

# DETERMINATION OF VOLATILES, TOCOPHEROLS AND COLOUR CHANGES IN AROMATISED OILS WITH MARJORAM (*ORIGANUM MAJORANA* L.)

**Vilma Kraujalyte<sup>\*1</sup>, Mirjana Andjelkovic<sup>2</sup>, Roland Verhé<sup>2</sup>,  
Petras Rimantas Venskutonis<sup>1</sup>**

<sup>1</sup>*Faculty of Chemical Technology, Kaunas University of Technology, Kaunas, Lithuania*

<sup>2</sup>*Faculty of Bioscience Engineering, Gent University, Gent, Belgium*

\*e-mail: [salavija@yahoo.com](mailto:salavija@yahoo.com)

## Abstract

Grape seed oil was aromatised by mixing it with dried and ground marjoram herb. Headspace volatiles of marjoram and aromatised oil were assessed by solid phase microextraction – gas chromatography – mass spectrometry (SPME-GS-MS).  $\gamma$ -Terpinene (14.6 %), 4-terpineol (11.7%),  $\alpha$ -terpinene (11.5%),  $\beta$ -phellandrene (11.1%), sabinene (11.0%) and  $\alpha$ -thujene (7.0%) were major constituents among 22 compounds identified in dry marjoram headspace. However only 10 hydrocarbon terpenes were detected in aromatised oil headspace; their concentrations increased when higher amount of herb (6% compared to 3%) was added and kept longer time (4 weeks compared to 48 hours and 1 week). Percentage composition of volatiles was almost similar for all oil samples. Two tocopherols isomers,  $\alpha$ - and  $\gamma$ - were found in oil samples, the major being  $\alpha$ -tocopherol (241.8–274.3 mg kg<sup>-1</sup>). The concentration of tocopherols significantly increased in the oils aromatised with marjoram. Colour of oils was evaluated using CIELAB method; maceration of oil with herb additives resulted in the changes of a\* and b\* colour characteristics of oil. Most likely, these changes were due to the migration of chlorophyll and carotene type pigments from herb to the oil.

**Key words:** Marjoram, headspace volatiles, tocopherols, grape seed oil

## Introduction

Lipid rich foods, fats and oils deteriorate during long term storage and/or heating. Deterioration process proceeds through several degradation reactions. Using of antioxidants is one of the ways to inhibit oxidation. Antioxidants in terms of their origin can be classified in two main groups, synthetic and natural. The most popular antioxidants in food production are synthetic phenols, such as BHT, BHA, TBHQ and PG. However, there is some uncertainty regarding health risks associated with the consumption of synthetic additives. Therefore, the interest in natural additives has been increasing during last decades (Yanishlieva et al, 2006). Since the ancient times various parts of plants and their extracts have been used for food flavouring and preservation (Hinneburg et al., 2006; Pokorny 2001; Nakatani, 1997). Lamiaceae family plants containing phenolic and volatile compounds were extensively studied as a source of natural antioxidants (Fecka and Turek, 2008; Politeo et al., 2006; Triantaphyllou et al., 2001).

The composition of marjoram volatile compounds was previously studied and 4-terpinenol,  $\gamma$ -terpinene,  $\alpha$ -terpinene, sabinene,  $\beta$ -phellandrene and *p*-cymene were reported as the most important volatiles in marjoram essential oil. However, it was shown that chemical composition of marjoram is rather variable and depends on geographical origin, plant vegetation phase and handling procedures of harvested herb (Baranauskienė et al., 2006; Politeo et al., 2006).

Seed oils are good sources of tocopherols. The level of individual tocopherols in seed oil also depends on many factors. For instance, it was reported that tocopherol content in grape seed oil was dependant on the production region, oil quality and some other factors (Beveridge et al., 2005; Crews et al., 2006).

The aim of this work was to evaluate the changes of volatile compounds, tocopherols and colour during aromatization process of grape seed oil with marjoram herb.

