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Varietal and processing effects on the volatile profile of Australian olive oils

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Abstract

The volatile profile of virgin olive oils was established using SPME and gas chromatography(-mass spectrometry). The major volatile in approximately 50% of the oils was *E*-hex-2-enal in contrast with European oils. The minor contribution of C5 compounds to the volatile profiles also contrasted with data on European oils. Hierarchical Cluster Analysis (HCA) implicates variety as the single-most important factor in determining volatile profile whilst malaxation time and temperature exerted a minor secondary effect on the volatile profile.

Keywords: Olive oil; Volatile compounds; *E*-hex-2-enal; Aroma; Malaxation

1. Introduction

The complex flavour of virgin olive oil can be attributed to the phenolic content and profile of volatile compounds (Morales, Rios, & Aparicio, 1997). The latter includes various aldehydes, alcohols, furans, acids, ketones and esters (Aparicio, Morales, & Alonso, 1997) that have been determined by a variety of techniques including variants of headspace analysis and gas chromatography (Angerosa, Digiacinto, Vito, & Cumitini, 1996; Aparicio, Morales, & Alonso, 1996; Morales, Aparicio, & Rios, 1994). Hexanal, *E*-hex-2-enal, hexan-1-ol, and 3-methylbutan-1-ol are the major volatile compounds of European olive oils (Kiritsakis, 1998). These compounds are not present in the olive fruit in significant concentrations and lipoxygenase (LOX) pathways for the formation of C6 aldehydes, alcohols and esters from linoleic and linolenic acids have been delineated (Angerosa, Basti, & Vito, 1999).

The volatile composition of olive oil depends on the levels and activity of the enzymes involved in the various pathways (Angerosa et al., 1999) which are genetically determined (Campeol, Flamini, Chericoni, Catalano, & Cremonini, 2001). Other factors that influence the volatiles are climate and soil type (Ranalli, De Mattia, Patumi, & Proietti, 1999) and ripening cycle of the fruit (Ranalli, Tombesi, Ferrante, & Demattia, 1998). The effect of processing equipment has also been studied (Angerosa, Mostallino, Basti, Vito, & Serraiocco, 2000; Di Giovacchino, Costantini, Serraiocco, Surricchio, & Basti, 2001; Ranalli & Angerosa, 1996; Ranalli. Costantini, De Mattia, & Ferrante, 2000) as well as the effects of the processing variables of malaxation[1] time and temperature. However, the effect of these variables on volatile profiles is ambiguous. For instance, Ranalli, Contento, Schiavone, and Simone (2001) demonstrated for the varieties, *Caroleo*, *Leccino* and *Dritta*, that increasing temperature led to a decrease in the levels of most volatiles, especially *E*-hex-2-enal, hexanal and *E*-hex-2-en-1-ol. On the other hand, the results reported by Angerosa, Mostallino, Basti, and Vito (2001) are more variable. For cv *Coratina*, levels of *E*-hex-2-enal and *E*-hex-2-en-1-ol increased with temperature, whereas, for cv *Frantoio*, the level of *E*-hex-2-enal decreased, while *E*-hex-2-en-1-ol levels showed a variable response to temperature. The levels of hexanal generated by these two varieties at different tempera-

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[1] Malaxation is the “slow and continuous stirring of the olive paste, which increases the percentage of the ‘free oil’ by helping the droplets of oil to merge into large drops and by breaking up the oil/water emulsion“ (Di Giovacchino, 2000).

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tures also showed complex behaviour. Levels generally decreased with temperature for cv *Coratina*, while there were no clear trends for cv *Frantoio*. A direct comparison between the Ranalli and Angerosa studies is not possible because of the different processing methods and different varieties, but these reports highlight the fact that there is still much to be understood in the relationship between processing and volatile production.

Such a complex interplay of factors requires the development of a systematic approach for investigating the development of volatile compounds in virgin oils. This paper presents data for the volatile profile of a number of commercial and laboratory-produced oils. It investigates the feasibility of using solid phase microextraction and gas chromatography for this purpose. Results demonstrate that the profiles of some Australian oils are distinctly different from those of European oils as has been demonstrated (Ryan, Antolovich, Herlt, Prenzler, Lavee, & Robards, 2002) for the phenolic profiles.

2. Materials and methods

2.1. Standards

E-hex-2-enal (Merck), heptan-1-ol, hexanal, heptanal, hexyl acetate, *E*-oct-2-enal, *E,Z*-deca-2,4-dienal, (Aldrich), octanal, nonanal, *n*-alkanes C8 to C14 (Sigma), and hexan-1-ol (Riedel de Haen) were used without further purification to determine retention data.

2.2. Oil samples

2.2.1. Commercial oils

Oil samples were obtained from two processors in the Riverina Region of South Western New South Wales and are described in Table 1. Oils were produced in either the 2000 or 2001 harvest season from fruit grown locally and processed within 1 day of harvest.

2.2.2. Laboratory processed oils

Oil samples were produced from *Corregiola* fruit (4 kg) within 1 day of harvest on an Abencor laboratory scale (1 kg) oil mill—hammer mill, malaxer, and centrifuge.

