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APPLICATION OF THE GAS CHROMATOGRAPHY – MASS SPECTROMETRY COUPLED WITH MULTIVARIATE ANALYSIS TO EVALUATE THE QUALITY OF *MENTHA PIPERITA* ESSENTIAL OIL MODEL SAMPLES BLENDED WITH *MENTHA SPICATA* ESSENTIAL OIL

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ABSTRACT

This study investigated the effectiveness of gas chromatographic – mass spectrometry (GC-MS) analysis with subsequent multivariate analysis for evaluation of the quality of *Mentha piperita* oil blended with *Mentha spicata* oil. The characteristic volatile compounds of *M. piperita* oil, menthol, menthone, isomenthone and menthyl acetate, showed good representation. Carvone was the predominant compound in *M. spicata* oil followed by β-caryphyllene and 1, 8-cineole. Model samples of *M. piperita* oil blended with 5%- 50% (v/v) *M. spicata* oil were prepared and subjected to GC-MS and odour sensory analysis. The increase in the level of *M. spicata* oil in *M. piperita* oil model samples revealed a gradual decrease in the most potent odorants of *M. piperita* oil and remarkable increase in the characteristic volatiles of *M. spicata*. The decrease in the odour sensory quality of the model samples was coincident with the decrease in the relative concentration of menthol and menthone. However, this decrease showed dramatic trend at high levels (30%-50% v/v) of *M. spicata* oil. The GC-MS analysis data was subjected to principle component analysis (PCA) and cluster analysis. The results revealed the efficiency of these tools of analysis for accurate detection of *M. spicata* oil in *M. piperita* oil even at low level (5%v/v).

Keywords: *Mentha piperita*, *Mentha spicata*, Essential oil, PCA, Cluster analysis, Odour sensory analysis.

INTRODUCTON

Mentha, commonly known as mint, is one of the most common herbs which have been known for its medicinal and aroma therapeutic properties since ancient times. The ancient Egyptians, Greeks and Romans used mint as flavouring agent for food and as medicine (Kumar et al., 2011). The essential oils of mint have been used as perfumes, food flavours, deodorants, pharmaceuticals and insecticides (Kumar et al., 2011), antibiotic (Emami et al., 2012), antimicrobial (Yadegarinia et al., 2006) and antioxidant (Kizil et al., 2010). *Mentha* is the most important genus in *Lamiaceae* family because it contains a number of taxa, the essential oils of which have high economic value. The amount of the oils produced annually is over 23,000 metric tons with a value exceeding $ 400 million (Lawrence, 2006). This makes them the most economically important essential oils produced.

The genus *Mentha* includes five sections and 25-30 species (Harly and Brighton, 1977). However in Egypt *Mentha piperita* L (*M. piperita*) and *Mentha spicata*L. (*M. spicata*) are the most widely cultivated for the commercial oil production. These two aromatic plants are closely related mint species and even minor variation in the essential oils from different sources can significantly alter their flavour. Consequently, careful analysis of volatile components of both aromatic plants is important to ensure their quality and authenticity.

Peppermint (*M. piperita*) oil is one of the most popular and widely used essential oils, mostly because of its main components menthol and menthone (Gul, 1994; Díaz-Maroto *et.al.*, 2008). Menthol is a waxy, crystalline substance has a pleasant taste. It is used for various medical purposes while menthone is used in perfumery and as a flavour agent. Normally, essential oil of *M. piperita* has 30% - 55% of menthol while composition of menthone is between 14% and 32% (ESCOP, 1997).International demand for peppermint oil has increased in the past few years. Among the peppermint of different origins studied, peppermint of USA and Egypt origin contains the highest menthol and gives optimum oil yield (Kumar et al., 2011).

Spearmint (*M. spicata*) is intensively cultivated for its essential oil. The leaves, herbs and essential oil of *M. spicata* were used much earlier than those of peppermint (Hornok, 1992). The ground fresh biomass and

dried leaves of the plant are used as spice and herbal tea, and cultivated commercially in the entire world. Spearmint oil has also economic importance and is used in perfumery, confectionary and pharmaceutical preparations. Carvone is the main component of the essential oil of *M. spicata*, for which it is widely used as spices. It constitutes 50% - 65% of its total monoterpenes composition (Kokkini *et al.,* 1995). Other major components of *M. spicata*oil are limonene, and 1, 8- cineole (Kokkini et at., 1995; Telci *et al.,* 2010). The composition of essential oil of *M. spicata* has been extensively investigated. For example carvone and menthone – rich oils (Sticher *et al.,* 1968), carvone and neodihydrocarveol – rich oils (Nagasawa et al., 1976a,b), dihydrocarveol and carvone – rich oils (Sivropulou *et.al.*,1995), carvone and linalool – rich oils (Hadjiakhoondi et al., 2000), high carvone – rich oils and lower limonene –rich oils (Edris *et al.,* 2003; XiaoHua *et al.,* 2012).

