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Volatile components of Zalema white wines

M. José Gómez-Míguez [a], Juan F. Cacho [b], Vicente Ferreira [b], Isabel M. Vicario [a], Francisco J. Heredia [a,*]

[a] Laboratory of Food Colour and Quality, Department of Nutrition and Food Science, Faculty of Pharmacy, University of Seville, 41012 Seville, Spain
[b] Department of Analytical Chemistry, Faculty of Science, University of Zaragoza, 50009 Zaragoza, Spain

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Abstract

The volatile composition of young white wines from *Vitis vinifera* cv. Zalema, an autochthonous grape variety in Huelva (southern Spain), has been studied by gas chromatography-olfactometry (GC-O) and techniques of quantitative analysis. This is the first time that an olfactometric analysis has been reported in wines made from this grape variety. The quantitative chemical study has shown 71 volatile compounds, of which 23 were in concentrations above their thresholds. On the basis of the odour activity values (OAVs), the most potent odorants were fermentative compounds, mainly fatty acids and their ethyl esters. Two norisoprenoids, β-damascenone and β-ionone, two alcohols (isoamyl alcohol and β-phenylethanol), three volatile thiols, 4-mercapto-4-methyl-2-pentanone, 3-mercaptohexyl acetate and 3-mercapto-1-hexanol, and two carbonyl compounds (acetaldehyde and phenylacetaldehyde) also exhibited OAVs > 1. The GC-O study corroborated these results, showing that five esters (isoamyl acetate, ethyl hexanoate, ethyl butyrate, ethyl isovalerate and ethyl octanoate), isoamyl alcohol and β-damascenone can be considered as the most powerful odorants of Zalema wines.

Keywords: Analysis; Aroma; Gas chromatography-olfactometry; White wine; Zalema

1. Introduction

In recent sensory studies based on consumer preferences, the flavour of wine was found to be one of the most important attributes considered when buying wines (Yegge & Noble, 2001). But the flavour of a wine presents an extremely complex chemical pattern in both qualitative and quantitative terms. Over 1000 volatile compounds have been found in wines, with a wide concentration range varying from hundreds of mg/l to the μg/l or ng/l level. It is well known that the chemical compounds responsible for wine aroma are mainly alcohols, esters, acids, aldehydes and ketones, of which esters are particularly important (Rapp & Mandery, 1986). However, the particular importance of each compound to the final aroma is related to its odour perception threshold, which is defined as the lowest concentration that can be detected by smelling. Therefore, the concentration/threshold ratio, known as the "odour activity value" (OAV), allows us to estimate the contribution of a specific compound to the aroma of a wine. However, most of the volatile compounds are found at concentrations near or below their individual sensory thresholds. This complexity has made it almost compulsory to begin any wine aroma research with gas chromatography-olfactometry (GC-O) analysis, a technique which helps to establish the most important odorants. Of the analytical tools that correlate sensory and instrumental analysis, GC-O seems to be the most appropriate technique because, thanks to this technique, the human and electronic responses are combined to maximize the available detection capabilities (Mayol & Acree, 2001). Recent studies of wines based on GC-O have identified almost all of the most important wine odorants (Aznar, López, Cacho, &

* Corresponding author. Tel.: +34 954556761; fax: +34 954557017.
E-mail address: heredia@us.es (F.J. Heredia).

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Ferreira, 2001; Ferreira, Aznar, López, & Cacho, 2001; Guth, 1997a) and previously published articles have reported that intensity data in GC-O techniques provide useful information (Ferreira, Ortín, Escudero, López, & Cacho, 2002; Guth, 1997b).

Zalema is an autochthonous white grape variety of *Vitis vinifera* grown exclusively in Huelva (southwest of Spain), where it represents over 90% of the overall production. The production of monovarietal young wines with this grape is frequent. Nowadays careful wine making, with early harvests and exhaustive fermentation control, are producing good-quality young white wines from this variety.

To our knowledge, only one scientific study on the volatile composition of Zalema wines has been previously published (Hernanz, Heredia, Beltran, & Recamales, 1999). The study reported that the aroma of Zalema wines is mainly composed of higher alcohols and esters formed through the fermentation process, which provide "fruity", "clean" and "fresh herb" flavours.

Therefore, given the lack of information about the aromatic profile of these wines, the aim of the present work is to study more in depth the volatile composition of Zalema white wines, on the basis of GC-O analysis and odour activity values (OAVs), in order to elucidate the most potent aroma compounds of these wines. The GC-O method used in this work is a novel technique of which one of the main features is that the extract is prepared by a sensitive dynamic headspace sampling technique.

2. Materials and methods

2.1. Wine samples

A total of nine bottles of Zalema white wines were analysed in this study. They were kindly donated by three different wineries (3 manufacturers × 3 bottles). All of them were elaborated using standard wine-making practices, established by the Regulating Council for the *Condado de Huelva* Denomination of Origin. The samples (2002 vintage) were taken five months after wine making and then analysed.