2.3. Solid phase microextraction

Oil samples (7 ml) were placed in Reactivials (Supleco, capacity 10 ml) and sealed with a septum. The sealed vial was placed in a thermostatted oven at 40 °C and, after equilibration, the SPME needle [100 μm polydimethylsiloxane (PDMS) fibre, Supelco] was inserted through the septum and left in the headspace for 30 min. The fibre was withdrawn and immediately injected into the gas chromatograph.

2.4. Gas chromatography

Analyses of volatile compounds were performed using a Varian Star 3400 CX gas chromatograph (Varian, Melbourne, Australia). Column temperature was programmed as follows: initial temperature 40 °C for 4 min, increased to 200 °C at 5 °C/min, with a final isothermal period of 10 min. Separation of commercial oils was achieved on a SGE DB-5 column (length 30 m, 0.25 mm i.d., film thickness 0.25 μm) using nitrogen carrier gas at a flow rate of 2 ml/min (pressure 14 psi). Laboratory-processed oils were separated under the same conditions using a Phenomenex ZB-5 column (length 30 m, 0.25 mm i.d., film thickness 0.25 μm). Injection was performed by thermal desorption via a splitless injector for 1 min at 250 °C. Following injection, the fibre was cleaned in a separate injection port for 10 min at the same temperature prior to re-use. Data were recorded and analysed using STAR chromatography software. The FID detector was maintained at 300 °C

Quantification was performed by external calibration as follows: a series of stock standards were prepared by weighing a single drop of appropriate standard (ca. 2 mg) into a known mass (ca. 7 g) of stripped light olive oil (produced no interfering GC peaks). Calibration curves were prepared by diluting known amounts (typically 0.3 to 1.5 ml) of stock standard in a further 7 g of light oil and injecting as described above.

2.5. Gas chromatography–mass spectrometry

Volatiles were analyzed by thermal desorption in the injection port of a Varian 3400 CX gas chromatograph (Varian, Melbourne, Australia) coupled with a Saturn 2000 ion trap mass spectrometer using the same chromatographic conditions as described for ‘gas chromatography’.

The electron impact ionization (EI) mode with automatic gain control (AGC) was used for MS. The electron multiplier voltage for MS was 1850 V, AGC target was 25, 000 counts, and filament emission current was 15 μA with the axial modulation amplitude at 4.0 V. The ion trap temperature was maintained at 250 °C and the manifold temperature was maintained at 60 °C. The temperature of the transfer line, interfacing the GC and MS, was set at 250 °C. Mass spectral scan time from m/z 35 to 450 was 0.8 s (using 2 microscans). Background mass was set at 45 m/z.

2.6. Statistical analysis

Concentration data are reported as the mean of triplicate determinations; confidence limits were below 10%. Hierarchical Cluster Analysis (HCA) was performed on gas chromatographic data using S-Plus

Version 6.0 for Sun SPARC (MathSoft Inc., 2000). Agglomerative clustering (AGNES) and divisive clustering (DIANA) techniques were employed. AGNES starts with each object as a separate cluster and the two clusters with the smallest dissimilarity are merged. The process repeats until a single large cluster forms. DIANA starts with one large cluster and divides them until all objects have been separated into individual clusters.

Preprocessing of the data involved scaling and transforming the raw GC ASCII data (time vs peak heights) for the 40 samples. Statistical analysis of the volatile profiles was restricted to the region from 0.8 to 30 min (retention index 700 to greater than 1400) as few

Table 1
Olive oils examined in the study showing details of the fruit and processing

Sample number	Processing date	Variety[a]	Maturity	Malaxation time (min)	Oil%
1	2000 season[b]				
2	22 May	Verdale	Unknown	50	10.1
3	22 May	Verdale	Unknown	50	10.1
4	22 May	Corregiola	60% green	40	13.3
5	25 May	Frantoio	Unknown	60	15.3
6	2 August	Sevillano	24.5	60	11.5
7	31 May	Mixed[c]	100% black	30	17.1
8	5 June	Mixed	95% black	45	7.8
9	8 June	Mixed	80% black	45	16.3
10	8 June	Corregiola	95% green	60	13.8
11	14 June	Mixed	95% black	50	12.2
12	21 June	Mixed	85% black	50	18.2
13	29 June	Mixed	95% black	60	17.9
14	20 July	Manzanillo	100% black	75	13.1
15	27 July	Corregiola	60% green	60	16.8
16	27 July	Mixed	90% black	90	29.1
17	27 July	Ferals	90% black	75	25.3
18	8 August	Corregiola	90% black	60	13.9
19	11 August	Manzanillo	100% black	60	9.5
20	2 August	Mixed	95% black	60	17.4
21	8 August	Mixed[d]	100% black	65	14.2
22	11 August	Unknown	90% black	60	12.7
23	16 May	Mixed	50% green	Not recorded	10.0
24	26 June	Mixed	100% black	40	17.7
25	26 June	Paragon	100% black	45	47.4[e]
26	29 June	Corregiola	70% green	60	18.1
27[f]	11 August	Unknown	90% black	60	12.7
28[g]	26 June	Paragon	100% black	45	47.4
29	3 July	Manzanillo	80% black	60	10.6
30[h]	3 July	Manzanillo	80% black	60	10.6
31	7 July	Corregiola	85% green	60	16.7
32	10 July	Manzanillo	100% black	50	13.9
33	2000 season				
34	2000 season				
35	2000 season				
36	Rancid				
37	16 May	Verdale	75% green		9.0
38	16 May	Unknown	60% green		10.0
39	5 July	CSU—mixed	100% black		18.0
40	28 June	CSU—mixed	100% black		18.0

[a] Variety as supplied by processor.

