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# Evaluation and Identification of Volatile Compounds of Some Promising Strawberry Genotypes Using HS-SPME Technique by GC/MS

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**Abstract:** Ten strawberry (*Fragaria x ananassa.* Duch.) genotypes (Osmanlı, Camarosa and 8 promising hybrids) were identified for their volatiles using SPME (Solid phase micro extraction) techniques by GC/MS and determined relationships among them using volatile compounds data also. Osmanlı, the most aromatic variety, was observed to accumulate high levels of esters compared to the other strawberry genotypes. The ester compounds were detected highly in all genotypes except Camarosa variety. Among the experimentel genotypes hybrid numbers 11 and 17 had the same aroma profiles.

**Key words:** Volatile compounds · HS-SPME · strawberry · GC/MS

## INTRODUCTION

Delicate in flavor and rich in vitamins, antioxidants and minerals, strawberry is now a favored food in the diets of millions of people around the globe. This extensive popularity reached such a high level through the efforts of breeders who developed varieties [1, 2]. The cultivated strawberry is grown throughout the world and its production and growth areas increase year by year. During the last decade, strawberry production in Turkey increased gradually and reached approximately 150 thousand tons. Strawberries in this country are grown using cultivars imported notably from the US, Spain, Italy and some other European countries. However, such imported cultivars are rather poor in flavor, yet are high in yields and firmness, big in size and resistant to many diseases. Turkey has a local variety known as Osmanlı which is very rich in volatile aromatic substances yet rather small in size (2-3 g fruit$^{-1}$ weight), pink in color, rather soft and not suitable for transportation. Moreover, its flowers are male sterile and the yield per plant is very low. Therefore, Osmanlı can not be considered as a commercial cultivar. However, one of its best advantages is that emasculation is not needed when used as a breeding material for crossings. To combine desirable characteristics in local and foreign cultivars, a cross-breeding program was started twenty years ago [3]. It was aimed to develop very rich in volatile aromatic substances. This breeding program was started with a major objective to develop new strawberry varieties, especially, well adopted and able to provide a high productive and high standards.

Fruit aroma and taste are the result of a special assortment and mixture of different metabolites. The different proportions of the volatile components often determine aroma properties. The flavor of cultivated strawberries is mainly determined by a complex mixture of esters, aldehydes, alcohols and sulfur compounds [4, 5]. The study of flavor on a more analytical and scientific basis was achieved by the development and application of modern analytical techniques. Therefore, the qualitative as well as quantitative composition of strawberry flavor is now well known [5]. The techniques used for isolation, concentration and identification of strawberry flavor compounds have often a profound influence in the results obtained in determining volatile composition. Chemical and physical properties of different volatiles vary; and this may influence the results obtained in the determination of volatiles depending on the technique used. Thus, different determination techniques might cause alterations in the apparent overall aroma composition and usually only approximate quantitative determinations of the volatiles can be made. Furthermore, formation of new compounds before and during the analysis is possible [6]. Quality control can be difficult if appropriate techniques are not used. Solvent extraction is

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extensively used in aroma compound analysis [7, 8]. Nevertheless, a rapid, simple and inexpensive extraction and concentration technique of determinants of fruit aromas can be very useful. One such technique is a sample preparation technique called Solid Phase Micro Extraction (SPME) [9]. SPME is a solvent-free, inexpensive, rapid and versatile technique for the extraction of organic compounds. It consists of a fused-silica fiber, coated with a polymeric stationary phase introduced into a liquid or gas sample. The technique involves two processes: (1) adsorption of the analytes of the sample and (2) thermal desorption of the analytes in a gas chromatograph. This technique has been used by several authors for the analysis of volatile compounds in food samples [10-12] demonstrating its utility for flavor analysis. Hakala *et al.* [13], reported that the headspace composition correlates practically to the odor of the food due to the same sampling technique as used in quantitative sensory profiling.