The commercial designation of essential oils is not always an unequivocal indication of either source or quality. This is particularly the case with peppermint oils where there is not only a wide range of materials available but also a broad spectrum of application parameters. Due to the high price of peppermint oil; it is often adulterated with the cheaper mint oil, *M. arvensis* L. Oil (Spencer et al., 1997). However, in Egypt *M. arvensis* L. is not cultivated in commercial scale, whereas the cultivated area with *M. spicata* (881 acre) is 5.6 fold higher than that cultivated with peppermint (156 acre) and the total production of the two herbsare9736 and 3405 tons , respectively. So blending peppermint oil with spearmint oil will lead to a low price product. The quality and suitability for use of blended peppermint oil should be judged by odour sensory analysis supported by the chromatographic analysis combined with mass spectroscopy (GC-MS) (Jirovetz *et al.,* 2002).

The multivariate analysis (MVA)statistical analysis for processing chromatographic data has been shown to be an efficient tool for classification, searching, similarities and detection of adulteration of fixed oils with less expensive oil (Mildner-Szkudlarz *et al.,* 2003; Biswas *et al.,* 2004; Capote et al., 2007; Mildner-Szkudlarz and Jeleń 2008) and it shows promise for routine quality control. However, as far as the authors are aware no study could be found concerning using MVA of the chromatographic data of the blended essential oils.

The morphology and overall aroma of Peppermint and spearmint are similar (Diaz- Maroto *et.al.,* 2008). However, their volatile composition is completely different. Therefore, the main objective of the present study was to develop a technique based on volatile compound profiles comparison using MVA including principal component analysis (PCA) for detection the quality of peppermint oil blended with spearmint oil. To achieve this aim, seven model samples containing peppermint oil mixed with different levels (0, 5, 10, 20, 30, 40 and 50% v/v) of spearmint oil were prepared and subjected togas chromatographic-mass spectrometric (GC-MS) analysis followed by PCA. A correlation between the odour quality of the blended model samples and the relative concentrations of menthone and menthol, the potent odorants of peppermint oil, was established.

MATERIALS AND METHODS

PLANT MATERIALS AND CHEMICALS

Air dried leaves of the two mint species, *Mentha Piperita* L.(*M. piperita*) and *Mentha spicata* L. (*M. spicata*) were purchased from Sekam Company, Sharkia, governorate, Egypt, which produce organic plants. The drying process was carried out at ambient temperature with final moisture content 10%. Authentic compounds and standard paraffins (C6 – C22) were purchased from sigma-Aldrich Co. (St. Louis, MO, USA). All other chemicals were of analytical grade.

EXTRACTION OF ESSENTIAL OILS AND PREPARATION OF THE BLENDED MODELSAMPLES

The two air dried mint species peppermint (*M. piperita*) and spearmint (*M. spicata*) were subjected to hydrodistillation for 3 hours using Clevenger type apparatus. The oil phase were separated and dried over anhydrous sodium sulphate. The collected oils were kept in dark brown sealed glass vials at 4°C until analysis (Telci *et al.,* 2010). The peppermint oil was blended with spearmint oil at six different levels 5, 10, 20, 30, 40 and 50% (v/v).

GAS CHROMATOGRAPHY (GC) ANALYSIS

GC analysis was performed using a Perkin Elmer Auto System XL equipped with a flame ionization detector (FID). A fused silica capillary column DB5 (60m x 0.32 mm id) was used. The oven temperature was maintained initially at 50°C for 5 min, and then programmed from 50 to 250°C at a rate of 4°C/min. Helium was used as the carrier gas, at flow rate 1.1 ml/min. The injector and detector temperatures were 220 and 250°C, respectively. The retention indices (Kovats index) of the separated volatile components were calculated using hydrocarbons (C6-C22, Aldrich CO.) as references.

