Turkish Journal of Agriculture and Forestry

Volume 29 | Number 5 Article 7

1-1-2005

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Turk J Agric For
29 (2005) 383-390

Comparison of Methodologies for the Identification of Aroma Compounds in Strawberry

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Received: 12.01.2005

Abstract: Three strawberry (*Fragaria x ananassa*. Duch.) varieties commercially grown in Israel (Tamar, Yael and Malach) were studied for their volatile compositions. Two techniques were compared: headspace solid phase micro extraction (HS-SPME) and liquid-liquid extraction (organic solvent: *tert*-butyl methyl ether) by gas chromatography/mass spectrometry (GC/MS). The influences of techniques on the volatile compounds were tested by comparing the volatiles determined in the 3 varieties. Malach, the most aromatic variety, accumulates high levels of furanones and esters compared to the other varieties. Differences in the aroma profiles obtained by utiliszing different techniques were noted. HS-SPME was more suitable for the determination of very volatile and non-polar esters, while liquid extraction was more appropriate for the determination of the polar and less volatile furanones.

Key Words: Strawberry, volatiles, aroma, SPME, GC/MS

Çilekte Aroma Maddelerinin Tanımlanmasında Kullanılan Metodların Karşılaştırılması

Özet: Bu çalışmada İsrail'de ticari olarak yetiştiriciliği yapılan (*Fragaria x ananassa.* Duch) 3 çilek çeşidinde ("Tamar", "Yael" ve "Malach") uçucu aroma kompozisyonları incelenmiştir. Aroma bileşikleri Tepe Boşluğu Katı Faz Mikro Ekstraksiyon (HS-SPME) ile sıvı-sıvı ekstraksiyon tekniği (organik çözücü: *tert*-butil metil eter) olmak üzere 2 farklı ekstraksiyon tekniği ve Gaz Kromatografisi Kütle Spektrometresi (GC/MS) ile belirlenmiştir. Kullanılan ekstraksiyon tekniklerinin 3 çilek çeşidinde aroma kompozisyonları üzerine etkileri incelenmiştir. Malach çeşidi öteki çeşitlere gore ester ve furanon içerikleri bakımından daha zengin olduğu belirlenmiştir. Denemede farklı ekstraksiyon teknikleri kullanıldığında aroma profillerinde de farklılıklar olduğu dikkati çekmiştir. HS-SPME tekniğinin çok uçucu ve polar olmayan esterlerin tanımlanması için uygun olduğu sıvı ekstraksiyon tekniğinin ise daha çok polar ve uçuculuğu az olan furanon bileşiklerinin tanımlanması için uygun olduğu saptanmıştır.

Anahtar Sözcükler: Çilek, uçucu bileşikler, aroma, KFME, GC/MS

Introduction

Strawberry is a member of the genus *Fragaria* (Rosaceae). Wild strawberries (*Fragaria vesca* L.) are diploid species (2n = 14), whereas cultivated strawberries (*Fragaria x ananassa* Duch.) are octaploid (2n = 56) (Hancock and Luby, 1993). The increased ploidy levels of cultivated strawberries result in larger fruit and higher yield, which however, contain less characteristic strawberry flavour compounds (Hancock and Luby, 1993).

The volatile flavour compounds of strawberries have been studied during the last decades. The introduction of capillary gas chromatography, combined with mass spectrometry, has made it possible to identify several

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hundred compounds in a single gas chromatographic run. Like in all fruits, volatile compounds are responsible for the aroma and contribute to the flavour of fresh strawberries. Strawberry aroma is mainly influenced by a complex of up to 360 individual esters, aldehydes, ketones, alcohols, terpenes, furanones and sulphur compounds (McFadden et al., 1965; Latrasse, 1991; Larsen and Poll, 1992; Larsen et al., 1992). These compounds are found only in minute levels in the fruit but have a major impact on its quality (Buttery, 1981). Esters are both quantitatively and qualitatively the most abundant class of these compounds; 131 different ones have been identified in strawberry aroma (Latrasse, 1991). Esters provide the fruity and floral notes and they comprise from 25% to 90% of the total volatiles in fresh ripe fruit (Pyysalo et al., 1979; Schreier, 1980; Douillard and Guichard 1990, Ito et al., 1990). Alcohols account for as much as 35% of the volatiles, but normally contribute little to strawberry aroma (Larsen and Watkins, 1995). While terpenes normally comprise <10% of strawberry volatiles and sulphur compounds <2%, they both may contribute to strawberry aroma (Schreier, 1980; Drinck et al., 1981).

