

# The impact of both the season of collection and drying on the volatile constituents of *Origanum vulgare* L. ssp. *hirtum* grown wild in Croatia

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**Summary** Samples of *Origanum vulgare* ssp. *hirtum* were collected from the same geographic area in the south of Croatia at different seasons of growth. The maximum fluctuations found for the main components from fresh plant material were: thymol [149.2–1124.4 mg (100 g)<sup>-1</sup>], carvacrol [51.6–564.3 mg (100 g)<sup>-1</sup>], p-cymene [20.2–220.9 mg (100 g)<sup>-1</sup>] and  $\gamma$ -terpinene [50.1–217.5 mg (100 g)<sup>-1</sup>]. The oregano that was analysed belonged to a thymol/carvacrol chemotype. The season of collecting affected the qualitative and quantitative composition of the essential oil. The most impressive difference was the increase of p-cymene content in August. After the drying of the plant material, all samples showed a minor decrease in essential oil yields when compared with fresh plants. Drying, at room temperature, had no effect on the qualitative composition of oregano oil. Because of the variability of essential oil compositions from seasonally collected fresh and dried oregano, it would be important to check the quantity and quality of such components before usage.

**Keywords** Carvacrol, essential oil, gas chromatography–mass spectrometry,  $\gamma$ -terpinene, p-cymene, thymol.

## Introduction

*Origanum vulgare* L. ssp. *hirtum* is widely distributed in the Mediterranean basin and is used as a spicy herb under the name 'Greek oregano'. It is generally accepted that Greek oregano is of the highest quality (Lawrence, 1984; Fleisher & Fleisher, 1988). Oregano is of great economic importance but this is not only related to its use as a spice. Its essential oil has antimicrobial, cytotoxic, anti-oxidant and antifungal activity (Lagouri *et al.*, 1993; Sivropoulou *et al.*, 1996; Adam *et al.*, 1998). Seasonal changes in essential oil content and composition have been shown to exist in different species (Mastelić, 1995). Climatic factors, rates of plant metabolism, differentiation and secretory activity of glandular hairs affect synthesis and secretion of essential oils. The geographic variations found in the essential oils of *Oregano*

species from Greek and Italy have been intensively investigated (Vokou *et al.*, 1993; Russo *et al.*, 1998). Published results suggest that the pattern of geographic variations of oregano oils are related to different climatic conditions. However, there is no data for the chemical profile of this spice from Croatia, in different seasons.

The loss of volatiles in herbs and spices during drying depends mainly on drying conditions and the biological characteristics of plants. Different effects of drying can be distinguished, depending on the composition and content of volatile compounds. Some compounds evaporate during drying, whilst others are partially retained. It is reported that some can disappear completely. Some compounds arise as oxidation products during drying (Luning *et al.*, 1995). The effect of drying on the volatiles of *Thymus vulgaris* and *Salvia officinalis* was reported by Venskutonis (1997). Similarly the impact of methods of drying on the flavour quality of *Oreganum majorana* was

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investigated by Raghavan *et al.* (1997) whereas Rao *et al.* (1998) reported on the impact of drying on volatiles of *Rosmarinus officinalis*. However, we did not find any papers dealing with these changes brought about by drying and specifically related to the season in which *O. vulgare* ssp. *hirtum* was collected.

Consequently, the aim of this study was to test the hypothesis that seasonal variations would be greater than the impact of drying on the content and composition of essential oil *O. vulgare* L. subsp. *hirtum*, grown wild in Croatia.

## Materials and methods

### Origin of the samples

To avoid complications from different geographic and climatic ecosystems, the plant material was collected at varying seasons from the same geographic area in the south of Croatia (near Split). Three replicates were harvested for each collection period. The samples consisted of leaves and topical stalks with or without flowers, depending upon the season of collection. The voucher specimens of dried plant material are deposited in the Department of Organic Chemistry, Faculty of Chemical Technology, University of Split.

### Drying of plant material

Each sample was collected in triplicate, pooled and then divided in two parts: one was investigated as fresh plant material (100 g), and the other (100 g) was dried and then submitted to the same investigations. Air-drying of the plant material was performed for 10 days, in a shaded place at room temperature.