GAS CHROMATOGRAPHY – MASS SPECTROMETRY (GC-MS) ANALYSIS

The analysis was carried out by using a coupled gas chromatography Hewlett-Packard model (5890)/mass spectrometry Hewlett-Packard-MS (5970). The ionization voltage was 70 eV, mass range m/z 39-400amu. The GC conditions were carried out as mentioned above.

QUALITATIVE AND QUANTIATIVE ANALYSIS

Identification of the individual components was carried out by comparison of their GC retention indices (RI) with those of the authentic compounds or literatures (Adams, 1995) and / or by computer matching with mass spectral library (National Institute of Standards and Technology, NIST) data base. The relative amounts of individual components observed in total ion chromatograms were expressed as percent peak area against total peak area without FID response factor correction.

SENSORY EVALUATION

Odour sensory analysis was carried out on the essential oils isolated from *M. piperita* oil blended with 5% - 50% (v/v) *M. spicata* oil. The *M. piperita* oil was used as a reference sample. Each sample was placed in a

glass flask (diameter: 40 mm, volume 50 ml) sealed by a screw cap top. Assessors (10 assessors, Food Technology and Nutrition Division, National Research Center, Cairo, Egypt.) sniffed the samples after they were stopped for 20 min. at room temperature. The odour similarity of each sample as compared to *M. piperita* oil was evaluated (Fadel *et al.,* 2008) by each assessor using unstructured 10 cm line scale of 0.0 (no similarity) to 10.0 (identical).

STATISTICAL ANALYSIS

In this study, PCA was performed to interpret data structure. This method helps us to know which sample is different from others and investigate how the variations in the data are affected by different variables. Also the sample patterns can be detected by this analytical method. The principal components show the directions of maximum variance in the data and the loading of each of them explains the contribution of different variables to that PC. The cluster analysis is another toll that was used, in the present study, for data analysis and identification of the main groups of samples. The clustering by squared Euclidean distance is an appropriate metric to measure the dissimilarity between sets of samples. Partial least squares (PLS) analysis was used to build a model that was able to predict the quantitative information.PLS algorithm was based on linear regression method. Partial least squares are a method for constructing predictive models when the factors are many and highly collinear. The goal of PLS is to predict *Y* from *X* and to describe their common structure (Esbensen, 2004).

RESULTS AND DISCUSSION

ESSENTIAL OILS OF *MENTHA PIPERITA* L. AND *MENTHA SPICATA* L

The essential oil content of *M. spicata* (1.6% ± 0.08) has been found to be more than twice that of *M. piperita* (0.70% ± 0.04) on dry weight basis. This finding is in quite agreement with previous reported studies (Fadel and Eisa, 1994; Edris *et al.,* 2003). The essential oil content of *Mentha* plants is generally considered to be lower than 2% (Kokkini *et al.,* 1989; Lawrence, 1989).

Regarding the chemical composition of the essential oils of the two *Mentha* species, Table 1 shows the identified compounds, mode of identification and percentage obtained by GC/MS analysis. As shown in this table, the major compounds identified in *M. piperita*oil were menthol (50.85%), menthone (20.50%), carvone (10.94%) and 1,8-cineole (6.87%). Agarwal (2008) stated that, the chemical composition of *M. piperita*oil is characterized by the presence of oxygenated monoterpenes such as menthol, menthone, isomenthone, menthyl acetate, dihydrocarvacrol, menthofuran and 1,8-cineole.Menthol and menthone are reported to be the major components of *M. piperita* oil responsible for its various properties (Gul, 1994). Menthol, because of its pleasant taste, is extensively used for flavouring mouth washes, tooth paste, chewing gums and candies.

The qualitative evaluation of the peppermint oil, according to the *European pharmacopoeia*, is based on the relative amount of menthol (44% - 55%) free alcohols (as menthol), 15% -32% ketones (as menthone), 4.5% -10% ester (as menthyl acetate), 3.5% -14% cineole, 1.5% -10% isomenthone, 1% -9% menthofuran, 1% -5% limonene, no more than 4% pulegone, 1% carvone and 0.5% sesquiterpenes. In this respect it is evident from Table 1 that menthol, menthone, isomenthone and 1,8-cineole contents are compartible with the *Eropean pharmacopoeia*. Menthyl acetate content was quite low whereas limonene, menthofurane and pulegone were absent. Limonene was shown to be the precursor of both groups of cyclic *P*-menthane (C_2 or C_3 – substituted cyclic compounds) monoterpenes (Kjonaas and Croteau, 1983; Croteau, 1991).