## Materials and Methods

### *Materials and preparation of aromatised oil*

Refined grape seed oil was obtained from a specialized retailer in Belgium and stored in dark glass bottles at 4 °C. Dried marjoram herb (*Origanum majorana* L.) was purchased in a local market in Lithuania and stored in dark and dry place before analysis.

Aromatised oils were prepared by adding 3% and 6% (w/w) of marjoram herb to 100 ml grape seed oil and keeping the samples 48 hours, 1 and 4 weeks at 18±2 °C temperature. After aromatization the samples were filtered and kept in cool dark place before further analysis.

*Analysis of volatile compounds.* One g of marjoram, 2.5 g of grape seed oil and aromatised oils were weighed in vials and closed with a septum (Gerstel, Mulheim-an-der-Ruhr, Germany). Solid phase microextraction (SPME) and sample injection into a chromatograph was performed by using Gerstel MPS-2 autosampler. The samples were preheated for 2 min at 30 °C and headspace volatiles were extracted on DVB/CAR/PDMS fiber (Supelco, Inc., Bellefonte, PA, USA) during 60 min, at 30 °C followed by desorption for 5 min at 250 °C.

GC-MS analysis was performed on a Hewlett-Packard 6890 GC Plus coupled with a HP 5973MSD and equipped with a CIS-4PTV injector and HP5-MS capillary column (30 m length, 0.25 mm i.d., 0.25 µm film thickness). Injector operating in splitless mode was heated at 250 °C, GC oven temperature was programmed from 40 °C (2 min hold) to 150 °C at 5 °C min<sup>-1</sup> and from 150 to 250 °C at 10 °C min<sup>-1</sup> (2 min hold). Temperature of transfer line to MSD was 260°C. Helium was used as a carrier gas at 1 ml min<sup>-1</sup> velocity. MSD parameters were as follows: ionization EI 70 eV; acquisition parameters in full scan mode: scanned m/z 40–200 (0–20 min), 40–400 (>20 min). The analytes were identified by comparison of their mass spectra with mass spectral libraries (Nist'98 and Wiley 6<sup>th</sup>) and by comparing calculated GC peak retention index (IR) with data present in literature (Adams, 2007).

*Analysis of tocopherols.* Tocopherols were determined by HPLC equipped with UV diode array detector which was set at 292 nm wavelength (AOCS, 1999). Two g of oil were dissolved in 25 ml of LC-MS grade hexane and directly injected into a normal phase Altima Silica column (250 mm length 4.6 mm id, particle size 5 µm). Tocopherols were eluted in 20 min with hexane/isopropanol (99.5:0.5) at a flow rate 1.5 ml min<sup>-1</sup>. The concentrations of tocopherols were determined from a calibration curve which was plotted using data obtained by measurements of reference compounds.

*Colourimetric measurements.* The colour was evaluated on a CIELAB instrument under CIE Illuminant D65 by using 10 g of aromatised oil in a plastic Petri dish; each sample was measured ten times with spectrophotometer (CM-2600d/2500d Konica Minolta) connected to a computer.

*Statistical analysis.* Statistical analysis was made using software Microsoft Excel 2003. The results were compared by t-test and p-value.

## Results and Discussion

### *Volatile compounds in marjoram and aromatised oils*

Twenty-two volatile compounds (Table 1) were identified in dry marjoram herb. The major compounds were  $\gamma$ -terpinene (14.6%), 4-terpineol (11.7%),  $\alpha$ -terpinene (11.5%),  $\beta$ -phellandrene (11.1%), sabinene (11.0%) and  $\alpha$ -thujene (7.0%). Aromatisation of grape seed oil proceeds due to a migration of herb constituents into oil. The process depends on various parameters, such as temperature, surface area of plant material, contact time and the properties of constituents present in herb. Moreover, the release of dissolved in oil constituents from oil, their concentration in headspace and consequently possible impact on flavour of aromatised oil will depend on the solubility of individual constituents and their vapour pressure.