[b] Processing data is not available for the 2000 season.

[c] "Mixed" is fruit taken from both typically oil producing trees and those considered more for table olives.

[d] Processing records indicate that this may be the same fruit as used for oils 39 and 40, but oil 4 was processed with a 2-phase decanter and oils 39 and 40 with a stone mill and mat press.

[e] This was a small batch of fruit processed at the end of the day and contaminated with other oil increasing apparent yield.

[f] Same as oil 22.

[g] Same as oil 25.

[h] Same as oil 29.

volatiles eluted outside this region and those that did were relatively minor in amount. Moreover, polysiloxane peaks at 32, 34 and 37 min occurred in SPME fibre blank runs and were derived from decomposition of the SPME fibre. Studies are continuing to eliminate the problem of blank peaks, possibly involving the selection of another fibre type.

3. Results and discussion

The forty commercial oil samples came from normal processing operations of two small volume processors (less than 500 l per batch). The oils were obtained from commercially significant varieties grown in the Riverina region of NSW, Australia and processed by either 2-phase decanter or traditional mat press. From the sensory perspective, all oils belonged to the extra virgin olive oil grade at pressing. Oil 36 was stored for more than 12 months in a clear plastic container, exposed to heat and light to deliberately induce rancidity. Oils 1, 33, 34 and 35 were processed in 2000 and stored under conditions to preserve aroma until testing in 2001. All other oils were pressed and analyzed in 2001.

Given that varietal, geographic and processing effects can all influence the volatile fraction, a method was developed to survey a broad range of volatiles. SPME has widespread application in analysis of volatiles (Kataoka, Lord, & Pawliszyn, 2000; Steenson, Lee, & Min, 2002) but has had limited application to olive oils (Servili, Baldioli, Begliomini, Selvaggini, & Montedoro, 2000). In the analysis of volatiles of olive oil, dynamic headspace remains the preferred procedure (Ranalli et al., 2001) but this work demonstrates the suitability of SPME in this role. Moreover, SPME offered the advantages of solventless recovery of volatiles, ease of operation and particularly reduced sample preparation time (20–30 min vs 2–3 h). The anticipated range of volatiles was generally well resolved and gave a linear response over the range 0.01–0.1 mg g^{-1} oil with an r^2 exceeding 0.9 in all cases. Of the various SPME fibres, the broad selectivity of the PDMS (100 μm) fibre for a diverse range of volatile analytes has been demonstrated (Perera, Marriott, & Galbally, 2002) and was chosen for this study. Identification of volatiles was based on comparison of retention indices and EI mass spectra with those of authentic samples.

Published work has focussed on the C5, C6 compounds arising from the LOX pathway (Angerosa et al., 2001; Ranalli et al., 2001). In the present work this has been extended to less volatile compounds with retention indices exceeding 1400 on SE-54 (Reiners & Grosch, 1998). The inevitable price of this extension was decreased resolution of compounds eluting in the C5/C6 region of the chromatogram, although GC-MS distinguished compounds in this region. The focus on C5, C6 compounds has also been criticised by Reiners and Grosch (1998) who found that "heavier" volatiles, up to C10 (e.g. deca-2,4-dienal), are also significant components of the volatile aroma fraction.

The most notable feature of the data was the distinction from European oils in which *E*-hex-2-enal was the dominant volatile in the profile of all published oils (e.g. Ranalli et al., 2001). For the Australian oils involved in this study, *E*-hex-2-enal was the major volatile in less than 50% of the oils tested (Table 2). Table 2 lists all volatiles with a concentration exceeding 1.0 mg kg^{-1}. The C5 compounds reported elsewhere (e.g. Angerosa, Basti, & Vito, 1998, 2001) as significant contributors to the volatile profile were not quantitatively as important in these oils. Evidence indicates that C5 compounds are the result of activity of an additional branch of the LOX pathway (Angerosa et al., 2001). Thus, the observed differences in volatile profiles may signal a difference in the relative importance of these pathways in Australian and European oils.

Some volatiles were observed in significant concentrations (between 1 and 3 mg kg^{-1}) but in relatively few oils and are not quoted in Table 2. Representative chromatograms are shown in Fig. 1 using flame ionization detection (FID). These samples represent oils in which *E*-hex-2-enal was the dominant volatile (Fig. 1a) and one in which another compound was dominant (Fig. 1b). Chromatograms obtained with FID and mass spectral detection were similar but an important distinction was seen in the relative intensity of peaks, reflecting differences in relative sensitivities of the two common detectors for the same volatiles. A chromatogram of the rancid oil, sample 36, is shown in Fig. 1c. This oil contained many additional volatiles at much higher concentrations than other oils.