As no significant differences were found neither within the same winemaker nor between them, results in this work are expressed as mean of all samples.

2.2. Reagents and standards

All the reagents used were of analytical quality. Solvents: dichloromethane of HPLC quality (Fisher Scientific, Loughborough, UK), methanol of LiChrosolv quality (Merck, Darmstadt, Germany), absolute ethanol (Panreac, Barcelona, Spain). Pure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA). The reagents used were solid anhydrous ammonium sulfate and tartaric acid (ACS-ISO quality) (Panreac, Barcelona, Spain), LiChrolut resins, prepacked in 200, 400 and 1000 mg cartridges (Merck, Darmstadt, Germany), α,α,α-tris-(hydroxymethyl)-methylamine (Tris) 99.9% (Aldrich-España, Madrid, Spain), cysteine 99% and *p*-hydroxymercuribenzoic acid (Sigma, St. Louis, MO). The BHA (3-*tert*-butyl-4-hydroxyanisole) solution contained 1 g of this compound per 100 mg of ethanol. The chemical standards used were 2-butanol, 4-methyl-2-pentanol, 2-octanol and 4-hydroxy-4-methyl-2-pentanone, supplied by Merck (Darmstadt, Germany), PolyScience (Miles, USA), PolyScience (Miles, USA) and Aldrich (Gillingham, UK), respectively.

2.3. Quantitative analysis of aroma compounds

2.3.1. Major compounds (liquid–liquid microextraction and GC-FID analysis)

Quantitative analysis and identification of major compounds were carried out by the procedure described by Ortega, López, Cacho, and Ferreira (2001). In accordance with this method, 3 ml of wine and 7 ml of water were salted with 4.5 g of $(NH_4)_2SO_4$ and extracted with 0.2 ml of dichloromethane. The extract was then analyzed by GC with FID detection. A HP5890 series II gas chromatograph equipped with an HP7673A automatic sampler was used. The column (50 m × 0.32 mm and 0.5 μm film thickness) was a DB-Wax 20 from J&W Scientific (Folsom, CA, USA), preceded by a 2 m × 0.53 mm uncoated pre-column. The temperature program was as follows: 40 °C for 5 min, then raised at 3 °C/min up to 200 °C. Injector and detector were both kept at 250 °C. Carrier gas was H_2 at 3 ml/min, the split flow was 30 ml/min, and the injection (3 μl) was performed in split mode. Quantitative data were obtained by interpolation of relative peak areas in the calibration graphs built by the analysis of synthetic wines containing known amounts of the analytes. 2-Butanol, 4-methyl-2-pentanol, 4-hydroxy-4-methyl-2-pentanone and 2-octanol were used as internal standards. Each sample was extracted in triplicate.

2.3.2. Minor compounds (SPE and GC-ion trap-mass spectrometry)

Minor compounds were determined and identified by the method proposed by López, Aznar, Cacho, and Ferreira (2002). Wine (50 ml), containing 25 μl of BHA solution, were passed through a Lichrolut EN cartridge at around 2 ml/min. The SPE cartridge had been previously conditioned with 4 ml of dichloromethane, 4 ml of methanol and, finally, with 4 ml of a water–ethanol mixture (12%, v/v). The sorbent was dried by letting air pass through it (−0.6 Bar, 10 min). Analytes were recovered by elution with 1.3 ml of dichloromethane. The internal standard solution (25 ml, containing 2-octanol, 4-methyl-2-pentanol and 4-hydroxy-4-methyl-2-pentanone) was added to the eluted sample. The extract was then analysed by GC with ion trap MS. The GC was a Star 3400CX fitted to a Saturn 4 electronic impact ion trap mass spectrometer from Varian. The column used was a DB-WAXetr from J&W

(Folsom, CA, USA), 60 m × 0.25 mm with 0.5 μm film thickness, and was preceded by a 3 m × 0.32 mm uncoated (deactivated, intermediate polarity) precolumn. The carrier gas was He at 1 ml/min. The temperature program was as follows: 40 °C for 5 min, raised to 230 °C at 2 °C/min. A 1096 septum-equipped programmable injector (SPI) from Varian was used. The initial temperature of this injector was 30 °C for 0.6 min and was then raised to 230 °C at 200 °C/min. Three microlitres of sample were injected. A 35–220 *m*/*z* mass range was recorded. Quantitative data were obtained by interpolation of relative peak areas in the calibration graphs obtained from the GC–MS analysis of dichloromethane solutions containing known amounts of the analytes and of the internal standards. Each wine sample was extracted in triplicate.

2.3.3. 3-Mercaptohexyl acetate, 2-furfurylthiol, 3-mercapto-1-hexanol, 4-mercapto-4-methyl-2-pentanone, and 2-methyl-3-furanthiol (SPE and GC-ion trap MS analysis)

One gram of LiChrolut EN resin was dry-packed in a 6 ml polypropylene cartridge. Resins were conditioned with 10 ml of dichloromethane, 10 ml of methanol and then washed with 10 ml of an aqueous ethanol solution (13% ethanol v/v). Wine (200 ml) containing 200 μl of BHA solution were then passed through the resins at a maximum speed of 4 ml/min. The bed of resin was then washed with 200 ml of a solution of Tris (2.42 g/100 ml, 40% methanol v/v, pH 7.2) and dried, and finally the odorants were eluted with 10 ml of dichloromethane.