### Isolation of the essential oil

The essential oil was isolated from collections by hydrodistillation in a modified Clevenger type apparatus for 3 h. The essential oils that were obtained were dried over anhydrous sodium sulphate (Fluka Chemie, Buchs, Switzerland) and stored under nitrogen in sealed vials, at  $-20^{\circ}\text{C}$ . The yield of essential oil was determined by the gravimetric method and expressed as a mean value of analysed triplicates.

### GC-MS analysis of volatiles

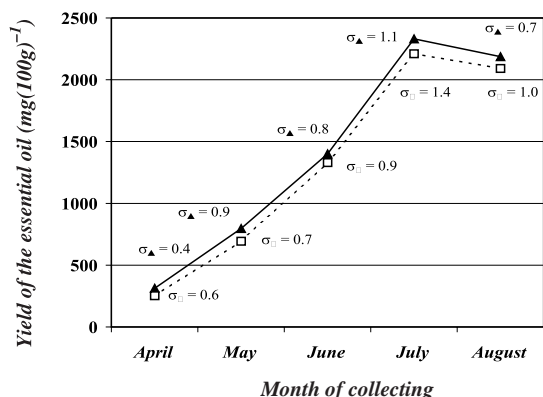
Volatiles were analysed using a Hewlett-Packard GC-MS system (GC 5890 series II, MSD 5971A; Hewlett Packard, Vienna, Austria), with two columns which had different stationary phase polarities. GC operating conditions were: column HP-20M (Carbowax 20M; Hewlett Packard, Vienna, Austria),  $50\text{ m} \times 0.2\text{ mm}$  i.d., film thickness  $0.2\text{ }\mu\text{m}$ , column temperature programmed for  $70^{\circ}\text{C}$  isothermal for 4 min, then increased to  $180^{\circ}\text{C}$  at a rate of  $4^{\circ}\text{C min}^{-1}$ ; column HP-101 (Methylsilicone; Hewlett-Packard, Vienna, Austria),  $25\text{ m} \times 0.2\text{ mm}$  i.d., film thickness  $0.2\text{ }\mu\text{m}$ , column temperature programmed for  $70^{\circ}\text{C}$  isothermal for 2 min, then increased to  $200^{\circ}\text{C}$  at a rate of  $3^{\circ}\text{C min}^{-1}$ ; carrier gas helium, flow rate  $1\text{ mL min}^{-1}$ , injector temperature  $250^{\circ}\text{C}$ , volume injected  $1\text{ }\mu\text{L}$ , split ratio 1:50. MS conditions: ionization voltage  $70\text{ eV}$ , ion source temperature  $280^{\circ}\text{C}$ , mass range 30–300 mass units (Miloš & Radonić, 1996; Mastelić & Kuštrak, 1997; Mastelić *et al.*, 1998).

### Identification and quantization of volatiles

Components were identified by their retention indices on columns HP-20M and HP-101; and by comparing their mass spectra with those in a commercial library (Wiley library, Mass Spectral Database, Hewlett-Packard, Vienna, Austria) as well as with spectra published by Adams (1995), and in some cases by using standards. The quantity of each compound identified in the individual sample was calculated in relation to the mean peak area of compounds identified on HP-20 M and HP-101 columns. All results are presented as the mean value of triplicate analysis. The content of all components are expressed as  $\text{mg (100 g)}^{-1}$  fresh plant material.

## Results and discussion

The yields of oregano essential oil obtained by the hydrodistillation of fresh plant material fluctuated from  $312.6$  to  $2331.0\text{ mg (100 g)}^{-1}$ , depending on the month of collection (Fig. 1). For measurements in all seasons, a S.D. ( $\sigma$ ) of better than 1.1 was achieved. The content of essential oil was the lowest in April and the highest during flowering



**Figure 1** Yields of the essential oil from seasonally collected plant material:  $\blacktriangle$  – fresh oregano,  $\square$  – dried oregano.

(July). The decrease of the oil content after flowering may be because of the fact that during August, the plant tissue was already becoming senescent and phenols might have undergone decomposition, perhaps preventing stress damage by acting as natural antioxidants (Russo *et al.*, 1998). The natural oil of fresh oregano was a light yellow colour.