One goal of this work was to identify whether volatile profiles of commercial oils were differentiated by cultivar and processing variables. Given the diversity of oils in this study, differing in cultivar, harvest dates, growers and processors, it was unknown whether statistical techniques would cluster oils or identify a continuum. Both, agglomerative nesting hierarchical clustering (AGNES) and divisive analysis hierarchical clustering (DIANA) were performed to classify the oils into groups formed by similar objects (Kaufman & Rousseeuw, 1990). All data were standardised before the statistical procedures were applied. In AGNES, at commencement of analysis, all cases are considered as separated clusters, and larger clusters are formed by grouping cases into ever increasing clusters until all cases are members of a single cluster. DIANA approaches clustering from the opposite direction, starting with all cases as one cluster and then splits them until there are many clusters. The Hierarchical clustering method is a useful preliminary technique, when it is not known how many groups or partitions are required to describe

Table 2
Concentrations of major volatile compounds in commercial olive oils as determined by SPME and GC with FID

Oil Number—	Concentration (mg kg^{-1} oil as hexanal)[a]														
Retention Index (SE-54)	Ethyl isobutyrate	Hexanal	*E*-hex-2-enal	Hexan-1-ol	Octanal	Hexyl acetate	*E*-oct-2-enal	Nonanal	*E*,*Z*-Nona-2,4-dienal	4-Ethyl guaiacol	*E*,*Z*-deca-2,4-dienal	*E*-4,5-epoxy-*E*-dec-2-enal	Damascenone	Unknown aldehyde I	Unknown aldehyde II
	779	800	855	885	1009	1020	1061	1105	1215	1265	1282	1370	1380	> 1400	> 1400
1	1.8	1.3	4.6			2.4		2.0	4.2	2.2		3.9	9.9	7.6	3.6
2	3.8	10.1	2.0		2.0	2.4		3.1		3.2	2.0	3.7	3.6	4.7	3.3
3	3.3	8.9	2.4		2.1	2.2		3.4	1.6	3.1	2.1	3.5	3.5	4.5	6.1
4	1.6	9.6	2.6		2.5	3.6	2.2	3.2	7.6	3.6	1.7	5.9	11.6	3.4	2.8
5	2.3	4.1	31.4	1.6	3.0	1.7		3.1	3.0	3.7		3.7	2.1	2.6	5.0
6		3.4	11.0		2.5	2.2		2.3	12.3	2.4		3.3	4.7	7.7	4.2
7	2.5	3.0	21.1	1.8	2.5	4.0		2.9	2.0	2.7	1.7	3.6	5.0	2.6	3.6
8	2.1	6.1	14.8		1.9	4.4	2.8	4.7	6.0	3.8	2.6	4.9	12.6	3.5	5.5
9	1.6	2.9	10.6		2.2	3.0		3.3	8.5	2.7	2.6	3.4	3.5	2.9	5.3
10	2.1	3.1	30.7		2.6			2.8		2.8	2.2	3.5	1.7	1.9	3.2
11	1.8	3.4	26.3		2.3	2.2		3.2	2.2	2.8	2.9	3.9	3.7	3.3	3.6
12	1.9	3.0	17.5		1.8	2.0		2.6	4.1	2.8	2.5	5.5	17.3	2.1	5.0
13	1.6	3.1	11.4			1.9	2.9	2.3	8.5	2.4	2.9	3.1	4.7	7.1	3.3
14		3.5	2.7	1.6				2.4	6.8	2.2	2.1	3.0	4.5	25.3	4.2
15	1.7	2.8	24.9	2.1	1.7			2.2		2.6	1.9	3.1	1.7	2.5	3.3
16	1.8	2.0	10.4	1.7	2.0			1.6	3.2	2.2		4.8	14.3	3.5	2.4
17	1.6	2.9	9.8			2.4		1.9	11.1	2.2		3.9	9.9	4.0	3.7
18	1.7	3.1	23.0	1.6	1.8			2.2	3.5	2.2	1.6	2.3	2.0	6.2	2.7
19		2.8						2.0	5.5	2.0		2.5	3.5	33.4	3.8
20	1.9	2.4	16.0	2.9	1.7			2.1	3.9	1.8		2.2	2.5	3.5	2.3
21		2.3	7.8	2.5			1.9	2.0	2.2	2.0	1.7	2.8	5.5	11.3	3.5
22	1.9	4.3						2.0	4.6	1.9		2.7	5.4	30.0	3.2
23	1.8	4.5	2.1	1.8				2.0	2.0	2.5		5.9	5.9	4.4	3.0
24	1.6	2.0	7.5	1.6		1.8	2.1	1.8	7.7	2.0		2.8	4.0	4.9	3.2
25	1.6	1.9	5.7	1.8				2.0	8.2	2.1		2.4	2.8	3.4	3.8
26	1.6	2.7	21.1					2.0	3.7	2.3		3.7	2.3	4.4	2.8
27	1.7	4.1						1.9	4.4	1.9		2.6	5.2	27.6	3.7
28	1.9	3.8	5.4	1.7				1.8	7.8	2.0		2.5	2.7	3.4	2.9
29	1.6	4.0	3.6					1.9	5.0	2.2		3.0	2.9	14.0	2.8
30	1.3	3.5	3.5					1.7	4.9	1.6		2.1	2.9	15.5	2.5
31		3.0	22.4					1.9	1.6	2.8	1.6	2.3	1.6	2.3	2.5
32	1.7	3.0	3.2	1.6				2.0	5.6	1.9		2.5	2.9	22.0	3.2
33	1.6	2.6	4.8		1.7			1.9	2.8	1.7		3.4	1.6	3.3	2.7
34	2.9	6.9	4.6	2.3	2.7	2.0		3.0	3.9	3.3	1.5	3.5	2.8	5.9	2.0
35	9.8	2.9	1.6	3.8	2.0	2.4	1.7	2.3	6.5	2.6		3.2	6.5	4.2	2.8
36		30.7	3.4	19.8	6.6	4.5	2.2	11.0	15.2	15.4	7.8	6.5	5.9		2.4
37	3.1	3.7	2.0	6.5	2.7	2.9		2.6	3.2	2.9	1.7	3.2	3.4	3.2	3.7
38	2.4	2.1	3.0	4.4		7.9		3.2	1.7	2.7	2.1	4.1	9.0	2.7	3.1
39	2.4	2.2	3.3	8.8	1.6		2.0	2.3	9.2	2.4	1.6	3.3	6.6	16.0	4.6
40	2.4	3.3	7.6	2.6	1.7		2.4	2.6	10.0	2.8	1.8	3.4	5.0	13.7	3.6

