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Short communication

## Study of volatiles in grapes by dynamic headspace analysis Application to the differentiation of some *Vitis vinifera* varieties

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### Abstract

The aim of our study was to determine the varietal aromas (free volatiles) from grapes by the analysis of isolated compounds according to the method proposed by Salinas and co-workers for wine samples. The results were applied to their differentiation. Grapes of the following varieties were studied: Monastrell, Tempranillo, Cabernet Sauvignon, Dyer Grenache (dark-purple), Airén, Chardonnay and Ugni Blanc. To 100 g of crushed grapes was added a fermentation inhibitor (gentamicine) to impede the formation of new aromas. Skins and juice were kept together for 1 h at 10°C. The samples were purged with He for 20 min at a flow of 84 ml/min and the volatiles were adsorbed in a metal tube packet with Tenax TA. The desorption was carried out by thermal desorption in a kit coupled to a gas chromatograph and a mass spectrometer. The application of cluster analysis to the volatiles was differentiated into three groups: one for white grapes, one for Monastrell, Tempranillo and Cabernet Sauvignon, and the other for Dyer Grenache. Hexyl acetate, benzyl alcohol, phenylethyl alcohol and benzaldehyde were the four discriminant variables used in the group differentiation. © 1999 Elsevier Science B.V. All rights reserved.

**Keywords:** Grapes; Fruits; *Vitis vinifera*; Food analysis

### 1. Introduction

The flavor of grapes, as of any other fruit, is made up of a great number of volatile compounds, among which may be a number of alcohols, esters, acids, terpenes and carbonyl compounds. Their respective concentrations are very low and vary considerably between varieties. Such is the case with certain terpenes whose concentrations are higher in aromatic varieties such as Moscatel (Muscat varieties) than in neutral varieties [1,2] to which most wine producing varieties belong.

The determination of volatiles in fruit involves preparation of sample prior to chromatographic

analysis in order to isolate the compounds from the complex of non volatile material and to determine their concentration. The most frequently used methods for isolation and concentration of flavor constituents from fruits involve extractions [3–8], distillation [9–13] and simultaneous distillation–extraction techniques [14,15]. However, all these techniques are time-consuming and involve excessive manipulation of the sample, which may lead to serious errors. Furthermore, new aromas may arise from the aromatic precursors already present in the samples or from chemical and biochemical reactions promoted by heat, pH and oxidation–reduction conditions. For this reason, the aromas analysed may not reflect the real aromas of the sample [16]. It is, therefore, desirable for analytical techniques to be used which would permit analysis of the volatile

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fraction of a fruit as faithfully as possible to reflect the aroma which naturally reaches the nose. Headspace analysis is such a technique, which makes it possible to analyse the volatile fraction without necessarily attiring it. The dynamic mode involves purging the sample in an inert gas much in the same way as we breathe in the natural fragrance of a fruit, and permits correlation with organoleptic studies.

Recently a dynamic headspace method has been developed which permits the analysis of the volatile fraction of a wine by purging with an inert gas followed by thermal desorption and gas chromatography [17,18]. The aim of the study described in this paper is to adapt this method to the analysis of the volatile fraction of grapes and to the differentiation of different varieties. Seven widely used wine producing varieties were analysed and cluster and principal component statistical treatments were applied.

## 2. Experimental

### 2.1. Plant material

Four red wine grape varieties (Monastrell, Tempranillo, Cabernet Sauvignon and Dyer Grenache) and three white wine varieties (Airén, Chardonnay and Ugni Blanc) were used. Healthy grapes were picked on the 30 September 1996 at an experimental field station belonging to the Albacete (Spain) Provincial Government.

### 2.2. Sample preparation

One hundred g of berries were crushed, to which was added gentamicine, a fermentation inhibitor (Sigma, Alcobendas, Spain) to prevent the formation of new aromas. Skins and juice were kept together for 1 h at 10°C and analysed immediately (skin+juice).

### 2.3. Headspace sampling

The method of Salinas and Alonso [18] was slightly modified for adaptation to the characteristics of the new matrix. To prevent alteration of the volatiles a lower working temperature of 27°C and

higher purge gas flow-rate of 84 ml/min for 20 min were chosen. Helium was used instead of nitrogen because it can be obtained in a purer state. The volatiles were trapped in 0.1703 g of previously conditioned Tenax TA (60 mesh, Alltech, IL, USA) contained in a metal tube of standard dimensions (Perkin-Elmer Hispania, Spain) connected to the instrumental device outline shown by this method.

