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Volatile compounds of selected white and black myrtle (*Myrtus communis L.*) types from Mediterranean region of Turkey

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Myrtle (*Myrtus communis* L.) is a valuable plant for consumption in various fields such as culinary, cosmetic, pharmaceutical, therapeutical and industrial traits, and has become an important plant in the Meditarranean region of Turkey. The volatile compounds of 7 myrtle fruits including white (4 types) and black (3 types) were compared using headspace/solid phase micro extraction-gas chromatography mass spectrometry (HS/SPME-GC/MS) techniques. Fruits were collected from Tarsus district of Mersin province in the Mediterranean region of Turkey. Thirty-one (31) compounds were detected and among the detected volatiles aldehydes, esters, alcohols, terpenes and other compounds were detected in various percentages in myrtle types. However, ester compounds were not detected in black myrtle types. Hexanal, alpha pinene, phenol and eucalyptol were detected both in white and black myrtle types in higher percentages.

Key words: Myrtle, *Myrtus communis* L., volatiles, headspace, solid phase micro extraction (SPME), gas chromatography-mass spectrometry (GC/MS).

INTRODUCTION

Myrtaceae (*Myrtus communis* L.) is an evergreen shrub belonging to the family of Myrtaceae growing spontaneously throughout the Mediterranean region of Turkey especially in natural pine forests, particularly in the Taurus Mountains and riversides and a typical annual shrub of the other Mediterranean countries such as Greece, Italy, especially Algeria, Tunisia, and Morocco (Davis, 1982). In Turkey, the people in the Mediterranean region have named myrtle fruits as 'hambeles', 'murt', 'mersin' and consume them for some medicinal purposes (Baytop, 1984). The oils of the fruits are also consumed both in the flavor and fragrance industries. Recent studies have focused on the healthy functions of aromatic and medicinal plants, which have antioxidant, antimicrobial, and mutagen properties due to the dietary intake of antioxidant compounds that are important for health (Duh et al., 1999). Recent studies showed that myrtle fruits have anti-hyperglycemic (Elfellah et al., 1984),antiseptic and anti-inflammatory traits (Al-Hindawi et al., 1989; Diaz and Abeger, 1987). Morever, different parts of the plant find various uses in food and cosmetic industries (Chalchat et al., 1998; Messaoud et al., 2005) and fruits are used in liquors preparation (Nuvoli and Spanu, 1996), while its leaves are used as a hop substitute in beer (Buhner, 1998).

Myrtle species are known to be very rich in aromatic plant due to the presence of high essential oil content in its leaf, flower, and fruit glands and play an important role for their antimicrobial, tonic and balsamic properties (De Laurentis et al., 2005). The chemical composition of the

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myrtle leaf essential oil belonging to the different regions and harvested at different periods has been widely studied (Bradesi et al., 1997; Chalchat et al., 1998; Gardeli et al., 2008; Messaoud et al., 2005) and the evaluation of the fruit essential oil composition have also been reported (Mazza, 1983; Mulas et al., 2000). Moreover, many phytochemical researches investigated at the same time the essential oil composition of leaves and fruits as well as the other parts of *M. communis* (Aidi et al., 2007; Boelens and Jimenez, 1992; Flamini et al.,2004; Gauthier et al., 1988; Jerkovic et al., 2002; Tuberoso et al., 2006), also. Messaoud et al (2011), reported that *M. communis L.* presents polymorphism based on fruit color and chemical characteristics. However, little has been undertaken on the volatile composition of myrtle fruit (Asif et al., 1979; Çakir, 2004). Thus far, there have been few attempts to study the essential oil composition, fatty acids and antioxidant activity in the fruits of myrtle, in Turkey (Özek et al., 2000; Serçe et al., 2010). Moreover, as far as we know, no information has been published with regard to the volatile composition of various myrtle types esspecially intensively grown myrtle area (Mersin) of Turkey using Headspace/solid phase micro extraction-gas chromatography mass spectrometry (HS/SPME-GC/MS) techniques. The methods used for the isolation, concentration and identification of fruit flavour compounds often have a profound influence on the results obtained in the volatile composition determination. The chemical and physical properties of the different volatiles vary, and this may influence the results obtained in volatile determinations depending on the method used. Thus, different determination methods might cause alterations in the apparent overall aroma composition, and usually only approximate quantitative determinations of the volatiles can be performed. Furthermore, the formation of new compounds before and during the analysis is possible (Rapp, 1982). Quality control can be difficult if inappropriate methods are used. Solvent extraction has been used extensively in aroma compound analysis (Hirvi and Honkanen, 1982; Hirvi, 1983). Nevertheless, a rapid, simple and inexpensive technique for extracting and concentrating the determination techniques of fruit aromas can be very useful. The SPME (Arthur and Pawliszyn, 1990) technique is a very suitable and a solvent-free, inexpensive, rapid and versatile technique for the extraction of volatiles. It consists of a fused-silica fibre, coated with a polymeric stationary phase introduced into a liquid or gas sample. The method involves 2 processes: partitioning of the analytes between the coating and the sample and the thermal desorption of the analytes into the gas chromatograph. This method has been used by several authors for the analysis of volatile compounds in food samples (Hawthorne et al., 1992; Pelusio et al., 1995; Türemiş et al., 2003; Kafkas and Paydaş, 2007; Kafkas et al., 2005; 2009).