[a] Standard error for repeat analyses of a single volatile in the same oil was less than 10% for all analytes and generally better than 4%. A blank space means that the compound was not detected at the level of 1.0 mg kg^{-1}.

the data. A limitation of the method is the inability to correct mistakes as the clustering occurs, once an agglomerative algorithm has joined data they cannot be split, while a divisive algorithm cannot rejoin data that has been previously separated (Everitt, Landau, & Leese, 2001). It is therefore not unusual to find minor differences between the results of agglomerative clustering and divisive clustering.

A useful representation of a clustering technique is a dendrogram (Fig. 2) in which the similarity of samples is shown graphically. Both clustering techniques produced the same groups with the exception of oils 9, 33 and 39. Oils 2 and 3, 22 and 27, 25 and 28, that were identical oils (Table 1), were placed in the same groups. Samples that were linked at the first steps of agglomeration came from oils containing a high concentration of *E*-2-hexenal in the case of the cluster containing oils 5, 7, 10, 11, 15, 18, 26 and 31. This cluster comprised all oils derived from the cultivar *Corregiola* plus some other oils. All oils processed from *Manzanillo* fruit were clustered separately and once again were contained within a single cluster but also with some oils obtained from other

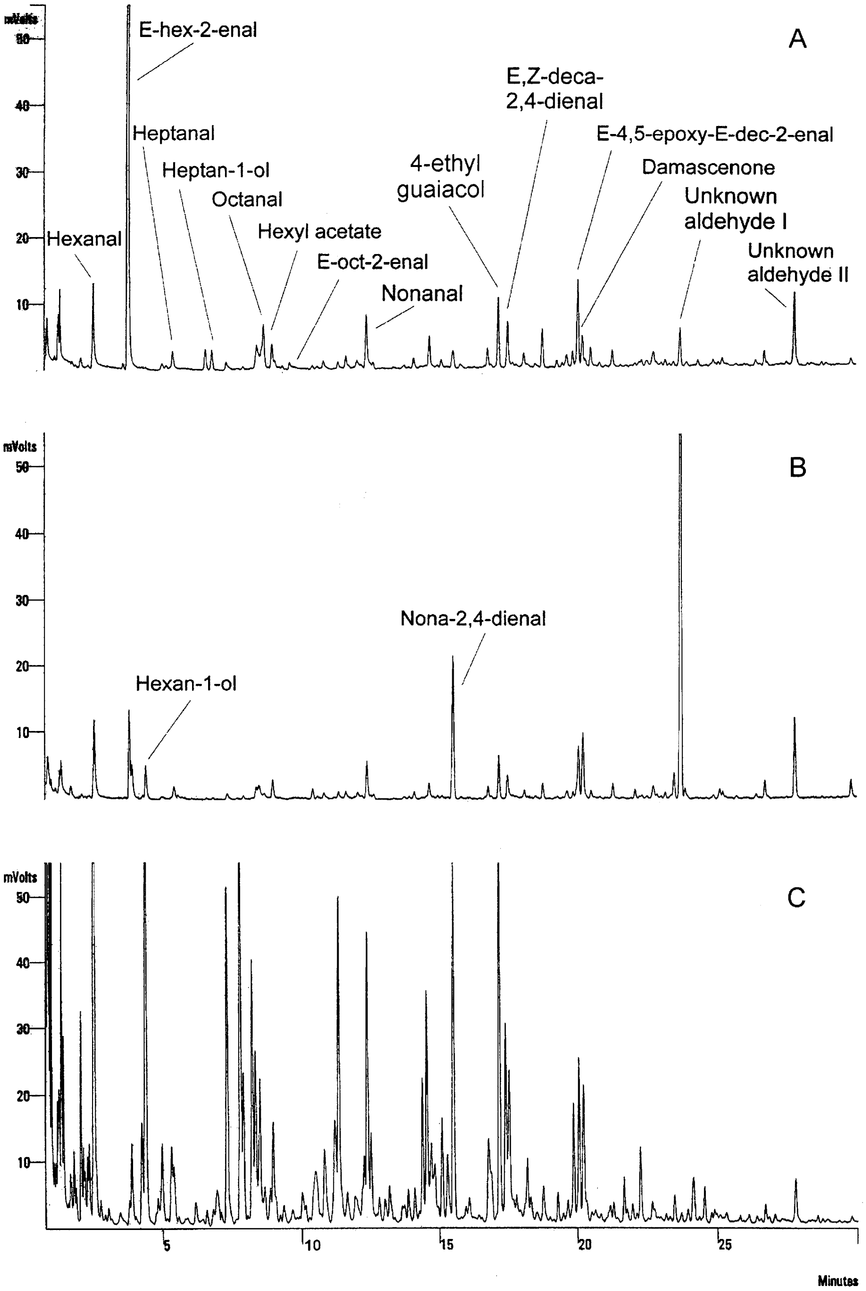


Fig. 1. Representative chromatograms for (a) oil 10 and (b) oil 32 in which *E*-hex-2-enal or another compound is the dominant volatile, respectively. Detection was by FID with other conditions as described under Materials and methods. In (c), the chromatogram of oil sample 36, which was rancid, is shown for comparison. Chromatograms are shown between 0.8 and 30.0 min at constant attenuation rather than being normalized.

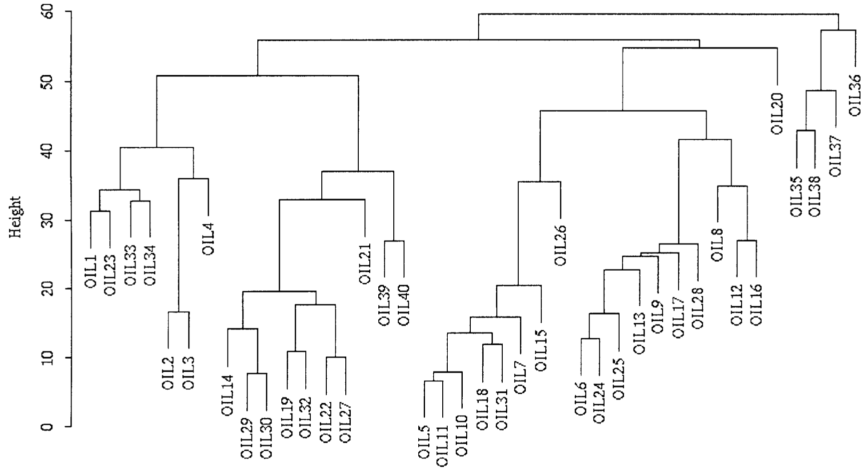