It is important to note that only lower concentration of menthofuran or pluegone is acceptable in commercial oils as their quantity in the oil defines the quality of peppermint oil (Dwivedi et al., 2004).The considerable high content of carvone, in peppermint oil (Table 1) may be attributed to the drying process. Machhour et al., (2011) correlated the high content of carvone and absence of menthol and menthone in peppermint oil to many factors such as drying process. The composition of the essential oils extracted from fresh and dried peppermint by using three drying processes namely, sun drying, air drying and oven drying have been compared by Asekun *et al.* (2007). Their results showed an important effect of the dying methods on the composition of essential oils because huge differences on the quality and quantity in the essential oils extracted from the different specimens were found. Only four sesquiterpenes (β-Bourbonene, β-Elemene, β-Caryophyllene and β-Muurolene) were detected in peppermint oil with total concentration 2.27% (Table 1) which is in agreement with previous studies (Umezu et al., 2001; Dwivedi et al., 2004; Yadegarinia et al., 2006).

Regarding *M. spicata* oil it was, as for *M. piperita* oil, quantitatively dominated by the monoterpenoids rather than sesquiterpenes. Among the identified oxygenated monoterpenes, carvone was the most abundant individual compound, followed by1, 8-cineole (6.52%).The chemical composition of the essential oil of *M. spicata* has been studied by different researchers; carvone was the major compound in all cases and is character impact compound in spearmint oil, followed by limonene and 1, 8-cineole (Barton et al., 1992; Marongiu *et al.,* 2001;Pino et al., 2001; Lawrence, 2007; Díaz-Maroto *et al.,* 2008).Carvone exists naturally in two different enanitomeric forms with R (-) carvone being the source of spearmint aroma and S (-) carvone be the source of the typical caraway aroma (Boelens *et al.,* 1993). As shown in Table 1the identified non-oxygenated monoterpenes were present in low concentrations, such as α-pinene (0.25%), myrcene (0.37%), α-phellandrene (0.18%). In the present study limonene content (2.28%) was lower than that recorded in previous studies (Kokkini et al., 1995; Chauhan *et al.,* 2009). In contrast, 1, 8-cineole was present at higher concentration (6.52%).The carvone derivative, *trans*-carvyl acetate was present at substantial concentration (4.57%).This compound may exerts an effect on the overall spice aroma (Díaz- Maroto *et.al.,* 2003).Oil of spearmint available in the Egyptian market was found to be characterized by high carvone content (73.18%) and lower limonene (5.00%) and 1, 8-cineole (5.09%) (Edris *et al.,* 2003).

Table I. Volatile compounds identified in spearmint oil *Mentha spicata* (MS) and peppermint oil *Mentha piperita* (MP) blended with different levels of MS

