Santos-Gomes and Fernandes-Ferreira, 2001), not so the quantitative content. The constituents alpha-pinene, beta-pinene, 1,8 cineole, alpha-thujone, beta-thujone, camphor, borneol, alpha-humulene, viridiflorol, and manool account at least 81% of weight in all samples. This quantitative composition of the samples is quite related to samples reported in Arouca, Portugal (Santos-Gomes and Fernandes-Ferreira, 2001) and New Zealand (Santos-Gomes and Fernandes-Ferreira, 2001) and meets the requirements of ISO 9909 (1999) standard for all compounds except camphene.

Tab. 3 presents the average values (%) found for the main constituents in each phenological period.

Tab. 3. Content (%) of each major constituent in all phenological phases

	VS	IF	FF	AF
alpha-pinene	4.8	4.8	4.7	4.3
beta-pinene	2.6	1.3	1.2	2.4
1,8 cineole	8.7	4.7	3.5	9.8
alpha-thujone	40.1	48.3	46.5	39.5
beta-thujone	4.1	5.8	5.6	4.7
camphor	4.1	8.3	8.0	12.1
borneol	1.3	3.8	3.7	1.0
alpha-humulene	7.3	4.0	3.8	3.3
viridiflorol	12.2	12.6	4.3	3.4
manool	1.2	4.5	0.1	1.1

VS = Vegetative Stage; IF = Initial Flowering; FF = Full Flowering; AF = After Flowering

The results show a chemotype with a proportion of alpha and beta thujone of 10:1, having high (44 - 54%) total thujone (alpha and beta) content, according to Perry *et al*. (1999).

The percentage of each major constituent in the oil was found to vary during all the phenological periods. It was observed that alpha-pinene, alpha-thujone, beta-thujone, borneol, viridiflorol and manool have a higher percentage during initial flowering, while cineole and camphor show a higher value after full flowering. Alpha-humulene reaches its higher percentage during the vegetative stand period.

The variation of alpha-thujone is different to that described by Asllani (2000) and Santos-Gomes and Fernandes-Ferreira (2001), where the maximum value is reported during the vegetative stand.

In conclusion, the best moment for harvesting is the initial flowering period, if the objective is a high content in alpha-thujone, and during full flowering, if the objective is a high yield in essential oil.

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