cultivars. Oils produced from fruit harvested in May generally formed distinct clusters showing some similarity to the rancid oil, 36. Nevertheless, the latter was placed in a group distinct from all other oils.

The "*Corregiola* cluster" was characterised by a dominance of *E*-hex-2-enal. The statistical procedures were able to distinguish between oils that had greater than 20 mg kg^{-1} *E*-hex-2-enal (5,7,10,11,15,18,26,31) and those that had less than 20 mg kg^{-1} *E*-hex-2-enal (8,9,12,16,20). In fact AGNES grouped these latter oils in a separate cluster, whereas they appeared on a separate subgroup of a main branch with DIANA. All of these oils typically had 1.5–3.0 mg kg^{-1} octanal—a compound that was not present in most other oils.

The "*Manzanillo* cluster" (oils 14, 19, 22, 27, 29, 30, 32) was characterised by a compound that had a retention index greater than 1400 (Table 2; Unknown aldehyde I). Typically for these oils, the hexanal level exceeded that of *E*-hex-2-enal. A further cluster (oils 21, 39, 40), containing oils from mixed fruit, was characterised by intermediate levels of *E*-hex-2-enal (5–10 mg kg^{-1}) and nona-2,4-dienal in approximately the same concentrations.

The oils used in this study covered several varieties that were harvested on different dates from the same groves. For instance, *Manzanillo* oils 14, 19, 29, 30 and 32 were harvested from a grove near Wagga Wagga in South Western New South Wales on 3, 10, 20 July and 11 August 2001, respectively. These oils appear in the same cluster at a dissimilarity level of 20 for the AGNES plot (Fig. 2). Similar results were observed with oils 12 and 16 harvested on 21 June and 27 July. *Corregiola* oils 10, 26, 31 and 15, harvested on 8 and 29 June and 7 and 27 July, showed less similarity but were still clustered within in the same group. These data suggest that harvest date did not exert a significant