The methods used for the isolation, concentration and identification of strawberry 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 determinants of fruit aromas can be very useful. One such technique is a new sample preparation method called solid phase micro extraction (SPME) (Arthur and Pawliszyn, 1990). SPME is a solvent-free, inexpensive, rapid and versatile method for the extraction of organic compounds. 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), demonstrating its utility for flavour analysis.

In our previous study (Lavid et al., 2002), we reported only the levels of furaneol, methoxyfuraneol and total volatiles contents of 3 strawberry varieties using liquid-liquid extraction (solvent: tert-butyl methyl ether (t-BME)). In this study, we report the headspace solid phase micro extraction (HS-SPME) technique and compare it with the liquid-liquid extraction technique (using t-BME as a solvent) in detail to identify the aroma compounds of 3 Israeli commercial varieties.

Materials and Methods

Three commercial Israeli strawberry varieties Yael, Tamar and Malach were grown in the greenhouse at the Volcani Research Centre, and harvested at different maturation stages (green, pink and ripe) for aroma compound analysis. The analyses were performed in the Newe Ya'ar Research Centre. Two different extraction techniques were used. HS-SPME was applied to ripe fruits, whereas tert-butyl methyl ether extraction was applied to the green, pink and ripe fruits. The same lot of ripe berries was partitioned to be used for both techniques to allow for more significant comparisons between the methods.

Analysis of Volatiles

Solid Phase Micro-Extraction (SPME)

The fruit flesh was homogenised in a food processor and 10 g of the homogenate were diluted with 2 ml of NaCl saturated aqueous solution, and immediately headspace sampling was conducted on 65 µm fused silica fibres coated with polydimethylsiloxane/divinylbenzene (PDMS/DVB) (Supelco). The SPME fibre was inserted into a 20 ml vial containing the strawberry homogenate. After 30 min at 65 ºC, and under stirring, the SPME syringe was introduced into the injector in the port of the GC-MS apparatus for further analysis.

Solvent extraction in tert-Butyl Methyl Ether (t-BME)

Green, pink and ripe fresh strawberries (1 kg) devoid of the calyx were homogenised with Celite and NaCl using a food processor. From this homogenate 30 g samples were extracted with 50 ml of tert-butyl methyl ether containing internal standard (10 mg of isobutyl benzene) by shaking for 30 min. The organic phase was dried on anhydrous Na_2SO_4 and evaporated under nitrogen to 1 ml (Lewinsohn et al., 2001).

GC-MS Analysis

Volatile compounds were analysed on an HP-GCD apparatus equipped with an HP-5 MS (30 m x 0.25 mm x 0.25 µm) fused-silica capillary column. Helium (1ml min-1) was used as a carrier gas. The injector temperature was 250 ºC, set for splitless injection. The oven conditions were set to 50 ºC for 1 min and then the temperature was increased to 200 ºC at a rate of 4 ºC min^{-1}. Thermal desorption was allowed for 1.5 min. The detector temperature was 280 ºC. The components were identified by comparison of mass spectra and retention time data with those of authentic samples and complemented with a NIST GC-MS library. The quantitative analyses were determined using isobutylbenzene as an internal standard (Shalit et al., 2001).

