

Aroma compounds and chemical content of four selected mulberry genotypes using gas chromatography (gc-ms) in Turkey

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Abstract

The objective of this work was to identify and quantify the volatile compounds, pH, and soluble solid content (SSC) in four mulberry genotypes. The volatile compounds were extracted by solid phase microextraction (SPME). The pH results of the four mulberry genotypes were changed from 5.99 to 6.47. Soluble solid matter (SSM) of A5 genotype (*Morus alba* L.) had the highest rate of 14.6%, followed by S7 (*Morus rubra* L.) with %14.04, P1 genotype (*M. alba* var. *laevigata*) with %13.24, and S6 (*Morus rubra* L.) with %12. Identification and quantification of aroma components in the four mulberry genotypes were performed by gas chromatography using a mass selective detector (GC-MS). We determined 21 aroma compounds (2 esters, 3 alcohols, 1 aldehydes, 4 ketones, 11 acids) in P1 genotype, 20 aroma compound (4 alcohols, 1 aldehydes, 2 ketones, 12 acids, 1 other compound) in S7 genotype, 14 compound (1 ester, 1 alcohol, 0 aldehydes, 2 ketones, 10 acids) in A5 genotype, and 16 aroma compound (1 ester, 3 alcohols, 0 aldehydes, 1 ketones, 11 acids) in S6 genotype. Esters, terpenes, aldehyde, and ketones were identified to be the small classes of aroma compounds in the mulberry fruit samples. Alcohols were the most considerable group of volatile compounds in mulberry. Among the alcohols, 2-(2-ethoxy ethoxy) ethanol was the most abundant component in fruits of four mulberry genotypes. In addition to alcohols, one of the most extensive aroma compounds, which are acids, account for one of the most significant proportions of the total aroma composition in terms of the number of aroma components. This study provides valuable information about the volatile compounds in mulberry fruits. This information can be used to develop new cultivars of mulberry fruits with improved aroma.

Key words

Mulberry, SPME, *Morus alba* L., *Morus rubra* L., *Morus alba* var. *laevigata*, SSM, pH.

Introduction

Mulberry, which belongs to the genus *Morus* in the family *Moraceae*, is cultivated in the wild or cultivated in many countries for its foliage. It is an important food source for silkworms (*Bombyx mori*). This plant includes twenty-four species. Generally, there are three types of mulberries, such as white (*Morus alba*), black (*M. nigra*), and red (*M. rubra*). But, among them, *Morus alba* L. is prevalent (Butt et al., 2008; S, Ercisli et al., 2007). However, the colour of mulberry fruit cannot be used to determine mulberry species. Mulberries are widely distributed in diverse regions changing from tropical to temperate, which shows their high adaptability to diverse environmental conditions (S, Ercisli et al., 2007). Turkey is one of the most important centres for mulberry cultivation and has a long history of cultivation. The most commonly cultivated mulberry species with edible fruits in Turkey are *Morus nigra*, *Morus rubra*, *Morus alba* and *Morus laevigata* (Özgen et al., 2009; Yıldız, 2013). In Turkey, mulberry fruit is often eaten fresh, dried, or made into molasses and jam because of its delicious taste, pleasant colour, low calorie content, and high nutritional value. For centuries, mulberry fruit has also been used in folk medicine for its pharmacological effects, including reducing fever, treating sore throat, protecting the liver and kidneys, improving vision, and lowering blood pressure in Turkey (Sezai Ercisli, 2004; Yang et al., 2014). The rich gene pool of mulberry in Turkey remains crucial as farmers and rural communities, especially in developing countries, have contributed significantly to the creation, conservation and availability of wild mulberry genetic resources in the past and in the present (Choudhary et al., 2013; Sezai Ercisli, 2004; Yang et al., 2014). Because of its nutritional value, mulberry fruit is consumed in fresh and processed forms. Mulberry fruit can be used in various forms, such as jam, syrup, pulp, ice cream, vinegar, and concentrate alcohol. In addition, mulberry leaves and organs are used pharmacologically throughout the world, particularly in China (Koyuncu, 2004). According to Chinese medicine, they are effective in monitoring diseases due to their sedative effect. They act by relieving the symptoms of fever, sore throat, and cough, and by protecting the liver, improving vision, facilitating urine flow, and lowering blood pressure (Li et al., 2009). Recent studies have shown that mulberry has significant effects on human nutrition and health through its constituents, such as organic acids, phenols, and sugars (S, Ercisli et al., 2007; Koyuncu, 2004; Özgen et al., 2009; Zhang et al., 2008). Some mulberry species' chemical composition and nutritional potential have been reported in studies worldwide (Darias-Martín, 2003; S, Ercisli et al., 2007; Gerasopoulos, 1997; Mohammad Imran et al., 2010; Xing et al., 2018). Only a few mulberry species have been studied for their edible fruits (*Morus*