Compound	RI [a]	MS	MP	Concentration percentage of MS in MP						Method of Identification [b]
				5%	10%	20%	30%	40%	50%	
α-Pinene	935	0.25 ± 0.01*	0.43 ± 0.01	0.42 ± 0.03	0.39 ± 0.03	0.38 ± 0.01	0.37 ± 0.02	0.36 ± 0.01	0.35 ± 0.01	RI, MS, St
Sabinene	979	---	0.14 ± 0.01	0.14 ± 0.01	0.13 ± 0.00	0.12 ± 0.01	0.11 ± 0.01	0.09 ± 0.01	---	RI, MS, St
β-Myrcene	989	0.37 ± 0.00	0.78 ± 0.04	0.75 ± 0.04	0.70 ± 0.07	0.69 ± 0.03	0.67 ± 0.02	0.61 ± 0.02	0.57 ± 0.02	RI, MS, St
α-Phellandrene	1004	0.18 ± 0.07	---	---	---	---	0.05 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	RI, MS
D-Limonene	1035	2.28 ± 0.19	---	1.15 ± 0.06	1.36 ± 0.25	1.48 ± 0.17	1.63 ± 0.08	1.76 ± 0.02	1.90 ± 0.06	RI, MS, St
1,8-Cineole	1037	6.52 ± 0.15	6.87 ± 0.02	5.89 ± 0.35	5.50 ± 0.39	5.75 ± 0.09	6.06 ± 0.09	6.24 ± 0.29	6.72 ± 0.05	RI, MS, St
Menthone	1159	---	20.50 ± 0.06	17.30 ± 0.41	15.99 ± 0.24	13.13 ± 0.17	12.49 ± 0.10	11.56 ± 0.53	9.96 ± 0.34	RI, MS, St
iso-Menthone	1164	---	3.28 ± 0.07	3.07 ± 0.16	2.94 ± 0.04	2.55 ± 0.06	2.41 ± 0.03	2.17 ± 0.02	1.77 ± 0.08	RI, MS, St
Borneol	1170	1.85± 0.06	0.53 ± 0.04	0.86 ± 0.04	0.81 ± 0.11	0.95 ± 0.02	0.99 ± 0.02	1.13 ± 0.02	1.23 ± 0.03	RI, MS
L-(-)-Menthol	1175	---	50.85 ± 0.60	49.06 ± 0.77	47.07 ± 0.74	44.77 ± 1.35	39.11 ± 0.82	34.34 ± 0.69	27.51 ± 0.24	RI, MS, St
cis-Carveol	1229	1.42 ± 0.06	---	---	0.09 ± 0.01	0.27 ± 0.01	0.43 ± 0.02	0.59 ± 0.01	0.69 ± 0.01	RI, MS
R-(-)-Carvone	1245	58.78 ± 0.59	10.94 ± 0.57	14.15 ± 0.36	16.63 ± 1.10	19.71 ± 0.95	23.33 ± 0.40	26.87 ± 0.94	32.93 ± 0.38	RI, MS, St
trans-Menthylacetate	1270	---	2.14 ± 0.06	1.98 ± 0.07	1.98 ± 0.09	1.60 ± 0.07	1.46 ± 0.02	1.22 ± 0.01	1.08 ± 0.03	RI, MS
Carvacrol	1299	1.06 ± 0.01	---	---	0.05 ± 0.01	0.09 ± 0.01	0.12 ± 0.01	0.38 ± 0.02	0.50 ± 0.03	RI, MS
Dihydrocarveol acetate	1302	---	1.27 ± 0.01	1.28 ± 0.06	1.26 ± 0.03	1.17 ± 0.12	0.94 ± 0.02	0.72 ± 0.02	0.68 ± 0.02	RI, MS
Piperitenone	1310	0.35 ± 0.04	---	---	---	---	0.07 ± 0.01	0.14 ± 0.01	0.22 ± 0.01	RI, MS
trans-Carvyl acetate	1338	4.57 ± 0.07	---	0.25 ± 0.08	0.44 ± 0.01	0.90 ± 0.02	1.47 ± 0.04	1.88 ± 0.05	2.24 ± 0.03	RI, MS
Geranyl acetate	1382	0.36 ± 0.04	---	---	---	---	0.10 ± 0.01	0.15 ± 0.01	0.18 ± 0.01	RI, MS, St
β-Bourbonene	1385	3.91 ± 0.03	0.36 ± 0.01	0.68 ± 0.04	0.72 ± 0.01	1.09 ± 0.02	1.53 ± 0.09	2.05 ± 0.04	2.27 ± 0.06	RI, MS
β-Elemene	1393	1.32 ± 0.01	0.17 ± 0.01	0.23 ± 0.05	0.29 ± 0.01	0.41 ± 0.01	0.52 ± 0.04	0.68 ± 0.01	0.71 ± 0.01	RI, MS
trans-Jasmone	1396	0.27 ± 0.01	---	---	---	---	0.09 ± 0.01	0.11 ± 0.01	0.13 ± 0.01	RI, MS
β-Caryophyllene	1424	6.97 ± 0.08	1.55 ± 0.04	1.89 ± 0.07	2.09 ± 0.05	2.67 ± 0.04	2.89 ± 0.11	2.97 ± 0.06	3.47 ± 0.14	RI, MS, St
Aromadendrene	1440	0.46 ± 0.01	---	---	---	---	---	0.12 ± 0.01	0.21 ± 0.01	RI, MS
α-Humulene	1458	0.76 ± 0.18	---	---	---	0.10 ± 0.02	0.20 ± 0.02	0.29 ± 0.01	0.37 ± 0.01	RI, MS
Germacrene-D	1480	0.61 ± 0.11	---	---	0.10 ± 0.01	0.14 ± 0.03	0.19 ± 0.02	0.22 ± 0.00	0.27 ± 0.02	RI, MS
β-Selinene	1482	0.43 ± 0.20	---	---	0.08 ± 0.02	0.14 ± 0.04	0.18 ± 0.01	0.20 ± 0.01	0.22 ± 0.01	RI, MS
Bicyclogermacerene	1495	0.32 ± 0.13	---	---	---	0.03 ± 0.01	0.08 ± 0.01	0.11 ± 0.01	0.16 ± 0.01	RI, MS
β-Muurolene	1499	0.82 ± 0.30	0.19 ± 0.10	0.19 ± 0.02	0.21 ± 0.01	0.24 ± 0.02	0.27 ± 0.02	0.34 ± 0.02	0.38 ± 0.03	RI, MS
β-Bisabolene	1506	0.30 ± 0.12	---	---	---	---	0.08 ± 0.01	0.10 ± 0.01	0.17 ± 0.01	RI, MS
γ-Cadinene	1515	0.53 ± 0.07	---	---	0.13 ± 0.02	0.18 ± 0.05	0.19 ± 0.01	0.23 ± 0.01	0.25 ± 0.02	RI, MS