The GC–MS analysis of isolated oregano essential oil revealed a total of 28 compounds that include monoterpenes and small amounts of sesquiterpenes. The compounds are listed in order of their elution time on HP-20M column in Table 1. The oils were predominately oxygenated monoterpenoids. The seasonal fluctuations of the main components from fresh oregano were as follows: thymol [149.2–1124.4 mg (100 g)<sup>-1</sup>;  $\sigma < 2.0$ ], carvacrol [51.6–564.3 mg (100 g)<sup>-1</sup>;  $\sigma < 1.5$ ], p-cymene [20.2–220.9 mg (100 g)<sup>-1</sup>;  $\sigma < 0.9$ ] and  $\gamma$ -terpinene [50.1–217.5 mg (100 g)<sup>-1</sup>;  $\sigma < 0.9$ ]. The seasonal fluctuations of other quantitatively important volatiles from fresh oregano were:  $\alpha$ -thujene [8.5–92.3 mg (100 g)<sup>-1</sup>;  $\sigma < 0.8$ ],  $\beta$ -myrcene [25.9–45.5 mg (100 g)<sup>-1</sup>;  $\sigma < 0.8$ ], caryophyllene [2.6–37.2 mg (100 g)<sup>-1</sup>;  $\sigma < 0.7$ ],  $\beta$ -pinene + sabinene [8.3–28.7 mg (100 g)<sup>-1</sup>;  $\sigma < 0.8$ ] and  $\beta$ -bisabolene [2.2–15.3 mg (100 g)<sup>-1</sup>;  $\sigma < 0.6$ ]. There is a similarity when these essential oil compositions are compared with some other plants from the *Lamiaceae* family, like *Satureja montana* and *T. vulgaris* (Mastelić, 1995). When presented as percentages, the seasonal variations of analysed

compounds appear to be rather random. Despite the striking quantitative differences of the major four essential oil components, the sum of their percentage composition was almost equal in different seasons (83.7–86.8%, Fig. 2), this was also reported by Kokkini *et al.* (1994). The most striking seasonal difference found when analysing fresh oregano was the high concentration of p-cymene in August [220.9 mg (100 g)<sup>-1</sup>;  $\sigma = 0.7$ ], also reported by Kokkini *et al.* (1996). An increase in the thymol percentage with a decreasing carvacrol content (and *vice versa*) in all samples indicates a probable connection between the biosynthetic pathways of these major components and supports a finding which indicates that the formation of thymol from  $\gamma$ -terpinene is *via* p-cymene (Poulose & Croteau, 1978). According to Russo *et al.* (1998), there are two main chemotypes of oregano (either the thymol or the carvacrol chemotype) but there are also two intermediary chemotypes (thymol/carcacrol and carvacrol/thymol). The environmental conditions are able to influence which biosynthetic pathway (thymol or carvacrol) may be dominant. The results reported here suggest that the oregano that we analyzed belonged to a thymol/carcacrol chemotype.

After drying, all samples of the plant material showed a minor decrease in essential oil yields when compared with fresh plant material at the same stage of plant development, Table 1. After drying, the yield of essential oil from dried material ranged from 252.5 to 2210.1 mg (100 g)<sup>-1</sup>, depending on the month of collection (Fig. 1). The calculated sample S.D. (triplicates analysis of yield) varied from  $\sigma = 0.6$ ,  $\sigma = 1.4$ . The majority of volatile compounds were retained under the drying conditions that were used. The dry matter loss was relatively high for all samples, especially for younger plants. After drying the plant material, the oil that we isolated was a darker coloured when compared with oil from fresh plants.

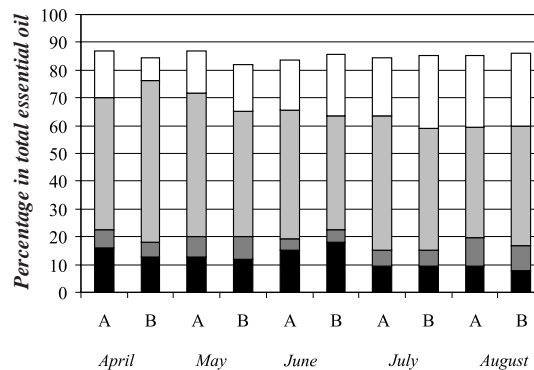
The dried plant material was made up of four main components, which are also found in fresh oregano, but with different proportions [thymol 147.5–971.0 mg (100 g)<sup>-1</sup>,  $\sigma < 1.9$ ; carvacrol 20.0–581.9 mg (100 g)<sup>-1</sup>,  $\sigma < 1.4$ ;  $\gamma$ -terpinene 32.0–242.0 mg (100 g)<sup>-1</sup>,  $\sigma < 0.6$ , p-cymene 13.5–189.3 mg (100 g)<sup>-1</sup>,  $\sigma < 0.7$ ]. Other quantitatively important fluctuations in amounts of volatiles