Eating quality is one of the most important character for determining the value of new varieties. For this reason, a strawberry breeding programme was initiated at the University of Cukurova, Faculty of Agriculture, Department of Horticulture in 1984 [3]. Strawberry production are done using Camarosa variety due to high yielded and desirable flesh firmness characteristics, but having weak aroma. So, it is condemned by the consumers. The aim of this paper is to characterize promising strawberry hybrids in terms of aroma profiles using HS-SPME extraction technique by GC/MS and to determine relationships among them using volatile compounds data.

## MATERIALS AND METHODS

**Materials:** Strawberries (*Fragaria x ananassa*, Duch., Rosaceae) were grown in the implementation field of the Department of Horticulture, Faculty of Agriculture, University of Cukurova during 2001-2002 growing period. In the experiment, 8 hybrids were numbered as 3, 5, 6, 8, 11, 12, 13 and 17 and cultivar Osmanlı were used as plant materials. The plants were grown in walk-in plastic tunnels. The fruits of experimental genotypes were harvested at ripe maturation stage and immediately treated with liquid nitrogen and stored -80°C until aroma extractions. Ripe frozen strawberries (1 kg) devoid of the calyx were separated into three replicates and each replicate was homogenized in a food processor with a mixture of Celite and NaCl. The same homogenate was used for each extraction technique: HS-SPME, Im- SPME. The chromatography standards, all reagents and solvents were purchased from Sigma Chemical Co. (St Louis, MO).

**Extraction of aroma compounds:**
**Head Space-Solid Phase Micro Extraction (HS-SPME):** The SPME fiber was inserted into a 20 ml glass vial containing 5 g strawberry homogenate. A Supelco holder (Bellafonte, PA, USA) and fibre 100 μm polydimethylsiloxane (PDMS)-coated fused-silica fibre were used holder coated with fused-silica fibre was used. Samples were held in place for 30 min at 30°C. During this time the strawberry samples were stirred with a magnetic stirrer. After equilibration the fiber was removed from the sample and the analytes were thermally desorbed in the injector port of the gas chromatograph. Thermal desorption was performed in the injector glass liner at 250°C for 2 min.

**GC-MS analysis:** Flavor compounds of strawberries were analysed using Shimadzu GC-MS QP 5050A. In this system, nonpolar CPSil5CB column (25 m × 0.25 mm i.d., 0.4 μm thickness) was used, with helium as the carrier gas. The GC oven temperature here was kept at 60°C and programmed to 260°C at a rate of 5°C/min and then kept constant at 260°C for 40 min. Split flow was adjusted to 50 ml $min^{-1}$. The injector temperature was at 250°C. The MS was taken at 70 eV. The mass range was m/z 30-400. A library search was carried out using the Wiley GC-MS Library as well as the House-Baser Library of Essential Oil Constituents. The mass spectra were also compared with those of reference compounds and confirmed with the aid of retention indices from published sources. Relative percentage amounts of the separated compounds were calculated from total ion chromatograms through a computerized integrator.

**Data analysis:** Volatile compounds determined in strawberry genotypes were scored as present (1) or absent (0). The Jaccard similarity matrix was calculated using numerical taxonomy and multivariate analysis system (NTSYSpc) 2.11V package software [14]. UPGMA analysis was performed to construct a dendrogram.

## RESULTS AND DISCUSSION

The characteristic flavor of strawberry fruit is a complex interaction between a large number of volatile and non-volatile components. The non-volatile compounds, for example, sugars and acids, are