Bornyl acetate	1532	0.34 ± 0.08	---	---	---	0.03 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	0.17 ± 0.01	RI, MS
trans-Nerolidol	1534	0.53 ± 0.09	---	---	---	0.04 ± 0.01	0.12 ± 0.02	0.16 ± 0.01	0.21 ± 0.01	RI, MS
α-Cadinene	1538	0.88 ± 0.01	---	---	0.16 ± 0.03	0.23 ± 0.02	0.37 ± 0.01	0.40 ± 0.01	0.44 ± 0.04	RI, MS
Elemol	1550	0.58 ± 0.32	---	---	0.11 ± 0.02	0.18 ± 0.03	0.21 ± 0.01	0.23 ± 0.00	0.25 ± 0.01	RI, MS
Spathuteuol	1575	0.73 ± 0.02	---	0.15 ± 0.01	0.23 ± 0.03	0.25 ± 0.02	0.28 ± 0.02	0.30 ± 0.01	0.34 ± 0.02	RI, MS
Caryophyllene oxide	1581	0.28 ± 0.04	---	0.19 ± 0.05	0.18 ± 0.03	0.21 ± 0.01	0.21 ± 0.01	0.24 ± 0.02	0.24 ± 0.01	RI, MS
epi-α-Cadinol	1641	0.96 ± 0.06	---	0.21 ± 0.06	0.20 ± 0.04	0.29 ± 0.03	0.35 ± 0.02	0.40 ± 0.01	0.51 ± 0.02	RI, MS
α-Cadinol	1655	0.11 ± 0.03	---	---	---	---	---	0.03 ± 0.01	0.05 ± 0.01	RI, MS
Farnesol (E,Z)	1696	0.92 ± 0.03	---	0.16 ± 0.01	0.15 ± 0.03	0.23 ± 0.07	0.36 ± 0.02	0.45 ± 0.01	0.52 ± 0.03	RI, MS

*Values expressed as relative area percentages;[a] Retention indices;[b]Compounds identified by GC-MS (MS) and / or retention index on DB5 (RI) and /or by comparison of MS and RI of standard compound (St) run under similar GC-MS conditions; ---, Not detected.

Twenty sesquiterpenes were identified in the spearmint oil in the present study, with total concentration of (21.69%). β-Caryophyllene comprised the highest content (6.97%) followed by β-bourbonene (3.91%) and β-elemene (1.32%). These results are in agreement with those reported in previous studies (Díaz-Maroto *et al.*, 2003). The total yield of sesquiterpenes identified in the essential oil of spearmint type grown under different climatic conditions in Turkey varied from 9.45% to 16.57%of the total oil (Telci *et al.*, 2010).

COMPOSITION OF *M. PIPERITA*OIL (MP) BLENDED WITH *M. SPICATA*OIL (MS).

Table 1 show the volatile compounds identified in *M. piperita* oil blended with different levels ofMS oil (5% – 50 %, v/v). As shown in the Table the compounds which are characteristic to *M. spicata*oil such limonene and carvone derivatives, *cis*-carveol, carvacrol and *trans*-carvylacetate were absent in *M. piperita* oil. In addition, eighteen sesquiterpenes were detected only in *M. spicata* oil. Therefore, the presence of these compounds might be used as marker to detect blending of MP with MS oil. Limonene and *trans*-carvylacetate were identified in model sample MP with 5% (v/v) MS whereas *cis*-carveol and carvacrol were detected in MP samples with 10% (v/v) MS. The sesquiterpenes were identified in samples of MP oil with variable levels of MS oil (Table 1).