**Table 1** Qualitative and quantitative composition of seasonally collected *Origanum vulgare* (L.) ssp. *hirtum* from fresh and dried plant material. The amounts are expressed as the mean value of three replicates for all collecting periods

No.	Identified compound	Content [mg (100 g) <sup>-1</sup> fresh plant material]												Mode of identification
		April		May		June		July		August		B ± σ		
		A ± σ	B ± σ	A ± σ	B ± σ	A ± σ	B ± σ	A ± σ	B ± σ	A ± σ	B ± σ			
<b>Hydrocarbons</b>														
1.	α-Thujene	8.5 ± 0.5	6.2 ± 0.2	16.7 ± 0.3	16.8 ± 0.4	43.4 ± 0.1	41.8 ± 0.5	87.3 ± 0.8	69.2 ± 0.6	92.3 ± 0.5	69.8 ± 0.4	1 <sub>1</sub> , 1 <sub>2</sub> , MS		
2.	β-Pinene + Sabinene	8.3 ± 0.3	7.8 ± 0.2	28.7 ± 0.7	25.5 ± 0.5	11.2 ± 0.3	3.8 ± 0.3	9.2 ± 0.3	7.7 ± 0.3	13.1 ± 0.8	10.5 ± 0.6	1 <sub>1</sub> , 1 <sub>2</sub> , MS		
3.	δ-3-Carene	–	–	4.0 ± 0.3	2.8 ± 0.2	–	–	–	–	t	t	1 <sub>1</sub> , 1 <sub>2</sub> , MS		
4.	α-Terpinene	–	–	–	–	55.4 ± 0.2	49.6 ± 0.7	76.3 ± 0.9	55.9 ± 0.4	–	–	1 <sub>1</sub> , 1 <sub>2</sub> , MS		
5.	β-Myrcene	–	–	–	–	25.9 ± 0.3	20.5 ± 0.4	45.5 ± 0.8	38.0 ± 0.4	37.2 ± 0.5	35.5 ± 0.3	1 <sub>1</sub> , 1 <sub>2</sub> , MS		
6.	β-Phelandrene	–	–	–	–	5.6 ± 0.3	–	–	–	21.9 ± 0.3	20.9 ± 0.3	1 <sub>1</sub> , 1 <sub>2</sub> , MS		
7.	γ-Terpinene	50.1 ± 0.3	32.0 ± 0.3	102.0 ± 0.3	81.4 ± 0.4	211.4 ± 0.5	242.0 ± 0.6	217.5 ± 0.9	207.5 ± 0.3	205.6 ± 0.6	163.1 ± 0.3	1 <sub>1</sub> , 1 <sub>2</sub> , MS		
8.	p-Cymene	20.2 ± 0.4	13.5 ± 0.4	56.6 ± 0.4	56.5 ± 0.3	58.8 ± 0.6	58.3 ± 0.2	135.2 ± 0.9	125.3 ± 0.4	220.9 ± 0.7	189.3 ± 0.6	1 <sub>1</sub> , 1 <sub>2</sub> , MS		
9.	Terpinolene	–	–	1.5 ± 0.43	1.2 ± 0.1	–	–	–	–	5.2 ± 0.4	3.8 ± 0.4	1 <sub>1</sub> , 1 <sub>2</sub> , MS		
10.	Alloocymene	–	–	–	–	2.8 ± 0.4	–	–	–	–	0.1 ± 0.1	1 <sub>1</sub> , 1 <sub>2</sub> , MS		
11.	γ-Elementene	0.7 ± 0.4	0.5 ± 0.1	2.1 ± 0.4	2.5 ± 0.2	–	–	–	–	4.4 ± 0.43	–	1 <sub>1</sub> , 1 <sub>2</sub> , MS		