There is little information with regard to volatile composition of fruits of myrtle types from different regions of the world and no information from the intensively grown myrtle region of Turkey especially with the use of HS/SPME-GC/MS technique. Consequently, this paper was aimed at detecting volatile compounds using HS/SPME-GC/MS techniques in 7 myrtle types (white and black) from Tarsus/Mersin provinces of Meditarrenan region of Turkey.

MATERIALS AND METHODS

The fruit samples of the white and black fruits of myrtle types were harvested in the middle of September, 2011 from Tarsus district of Mersin provinces in the Mediterranean region of Turkey. Myrtle type numbers; 1, 2, 3, 4 and 5 were collected from the region called “Tarsus Dam”; numbers 8 and 16 ypes were “Yeşil mahalle” of Tarsus district. The fruit colors of myrtle types were white except types 4 and 5. The fruit color was black in types 4 and 5. The fruits were harvested and immediately transferred into the laboratory of Biotechnology Research and Experimental Center at University of Çukurova using portable refrigerators during transportation approximately in 2 h.

Fruit volatiles were extracted by HS/SPME using 250 g of fruits in each replicate. Triplicate extraction and analysis were done (Kafkas and Paydaş, 2007). The fruit flesh was homogenized in a food processor and 5 g of the homogenate was diluted with 2 ml of NaCl saturated aqueous solution and immediately headspace sampling was conducted on 100 µm fused silica fibres coated with polydimethylsiloxane/divinylbenzene (CAR/PDMS) (Supelco). Volatile compounds were analyzed with an automatic HS-40 Head Space Autosampler (Agilent 7890A GC) with split splitless inlet MSD system combined with Combi PAL autosampler system. Needle temperature was 120°C, extraction was done during 30 min and at 35°C temperature conditions. HP-5 MS (30 m × 0.25 mm × 0.25 µm) fused-silica capillary column was used. Helium (1 ml/min) was used as a carrier gas. The injector temperature was set at 250°C, for split-less injection. The oven conditions were set at 50°C for 1 min and then the temperature was increased to 200°C at a rate of 4°C/min. Thermal desorption was allowed for 1.5 min. The detector temperature was 280°C. The components were identified by comparison of mass spectra and retention time data with those of authentic samples and complemented with an identify by doing a NIST, Wiley, Flavor libraries search of the acquired mass spectral data.

RESULTS AND DISCUSSION

The results of volatile compounds in fruits of 7 myrtle types are shown in Table 1. Thirty-one (31) volatile compounds were identified in fruits, including esters, alcohols, terpenes, aldehydes and other compounds. Hexenal was detected in various percentages in myrtle types. The lowest hexanal percentage was detected in type 2, while the highest was detected in type 4. Four (4) ester compounds were detected in white myrtle types, whereas no ester was identified in black myrtle types. Linanyl butyrate and linanyl acetate were detected with higher percentages than isobutyl butyrate and carbonic acid dodecyl iso butyl ester. Alcohols were detected as major compounds except type 16, whereas, terpen compounds were detected as major compounds in type16. Among the detected terpene compounds, alpha pinene

Table 1. The volatile compounds of white and black myrtle fruits detected by HS/GC/MS techniques (%).