### 2.4. Analysis of volatile compounds

The packed tube was introduced into a thermal desorber two-stage TD-4 (Perkin-Elmer Hispania) coupled to a gas chromatograph HP 6890 (Hewlett-Packard, Palo Alto, CA, USA) and a mass spectrometer HP 5973. Programmed conditions were: thermal desorption: oven temperature: 300°C, desorption time: 4 min, cold trap low temperature: -30°C, cold trap high temperature: 350°C, injection time: 40 s, transfer line temperature: 200°C, trap adsorbent: Tenax TA (0.02 g±0.1 mg). Gas chromatography: gas carrier: helium 30 p.s.i. (1 p.s.i.=6894.76 Pa), temperature programme: 50°C→180°C (2 min), ramp: 2.5°C/min, temperature detector: 230°C, split ratio: 1:50. Mass spectrometry EI: 70 eV, rate  $m/z$  40–450 u, library: National Institute of Standards and Technology (NIST)

The chromatograph was equipped with an SGE 50 mm×0.22 mm I.D. fused silica capillary column coated with a 0.25- $\mu$ m layer of cross-linked BP-21. Sample components were identified by NIST library and by comparison with the mass spectrum of the commercial standards. Quantification was carried out by the external standard method using the calibration graphs of the corresponding volatiles supplied by Sigma–Aldrich (Alcobendas, Madrid, Spain), Merck (Darmstadt, Germany) and Chem Service (West Chester, CA, USA). The internal standard method was not considered suitable since the method randomly adsorbed the methyl octanoate used by Salinas and Alonso [18] as internal standard in wines. Analyses were carried out in triplicate.

### 2.5. Statistical treatment

To ascertain whether the analysed volatiles permitted the grape varieties to be differentiated, a cluster analysis was first made to group similar varieties.

Discriminant analysis was then applied to identify the most discriminatory variables, using the SPSS statistical package for windows, version 7.5, 2s [19].

### 3. Results and discussion

The different chromatographic profiles obtained

Table 1  
Concentrations ( $\mu\text{g/l}$ ) of volatiles

Group	Monastrell (mean/RSD)	Tempranillo (mean/RSD)	Grenache (mean/RSD)	Cabernet (mean/RSD)	Airen (mean/RSD)	Chardonnay (mean/RSD)	Ugniblanco (mean/RSD)
<i>C<sub>6</sub></i>							
1-Hexanol	708.82/7.6	498.10/8.5	1210.70/4.5	1081.71/6.3	367.95/2.4	439.49/8.4	311.04/9.4
( <i>cis</i> ) 3-Hexen-1-ol	32.09/8.9	33.96/7.6	27.87/6.3	48.29/11.2	338.08/3.1	229.76/5.8	60.79/16.6
( <i>trans</i> ) 2-Hexenal	1720.47/8.1	1164.20/14.3	133.55/2.3	1493.49/5.5	505.39/9.7	786.22/7.7	1081.77/7.3
Total	2461.38	1696.26	1372.12	2623.49	1211.42	1455.47	1453.60
<i>Terpenols</i>							
Linalool	0.84/9.3	1.16/8.5	0.48/7.4	1.22/8.6	n.d.	n.d.	n.d.
Geraniol	14.06/5.5	0.00/0	0.73/17.5	7.21/8.8	n.d.	n.d.	n.d.
Total	14.90	1.16	1.21	8.43	n.d.	n.d.	n.d.
<i>Alcohols</i>							
Benzyl alcohol	89.57/10.7	41.39/9.4	n.d.	76.08/14.5	n.d.	27.35/9.1	20.01/18.8
Phenylethyl alcohol	1270.93/7.8	226.05/17.2	1635.38/8.7	456.78/9.1	43.45/10.3	175.11/9.5	148.13/10.1
1-Pentanol	81.29/3.7	24.74/2.3	79.82/4.1	57.59/2.7	n.d.	n.d.	38.53/5.3
1-Heptanol	2.25/8.1	n.d.	3.82/5.7	n.d.	n.d.	n.d.	3.87/14.2
1-Octanol	3.35/4.3	1.35/7.8	2.31/5.6	1.29/5.2	1.01/3.5	1.52/7.4	1.45/2.2
1-Octen-3-ol	4.00/10.2	5.36/12.3	3.82/8.5	5.36/9.4	1.31/4.3	2.59/9.3	3.63/14.2
Total	1451.39	298.89	1725.15	597.10	45.77	26.57	215.62
<i>Esters</i>							
Ethyl hexanoate	1.75/2.3	n.d.	0.98/5.7	1.57/3.1	n.d.	n.d.	n.d.
Hexyl acetate	12.01/5.2	n.d.	0.69/3.4	2.32/3.7	n.d.	n.d.	1.36/1.8
Ethyl heptanoate	0.90/3.9	n.d.	0.55/6.5	1.20/4.8	n.d.	n.d.	0.42/2.0
Ethyl octanoate	n.d.	4.49/2.8	10.78/1.3	6.66/3.2	1.15/4.1	6.93/2.7	3.36/6.5
Ethyl decanoate	1.33/8.7	n.d.	1.68/8.2	0.98/9.2	0.09/14.1	0.15/11.6	0.16/12.0
Ethyl dodecanoate	16.33/3.4	n.d.	6.26/4.7	2.68/5.4	n.d.	n.d.	n.d.
Total	32.33	4.49	20.94	15.41	1.24	7.08	5.30
<i>Acids</i>							
Isobutyric	475.42/7.3	279.81/12.6	151.15/8.3	174.69/9.6	n.d.	532.43/8.9	258.37/8.2
Hexanoic	112.43/12.4	8.31/16.6	94.87/9.5	76.06/10.3	78.28/11.1	3.91/12.9	n.d.
Heptanoic	53.08/6.8	n.d.	n.d.	n.d.	17.9/5.4	n.d.	n.d.
Octanoic	1114.80/5.6	47.30/7.6	1721.30/6.2	357.65/7.9	76.50/5.2	59.21/6.3	40.08/11.8
Total	1755.73	335.42	1967.32	608.40	171.97	595.55	298.45
<i>Carbonilic</i>							
Benzaldehyde	11.28/3.5	5.79/2.9	4.59/2.3	13.19/3.8	2.53/5.0	6.02/3.1	5.26/4.4
Decanal	1.42/6.7	n.d.	n.d.	n.d.	3.84/4.7	n.d.	n.d.
Total	12.70	5.79	4.59	13.19	6.37	6.02	5.26
<i>Others</i>							
Limonene	9.41/3.7	11.23/6.9	7.07/4.1	8.32/2.2	7.57/3.4	12.49/3.1	5.85/2.9