12.	Caryophyllene	2.6 ± 0.3	3.7 ± 0.4	8.1 ± 0.3	9.2 ± 0.4	28.0 ± 0.5	20.5 ± 0.4	31.9 ± 0.7	27.6 ± 0.4	37.2 ± 0.4	33.5 ± 0.4	1 <sub>1</sub> , 1 <sub>2</sub> , MS		
13.	α-Humulene	0.8 ± 0.1	0.6 ± 0.1	1.4 ± 0.2	1.6 ± 0.2	4.1 ± 0.4	t	3.9 ± 0.4	4.6 ± 0.3	8.3 ± 0.3	7.9 ± 0.2	1 <sub>1</sub> , 1 <sub>2</sub> , MS		
14.	β-Bisabolene	3.4 ± 0.2	3.7 ± 0.3	4.4 ± 0.6	8.0 ± 0.4	4.2 ± 0.3	11.3 ± 0.5	2.2 ± 0.4	13.5 ± 0.7	15.3 ± 0.4	20.9 ± 0.5	1 <sub>1</sub> , 1 <sub>2</sub> , MS		
15.	β-Cubebene	–	2.0 ± 0.1	2.5 ± 0.2	1.0 ± 0.1	7.0 ± 0.3	4.0 ± 0.4	3.0 ± 0.2	5.6 ± 0.4	13.1 ± 0.4	8.4 ± 0.4	1 <sub>1</sub> , 1 <sub>2</sub> , MS		
16.	δ-Cadinene	1.0 ± 0.1	0.2 ± 0.1	1.7 ± 0.3	2.1 ± 0.2	–	–	–	–	4.6 ± 0.4	5.6 ± 0.4	1 <sub>1</sub> , 1 <sub>2</sub> , MS		
<b>Oxygen containing compounds</b>														
17.	1-Octen-3-ol	3.4 ± 0.2	3.5 ± 0.1	6.2 ± 0.1	4.7 ± 0.2	11.9 ± 0.2	13.2 ± 0.3	21.7 ± 0.5	20.1 ± 0.2	12.9 ± 0.4	12.1 ± 0.5	1 <sub>1</sub> , 1 <sub>2</sub> , MS		
18.	Linalol	0.1 ± 0.1	–	t	–	–	–	t	–	t	t	1 <sub>1</sub> , 1 <sub>2</sub> , MS		
19.	Bornyl acetate	–	–	–	–	–	4.0 ± 0.3	–	–	10.9 ± 0.2	10.4 ± 0.3	1 <sub>1</sub> , 1 <sub>2</sub> , MS		
20.	Terpinen-4-ol	–	–	t	–	–	–	5.1 ± 0.5	6.2 ± 0.4	8.7 ± 0.5	4.2 ± 0.2	1 <sub>1</sub> , 1 <sub>2</sub> , MS		
21.	Pulegone	2.2 ± 0.2	0.5 ± 0.1	7.4 ± 0.1	9.2 ± 0.2	–	t	–	–	–	–	1 <sub>1</sub> , 1 <sub>2</sub> , MS		
22.	Methylthymyl ether	3.3 ± 0.2	2.3 ± 0.1	4.1 ± 0.2	4.6 ± 0.1	0.1 ± 0.3	–	–	–	–	–	1 <sub>1</sub> , 1 <sub>2</sub> , MS		
23.	2-iso-Propyl-1-methoxy-4-methyl benzene	0.3 ± 0.1	0.2 ± 0.1	1.4 ± 0.1	0.9 ± 0.2	–	–	1.1 ± 0.2	1.0 ± 0.5	3.9 ± 0.4	6.3 ± 0.3	1 <sub>1</sub> , 1 <sub>2</sub> , MS		
24.	α-Terpineol	–	–	–	–	1.4 ± 0.5	1.3 ± 0.2	–	–	2.6 ± 0.3	–	1 <sub>1</sub> , 1 <sub>2</sub> , MS		
25.	Borneol	2.3 ± 0.5	1.9 ± 0.4	7.2 ± 0.5	6.5 ± 0.3	11.2 ± 0.5	10.2 ± 0.2	16.4 ± 0.5	17.2 ± 0.6	8.7 ± 0.2	7.9 ± 0.3	1 <sub>1</sub> , 1 <sub>2</sub> , MS		