AGNES HCA plot



DIANA HCA plot

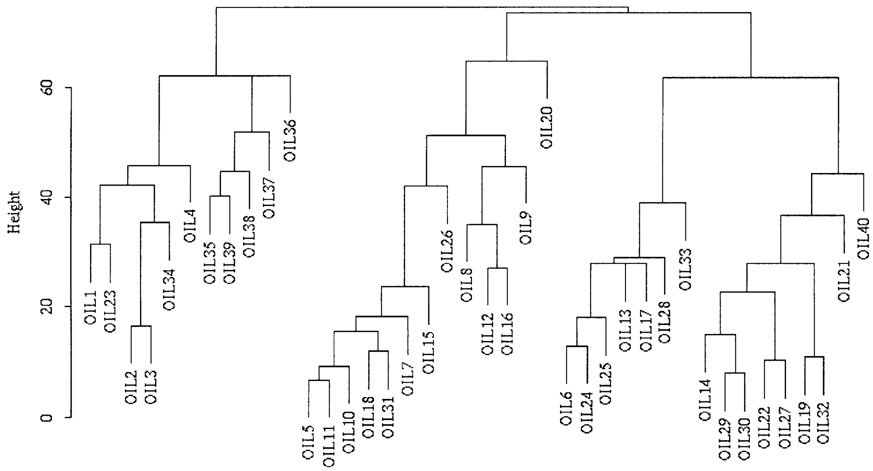


Fig. 2. AGNES and DIANA HCA plot of chromatographic profiles (FID, scaled) from retention times 0.8 to 30 min.

Table 3
Effect of malaxation time and temperature on hexanal and *E*-hex-2-enal contents of laboratory-processed oils

Malaxation conditions—Time (min) / Temperature (°C)	Concentration (mg kg^{-1} oil as hexanal)	
	Hexanal	*E*-hex-2-enal
15/25	3.5±0.8	25.4±3.9
15/35	2.7±0.8	22.4±4.8
60/25	3.9±1.0	27.5±2.9
60/35	3.1±0.6	23.9±2.7

influence on the volatile profile whilst variety was the single-most important factor in determining volatile profile.

Commercial oils were obtained from routine processing that did not allow systematic variation in malaxation time and temperature. However, the effect of these variables on volatile profile remains controversial and was investigated using laboratory-processed oils (scale: 1 kg) from cv *Corregiola*. Temperatures of 25 °C and 35 °C were used with malaxation times of 15 min and 60 min. The major peak in these laboratory-processed oils was *E*-hex-2-enal with lesser amounts of hexanal (Table 3), consistent with the data for commercially processed oils from this cultivar. An increase in malaxation temperature was associated with a reduction in the amount of *E*-hex-2-enal and hexanal whereas the level of both volatiles increased when malaxation time was increased from 15 to 60 min. These changes, however, were not statistically significant ($P < 0.05$), with the exception of decreasing hexanal formation associated with increased malaxation temperature. The decrease in concentration of hexanal with increasing temperature is consistent with Ranalli's results for three cultivars (Ranalli et al., 2001) and Angerosa's results (Angerosa et al., 1998) for cv *Coratina*, but not with cv *Frantoio*, a cultivar closely related to cv *Corregiola*. A decrease in volatile formation with increased malaxation temperature can be attributed to inactivation of hydroperoxide lyases (Salas & Sánchez, 1999) which are critical to formation of the C6 volatiles. For a given cultivar, the competing influences of malaxation time and temperature must be optimized to achieve the most desirable volatile profile.

4. Conclusions

Statistical analysis of the volatile profiles, which was performed blind, to avoid any bias, provided clear clustering of oils from cv *Corregiola* and cv *Manzanillo*, and separation of these oils from oils produced with mixed fruit. Varietal character of the oil produced the principal dissimilarities in volatile profile in the statistical clustering. Moreover, clustering produced identification of oils of unknown origin, consistent with observations made by the processing facility regarding behaviour of the oil. There were differences in clustering for oils produced from the same grove at different harvest dates, but these differences were too random to allow any broad conclusions regarding effect of harvest date on volatile production.

Variations in malaxation time and temperature produced changes in the volatile profile consistent with previous reports; increased temperature and decreased time led to a reduction in the amount of volatiles produced. However, dramatic differences in the major volatile component of oils were observed compared with data on European oils; less than 50% of the Australian oils contained *E*-hex-2-enal as the major component. One of two other compounds (damascenone and Unknown aldehyde I) dominated the chromatograms of most of the remaining oils.

The results of this project indicate the utility of SPME/GC analysis, coupled with statistical analysis, as a technique to analyze for volatiles in olive oils. The profiles of volatiles adsorbed by the fibre provide very reproducible extractions for GC analysis for a single fibre.

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References

Angerosa, F., Basti, C., & Vito, R. (1998). Biogeneration of volatile compounds in virgin olive oil: Their evolution in relation to malaxation time. *Journal of Agricultural & Food Chemistry*, *46*, 2940–2944.

Angerosa, F., Basti, C., & Vito, R. (1999). Virgin olive oil volatile compounds from lipoxygenase pathway and characterization of some Italian cultivars. *Journal of Agricultural & Food Chemistry*, *47*, 836–839.

Angerosa, F., Digiacinto, L., Vito, R., & Cumitini, S. (1996). Sensory evaluation of virgin olive oils by artificial neural network processing of dynamic head-space gas chromatographic data. *Journal of the Science of Food & Agriculture*, *72*, 323–328.

Angerosa, F., Mostallino, R., Basti, C., & Vito, R. (2001). Influence of malaxation temperature and time on the quality of virgin olive oils. *Food Chemistry*, *72*, 19–28.