This organic phase was extracted with four successive additions of 1 ml of a 1 mM *p*-hydroxymercuribenzoate solution in Tris at pH 7.2. The four aqueous phases were combined and added with 600 μl of a 200 mM cysteine solution in Tris at pH 7.2. The aqueous solution was then extracted with three successive additions of 0.8, 0.4 and 0.4 ml of dichloromethane. The three organic phases were combined and added with 40 μl of the internal standard solution (2-octanol (100 ppm in dichloromethane)). Finally, the extract was concentrated to 100 μl by heating at 48 °C.

The extract (20 μl) was analysed by GC–MS. The GC was a CP3800 fitted to a Saturn 2200 electronic impact ion trap mass spectrometer from Varian. The column was a DB-WAXetr from J&W (Folsom, CA, USA), 60 m × 0.25 mm × 0.25 μm. The carrier was He at 1 ml/min. The temperature program was the following: 40 °C for 5 min, then raised to 170 °C at 2 °C/min and, finally, to 230 °C at 20 °C/min. A 1079 PTV injector from Varian (NY, USA) was used under the following injection program: initial 40 °C for 0.60 min and then raised to 250 °C at 100 °C/min. The purge valve was opened the first 0.4 min and then closed until 4.8 min. MS acquisition was carried out in selected ion storage (SIS) mode of an ionic range from 73 to 134 *m*/*z* for 2-methyl-3-furanthiol and 4-mercapto-4-methyl-2-pentanone and from 70 to 135 for 3-mercaptohexyl acetate, 2-furfurylthiol, and 3-mercapto-l-hexanol. The *m*/*z* quantitative fragments were 114, 75, 88, 81, and 82 *m*/*z*, respectively. Each wine sample was extracted in triplicate.

2.4. GC-O analysis

GC-O analysis was carried out on the wine which had been rated highest in a previous sensory analysis. In this way, the chosen wine reached the highest scores in relation to fruity notes (pear, apple, banana, etc.) and fresh notes (citric, herbaceous), while notes of oxidation (rancid, overriped fruit, cauliflower, sulphurous) were the lowest scored, compared to the other wines.

2.4.1. Preparation of extract

Wine extract was obtained by a dynamic headspace sampling technique. (Campo, Ferreira, Escudero, & Cacho, 2005). According to these authors, this headspace strategy makes it possible to obtain simpler and cleaner olfactograms than those obtained when extracts are obtained by other methods, such as solid-phase extraction. Furthermore, this technique allows us to obtain higher differences in GC-O scores, which facilitates the ranking of odorants according to their potential importance.

A standard SPE cartridge (0.8 cm internal diameter, 3 ml internal volume) filled with 400 mg of LiChrolut EN resin was first washed with 2 ml of methanol and 20 ml of dichloromethane and then dried by letting air pass through for 15 min. At the same time, a mixture of 80 ml of wine and 20 ml of ''synthetic saliva'' solution (containing 0.168 g $NaHCO_3$, 0.048 g K_2HPO_4, 0.166 g KH_2PO_4 and 0.088 g NaCl per 100 ml) was poured into a bubbler flask. The SPE cartridge was placed on the top of the bubbler flask, to which a stream of nitrogen was also connected. The stream of nitrogen was passed for 200 min, at around 100 ml/min, through the fritted tubing into the wine and saliva solution. Volatile wine constituents released in the headspace were transporting through the bubbler sidearm to the cartridge, where they were trapped. During the extraction, the mentioned system (a purge-and-trap system) was placed in a water bath at 37 °C. This system represents an ''artificial mouth'', where the artificial saliva represents the retronasal perception of the odorants (Campo et al., 2005; Roberts & Acree, 1996). The trapped volatiles were eluted from the trap (the cartridge) with 3.25 ml of dichloromethane, and the extract was finally concentrated to a final volume of 0.2 ml under a stream of N_2.

2.4.2. Sniffing

The concentrated extract of the wine was used in the GC-O analysis: a ''posterior intensity method''. This GC-O technique is based on the measurement of the intensity of the eluted odours by using a single posterior rating scale. This technique is useful for discovering the most powerful odorants into a sample (Van Ruth & O'Connor, 2001). Sniffings were carried out using an 8360GC (Fisons Instruments) equipped with a flame ionization detector (FID)

and a sniffing port (ODO-1 from SGE, Melbourne, Australia) connected by a flow splitter to the column exit. The split ratio used between the FID and the sniffing port was 1:1. The column used was a DB-Wax from J&W (Folson, CA, USA), 30 m × 0.32 mm with 0.5 μm film thickness. The carrier gas was H_2 at 3 ml/min. One microlitre was injected in splitless mode. Injector and detector were both at 250 °C. The temperature program was the following: 40 °C for 5 min, then at 4 °C/min up to 100 °C and, at 6 °C/min up to 200 °C for 15 min, then raised to 230 °C at 50 °C/min.