26. Thymyl acetate	149.2 ± 0.7	147.5 ± 0.8	—	—	—	—	1.4 ± 0.2	1.3 ± 0.3	2.3 ± 0.2	—	6.6 ± 0.3	6.3 ± 0.4	$I_1$ , —, MS
27. Thymol	51.6 ± 0.8	20.0 ± 0.5	413.9 ± 0.5	312.7 ± 0.9	646.1 ± 1.3	542.6 ± 1.0	1124.4 ± 2.0	971.0 ± 1.9	870.5 ± 1.6	901.3 ± 1.3	870.5 ± 1.6	901.3 ± 1.3	$I_1$ , $I_2$ , MS
28. Carvacrol	—	—	119.6 ± 1.0	117.7 ± 1.3	254.1 ± 0.9	294.6 ± 1.1	486.7 ± 1.5	581.9 ± 1.3	564.3 ± 1.3	550.0 ± 1.4	564.3 ± 1.3	550.0 ± 1.4	$I_1$ , $I_2$ , MS
100 g of fresh material	—	—	—	—	—	—	—	—	—	—	—	—	—
drying produced	—	—	—	—	—	—	—	—	—	—	—	—	—
dried material [g] ± σ	17.3 ± 0.8	30.9 ± 0.7	38.8 ± 0.8	45.2 ± 0.9	45.0 ± 0.5	—	—	—	—	—	—	—	—

A – fresh plant material, B – dried plant material, σ – S.D., not detected,  $I_1$  – retention indices on HP-20M column,  $I_2$  – retention indices on HP-101 column, MS – mass spectra, t – traces [ $< 0.1$  mg (100 g) $^{-1}$  fresh material].



**Figure 2** Concentration (%) of four main components in total essential oil: A – fresh oregano, B – dried oregano; □ – karvakrol, ■ – timol, ▒ – p-cymene, ■ – γ-terpinene.

found in dried oregano were: α-thujene [6.2–69.8 mg (100 g) $^{-1}$ ;  $\sigma < 0.6$ ], β-myrcene [20.5–35.5 mg (100 g) $^{-1}$ ;  $\sigma < 0.4$ ], caryophyllene [3.7–33.5 mg (100 g) $^{-1}$ ;  $\sigma < 0.5$ ], β-pinene + sabinene [7.8–25.5 mg (100 g) $^{-1}$ ;  $\sigma < 0.6$ ] and β-bisabolene [3.7–20.9 mg (100 g) $^{-1}$ ;  $\sigma < 0.7$ ]. The results from Table 1 demonstrate that the drying of the plant material had no effect on the qualitative composition of oregano essential oil. The changes found in quantitative composition of each identified compound were complex, especially for those major intermediates involved in the phenolic biosynthetic pathway (Poulose & Croteau, 1978). It is notable that the content of monoterpene hydrocarbons always decreased after drying the plant (Table 1). Only the amount of sesquiterpene β-bisabolene increased constantly with drying. The continual decrease (with drying) of thymyl-acetate and 2-iso-propyl-1-methoxy-4-methyl benzene was particularly noteworthy amongst the oxygen containing compounds.

In conclusion, the seasonal variability of the components of the essential oil, from both fresh and dried plant material, is large enough to dictate that samples should be checked, for both quantity and quality, before use if these parameters are important. Different times of harvesting combined with drying are the root causes of these differences. The seasonal variations in the essential oil quantitative composition are remarkable. However it should be taken into account that oregano can be devoid of its characteristics odour when the plants are collected in August (this is the result of high concentrations of p-cymene). Air-drying can be

recommended as a suitable method for preservation of oregano, although it results in an oil yield decrease. The oil yield decrease (after drying) was associated with a decrease in monoterpene hydrocarbons, drying of oregano had no effect on qualitative oil composition. Adaptation of drying methods could be used to improve the quantitative oil changes in dried plant material, these adaptations in the methods merit further investigation.