alba, *Morus indica*, *Morus nigra*, and *Morus laevigata*) and wood (Imran et al., 2010). In the research on mulberries, properties of chemical and pomological were usually studied. (Gundogdu et al., 2011) studied the biochemical content (the content of organic acids, phenolic compounds, sugars, vitamin C (ascorbic acid), and antioxidant capacity) of fruits of white mulberry (*Morus alba* L.), black mulberry (*Morus nigra* L.), and red mulberry (*Morus rubra* L.) fruits cultivated in the east of Turkey. Some researchers determined some biochemical parameters (dry matter, total sugars, total acidity, ascorbic acid, and pH) of black mulberries from different regions of Turkey (Elmaci et al., 2005; S, Ercisli et al., 2007; Koyuncu, 2004; Lale et al., 1996). In the other detailed study, the polyphenolic profile of 11 *Morus alba* fruits grown in the Vojvodina region of northern Serbia was investigated using ultra-high performance liquid chromatography (UHPLC) to identify and quantify polyphenols (Natić et al., 2015). In addition, some researchers found numerous flavonoids in the genus *Morus* (Chu et al., 2006; Ercisli et al., 2008; Özgen et al., 2009). In the earlier study, the flavour characteristics of three cultivars of black mulberry (*Morus nigra*) from the Aegean region of Turkey were determined a total of 18 flavour compounds in the three cultivars using GC/MS (Elmaci et al., 2005). Zhu et al., (2018) investigated the volatile compounds of mulberries taken from three cultivars (*M. nigra*, Y1, M. Macroua, Y2 and M. Alba, Y3) using different techniques, found that 41, 37 and 41 compounds, respectively. In a comparative study of aromatic compounds in fruit wines from raspberries, strawberries, and mulberries in the central region of Shaanxi, The authors found that 27, 30, and 31 odorants were detected, respectively. In addition, They found that alcohols formed the most abundant group, followed by esters and acids (Feng et al., 2015). However, the aroma content in mulberries has yet to be adequately studied. Moreover, to our knowledge, there are no comparative studies on the aroma composition using GC-MS in the mulberries. The aim of this study is to investigate the aroma compounds of 4 different mulberry genotypes selected from the Mediterranean region and Eastern Anatolia, which occupy an important place in mulberry cultivation in Turkey.

Material

Fruit samples were collected at harvest time from one genotype of white mulberry (*M. alba* L.), one genotype of purple mulberry (*M. nigra* L.), and two genotypes of red mulberry (*M. rubra* L.) grown in the experimental plot at the Horticultural Application and Research Farm of Cukurova University in Adana. This cultivation area was established with the support of Tubitak

projects on an area of two decades. The projects were carried out in 2021 and aimed to select promising mulberry genotypes from the Mediterranean region and Eastern Anatolia. Approximately one gramme of fruit samples from each genotype were stored at -20 °C before analysis.