Eight trained judges performed olfactometric analysis of the extract. All of them were extensive experienced in GC-O. Each judge evaluated the wine extract once in two time segments of 30 min in order to avoid fatigue. Each judge carried out one session per day. Judges were asked to measure the overall intensity of each perceived odour by using a 0–3 scale (0 = not detected; 1 = extremely weak odour; 2 = clear odour; 3 = intense odour) with seven possible scores (half values allowed). The eight intensity scores obtained for each odorant in the wine extract were averaged to give the mean intensity score for the odorant in the sample.

The identification of the odorants was carried out by comparison of their olfactory descriptions, their chromatographic retention index (RI), and MS spectra with those of pure reference compounds.

3. Results and discussion

3.1. Quantitative analysis

Quantitative data of the volatile compounds found in the monovarietal white wines from Zalema variety are shown in Table 1. The data are expressed as means (μg/l) of the GC analyses of triplicate extractions and they correspond to the average of the analysed wines. Table 1 also shows the perception thresholds and their corresponding calculated OAVs for each aroma compound identified.

Improvement in the analytical method used to extract the volatile compounds from these wines has allowed us to identify and quantify a higher number of volatile compounds in Zalema white wines: from 24 free aroma compounds, previously reported by Hernanz et al. (1999), to 71 compounds (Table 1), including carbonyl compounds, norisoprenoids, esters, alcohols, acids, phenols, terpenes, and thiols. They have been positively identified and quantitatively determined. Among the compounds found, well-known by-products of yeast metabolism were the most abundant substances. Thus, volatile compounds which reached the highest levels were, respectively, alcohols, acids (mainly C_4–C_{12} fatty acids) and esters (mean total concentration = 196.25 mg/l, 39.85 mg/l and 31.28 mg/l, respectively). Higher alcohols and esters, produced during alcoholic fermentation, play an important role in the flavour of wines, depending on the types of compounds and their concentrations (Valero, Moyano, Millán, Medina, & Ortega, 2002).

Alcohols are quantitatively the largest group of volatile compounds in Zalema wines, in accordance with previously published results, that indicate that alcohols represent 80–90% of the aromatic content of wines (Usseglio-Tomasset, 1998). These compounds can be recognized by their strong and pungent smell and taste and they are related to herbaceous notes. At concentrations above 400 mg/l, they are regarded as negative quality factors (Rapp & Versini, 1991). However, the total concentration of higher alcohols in Zalema wines analysed was below 200 mg/l (mean total = 196.35 mg/l). As can be seen in Table 1, among the alcohols, Zalema wines contained high amounts of isoamyl alcohol, β-phenylethanol and isobutanol. Alcohols with six carbon atoms, which supply "vegetal" and "herbaceous" nuances to the wine, usually have a negative effect on wine quality when their concentration is above their odour threshold values (Ferreira et al., 1995). However, these compounds (1-hexanol and *Z*-3-hexen-1-ol) were found at concentrations under their odour threshold values in the analysed Zalema wines.

Fatty acids have been described with fruity, cheese, fatty, and rancid notes (Rocha, Rodrigues, Coutinho, Delgadillo, & Coimbra, 2004). Among these compounds, decanoic acid, octanoic acid and hexanoic acid were present at high concentrations (Table 1).

In terms of the number of components identified, esters represent the largest group (20 individual compounds). As can be seen in Table 1, the highest levels were observed for ethyl lactate, ethyl acetate, diethyl succinate, isoamyl acetate, ethyl hexanoate and ethyl octanoate (mean values = 19.43 mg/l, 5.00 mg/l, 2.59 mg/l, 1.09 mg/l, 0.78 mg/l and 0.77 mg/l, respectively). These compounds are important in young wine aroma and are among key compounds in the fruity flavours of wines (Rapp & Mandery, 1986). It can be seen that the ethyl esters of fatty acids were more abundant than the acetates of higher alcohols. This fact means that, according to Ferreira, Fernández, Peña, Escudero, and Cacho (1995), the fruity character attributed to the aroma of Zalema wines is mainly related to tree fruit aroma notes (apple, pear, peach, cherry, etc.).

Some compounds considered off-flavours and related to young white wine oxidation, such as acetaldehyde and other carbonyl compounds, have also been detected (Table 1). Acetaldehyde has been found in high levels in relation to other carbonyl compounds, but its mean content was lower than in other non-oxidized white wines previously studied (30–100 mg/l) (Escudero, Asensio, Cacho, & Ferreira, 2002).

Lactones could be formed as artefacts from the chromatographic injection of their corresponding acids, or even by their silica catalyzed cyclisation. However, Ferreira, Fernández, Gracia, and Cacho (1995) have demonstrated that the lactones detected in wine extracts are mainly natural products of wines. The most abundant lactone in Zalema wines was γ-butyrolactone. This compound is associated with fruity, butter and rubber descriptors (Rocha et al., 2004).