Menthol, menthone, iso-menthone and menthylacetate, the main characteristic compounds of MP, were absent in MS oil and their contents showed significant decrease, in the model MP samples, by increasing the level of MS (Table 1).Five monoterpenes, (α-pinene, myrcene, 1, 8-cineole, borneol and carvone) and four sesquiterpenes (β-bournonene, β-elemene, β-caryphyllene and β-muurolene) were detected in both oils; however their total yields were higher in MS than MP oil. Among these compounds carvone showed significant increase in the blended samples, it comprised the highest concentration 32.93% of the total volatiles in sample MP with 50% (v/v) of MS.

The chromatographic (GC/MS) data of the investigated samples were subjected to PCA analysis. Fig. 1 shows the PCA score plot of MS, MP and six MP oil model samples. *M. piperita* oil with additions of 5, 10, 20, 30, 40 and 50% (v/v) of *M. spicata* oil has been marked as 5% MS, 10% MS, 20%MS, 30%MS, 40%MS and 50%MS, respectively. The results revealed the efficiency of this tool of analysis for detecting the added MS oil even with the lowest level (5% MS).According to Fig. 1, all variance in data is explained by PC1 and shows the large variance in samples. The first principal component separates MP, 5%MS, 10%MS, 20%MS, and 30%MS on the right side from 40%MS, 50%MS, and MS on the left side of score plot. It means all different levels of MS can be distinguished.

The average linkage clustering, Fig. 2, illustrates different clusters. The level of similarity between the samples is the key of merging different samples or clusters. The most similar sample to 5%MS is 10%MS. These two samples make a group. The next most similar sample to this group, is 20%MS that merge into this group and so on. Fig. 2 shows two main clusters which one cluster includes 50%MS, 40%MS, and 30%MS, while the other cluster includes MP, 20%MS, 10%MS, and 5%MS.

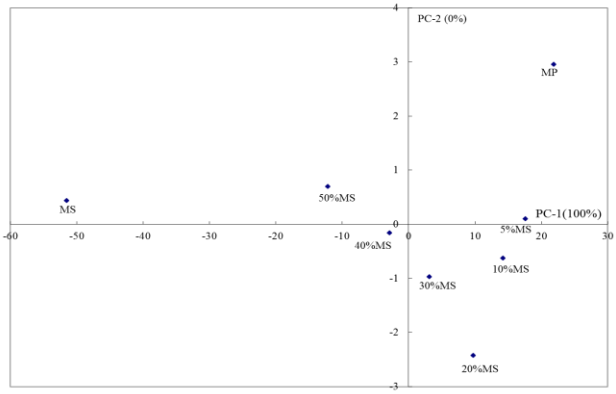

Figure 1. Principle component analysis (PCA) score plots of *Mentha spicata* oil (MS) and *Mentha piperita* oil (MP) blended with different levels (5% - 50% v/v) of *Mentha spicata* oil (MS).

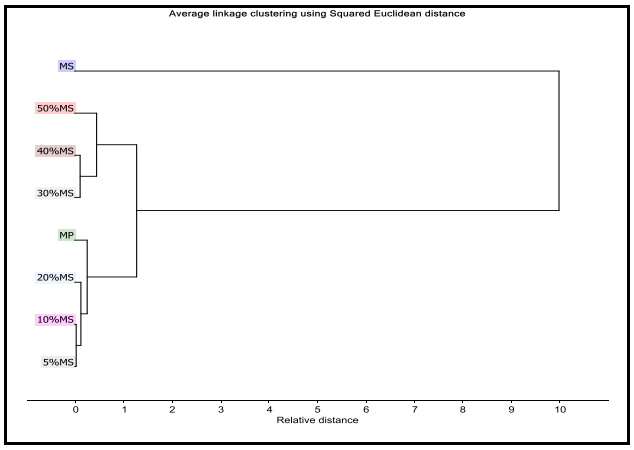

Figure 2. Cluster analysis based on gas chromatographic – mass spectrometric (GC-MS) analysis of *Mentha spicata* oil (MS) and *Mentha piperita* oil (MP) blendedwith different levels (5% - 50% v/v) of *Mentha spicata oil* (MS).