Table 1

## Volatile compounds in marjoram

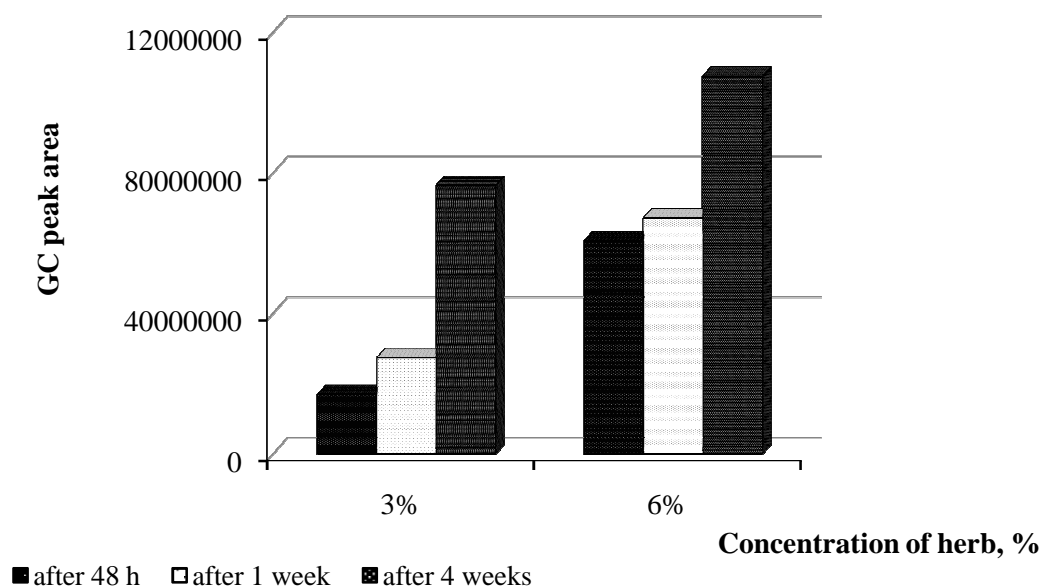
Compound	RI calculated	RI literature <sup>a</sup>	GC peak area $\times 10^7$	% of PA
$\alpha$ -thujene	926	931	15.6	7.0
$\alpha$ -pinene	931	939	3.1	1.4
sabinene	973	976	24.4	11.0
myrcene	993	991	9.2	4.2
$\alpha$ -phellandrene	1004	1005	7.3	3.3
$\alpha$ -terpinene	1017	1018	25.2	11.5
<i>p</i> -cymene	1024	1026	10.1	4.6
$\beta$ -phellandrene	1029	1031	25.1	11.1
<i>E</i> - $\beta$ -ocimene	1049	1050	1.2	0.5
$\gamma$ -terpinene	1060	1062	32.0	14.6
<i>cis</i> -sabinene hydrate	1069	1065	13.8	6.2
terpinolene	1087	1088	10.9	4.9
camphor	1143	1143	2.3	1.1
4-terpineol	1180	1179	25.8	11.7
<i>E</i> -dihydrocarvone	1198	1200	3.8	1.7
carvone	1244	1242	0.5	0.3
anethole	1285	1283	1.6	0.7
copaene	1379	1376	0.2	0.1
$\beta$ -caryophyllene	1421	1418	6.8	3.1
$\alpha$ -humulene	1457	1454	0.7	0.3
curcumene	1486	1482	1.3	0.6
$\beta$ -bisabolene	1511	1510	tr	tr

<sup>a</sup>-Adams. (2007) Identification of essential oil components by gas chromatography/ mass spectrometry. tr= traces, <0.05