Method

Total soluble solids (%)

The juices of mulberry fruits belonging to the selected types were squeezed, and the amounts of total soluble solids were determined in three replicates using a hand refractometer

pH meter measurement

The juices of mulberry fruits belonging to the selected types was measured with a glass electrode pH meter with 3 replications.

Sample preparation

About 1 g of the fruit samples were weighed, immediately into a 20 ml headspace vial, including 1 mL NaCl saturated solution to hamper any enzyme reaction.

Identification of aroma compounds

The volatile compounds of the mulberry samples were extracted by solid-phase microextraction (SPME: polydimethylsiloxane-divinylbenzene DVB/CAR/PDMS 50/30 µm) were supplied by Supelco (Bellefonte, PA, USA). One gram of the homogenized mulberry samples was weighed, and 1 mL of CaCl₂ was added to a headspace vial and incubated at 40°C for 30 minutes. Identification and quantification of aroma compounds were performed using a gas chromatography-mass spectrometer (GC/MS, Shimadzu GC-2010 Plus) equipped with a HP -An Innowax Agilent column (30 m × 0.25 mm i.d., 0.25 µm thickness), and helium was used as the carrier gas at a linear rate of 1.0 mL/min. The temperature of the GC oven was maintained at 40 °C and programmed to 260 °C at a rate of 5 °C/min and then held constant at 260 °C for 40 min. Head Space technique SPME The temperature of the injector was set at 250 °C. The MS was recorded in electron impact ionization (EI) at 70 eV. The mass range was m/z 30-400. A library search was performed using the commercial libraries of Wiley, NIST, and Flavor GC-MS. Mass spectra were also compared with those of reference compounds and confirmed with retention indices from published sources. Relative percentages of separated compounds were calculated from total ion chromatograms.

Statistical analysis

All the result data were processed with the JMP (v8.00, SAS Institute Inc., USA) package program. All results were given as the mean ± standard error (SE) and Least Significant Difference test (LSD test) and 5% significance level were used to determine different groups.

Result and Discussion

Total soluble content (%) and pH

The results of the four mulberry genotypes of the total soluble content amounts are given in fig 1. A5 genotype (*Morus alba* L.) had the highest rate of 14.6%, followed by S7 (*Morus rubra* L.) with %14.04, P1 genotype (*M. alba* var. *laevigata*) with %13.24, and S6 (*Morus rubra* L.) with %12. The pH values of the four mulberry genotypes were changed from 5.99 to 6.47. Considering the results, the fruits of A5 (*M. alba* L.) and S7 (*Morus rubra* L.) can be suggested for processing due to their higher TSS. In contrast, genotype P1 (*M. alba* var. *laevigata*) can be suggested for fresh fruit production because it bears attractive, larger fruit. In previous studies, some authors determined 15.27-30.80% in TSS and 3.60-5.65 in pH of fruits of *M. alba*, *M. rubra*, and *M. nigra* from different regions of Turkey (Aslan, 1988; Burğut et al., 2006; Cam, 2000; S, Ercisli et al., 2008; Erkalçeli et al., 2015). TSS and pH results of our study were parallel to the results of these studies.

Aroma composition of four mulberry genotypes with using GC-MS

The results of the aroma component analysis of four mulberry genotypes are summarized as percent (%) in Table 1. The results of our study have been studied that the four mulberry genotypes had various volatile profiles. Method of SPME fibre coupled with GC-MC on four mulberry genotypes revealed 21, 20, 14 and 16 volatile compounds in P1, S7, A5, and S6 genotypes, respectively (Table 1). The chemical groups of volatile compounds found in all fruits were: Aldehydes, alcohols, ketones, esters, terpenes and other compounds (Fig. 1). We determined 21 aroma compounds (2 esters, 3 alcohols, 1 aldehydes, 4 ketones, 11 acids) in P1 genotype, 20 aroma compound (4 alcohols, 1 aldehydes, 2 ketones, 12 acids, 1 other compound) in S7 genotype, 14 compound (1 esters, 1 alcohol, 0 aldehydes, 2 ketones, 10 acids) in A5 genotype, and 16 aroma compound (1 esters, 3 alcohols, 0 aldehydes, 1 ketones, 11 acids) in S6 genotype (Table 1). Table 1 shows that 2-(2-ethoxyethoxy)-ethanol was highest in all mulberry samples. 2-(2-Ethoxyethoxy)-ethanol, diphenyl-methanone, hexadecanoic acid, hexanoic acid, and 2,4-diisocyanato-1-methyl-benzene had higher abundances than other compounds.