Table 1
Quantitative data, odour thresholds and odour activity values

Compound	Average content (μg/l)	Odour threshold [a] (μg/l)	OAV[b,*]
Carbonyl compounds			
Acetaldehyde	2962.7 ± 173.8	500[1]	**5.93**
Acetoin	2992.1 ± 159.3	150000[8]	0.02
Diacetyl (2,3-butanedione)	81.8 ± 44.4	100[2]	0.82
Furfural	60.4 ± 33.1	14100[2]	0.00
Phenylacetaldehyde	1.5 ± 0.7	1[*]	**1.54**
5-methylfurfural	8.9 ± 2.0	20000[8]	0.00
5-hidroxymethylfurfural	90.7 ± 49.6	unknown	unknown
β-damascenone	0.7 ± 0.6	0.05[1]	**14.82**
β-ionone	0.2 ± 0.1	0.09[2]	**2.43**
Esters			
Ethyl isobutyrate	220.1 ± 71.2	15[2]	**14.68**
Ethyl isovalerate	14.1 ± 9.4	3[2]	**4.70**
Ethyl 3-hydroxybutyrate	127.9 ± 77.1	20000[*]	0.01
Ethyl acetate	5000.0 ± 0.0	12264[1]	0.41
Isoamyl acetate	1090.9 ± 100.1	30[1]	**36.36**
Phenylethyl acetate	213.8 ± 90.8	250[1]	0.86
Ethyl butyrate	374.8 ± 38.3	20[2]	**18.74**
Ethyl hexanoate	783.3 ± 77.5	14[2]	**55.95**
Ethyl octanoate	773.3 ± 45.0	5[2]	**154.67**
Ethyl decanoate	544.4 ± 26.0	200[2]	**2.72**
Ethyl lactate	19427.7 ± 36.9	154636[8]	0.13
Diethyl succinate	2586.1 ± 120.2	200000[8]	0.01
Ethyl 2-methylbutyrate	11.2 ± 4.4	18[2]	0.62
Butyl acetate	5.6 ± 2.4	1880[8]	0.00
Isobutyl acetate	70.9 ± 47.6	1600[8]	0.04
Ethyl cinnamate	0.1 ± 0.3	1.1[2]	0.16
Ethyl dihydrocinnamate	1.1 ± 1.2	1.6[2]	0.72
Ethyl furoate	20.8 ± 12.9	16000[4]	0.00
Ethyl vanillate	6.6 ± 2.1	990[4]	0.01
Methyl vanillate	2.7 ± 0.9	3000[4]	0.00
Alcohols			
1-butanol	2065.7 ± 113.8	150000[8]	0.01
Isobutanol	20639.0 ± 350.8	40000[1]	0.52
Isoamyl alcohol	149528.3 ± 730.8	30000[1]	**4.98**
β-phenylethanol	22547.9 ± 425.1	14000[2]	**1.61**
1-hexanol	824.3 ± 86.29	8000[2]	0.10
Z-3-hexenol	353.3 ± 25.9	400[2]	0.88
Benzyl alcohol	36.6 ± 17.2	200000[*]	0.00
Methionol	252.1 ± 80.8	1000[1]	0.25
Furfuryl alcohol	10.90 ± 3.3	2000[6]	0.01
Acids			
Propanoic acid	1621.1 ± 99.3	8100[2]	0.20
Butyric acid	995.9 ± 90.9	173[2]	**5.76**
Isobutyric acid	410.6 ± 40.2	230[2]	**1.79**
Isovaleric acid	349.3 ± 35.7	33.4[2]	**10.46**
Hexanoic acid	9499.1 ± 209.3	420[2]	**22.62**
Octanoic acid	9766.8 ± 218.7	500[2]	**19.53**
Decanoic acid	16861.3 ± 6076.2	1000[2]	**16.86**
2-methylbutyric acid	233.4 ± 43.3	50[6]	**4.67**
Phenylacetic acid	103.6 ± 15.7	1000[9]	0.10
Benzoic acid	9.0 ± 2.1	1000[*]	0.01
Volatile Phenols			
Guaiacol	0.7 ± 0.3	9.5[7]	0.07
Isoeugenol II	1.0 ± 0.3	6[*]	0.17
4-ethylguaiacol	0.1 ± 0.1	33[7]	0.01
4-vinylphenol	105.5 ± 13.1	180[7]	0.59
4-vinylguaiacol	462.8 ± 44.1	1100[7]	0.42
4-allyl-2,6,-dimethoxyphenol	3.4 ± 1.7	1200[6]	0.00
m-cresol	1.0 ± 0.7	68[2]	0.05
o-cresol	1.8 ± 0.3	31[4]	0.06
Vanillin	6.4 ± 2.6	60[*]	0.11
Acetovanillone	62.6 ± 5.4	1000[*]	0.06