The number of compounds detected in headspace of aromatised oils was from 8 to 12. For instance, 8 compounds were detected when oil was kept 48 hours with 3% of herb, while this number increased to 9 and 12 when the time was increased to 1 and 4 weeks, respectively. When higher amount of herb (6%) was added to the oil, 12 compounds were detected independently on the maceration time. The headspace profiles of aromatised oils were different from those of the marjoram herb. All identified in the headspace of aromatised oils compounds were hydrocarbon monoterpenes, sabinene and  $\gamma$ -terpinene being the major quantitatively constituents. Other detected compounds were  $\alpha$ -thujene,  $\alpha$ -terpinene,  $\alpha$ -pinene, myrcene,  $\alpha$ -phellandrene, *p*-cymene,  $\beta$ -phellandrene and terpinolene. The percentage of monoterpenes in headspace was quite similar in all oil samples: GC peak area of sabinene was 19.7–25.5%,  $\gamma$ -terpinene 13.9–15.0%,  $\alpha$ -thujene 11.4–13.4%,  $\alpha$ -terpinene 8.7–12.6%. The same hydrocarbon monoterpenes were major constituents in herb headspace, however, important oxygenated compounds, 4-terpineol and *cis*-sabinene hydrate (Table 1) were not detected among oil headspace volatiles. Most likely, oxygenated constituents as possessing polar hydroxyl groups were less soluble in grape seed oil. It is in agreement with Adams et al. (2011) who recently reported that hydrocarbon monoterpenes were dominating in headspace of aromatised with herbal material oil.

Although percentage composition of aromatised oil headspace volatiles was quite similar, the concentration of detected compounds, as it can be judged from their peak area, increased with increasing the amount of added to oil herb and maceration time (Figure 1). It indicates that

diffusion of marjoram volatile components to oil is a long process. For instance, the total amount of headspace volatiles in oil kept with 3 % of marjoram during 4 weeks was higher 4.5 times than that in oil macerated 48 hours.



**Figure 1. Total content of headspace volatile compounds (total GC peak area) above grape seed oil aromatised with marjoram**

#### *Effect of aromatisation on the concentration of tocopherols*

Tocopherols are important oil soluble antioxidants naturally present in oil bearing seeds, which are also present in other plant materials including aromatic herbs. Gómez-Coronado et al. (2004) reported that 100 g of fresh marjoram leaves contain 32.3 mg  $\alpha$ -tocopherol and 0.42 mg  $\gamma$ -tocopherol. In our study  $\alpha$  and  $\gamma$ -tocopherols were found in analysed grape seed oils (Table 2). The concentration of tocopherols increased in aromatised oils, which indicates migration of tocopherols from herb to oil during maceration. It may be observed that this process continues during all time of maceration; e.g. significant increase ( $p > 0.05$ ) in  $\gamma$ -tocopherol concentration was observed after 4 weeks as compared to 48 hours and 1 week. The concentration of tocopherols was also dependant on the added herb amount.