Table 1: Volatiles identified in ten strawberry genotypes by headspace solid phase micro extraction (HS-SPME, relative percentages) and polar GC/MS Column. Values marked with * indicate % of total

| | | Genotypes | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Compounds | RRI | 3 | 5 | 6 | 8 | 11 | 12 | 13 | 17 | *Osmanlı* | Camarosa |
| Esters | | | | | | | | | | | |
| Methyl butyrate | 986 | 1.9 | 5.4 | 5.4 | 0.7 | 1.3 | 1.0 | 7.6 | 0.9 | 0.4 | 4.4 |
| Ethyl butyrate | 1046 | 1.2 | 3.7 | 3.6 | tr | 1.2 | 0.8 | 1.4 | 0.4 | 2.6 | 1.7 |
| Methyl hexanoate | 1197 | 2.4 | 14.6 | 6.3 | 12.9 | 3.3 | 6.3 | 21.8 | 2.1 | 12.8 | tr |
| Ethyl hexanoate | 1244 | 0.6 | 8.4 | 3.8 | 5.3 | 6.0 | 7.4 | 20.0 | 4.3 | 58.1 | tr |
| Hexyl acetate | 1282 | 3.9 | 1.3 | 8.3 | 2.8 | 2.8 | 2.8 | 2.6 | 2.6 | 2.0 | - |
| (E)-2-hexenyl acetate | 1340 | 1.6 | 3.1 | 4.5 | 4.2 | 1.0 | 3.1 | 5.3 | 0.2 | 0.2 | tr |
| Butyl hexanoate | 1427 | - | - | 1.5 | - | 2.5 | - | - | 0.6 | - | 2.2 |
| Hexyl butyrate | 1424 | - | - | - | - | - | - | - | - | 0.9 | - |
| Methyl octanoate | 1399 | - | 0.8 | - | 0.9 | - | 0.6 | - | - | 1.9 | - |
| Ethyl octanoate | 1444 | - | - | - | 1.6 | 0.2 | 0.7 | - | 0.5 | 3.1 | tr |
| Octyl acetate | 1483 | 13.5 | 3.3 | 6.4 | 15.3 | 7.1 | 9.5 | - | 6.9 | | - |
| Octyl butyrate | 1623 | 15.6 | 2.7 | 5.4 | 3.2 | 14.3 | 2.6 | - | 13.0 | 0.1 | - |
| Octyl-2-methyl butyrate | 1634 | 0.7 | 0.8 | tr | 10.4 | 1.9 | 2.6 | - | 0.3 | | |
| Hexyl hexanoate | 1617 | - | - | - | - | - | - | - | - | 1.1 | - |
| Octyl isovalerate | 1654 | 4.3 | 2.6 | 5.1 | 3.8 | 7.6 | 3.3 | - | 0.5 | - | - |
| Decyl acetate | 1687 | 0.5 | 1.3 | - | 1.4 | 1.7 | 1.8 | - | tr | - | - |
| Octyl hexanoate | 1829 | 5.3 | 5.4 | 1.4 | 7.7 | 13.0 | 3.5 | - | 11.8 | 0.9 | - |
| Decyl butyrate | 1830 | 0.7 | - | 1.0 | - | 1.5 | - | - | 1.0 | - | - |
| Total Esters | | 52.2 | 53.4 | 53.5 | 70.2 | 65.4 | 46.0 | 58.7 | 45.1 | 84.1 | 8.3 |
| Total Esters (%)* | | 80.4 | 97.9 | 68.5 | 86.2 | 73.8 | 56.9 | 66.7 | 46.8 | 88.0 | 8.3 |
| Aldehydes | | | | | | | | | | | |
| Furfural | 1479 | - | - | - | - | 0.3 | 0.6 | - | 0.6 | - | 1.8 |
| 5-hydroxy methyl furfural | 1992 | 1.4 | - | - | - | 11.5 | 21.8 | - | 38.9 | 9.9 | 89.7 |
| Total Aldehydes | | 1.4 | - | - | - | 11.8 | 22.4 | - | 39.5 | 9.9 | 91.5 |
| Total Aldehydes (%)* | | 2.2 | - | - | - | 13.3 | 27.7 | - | 41.0 | 10.3 | 91.6 |
| Alcohols | | | | | | | | | | | |
| Hexanol | 1360 | 5.4 | - | - | - | - | - | - | - | - | - |
| Octanol | 1562 | 3.2 | - | - | | | | | | | |
| Total Alcohols | | 8.6 | - | - | - | - | - | - | - | - | - |
| Total Alcohols (%)* | | 13.3 | - | - | - | - | - | - | - | - | - |
| Terpenes | | | | | | | | | | | |
| Limonene | 1203 | 2.6 | 1.2 | 8.4 | 2.1 | 1.7 | 3.8 | 10.8 | 0.3 | 0.5 | tr |
| Linalool | 1553 | - | - | 1.5 | - | - | - | - | - | - | - |
| (E)-Nerolidol | 2050 | - | - | 1.8 | 8.0 | 2.0 | 8.6 | 18.5 | 6.7 | - | - |
| Total Terpenes | | 2.6 | 1.2 | 11.7 | 10.1 | 3.7 | 12.4 | 29.3 | 7.0 | 0.5 | - |
| Total Terpenes (%)* | | 4.0 | 2.1 | 14.9 | 12.4 | 4.1 | 15.3 | 33.2 | 7.2 | 0.5 | - |
| Lactones | | | | | | | | | | | |
| Gamma-decalactone | 2183 | - | - | 12.9 | 1.1 | 7.6 | - | - | 4.6 | 1.0 | - |
| Gamma-dodelactone | 2396 | - | - | - | tr | - | - | - | - | - | - |
| Total Lactones | | - | - | 12.9 | 1.1 | 7.6 | - | - | 4.6 | 1.0 | - |
| Total Lactones (%)* | | - | - | 16.5 | 1.3 | 8.5 | - | - | 4.7 | 1.0 | - |
| Total | | 64.9 | 54.6 | 78.1 | 81.4 | 88.5 | 80.8 | 88.0 | 96.2 | 95.5 | 99.8 |