Angerosa, F., Mostallino, R., Basti, C., Vito, R., & Serraiocco, A. (2000). Virgin olive oil differentiation in relation to extraction methodologies. *Journal of the Science of Food & Agriculture*, *80*, 2190–2195.

Aparicio, R., Morales, M. T., & Alonso, V. (1997). Authentication of European virgin olive oils by their chemical compounds, sensory attributes, and consumers attitudes. *Journal of Agricultural & Food Chemistry*, *45*, 1076–1083.

Aparicio, R., Morales, M. T., & Alonso, M. V. (1996). Relationship between volatile compounds and sensory attributes of olive oils by the sensory wheel. *Journal of the American Oil Chemists Society*, *73*, 1253–1264.

Campeol, E., Flamini, G., Chericoni, S., Catalano, S., & Cremonini, R. (2001). Volatile compounds from three cultivars of *Olea europaea* from Italy. *Journal of Agricultural & Food Chemistry*, *49*, 5409–5411.

Di Giovacchino, L. (2000). Technological aspects. In J. Harwood, & R. Aparicio (Eds.), *Handbook of olive: analysis and properties* (pp. 17–59). Maryland, USA: Aspen, Gaithersburg.

Di Giovacchino, L., Costantini, N., Serraiocco, A., Surricchio, G., & Basti, C. (2001). Natural antioxidants and volatile compounds of virgin olive oils obtained by two or three-phases centrifugal decanters. *European Journal of Lipid Science & Technology*, *103*, 279–285.

Everitt, B. E., Landau, S., & Leese, M. (2001). *Cluster analysis* (4th ed). Arnold, New York.

Kataoka, H., Lord, H. L., & Pawliszyn, J. (2000). Applications of solid-phase microextraction in food analysis [Review]. *Journal of Chromatography*. *A*, *880*, 35–62.

Kaufman, L., & Rousseeuw, P. J. (1990). *Finding groups in data*. Belgium: Wiley Interscience.

Kiritsakis, A. K. (1998). Flavor components of olive oil—a review. *Journal of the American Oil Chemists Society*, *75*, 673–681.

Morales, M. T., Aparicio, R., & Rios, J. J. (1994). Dynamic headspace gas chromatographic method for determining volatiles in virgin olive oil. *Journal of Chromatography*, *668*, 455–462.

Morales, M. T., Rios, J. J., & Aparicio, R. (1997). Changes in the volatile composition of virgin olive oil during oxidation—flavors and off-flavors. *Journal of Agricultural & Food Chemistry*, *45*, 2666–2673.

Perera, R. M. M., Marriott, P. J., & Galbally, I. E. (2002). Headspace solid-phase microextraction-comprehensive two-dimensional gas chromatography of wound induced plant volatile organic compound emissions. *Analyst*, *127*, 1601–1607.

Ranalli, A., & Angerosa, F. (1996). Integral centrifuges for olive oil extraction—the qualitative characteristics of products. *Journal of the American Oil Chemists Society*, *73*, 417–422.

Ranalli, A., Contento, S., Schiavone, C., & Simone, N. (2001). Malaxing temperature affects volatile and phenol composition as well as other analytical features of virgin olive oil. *European Journal of Lipid Science & Technology*, *103*, 228–238.

Ranalli, A., Costantini, N., De Mattia, G., & Ferrante, M. L. (2000). Evaluating two kinds of centrifuged virgin oils arising from continuous olive processing. *Journal of the Science of Food & Agriculture*, *80*, 673–683.

Ranalli, A., De Mattia, G., Patumi, M., & Proietti, P. (1999). Quality of virgin olive oil as influenced by origin area. *Grasas y Aceites*, *50*(4), 249–259.

Ranalli, A., Tombesi, A., Ferrante, M. L., & Demattia, G. (1998). Respiratory rate of olive drupes during their ripening cycle and quality of oil extracted. *Journal of the Science of Food & Agriculture*, *77*, 359–367.

Reiners, J., & Grosch, W. (1998). Odorants of virgin olive oils with different flavor profiles. *Journal of Agricultural & Food Chemistry*, *46*, 2754–2763.

Ryan, D., Antolovich, M., Herlt, T., Prenzler, P. D., Lavee, S., & Robards, K. (2002). Identification of phenolic compounds in tissues of the novel olive cultivar, Hardy's Mammoth. *Journal of Agricultural & Food Chemistry*, *50*, 6716–6724.

Salas, J. J., & Sánchez, J. (1999). The decrease of virgin olive oil flavor produced by high malaxation temperature is due to inactivation of hydroperoxide lyase. *Journal of Agricultural & Food Chemistry*, *47*, 809–812.

Servili, M., Baldioli, M., Begliomini, A. L., Selvaggini, R., & Montedoro, G. (2000). The phenolic and volatile compounds of virgin olive oil: relationships with the endogenous oxidoreductases during the mechanical oil extraction process. In V. Lanzotti, & O. Taglialatela-Scafati (Eds.), *Flavour and Fragrance Chemistry*. London: Kluwer.

Steenson, D. F., Lee, J. H., & Min, D. B. (2002). Solid phase microextraction of volatile soybean oil and corn oil compounds. *Journal of Food Science*, *67*, 71–76.