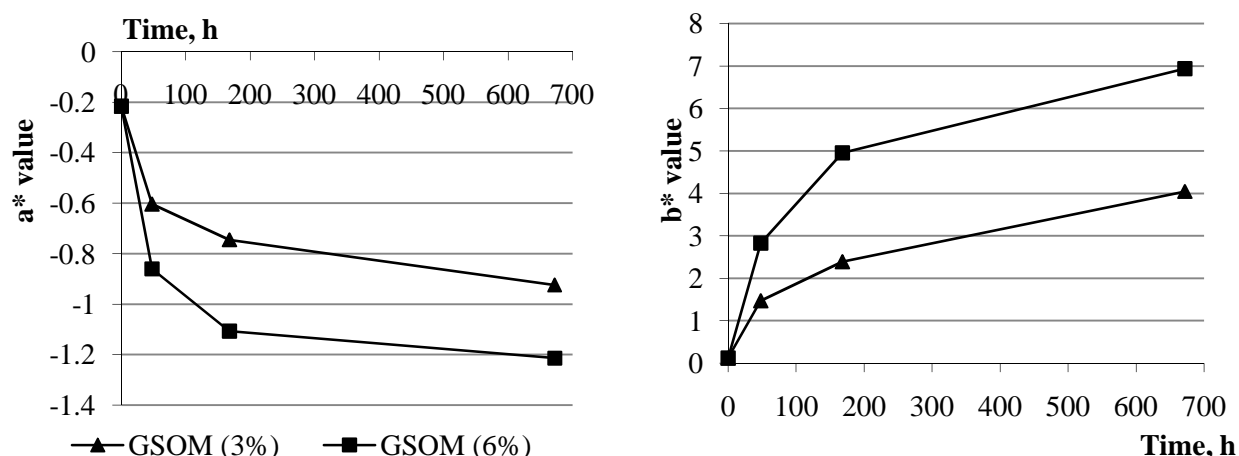
Table 2

**Concentration of  $\alpha$ - and  $\gamma$ -tocopherol, mg/kg in pure (GSO) and aromatised with marjoram (GSOM) grape seed oil**

Oil sample	$\alpha$ -tocopherol	$\gamma$ -tocopherol
GSO	232.6 $\pm$ 1.1 <sup>a</sup>	30.6 $\pm$ 0.9 <sup>a</sup>
GSOM (3%) 48h	241.8 $\pm$ 6.5 <sup>b</sup>	33.9 $\pm$ 1.7 <sup>b</sup>
GSOM (3%) 1w	248.1 $\pm$ 8.5 <sup>b</sup>	37.2 $\pm$ 5.2 <sup>b</sup>
GSOM (3%) 4w	257.6 $\pm$ 6.1 <sup>c</sup>	40.0 $\pm$ 3.9 <sup>c</sup>
GSOM (6%) 48	256.0 $\pm$ 11.7 <sup>c</sup>	38.3 $\pm$ 11.0 <sup>c</sup>
GSOM (6%) 1w	262.0 $\pm$ 6.3 <sup>c</sup>	45.9 $\pm$ 9.6 <sup>c</sup>
GSOM (6%) 4w	274.3 $\pm$ 1.3 <sup>d</sup>	59.0 $\pm$ 9.9 <sup>c</sup>

#### *Effect of aromatisation on oil colour*

Colour is important quality characteristic influencing food acceptability and depending on the presence of pigments (Escobar et al., 2007). Therefore, the measurements of spectrophotometric characteristics were performed in order to assess the impact of aromatisation on oil colour.



**Figure 2. Effect of marjoram maceration (extraction) time on CIELab – a\* and b\* values of grape seed oil**

An L\* value reflects relative lightness of oil. It may be observed that aromatisation did not have any effect on this colour characteristic; it was in the range of 60.9–61.9 for all samples. The negative a\* value is related to the greenish cast, which is related with chlorophyll content (Giacomelli et al., 2006). Obtained results show that a\* value during aromatisation significantly changed ( $p < 0.05$ ) and became more negative, in the course of maceration. It also depended on the amount of added to oil herb (Figure 2). The positive b\* value is related with the carotene content (Giacomelli et al, 2006). The b\* value of grape seed oil also significantly changed ( $p < 0.05$ ) during aromatisation and became more positive. These changes may be explained by the migration of marjoram pigments to oil.

## Conclusions

The results obtained in this study demonstrate that marjoram constituents, particularly volatile aroma compounds, tocopherols and pigments dissolve in grape seed oil during herb maceration (extraction). It seems, that this process proceeds rather slowly during the whole period of maceration and depends on the amount of added to oil herb. The main volatile compounds identified in the headspace of aromatised with marjoram oils were hydrocarbon terpenes, sabinene and  $\gamma$ -terpinene, while abundant in dried herb terpene alcohols 4-terpineol and sabinene hydrate were not detected in oil. The concentration of  $\alpha$ -tocopherols steadily increased in oils in the course of aromatisation. Such colour characteristics as a\* and b\* values were also influenced by marjoram herb; the changes of these characteristics were maceration time and herb concentration dependant.