Results and Discussion

The results of aroma analyses of 3 strawberry varieties by 2 different extraction methods are given in Table 1. Extraction with t-BME was performed at the green, pink and ripe maturation stages of the 3 cvs. whereas only ripe fruits were used when utilising the SPME technique. As seen in Table 1, only up to 10 esters were detected in ripe fruits when analysed by t-BME extraction, while up to 32 ester compounds were detected using HS-SPME. Therefore, the HS-SPME technique is more useful for detecting esters in strawberry. Based on t-BME extraction, a higher percentage of esters was found in Malach (1.58 µg g^{-1}) compared to Tamar (0.62 µg g^{-1}) and "Yael" (0.27 µg g^{-1}). Most of the esters are only found in ripe fruits (Figure 1). Tamar had a higher number of different esters in the t-BME extracts, and also a higher percentage of esters in the total volatiles (18% in t-BME, 83% by HS-SPME) than Yael (10.23% by t-BME, 81.2 by HS-SPME) and Malach (5.3% by t-BME, 70.1 by HS-SPME). Nevertheless, based on the results obtained by t-BME extracts, Malach contained more than 2-fold the levels of volatile esters (1.58 µg g^{-1} FW) compared to Tamar (0.62 µg g^{-1} FW) and Yael (0.27 µg g^{-1} FW). Utilizing SPME, Malach (73.3 %) had lower levels of volatile esters than Tamar (82.6%) and "Yael" (83.2%). Furthermore, many esters (methyl butanoate, ethyl butanoate, isopropyl butanoate, methyl hexanoate, butyl butanoate, ethyl hexanoate, hexyl acetate, (E)-2-hexenyl acetate, hexyl butanoate, (E)-2-hexenyl butanoate and octyl butanoate) were detected by HS-SPME in all varieties.

A similar study was performed by Hakala et al. (2001) using headspace SPME in 6 strawberry varieties. Methyl butanoate, ethyl butanoate, methyl hexanoate and ethyl hexanoate were found to be the most abundant ester compounds.

According to previous studies (Sanz et al., 1995; Perez et al., 1996; Wein et al., 2002), methoxyfuraneol and furaneol are the main contributors to the aroma of strawberries. Furanones are known derivatives of pentoses, and the incorporation of glucose into furanone and its derivatives have been demonstrated (Wein et al., 2001). The levels of furanones (2-amylfuran, methoxyfuraneol and furaneol) were about 20-fold higher in Malach, the most aromatic variety, compared to Yael and Tamar (Figure 1). Furan derivatives are higher in t-BME compared to in SPME extraction. Total furanones were detected at the highest level in Malach (70.7 %) of the total volatiles by t-BME (21.05 µg g^{-1}); 3.7 % by SPME), whereas Tamar (43.5% by t-BME (1.50 µg g^{-1}); 1.13 %) by SPME and Yael (9.5% t-BME (0.25 µg g^{-1}); 0.6% SPME) followed it in both extraction techniques.

Ripe strawberry fruits are able to metabolise a range of exogenous aldehydes to their corresponding alcohols and thereafter to various esters of endogenous acids (Mulders, 1973). In our experiments, aldehydes were detected in low concentration in Tamar and Yael fruits by HS-SPME, whereas higher levels were found in Malach by t-BME extraction. (E)-2-Hexanal was found at high levels when utilising both techniques and was also detected during the 3 maturation stages.

Alcohols also play an important role in ester biosynthesis. (Z)-3-Hexanol, hexanol, (E)-2-hexenol and 2-ethylhexanol were detected in t-BME extracts, whereas

Table 1. Volatiles identified in 3 strawberry varieties by headspace solid phase micro extraction (HS-SPME, relative percentages) and by solvent extraction (t-BME, μg g^{-1} FW) techniques, 1Values marked with ** indicate % of total.