Volatile compound	Type 1 (White)	Type 2 (White)	Type 3 (White)	Type 8 (White)	Type 16 (White)	Type 4 (Black)	Type 5 (Black)
Hexenal	15.87 ± 2.12	9.25 ± 3.12	29.2 ± 4.78	20.18 ± 5.11	11.23 ± 2.34	20.44 ± 5.09	16.64 ± 3.66
Isobutyl iso butyrate	0.71 ± 0.00	n.d.	n.d.	1.00 ± 0.01	0.49 ± 0.01	n.d.	n.d.
Linalyl butyrate	n.d.	8.24 ± 4.08	n.d.	5.15 ± 0.87	1.46 ± 0.14	n.d.	n.d.
Linanyl acetate	2.04 ± 0.07	n.d.	5.96 ± 1.09	4.02 ± 1.12	n.d.	n.d.	n.d.
Carbonic acid, dodecyl isobutyl ester	n.d.	n.d.	n.d.	0,88 ± 0.08	n.d.	n.d.	n.d.
Beta-selinene	0.5 ± 0.01	n.d.	n.d.	n.d.	0,77 ± 0.12	n.d.	n.d..
Alpha-selinene	n.d.	n.d.	n.d.	1,01 ± 0.07	0,62 ± 0.08	n.d.	n.d.
Alpha pinene	14.45 ± 1.29	18.03 ± 3.24	10.89 ± 2.41	15.09 ± 2.78	13.46 ± 2.56	9.85 ± 1.98	15.16 ± 3.77
Limonene	20.98 ± 278	5.25 ± 1.12	6.86 ± 1.27	4.93 ± 0.98	26.08 ± 5,87	n.d.	n.d.
Alpha-terpinene	1.6 ± 0.23	n.d.	n.d.	n.d.	5.48 ± 0.89	n.d.	n.d.
Valencene	n.d.	n.d.	n.d.	n.d.	0.13 ± 0.00	n.d.	n.d.
Alpha-phellandrene	0.47 ± 0.07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Myrcene	0.43 ± 0.02	n.d.	n.d.	0.33 ± 0.01	n.d.	n.d.	n.d.
Delta-3-carene	2.13 ± 0.13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Gamma-terpinene	0.52 ± 0.04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Neo-allo-ocimene	0.55 ± 0.08	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Beta-caryophyllene	0.29 ± 0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
p-cymene	n.d.	1.5 ± 0.78	1.41 ± 0.23	2.31 ± 0.90	n.d.	2.30 ± 0.14	2.56 ± 0.95
Beta-pinene	n.d.	n.d.	n.d.	n.d.	n.d.	1.59 ± 0.06	3.35 ± 1.02
Terpinolene	n.d.	1.06 ± 0.08	n.d.	0.89 ± 0.12	n.d.	n.d.	n.d.
Alpha-humulene	n.d.	0.73 ± 002	n.d.	0.53 ± 0.12	n.d.	n.d.	n.d.
Linalol	6.28 ± 0.17	4.78 ± 1.13	6.96 ± 2.02	4.90 ± 0.89	12.09 ± 3.12	4.19 ± 0.13	8.96 ± 3.56
4-Carvomenthenol	n.d.	n.d.	n.d.	n.d.	0.45 ± 0.12	n.d.	n.d.
3,7-Dimethyl-1,6-octadien-3-ol	n.d.	n.d.	n.d.	n.d.	1.13 ± 0.16	n.d.	n.d.
Nerolidol	n.d.	n.d.	n.d.	n.d.	2.91 ± 0.34	n.d.	n.d.
Phenol	5.68 ± 0.45	10.54 ± 1.23	5.94 ± 1.23	4.95 ± 1.03	1.72 ± 0.18	17.22 ± 3.09	13.99 ± 4.09
Eucalyptol	19.82 ± 2.35	35.88 ±	29.98 ± 4.23	28.69 ± 4.98	20.95 ± 5.89	39.97 ± 4.08	33.66 ± 6.57
Alpha-terpineol	0.74 ± 0.03	n.d.	n.d.	0.49 ± 0.01	n.d.	n.d.	n.d.
Nerol	0.23 ± 0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Geraniol	0.70 ± 0.05	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3,7-Dimethyl-acetate (E)-2,6-octadien-1-ol	n.d.	1.05 ± 0.02	n.d.	1.34 ± 0.98	n.d.	n.d.	n.d.