Table 1 (*continued*)

Compound	Average content (μg/l)	Odour threshold[a] (μg/l)	OAV[b,*]
Terpenes			
α-terpineol	25.7 ± 5.5	250[2]	0.10
Linalool	11.3 ± 1.1	25[2]	0.46
β-citronellol	2.2 ± 0.8	100[8]	0.02
Lactones			
γ-butyrolactone	3125.4 ± 105.2	unknown	unknown
δ-octalactone	15.2 ± 0.6	400[6]	0.04
δ-decalactone	23.2 ± 0.7	386[2]	0.06
γ-nonalactone	18.7 ± 2.5	30[2]	0.63
γ-decalactone	0.9 ± 0.5	88[2]	0.01
Thiols			
2-methyl-3-furanthiol	n.d.	0.005[5]	<0.1
4-mercapto-4-methyl-2-pentanone	0.005 ± 0.0	0.0008[5]	**6.25**
Furfurylthiol	n.d.	0.0004[5]	<0.1
3-mercaptohexyl acetate	0.024 ± 0.0	0.0042[5]	**5.71**
3-mercapto-1-hexanol	0.098 ± 0.0	0.060[5]	**1.63**

[1] Guth (1997b). The matrix was a 10% water/ethanol solution; [2] Ferreira et al. (2000). The matrix was a 11% water/ethanol solution containing 7 g/l glycerol and 5 g/l tartaric acid, with the pH adjusted to 3.4 with 1 M NaOH; [3] and [4] Ferreira et al. (2002), and López et al. (2002). The matrix was a 10% water/ethanol solution at pH 3.2; [5] and [8] Tominaga et al. (1998), and Etiévant (1991). Thresholds were calculated in a 12% water/ethanol mixture; [6] Van Gemert and Nettenbreijer (1977). The matrix was water: [7] Boidron et al. (1988). The matrix was a synthetic wine containing 12% ethanol, 8 g/l glycerol, and different salts; [9] Maga (1973); [*] Calculated in the Laboratory of Aroma Analysis and Enology, Department of Analytical Chemistry, University of Zaragoza, Spain. Orthonasal thresholds were calculated in a 10% water/ethanol mixture containing 5 g/l of tartaric acid at pH 3.2.
n.d., not detected.

[a] Reference from which the value has been taken is given in parentheses.

[b] Odour activity value calculated by dividing concentration by odour threshold value of the compound.

[*] In bold, compounds with OAV > 1.

Other remarkable compounds, such as volatile phenols, and norisoprenoids have also been detected. The volatile phenols play an important role in wine aroma. Ethylphenols are responsible for animal and smoky odours, while vinylphenols can be responsible for heavy pharmaceutical odours (Castro, Natera, Garacía, & García, 2003). As can be seen in Table 1, vinylphenols (4-vinylguaiacol and 4-vinylphenol) were the most abundant volatile phenols detected. This result corroborates that vinylphenols are the main phenols in white wines, while ethylphenols are more abundant in red wines (Boidron, Chatonnet, & Pons, 1988). Vinylphenols can be generated either from cinnamic acids as progenitors through yeast fermentation or as artefacts from the same acids in the GC injector (Boido et al., 2003). They have a characteristic "meaty smoky" odour (Falqué, Fernández, & Dubourdieu, 2001). The norisoprenoids detected and quantified in the wines were β-damascenone and β-ionone, the first of which was found at the highest levels (0.7 μg/l). This compound is related to flowery, sweet and fruity notes, while β-ionone supplies an aroma of violets.

The group of terpenes and thiols showed the lowest values in the Zalema wines analysed (mean total concentration = 39.20 μg/l and 0.127 μg/l, respectively). With reference to the detected free monoterpenes, α-terpineol was the most abundant (mean value 25.7 μg/l). With regard to volatile thiols, 3-mercapto-1-hexanol and 3-mercaptohexyl acetate were the most abundant thiols found in Zalema wines, in agreement with data reported for other red and white wines (Ortín, Ferreira, & Cacho, 2003).

3.2. Active odorants

From all the volatile compounds identified, those present at concentrations higher than their odour threshold are mainly considered as aroma-contributing substances. As can be seen in Table 1, 23 out of 71 components (32%) identified and quantified in the Zalema wines were found at concentrations higher than their corresponding threshold values (OAVs > 1). Therefore, only a few volatile compounds are potentially active odorants. The number of active odorants present in Zalema wines is similar to those found in other young white and rose wines (Escudero et al., 2004; Ferreira et al., 2002; López, Ortin, Pérez-Trujillo, Cacho, & Ferreira, 2003). As odour thresholds are affected by additive, synergic and antagonistic effects of the volatile compounds in a matrix, the identification of the most powerful odorants only on the basis of their OAV values should be considered as a tentative study.