RRI: Relative retention indices calculated against n-alkanes for polar column

responsible for the sweetness and tartness of the fruit; the volatile compounds, for example, esters and aldehydes, are the main components for producing the distinctive fruit flavor [15]. The results of aroma analyses of 10 strawberry genotypes by GC/MS techniques are given in Table 1.

The aroma profiles of 10 genotypes which were detected by HS-SPME and polar column are presented in

Table 2: Jaccard similarity matrix among strawberry genotypes

| | 3 | 5 | 6 | 8 | 11 | 12 | 13 | 17 | Osmanlı | Camarosa |
|---|---|---|---|---|---|---|---|---|---|---|
| 3 | - | | | | | | | | | |
| 5 | 0.72 | - | | | | | | | | |
| 6 | 0.62 | 0.63 | - | | | | | | | |
| 8 | 0.59 | 0.77 | 0.66 | - | | | | | | |
| 11 | 0.68 | 0.62 | 0.76 | 0.73 | - | | | | | |
| 12 | 0.66 | 0.77 | 0.59 | 0.80 | 0.81 | - | | | | |
| 13 | 0.38 | 0.46 | 0.47 | 0.44 | 0.40 | 0.44 | - | | | |
| 17 | 0.68 | 0.62 | 0.76 | 0.73 | 1.00 | 0.81 | 0.40 | - | | |
| Osmanlı | 0.45 | 0.52 | 0.45 | 0.57 | 0.52 | 0.57 | 0.44 | 0.52 | - | |
| Camarosa | 0.35 | 0.33 | 0.35 | 0.33 | 0.50 | 0.47 | 0.50 | 0.50 | 0.47 | - |