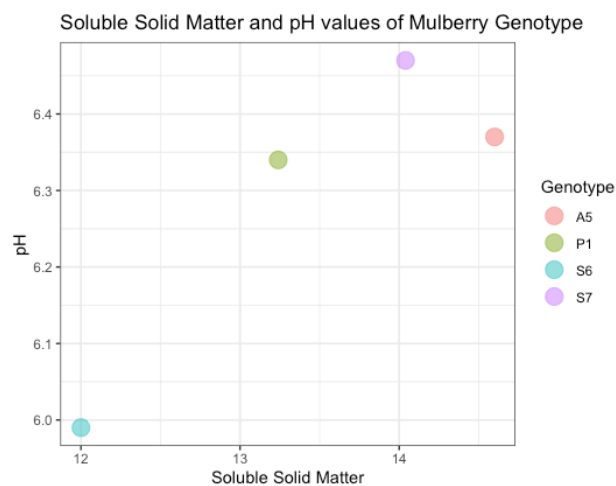


Fig 1. Some juice characteristics of four mulberry genotypes

Esters, terpenes, aldehydes, and ketones were shown to be the small classes of aroma compounds in the mulberry fruit samples. These groups were stated, which are given in Table 1. Esters included 1,2-Benzenedicarboxylic acid, bis(2-methyl propyl) ester, and Nonyl acetate. GC-MC analysis revealed that these compounds provide fruity, apple and red berry notes that form the essential aroma of mulberry. These results are consistent with the literature (Feng et al., 2015). Terpenes include 2,4-diisocyanato-1-methylbenzene, the major aroma components in all mulberry genotypes based on the combination of SPME (Fig 2). Only one aldehyde in four genotypes, which is 2,5-dimethyl-Benzaldehyde. Aldehydes are common and have been determined in earlier mulberry studies (Calín-Sánchez et al., 2013; Feng et al., 2015; Jiang et al., 2015). As shown in Table 1, alcohols were mulberry's most large group of volatile compounds. Among the alcohols, 2-(2-ethoxy ethoxy) ethanol was the most abundant component in mulberry fruit. Alcohols are formed by breaking amino acids, carbohydrates, and lipids (Antonelli et al., 1999; X. Li et al., 2014). Feng et al. (2015) noted alcohols were the largest group of aromatic compounds identified, with 25 compounds in mulberry. These results are consistent with our study. In addition to alcohols, the most abundant aroma compounds, acids account for one of the most significant proportions in the total aroma composition in terms of the number of aroma components (Fig 2). A total of 12 acids are 9,12-Octadecadienoic acid (Z, Z), Acetic acid, Decanoic acid, Dodecanoic acid, Formic acid, Heptadecene-(8)-Carbonic Acid-(1), Hexadecanoic acid, Hexanoic acid, Nonanoic acid, Octadecanoic acid, Octanoic acid and Tetradecanoic acid. There are few studies on the aroma composition of *Morus* species. (Zhang et al., 2011) determined thirty compounds in the fruits of *Morus alba*, but few of them matched those found in our study. The same situation is found in a study of (Göğüş et al., 2011). They reported 45 volatile compounds, but only a few were identified in the current study. These significant discrepancies in the results of studies could be because of a variety of factors, which are extraction methods, climate, soil and cultivation methods.