Compound	Tamar		Yael		Malach	
Esters	HS-SPME (%)	t-BME (μg g^{-1})	HS-SPME (%)	t-BME (μg g^{-1})	HS-SPME (%)	t-BME (μg g^{-1})
Methyl butanoate	5.35	-	7.15	-	4.31	-
Methyl 2-hydroxy butanoate	-	0.05	-	-	-	-
Methyl 3-methyl butanoate	0.41	-	0.28	-	0.63	-
Ethyl butanoate	20.40	-	13.97	-	19.67	-
Butyl acetate	0.46	-	0.97	-	0.96	-
Isopropyl butanoate	2.06	0.06	3.45	-	2.41	-
Ethyl 2-methyl butanoate	0.86	-	0.06	-	0.26	-
3-methyl butyl acetate	0.39	-	0.42	-	1.12	-
2-Methyl butyl acetate	0.66	-	0.23	-	0.21	-
Propyl butanoate	0.34	-	0.35	-	0.62	-
Ethyl pentanoate	0.13	-	0.06	-	0.20	-
Methyl hexanoate	4.96	0.10	4.60	-	6.16	0.35
Butyl butanoate	3.84	-	11.62	-	6.20	-
Ethyl hydroxybutanoate	-	0.04		-		-
Ethyl hexanoate	11.76	0.05	3.80	-	10.64	0.27
(Z)-3-Hexenyl acetate	0.20	-	0.46	-	0.41	-
Hexyl acetate	5.86	0.06	4.74	0.04	5.19	0.23
(E)-2-Hexenyl acetate	6.76	0.23	6.91	0.14	3.64	0.56
Isopropyl hexanoate	0.75	-	0.49	-	0.64	-
Butyl 3-methyl butanoate	0.08	-	0.45	-	0.49	-
Isopentyl butanoate	0.68	-	1.26	-	0.96	-
Hexyl isobutanoate	0.15	-	0.05	-	0.05	-
Pentyl butanoate	-	-	0.19	-	0.06	-
(E)-2-hexenyl propanoate	0.19	-	0.13	-	-	-
Methyl octanoate	0.17	-	0.09	-	0.16	-
Benzyl acetate	0.15	-	-	-	0.16	-
(Z)-3-Hexenyl butanoate	0.19	0.03	0.59	-	0.05	-
Butyl butanoate	-	-		-		-
Hexyl butanoate	6.30	-	6.79	-	1.59	-
(E)-2-hexenyl butanoate	7.08	-	9.05	-	0.50	-
Ethyl octanoate	0.16	-	-	-	0.24	-
Octyl acetate	0.33	-	0.06	-	0.48	-
Hexyl hexanoate	0.48	-	0.42	-	0.13	-
Octyl butanoate	1.82	-	2.36	-	2.18	-
Octyl 2-methylbutanoate	0.25	-	0.19	-	0.66	-
Total esters	83.22	0.62	81.19	0.27	70.98	1.58
% in total volatiles	85.88	17.97**	82.59	10.23**	73.34	5.30**
Aldehydes						
2- Heptanal	0.37		0.47		0.40	
(Z)-3-Hexenal	0.08		-		0.14	
(E)-2-Hexenal	8.49	0.56	10.56	0.63	13.39	1.33
Heptanal	-	-	-	0.02	-	-
Octanal	-		0.53		0.16	
Nonanal	0.21	0.03	0.26	0.03	0.13	0.03
(E)-2-nonenal	0.19		-		-	
Decanal	0.06	0.02	-	-	0.04	-
Total aldehydes	9.40	0.61	11.82	0.68	15.26	1.36
% in total volatiles	9.70	17.68**	12.02	25.76**	16.28	4.57**

Table 1. (Contunued).

Compound	Tamar		Yael		Malach	
Esters	HS-SPME (%)	t-BME (μg g^{-1})	HS-SPME (%)	t-BME (μg g^{-1})	HS-SPME (%)	t-BME (μg g^{-1})
Aromatic compounds						
Benzyl alcohol	-	-	-	0.07	-	0.05
Vanillin	-	0.10	-	-	-	0.15
Total aromatic compounds	-	0.10	-	0.07	-	0.20
% in total volatiles		2.89**	-	2.65**	-	0.67**
Acids	-	-	-	-	-	-
Butanoic acid	-	-	0.20	-	-	-
Hexanoic acid	-	-	0.15	-	-	-
Total acids			0.35			
% in total volatiles			0.36			
Alcohols						
(Z)-3-Hexenol	-	-	-	0.12	-	0.33
(E)-2-Hexenol	0.23		0.26	0.35	0.23	0.31
Hexanol	0.34		0.30	-	0.93	0.17
1-Octen-3-ol	-		-		0.14	
2-Ethyl hexanol	-	-	-	0.47	-	-
Total alcohols	0.57	0.00	0.56	0.94	1.30	0.81
% in total volatiles	0.59	0.00**	0.57	35.61**	1.34	2.72**
Lactones						
gamma-decalactone	0.89	0.62	1.30	0.36	1.44	3.82
gamma-dodecalactone	-	0.05	0.20	0.10	0.16	0.71
Total lactones	0.89	0.67	1.50	0.46	1.60	4.53
% in total volatiles	0.92	19.42**	1.53	17.42**	1.65	15.21**
Ketones						
2-Heptanone	-	-	0.22	-	0.12	-
Benzaldehyde	-	-	-	-	0.05	-
Total ketones	-	-	0.22	-	0.17	-
% in total volatiles	-	-	0.22	-	0.18	-
Furan derivatives						
2-Amylfuran	0.42		0.28		0.48	
Methoxyfuraneol	0.71	0.77	0.32	0.08	3.23	9.77
Furaneol	-	0.73	-	0.17	-	11.28
Total furan derivatives	1.13	1.50	0.60	0.25	3.71	21.05
% in total volatiles	1.66	43.48**	0.61	9.47**	3.83	70.66**
Terpenes						
Linalool	0.56	0.04	0.61	-	0.85	0.18
Geraniol	-	0.04	-	-	-	-
(E)-Nerolidol	-		0.50	-	0.45	0.14
Total terpenes	0.56	0.08	1.11	-	1.30	0.32
% in total volatiles	0.58	2.32**	1.13	-	1.34	1.07**
Total	96.90	3.45	98.30	2.64	96.77	29.79