According to the odour activity values (Table 1), the most important odorant of Zalema wines was ethyl octanoate, a compound associated with ripe fruits, pear and sweet notes (mean OAV = 155). Ethyl hexanoate was the next most significant compound (mean OAV = 56). In general, several fermentative compounds, mainly fatty acids and their ethyl esters were the most powerful odorants of these wines. This fact corroborates the typical fruity nuances associated to these wines. As a group, these compounds are able to exert a strong influence on wine aroma: they are responsible for a major part of the aroma

characteristics of a young white wine (Falqué & Fernández, 1999). These compounds have also been found as relevant volatile compounds in other Spanish white wines, as Albariño wines (OAVs < 10) (Falqué et al., 2001) or Maccabeo wines (5 < OAVs < 140) (Escudero et al., 2004). Within the group of the acetates of higher alcohols, only isoamyl acetate, with a characteristic "banana" odour, was found as an active odorant (OAV = 36.4). The presence of other esters, specifically ethyl acetate, phenylethyl acetate, ethyl 2-methylbutyrate and ethyl dihydrocinnamate, although exhibiting OAVs lower than one (0.2 < OAV < 1.0) also could contribute to the fruity character of Zalema wines. This is in accordance with Meilgaard's suggestion about the sensory contribution of a substance to the overall aroma: a compound should be considered as an aroma-contributing substance when its concentration is at least 20% of the threshold unit (OAV > 0.2) (Belitz & Grosch, 1999, Versini, Orriols, & Serra, 5; Versini et al., 1994).

With regard to alcohols, isoamyl alcohol and β-phenylethanol (mean OAVs = 4.98 and 1.61, respectively), were the only ones found contributing to Zalema wine aroma. These two alcohols are characterized by fruity and floral attributes, respectively.

The presence of norisoprenoids is considered to be a quality factor and typical for each variety, as they supply an agreeable scent of tobacco, fruits, tea, etc. (Schreier, 1984). The two norisoprenoids found in the analysed wines (β-damascenone and β-ionone) had OAVs higher than one. Therefore, although they were quantified in very low amounts, as their perception thresholds are very low, they play an important part in the wine aroma. β-Damascenone stood out with a mean OAV = 14.8 (Table 1). According to studies carried out by López, Ferreira, Hernández, and Cacho (1999), β-damascenone is a compound present at concentrations higher than its corresponding threshold in all wines. Particularly, in some white wines from the Canary Islands, such as Gual, Verdello, Marmajuelo, Listán and Malvasia wines, β-damascenone has been detected at high concentrations (OAVs > 100), which explains the aromatic descriptions (flowery, sweet, and fruity) attributed to these wines (López et al., 2003).

Some volatile thiols, such as 4-mercapto-4-methyl-2-pentanone, 3-mercaptohexyl acetate and 3-mercapto-1-

Table 2
Odour-active compounds found in the olfactometric study: gas chromatographic retention data (RI), olfactory description, chemical identity, and mean olfactometric intensities (0–3 scale, eight judges)

RI	Odour description[a]	Identity	Olfactometric intensity value
1136	Banana	Isoamyl acetate	2.69
1248	Green apple, anise	Ethyl hexanoate	2.56
1226	Sweet, fusel	Isoamyl alcohol	2.23
1052	Fruity, sweet	Ethyl butyrate	2.18
1082	Fruity, lemon, anise	Ethyl isovalerate	2.15
1845	Baked fruit, rape fruit	β-Damascenone	2.00
1007	Cream, sweet	Diacetyl	1.87
1448	Burned, beer	Ethyl octanoate	1.63
971	Sweet, strawberry	n.i.	1.55
1400	Grass	(*Z*)-3-hexen-1-ol	1.47
1284	Rage grape	Hexyl acetate	1.14
1685	Spicy, cheese	Isovaleric acid	1.16
1113	Green, fresh, fusel	Isobutanol	1.12
1464	Acid, spicy	Acetic acid	1.11
1067	Fruity, anise	Ethyl 2-methylbutyrate	1.11
1861	Baked fruit, chemical	n.i.	0.87
1660	Fresh, flowery	Phenylacetaldehyde	0.82
1388	Grass, pepper	4-Mercapto-4-methyl-2-pentanone	0.76
1000	Fruity, lemon	Ethyl isobutyrate	0.74
1842	Flowery, rose	2-Phenylethyl acetate	0.70
1645	Cheese	Butyric acid	0.62
2142	Meaty, chemical	n.i.	0.52
1319	Baked vegetable, kitchen	2-Methyl-3-furanthiol	0.50
1293	Shoe store	n.i.	0.50
1948	Flowery, pollen, perfume	2-Phenylethanol	0.48
1566	Citric, flowery, fresh	Linalool	0.47
1370	Grass	n.i.	0.41
2050	Skin, shoe store	n.i.	0.36
2095	Skin, burned	n.i.	0.36
1236	Green apple, fusel	n.i.	0.36
2380	Flowery	n.i.	0.34
2412	Flowery, anise	n.i.	0.34
1438	Cheese	n.i.	0.34
1443	Citric, fruity, flowery	n.i.	0.32

n.i., compound not identified.
[a] Odour description usually reported by at least two judges.

hexanol, and some carbonyl compounds (acetaldehyde and phenylacetaldehyde) were also at concentrations higher than their corresponding thresholds (Table 1).