## References

1. American Oil Chemist's Society (AOCS) (1999) AOCS Official Method Ce 8–89: Determination of tocopherols and tocotrienols in vegetable oils and fats by HPLC (5th ed.). In: *Official methods and recommended practices of the Am. Oil Chem. Soc.* Champaign, IL.
2. Adams, A., Kruma, Z., Verhé, R., De Kimpe, N., Kreicbergs, V. (2011) Volatile profiles of rapeseed oil flavored with basil, oregano, and thyme as a function of flavoring conditions. *J. Am. Oil Chem. Soc.*, 88, pp. 201–212.
3. Adams R. *Identification of essential oil components by gas chromatography/ mass spectrometry*, 4<sup>th</sup> edition. (2007) Allured Publishing Corporation, Carol Stream, USA.
4. Baranauskienė, R., Venskutonis, R., Dewettinck, K., Verhé, R. (2006) Properties of oregano (*Origanum vulgare* L.), citronella (*Cymbopogon nardus* G.) and marjoram (*Majorana hortensis* L.) flavors encapsulated into milk protein-based matrices. *Food Res. Int.*, 39, pp. 413–425.

5. Beveridge, T.H.J, Girard, B., Kopp, T., Drover, J.C.G (2005) Yield and composition of grape seed oils extracted by supercritical carbon dioxide and petroleum ether: varietal effects. *J. Agric. Food Chem.*, 53, pp. 1799–1804.
6. Crews, C., Hough, P., Godward, J., Brereton, P., Lees, M., Guet, S., Winkelmann, W. (2006) Quantitation of the main constituents of some authentic grape seed oils of different origin. *J. Agric. Food Chem.*, 54, pp. 6261–6265.
7. Escolar, D., Haro, M.R., Ayuso, J. (2007) The colour space of foods: virgin olive oil. *J. Agric. Food Chem.*, 55, pp. 2085–2093.
8. Fecka, I., Turek, S. (2008) Determination of polyphenolic compounds in commercial herbal drugs and spices from Lamiaceae: thyme, wild thyme and sweet marjoram by chromatographic techniques. *Food Chem.*, 108, pp. 1039–1053.
9. Giacomelli, L., Mattea, M., Ceballos, C. (2006) Analysis and characterization of edible oils by chemometric methods. *J. Am. Oil Chem. Soc.*, 83, pp. 303–308.
10. Gómez-Coronado, D.J.M., Ibañez, E., Rupérez, F.J., Barbas, C. (2004) Tocopherol measurement in edible products of vegetable origin. *J. Chromatogr.*, 1054, pp. 227–233.
11. Hinneburg, I., Dorman, H.J.D., Hiltunen, R. (2006) Antioxidant activities of extracts from selected culinary herbs and spices. *Food Chem.*, 97, pp. 122–129.
12. Nakatani, N. (1997) Natural antioxidants from spices and herbs. In: *Natural antioxidants: chemistry, health effect and applications*. Shahidi, F. (ed.) AOAC Press, Champaign, Illinois. pp. 64–75.
13. Pokorny J., Yanisliewa N., Gordon M. *Antioxidants in food: Practical applications*. (2001) CRC Press, Cambridge, England.
14. Politeo, O., Jukic, M., Milos M. (2006) Chemical composition and antioxidant activity of essential oils of twelve spice plants. *Croatia Chem. Acta*, 79, pp. 545–552.
15. Triantaphyllou, K., Blekas, G., Boskou, D. (2001) Antioxidant properties of water extracts obtained from herbs of the spices Lamiaceae. *Int. J. Food Sci. Nutr.*, 52, pp. 313–317.
16. Yanishlieva, N. V., Marinova, E., Pokorny, J. (2006) Natural antioxidants from herbs and spices. *Eur. J. Lipid Sci. Technol.*, 108, pp. 776–793.