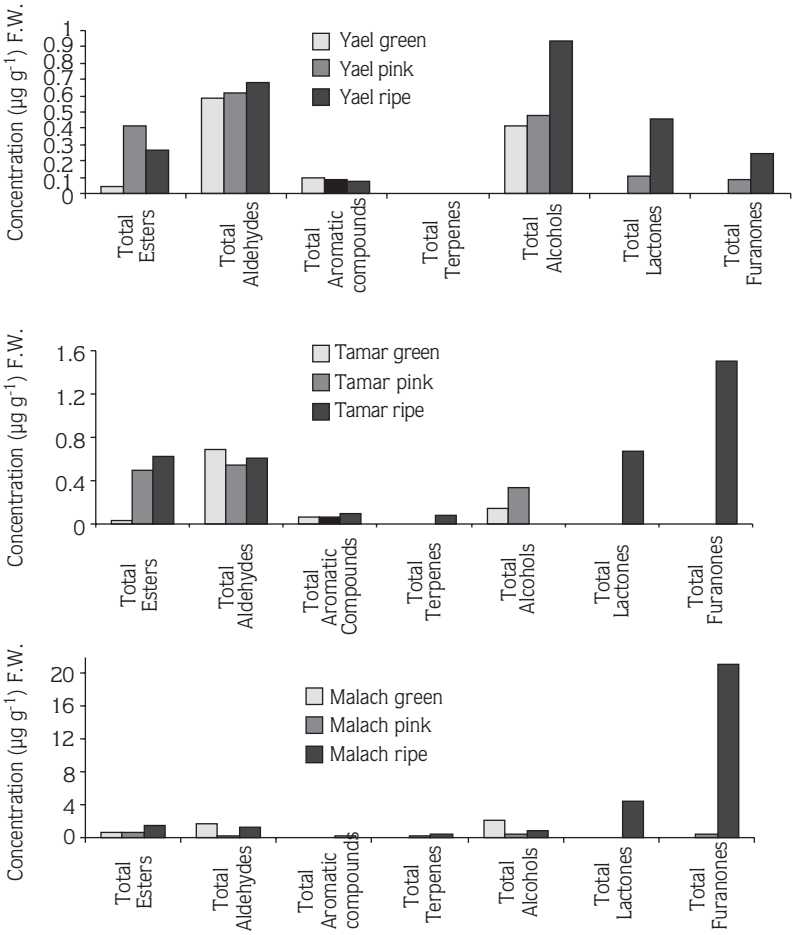

Figure 1. Major volatiles present in ripening strawberry fruits. Determinations were performed utilising the t-BME extraction technique. Means (n = 3) are depicted.