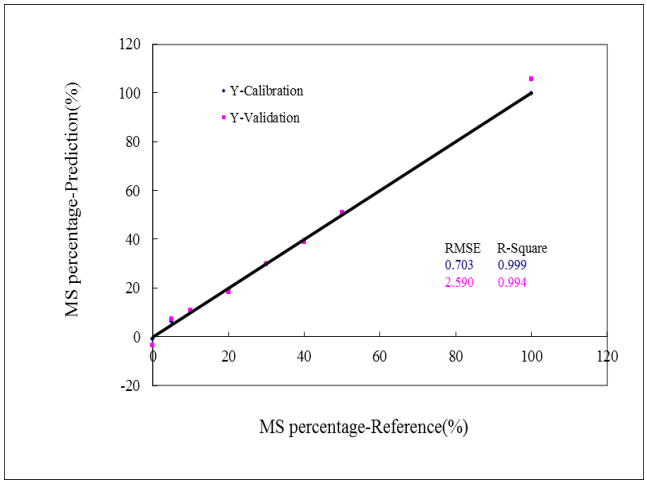

Figure 3. Partial least squares (PLS) plot of predicted blend of *Mentha piperita* oil (MP) with different levels (5% - 50% v/v) of *Mentha spicata* oil (MS).

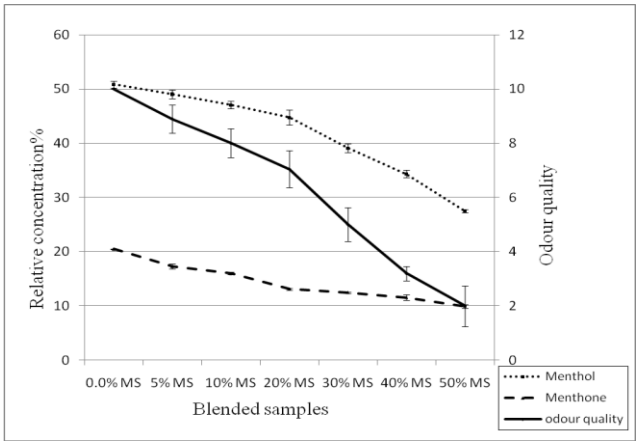

Figure 4. Variations in odour quality and relative concentrations of menthol and menthone in *Mentha piperita* oil (MP) blended with different levels (5% - 50% v/v) of *Mentha spicata* oil (MS).

The prediction result of constructed PLS model is shown in Fig. 3. The calibration curve indicates the reference and predicted MS percentages in different samples. The root means square error (RMSE) is about 2.59% and the coefficient of determination (R^2) is more than 0.99. Low RMSE shows the low uncertainty on predictions and high R^2 demonstrate how well our data fitted the calibration curve of PLS model.

EVALUATION OF THE ODOUR SENSORY QUALITY

As show in Fig. 4, blending of *M. piperita* oil with 5% - 50% (v/v) *M. spicata* oil has been led to a significant decrease ($P < 0.05$) in the odour quality of the *M. piperita* oil model samples. This decrease was consistent with the decrease in the relative concentrations of menthone and menthol, the main contributors to the characteristic odour of *M. piperita* oil. However, the odour quality of samples 30%MS– 50%MS showed a dramatic decrease. It is obvious from Fig.4 that the content of menthone in the samples with 20%-50% MS (13.13%–9.96%) were lower than the *European pharmacopoeia* requirements (15%- 32%). Menthol showed the same behavior, its relative concentrations in model samples with 30%– 50% (v/v) MS oil (39.11%– 27.52%) was lower than the required levels (44%– 55%).

CONCLUSION

Blending of *Mentha piperta* essential oil with variable levels of *Menthas picata* (5% -50%, v/v) revealed qualitative and quantitative variations in the volatile compound profiles. In the model MP samples, the decrease in the most characteristic volatile compounds of MP oil was associated with an increase in those of MS oil. However, at low level of blending 5% -20%, (v/v) the contents of the most potent odorants of *M. piperta* oil (menthol and menthone) were well within the limits recommended by *European pharmacopoeia*. The decrease in the scores of odour sensory quality of *M. piperta* samples was coincident with the decrease in the relative

concentrations of menthol and menthone. However, they showed remarkable decrease at high levels (30%- 50% v/v) of added MS oil.

Application of multivariate static analysis including PCA, cluster analysis and PLS for processing the data of GC-MS analysis of the model samples revealed the accurate detection of the added MS oil even with the lowest level (5% v/v). Therefore, this method could be used as efficient tool in quality assessment of essential oil.

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