n.d., Not detected.

was detected as a major compound. Limonene was detected in white myrtle types, whereas, this compound was not detected in black types. Eucalyptol was detected in higher percentages in black myrtle (types 4 and 5, respectively) compared to the white types (2, 3, 8, 16 and 1, respectively). Black and white myrtle types also differed in phenol percentages and detected 13.99 and 17.22% types 5 and 4, respectively. Due to the

anthocyanin contents, black types were found with Presence of higher phenol content.

In the previous studies, 47 volatile compounds were identified as fruit essential oils; 1,8-cineole (7.31 to 40.99%), geranyl acetate (1.83 to 20.54%), linalool (0.74 to 18.92%) and α-pinene (1.24 to 12.64%) were the main monoterpene compounds (Wannes et al., 2009). In this study, similar but higher percentages was detected based on linalool (4.19 to 12.09%) and alpha pinene (9.85 to 18.03%) compounds. The differences may be due to the use of various extraction methods and analysis techniques.

Martos et al. (2011), previously reported the essential oils of some Moroccan herbs including myrtle (*M. communis L.*) types by GC/MS technique. The same authors reported 1,8-cineole (40.37%) and α-pinene (21.82%) as major essential oil components. In this study, alpha-pinene and eucalyptol were detected in fruits of all myrtle types. 1,8 cineole was not detected; it may be due to the use of different materials. These results were different to those of Gauthier et al. (1988) who showed that there was an increase in the percentages of 1,8-cineole from 17 to 25% and myrtenyl acetate from 4 to 20% during fruit maturation of Moroccan *M. communis* var. italica. They also signalled the rainfall effect on mature fruit essential oil. In fact, after a rainfall period, there was a disappearance of α-pinene, an increase of myrtenyl acetate percentages from 2 to 43% and a decrease of 1,8-cineole and myrtenol proportions from 29 to 17% and 16 to 45%, respectively. Jerkovic et al. (2002) studied the changes in essential oil composition of Croatian myrtle fruit during its ripening but without mentioning the variety. They found that the main components were myrtenyl acetate (12.20 to 33.30%), 1,8-cineole, limonene (10.90 to 21.10%), α-pinene (4.00 to 15.30%) and linalool (4.70 to 7.70%). They also reported that there was a decrease of myrtenyl acetate contents during ripening period as obtained by Boelens and Jimenez (1992) for myrtle fruit essential oils from Spain. In agreement with Flamini et al. (2004) and Tuberoso et al. (2006) who studied the geographical variability of Italian fruit and leaf essential oils, the strong chemical variability in myrtle fruit volatile compounds could be ascribed not only to the geographical origin of the sample and its environmental conditions but also to the variety and genetic factors.

Conclusion

The results reported the essential oil composition of the white and black myrtle fruits in the Mediterranean region of Turkey. Fruit volatiles of myrtle types were differed in white and black myrtle types. Among the detected terpene compounds, α-pinene was detected as a major compound. Limonene was detected in white myrtle types, whereas, this compound was not detected in black types. It may be suggested that these differences could be due to the phenolic compounds especially in various anthocyanins. Eucalyptol was detected in a higher percentages in black myrtle (types 4 and 5, respectively) compared to the white types (2, 3, 8, 16 and 1, respectively). The proportions of the main essential oil compounds were detected as hexanal, alpha pinene, phenol and eucalyptol in both types. Although, esters and limonene was detected in a higher percentage in all white types, it was not detected in black types. So, essential oil of white and black myrtle fruits were characterized by the presence of many bioactive compounds which could have numerous applications in food, pharmaceutical, cosmetic and perfume industries. This results can be used for further studies to improve new myrtle types.

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