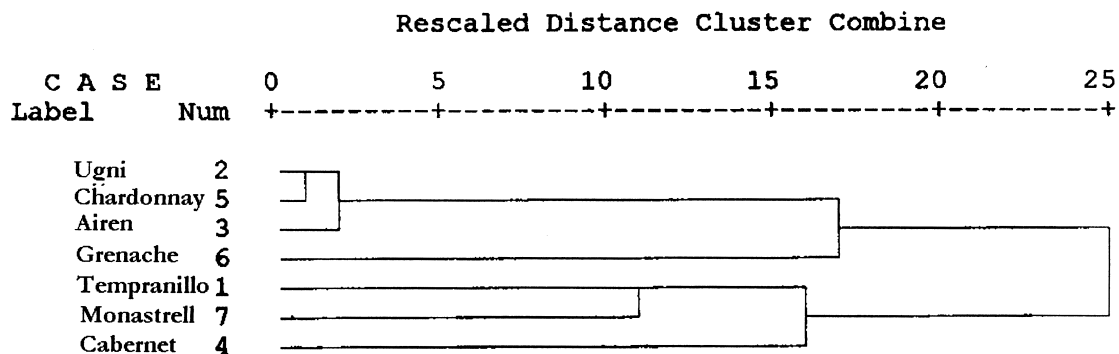


Fig. 1. Dendrogram.

indicate the different aromatic composition of the grapes and constitute a clearly defined “fingerprint” which can be used as a basis for varietal differentiation. Table 1 shows the concentration in  $\mu\text{g}/\text{l}$  of the volatiles present in the seven grape varieties analysed. The predominant compounds were  $\text{C}_6$ , whose formation is favoured by the joint action of oxygen and lipoxygenase type enzymatic systems [20]. These are followed by the acids, the most abundant of which is octanoic acid, and the alcohols, particularly phenylethyl alcohol. No free terpenols were detected in any of the white varieties analysed although previous studies of these same varieties showed that the addition of pectolytic enzymes to the grapes and hydrolysis at pH 3 encouraged the liberation of several terpenols [21]. Linalol and geraniol were only detected in the red varieties.

Cluster analysis permitted us to establish three groups as can be seen from Fig. 1. These groups corresponded to the white varieties, the red varieties (Monastrell, Tempranillo and Cabernet Sauvignon) and, on its own, Dyer Grenache. Discriminate analysis pointed to four variables (hexyl acetate, benzyl alcohol, phenylethyl alcohol and benzaldehyde) which provided a function which correctly grouped the grapes into the already established groups. Table 2 shows the coefficient for Fisher’s discriminant linear function.

Based on the volatile compounds analysed by dynamic headspace it was possible to establish different groups containing similar aromatic properties. These groups can be differentiated by the use of only four of the variables analysed.

Table 2  
Coefficients of Fisher

	Groups <sup>a</sup>		
	1	2	3
Hexyl acetate	-4.551	-6.525	-117.664
Benzyl alcohol	0.169	-1.442	-18.891
Phenylethyl alcohol	0.050	0.112	1.876
Benzaldehyde	-0.151	6.334	71.618
Constant	-11.623	-9.654	-1659.083

<sup>a</sup> Group 1=White varieties; group 2=Monastrell, Tempranillo and Cabernet Sauvignon; group 3=Dyer Grenache.

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