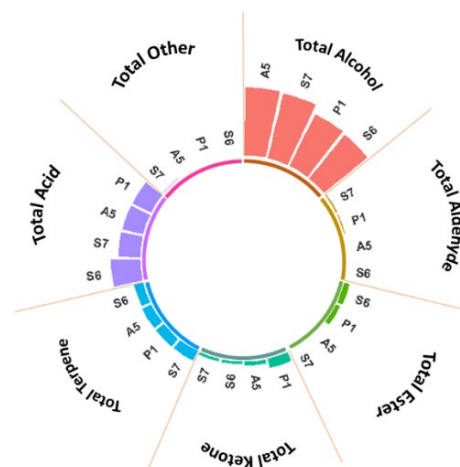


Fig 2. Circos graph plotting of main chemical groups in four mulberry genotypes

Table 1. Aroma compounds of four mulberry genotypes selected from Mediterranean region and Eastern Anatolia

	P1	S7	A5	S6
Esters				
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	0,46		0,35	
Nonyl acetate	3,86			5,23
Total Ester	4,32	0	0,35	5,23
Alcoholes				
1,4-Butanediol	0,45	0,4		0,73
2-(2-ethoxyethoxy)- Ethanol	54,14	60,76	65,44	53,5
Heptadecyl alcohol		1,99		0,44
Phytol	1,36	1,37		
Toplam Alcoholes	55,95	64,52	65,44	54,67
Aldehydes				
2,5-dimethyl- Benzaldehyde	0,79	1,14		
Total Aldehydes	0,79	1,14	0	0
Ketones				
Diphenyl- Methanone	0,46	1,87	3,3	2,69
Butyrolactone	0,65	0,58		
Civetone	6,11			
diphenyl- Methanone	1,83		0,59	
Total ketones	9,05	2,45	3,89	2,69
Acids				
9,12-Octadecadienoic acid (Z,Z)-	0,58	0,5	0,84	1,92
Acetic acid	2,99	2,11		0,41
Decanoic acid	1	0,57	0,89	0,91
Dodecanoic acid	0,89	0,49	0,91	0,86
Formic acid	2	1,13		
Heptadecene-(8)-Carbonic Acid-(1)		4,99	6,12	8,27
Hexadecanoic acid	6,22	5,32	6,83	10,83
Hexanoic acid	1,86	1,57	1,41	0,7
Nonanoic acid	1,82	0,55	0,97	0,44
Octadecanoic acid	0,91	1,02	1,2	2,12
Octanoic acid	1,1	0,99	0,93	1
Tetradecanoic acid	0,57	1,92	0,5	0,92
Total Acids	19,94	21,16	20,6	28,38
Terpenes				
2,4-diisocyanato-1-methyl-Benzene	9,97	10,21	9,72	9,01
Total Terpenes	9,97	10,21	9,72	9,01
Other compounds				
2,6-bis(1,1-dimethylethyl)-4-methyl-Phenol		0,52		
Total other compounds	0	0,52	0	0

Conclusion

In this study, SPME was used in conjunction with GC-MS to analyse the volatiles of four mulberry genotypes. It is a feasible technique to determine the quality and quantity of aroma components in mulberries. The results show that the four genotypes have different volatile profiles. The chemical groups of volatile compounds found in all fruits were: Aldehydes, alcohols, ketones, esters, terpenes and other compounds. The mulberries have been determined various aromatic substances Alcohols were the most considerable group of volatile compounds in mulberry. Among the alcohols, 2-(2-ethoxy ethoxy) ethanol was the most abundant component in mulberry fruit. In addition to alcohols, the most abundant aroma compounds, acids accounts for one of the most significant proportion of the total aroma. This study provides valuable information about the volatile compounds in mulberry fruits.

Statement of Conflict of Interest

The authors are declared that they have no conflict with this research article.

Author Contribution

M.A., and A.B: laboratory work, and field work, N.E.K., ÖFB; article writing

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