## References

- Adam, K., Sviropoulou, A., Kokkini, S., Lanaras, T. & Arsenakis, M. (1998). Antifungal activities of *Origanum vulgare* subsp. *hirtum*, *Mentha spicata*, *Lavandula angustifolia* and *Salvia fructinosa* essential oils against human pathogenic fungi. *Journal of Agricultural & Food Chemistry*, **46**, 1739–1745.
- Adams, R.P. (1995). *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*. Illinois: Alured Publishing Corporation.
- Fleisher, A. & Fleisher, Z. (1988). Identification of Biblical hyssop and origin of the traditional use of oregano group herbs in the Mediterranean region. *Economics and Botany*, **42**, 232–241.
- Kokkini, S., Karousou, R., Dardioti, A., Krigas, N. & Lanaras, T. (1996). Autumn essential oils of Greek oregano. *Phytochemistry*, **44**, 883–886.
- Kokkini, S., Karousou, R. & Vokou, D. (1994). Pattern of geographic variation of *Origanum vulgare* trichomes and essential oil content in Greece. *Biochemical Systematics and Ecology*, **2**, 517–528.
- Lagouri, V., Blekas, G., Tsimidou, M., Kokkini, S. & Boskou, D. (1993). Composition and antioxidant activity of essential oils from oregano plants grown wild in Greece. *Zeitschrift für Lebensmittel Untersuchung und Forschung*, **197**, 20–23.
- Lawrence, B.M. (1984). The botanical and chemical aspects of oregano. *Perfumer and Flavorist*, **9**, 41–51.
- Luning, P.A., Ebbenhorstseller, T., Derijk, T. & Rozen, J.P. (1995). Effect of hot air drying on flavour compounds of bell peppers (*Capsicum annum*). *Journal of the Science of Food and Agriculture*, **68**, 355–365.
- Mastelić, J. (1995). A study of the relations of terpenes and terpene glycosides of the aromatic plants belonging to the family Lamiaceae, *PhD Thesis*. Zagreb: University of Zagreb.
- Mastelić, J. & Kuštrak, D. (1997). Essential oil and glycosidically bound volatiles in aromatic plants. II. Rosemary (*Rosmarinus officinalis* L., Lamiaceae). *Acta Pharmaceutica*, **47**, 139–142.
- Mastelić, J., Miloš, M., Kuštrak, D. & Radonić, A. (1998). The essential oil and glycosidically bound volatile compounds of *Calaminta nepeta* (L.) Savi. *Croatica Chemica Acta*, **71**, 147–154.
- Miloš, M. & Radonić, A. (1996). Essential oil and glycosidically bound volatile compounds from Croatian *Cupressus sempervirens* L. *Acta Pharmaceutica*, **46**, 309–314.
- Poulose, A.J. & Croteau, R. (1978). Biosynthesis of aromatic monoterpenes. Conversion of  $\gamma$ -terpinene to p-cymene and thymol in *Thymus vulgaris* L. *Archives of Biochemistry and Biophysics*, **187**, 307–314.
- Raghavan, B., Rao, L.J., Singh, M. & Abraham, K.O. (1997). Effect of drying methods on the flavour quality of majoram (*Origanum majorana* L.). *Nahrung-Food*, **41**, 159–161.
- Rao, L.J., Singh, M., Raghavan, B. & Abraham, K.O. (1998). Rosemary (*Rosmarinus officinalis* L.) – impact of drying on its flavour quality. *Journal of Food Quality*, **21**, 107–115.
- Russo, M., Galletti, G.C., Bocchini, P. & Carnacini, A. (1998). Essential oil composition of wild populations of Italian oregano spice [*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart]: a preliminary evaluation of their use in chemotaxonomy by Cluster Analysis. I. Inflorescences. *Journal of Agricultural and Food Chemistry*, **46**, 3741–3746.
- Sivropoulou, A., Papanicolaou, E., Nikolau, C., Kokkini, S., Lanaras, T. & Arsenakis, M. (1996). Antimicrobial and cytotoxic activities of *Origanum* essential oils. *Journal of Agricultural and Food Chemistry*, **44**, 1202–1205.
- Venskutonis, P.R. (1997). Effect of drying on the volatile constituents of thyme (*Thymus vulgaris* L.) and sage (*Salvia officinalis* L.). *Food Chemistry*, **59**, 219–227.
- Vokou, D., Kokkini, S. & Bessiere, J.-M. (1993). Geographic variations of Greek oregano (*Origanum vulgare* ssp. *hirtum*) essential oils. *Biochemical Systematics and Ecology*, **21**, 287–295.