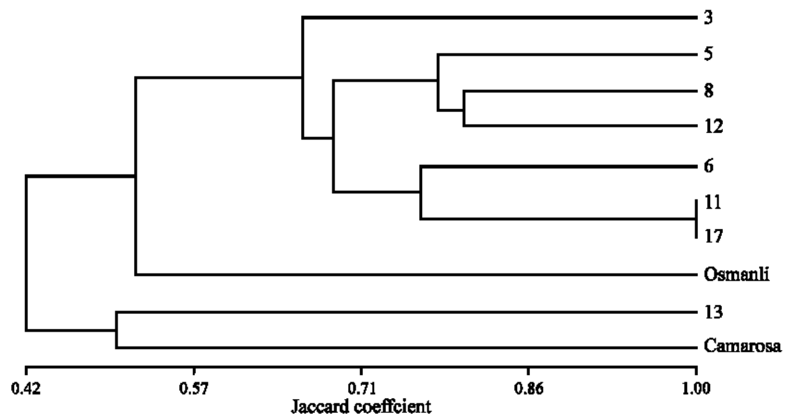


Fig. 1: UPGMA dendrogram of 11 strawberry genotypes. The data-base included 27 volatile compounds. UPGMA analysis was conducted by the NTSYSpc software using Jaccard similarity matrix

Table 1. In this technique, esters, aldehydes, alcohols, terpens and lactones were detected. The ester compounds were detected in all genotypes except for Camarosa, where aldehydes were found to be most abundant..Total ester compounds varied between 8.3% in Camarosa and 84.1% in *Osmanlı*. Among the aldehydes, furfural and 5-hydroxy methyl furfural compounds were detected with high percentages and the latter was detected in Camarosa with the highest level of 89.7%. Alcohols were detected only in Hybrid no. 3 as hexanol and octanol. In terms of terpenes, limonene was found to be the most abundant and detected in all genotypes except Camarosa. Linalool and (E)-Nerolidol were detected in some genotypes. Lactones, especially gamma-decalactone, were detected only in Hybrid nos 6, 8, 11 and 17 as well as in the Osmanlı cultivar, whereas gamma-dodelactone was found in trace amount only in Hybrid no. 8. As shown in Table 1, esters were found to be the most abundant compounds in all genotypes. Jacart similarity matrix data changed between 0.33 to 1.00. Hybrid nos 11 and 17 was found to be same aroma profile while hybrid no 13 was found to be very close to Camarosa variety in terms of volatile constiuents.

Watson *et al.* [15], reported that strawberry cultivars also differ in type and amount of volatiles they produce. Several authors reported that flavor differs in the growing season and at different locations [16, 17]. Kafkas *et al.* [12], compared HS-SPME and liquid-liquid extraction (with tert.-butyl methyl ether) techniques in three Israel commercial varietys using capillary column by GC/MS. The authors reported that HS-SPME was found to be more suitable for the determination of the very volatile and non-polar esters, while liquid extraction was found to be more appropriate for the determination of the polar and less volatile furanones.

The typical aroma of strawberries comes from not only one or a few impact aroma compounds, but also from numerous volatiles present at certain concentrations and in a particular balance among them. Thus, strawberry aroma is the result of the combined perception of many aromatic constituents [4]. Although over 360 compounds were identified in the aroma of strawberries, only a few volatiles (primarily methyl and ethyl esters) appear to be the most important contributors to strawberry aroma [5]. Therefore, breeding for high ester content of fruits is important. At this point, Hybrid nos 5, 11, 12 and 13 were found to be much closer to variety Osmanlı than the other genotypes. In this study, variety Osmanlı was found to be quite satisfactory in terms of aroma, yet not so in terms of fruit yield, firmness and commercial production but potential material for breeding studies especially in terms of having pleasant taste and aroma.

In summary, the data presented in this paper indicate that the identification of volatiles of strawberries is closely related to the varieties. Among the experimental genotypes Osmanlı was found to be the most aromatic variety and detected as accumulate high levels of esters compared to the other strawberry genotypes. The ester compounds were detected highly in all genotypes except Camarosa variety. According to the multivariate analysis system and UPGMA analysis hybrid numbers 11 and 17 had the same aroma profiles and hybrid no 13 was found to be very close to cultivar Camarosa.

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