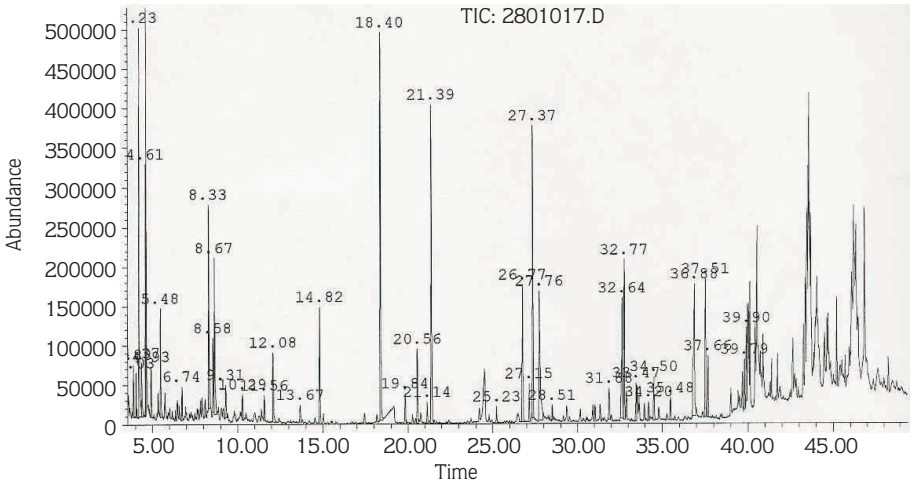

Figure 2. Gas-chromatographic patterns of the HS-SPME extracts of strawberry ripe fruits of Yael cv.

hexanol, (E)-2-hexenol and 1-octen-3-ol were detected in HS-SPME. The highest total alcohol levels were detected in cv. Malach (1.30 %), and it was 0.57% in Tamar and 0.56% in Yael. In t-BME extracts, the highest levels of alcohols were determined in cvs. Yael (0.94 µg g^{-1}) and Malach (0.81 µg g^{-1}). No volatile alcohols were detected in Tamar fruits.

Ketones were not detected by t-BME during the 3 maturation stages, whereas only 2-heptanone was detected by HS-SPME in Yael and Malach.

Lactones including gamma-decalactone and gamma-dodecalactone were detected utilizing both techniques. In the t-BME extracts the highest levels of lactones were detected in Malach (4.53 µg g^{-1};15.2%), followed by Tamar (0.67 µg g^{-1};19.4%) and Yael (0.46 µg g^{-1}; 17.4%). Utilising HS-SPME, higher levels of lactones were detected in Malach (1.60%) than in Yael (1.50%) and Tamar (0.89%), with levels of gamma-decalactone higher than those of gamma-dodecalactone. The results show that t-BME extraction is more useful than SPME for detecting lactones.

Monoterpenes were detected at very low concentrations utilising both techniques. In t-BME extraction, linalool was detected in Malach (0.18) and Tamar (0.04) but it was not found in Yael. Utilising HS-SPME, linalool was detected in all the varieties.

Aromatic compounds were detected at very low levels when utilising both techniques. Benzyl alcohol and vanillin were detected in the t-BME extracts, whereas benzaldehyde was detected in HS-SPME (Table 1).

The changes in the total esters, aldehydes, aromatic compounds, terpenes, lactones, and furanone levels of 3 commercial varieties during fruit maturation are shown in Figure 1. From the green through to the pink stage, the levels of total esters increased in all varieties. The levels of esters also increased in Malach and Tamar but decreased in Yael during the transition from the pink to the ripe stage Figure 2.

Total furanones sharply increased during the maturation stages, especially from the pink to ripe stage in Malach and Yael. In Tamar, furanones were detected only in the ripe stage. In the green maturation stage, no furanones were detected.

In all varieties, lactone levels increased during maturation. The highest concentration of lactones was detected in Malach, followed by Tamar and Yael. The concentrations of total aldehydes, aromatic compounds and alcohols also varied during maturation (Figure 1).

In conclusion, the choice of extraction technique is very important when determining the relevant aroma compounds in strawberries. Although both esters and furans contribute to the aroma of strawberries, HS-SPME was found to be more useful in detecting esters, aldehydes, terpenes and ketones, whereas t-BME extraction was more effective in detecting lactones, furan derivatives and alcohols in strawberry fruits. Malach, the most aromatic variety, accumulates relatively high levels of esters and furanones during fruit maturation compared with the other varieties examined.

Acknowledgements

This work was funded by the German-Israeli Foundation for scientific research and development (G.I.F. Research Grant No. G-591-113.12/98). Ebru Kafkas was partially supported by a fellowship from The Scientific and Technical Research Council of Turkey (TÜBİTAK). We would like to thank Zoharia Tanami for help in supplying fresh fruits. Publication of the Agricultural Research Organisation, Bet Dagan, Israel.

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