3.3. GC-O data

As mentioned above, the evaluation of the odour activity values must be considered as a preliminary step in order to establish the impact odorants of a wine. Further steps, such as GC-olfactometric studies, must be applied to confirm the impact of the active odorants already identified. The results from the olfactometric study carried out in this work are summarized in Table 2. The data in this table show the mean odour intensity scores given by the panel for each compound. A total of 34 odorants were detected in the GC-O experiment, 22 of which could be identified.

According to their olfactometric intensities (0–3 scale), the odorants could be divided into several groups (Table 2). The first group includes odorants that reached olfactometric intensities between 2 and 3 units, so they can be considered as the most intense odorants. This group is made up of four esters (mainly ethyl esters of fatty acids), one alcohol (isoamyl alcohol) and the β-damascenone. A heterogeneous second group, with olfactometric intensities between 1 and 2 units (clearly perceived odours), includes 9 compounds, from which one of them (RI 971) has not been identified. In this group, there are three esters, two alcohols, a fatty acid and a carbonyl compound (diacetyl). Finally, 19 compounds were found with intensity values below unit (weak odours), including some thiols and a terpene compound, linalool. Although 11 out of these 19 compounds have not been identified, it is not a relevant fact because most of them were perceived as very weak odours (mean olfactometric intensity values less than one).

Table 3 summarizes the most powerful odorants determined in Zalema wines on the basis of GC-O analysis and odour activity values. The olfactometric strategy used in this paper, that was carried out by a panel of eight tasters using a seven point quantitative scale, has been demonstrated to provide data of semiquantitative value (Ferreira, Pet'ka, Aznar, & Cacho, 2003). Therefore, it is expected that many compounds, that ranked high with respect to their olfactometric intensities, also give high OAV values (Table 3), such as ethyl hexanoate, isoamyl acetate, ethyl butyrate and β-damascenone. However, some compounds, mainly fatty acids, with OAVs > 1, were missed in the olfactometric study. This fact is not unusual since olfactometry requires a high degree of attention by the sniffers. Also the coelution of some compounds and the difficulties in measuring the intensity of the odour correctly when odorants appear in a complex and short area of the chromatogram must be considered (Ferreira et al., 2002). Another hypothesis that could explain the lack of compounds in the olfactometric study is that GC-O does not take into account matrix effects, which may have a large impact on odorant volatility and perception. On the other hand, some compounds were detected in the olfactometric experiment but their OAV values were lower than one. This group includes three acetates (hexyl acetate, ethyl 2-methylbutyrate and 2-phenylethyl acetate), two alcohols (*Z*-3-hexen-1-ol and isobutanol), a terpene compound (linalool), a carbonyl compound (diacetyl), a thiol (2-methyl-3-furanthiol) and acetic acid. This fact could be due to the fact that olfactometric data could overestimate the importance of a component as a result of the technique used in the preparation of the extract used in the olfactometric study. Nevertheless, it can be seen that most of them possessed OAVs near to one, which corroborates that compounds with OAVs > 0.2 can be considered as aroma-contributing substances (Belitz & Grosch, 1999, chap. 5; Versini et al., 1994).

Table 3
Potent odorants of Zalema white wines

Compound	OAV[a]	Olfactometric intensity value[b]
Compounds OAV > 1		
Ethyl octanoate	154.67	1.63
Ethyl hexanoate	55.95	2.56
Isoamyl acetate	36.36	2.69
Hexanoic acid	22.62	–
Octanoic acid	19.53	–
Ethyl butyrate	18.74	2.18
Decanoic acid	16.86	–
β-damascenone	14.82	2.00
Ethyl isobutyrate	14.68	0.74
Isovaleric acid	10.46	1.16
4-mercapto-4-methyl-2-pentanone	6.25	0.76
Acetaldehyde	5.93	–
Butyric acid	5.76	0.62
3-mercaptohexyl acetate	5.71	–
Isoamyl alcohol	4.98	2.23
Ethyl isovalerate	4.70	2.15
2-methylbutyric acid	4.67	–
Ethyl decanoate	2.72	–
β-ionone	2.43	–
Isobutyric acid	1.79	–
3-mercapto-1-hexanol	1.63	–
β-phenylethanol	1.61	0.48
Phenylacetaldehyde	1.54	0.82
Compounds OAV < 1 detected by GC-O		
Diacetyl	0.82	1.87
Z-3-hexenol	0.88	1.47
Hexyl acetate	–	1.14
Isobutanol	0.52	1.12
Acetic Acid	–	1.11
Ethyl 2-methylbutyrate	0.62	1.11
2-phenylethyl acetate	0.86	0.70
2-methyl-3-furanthiol	–	0.50
Linalool	0.46	0.47

[a] Odour activity value calculated by dividing concentration by odour threshold value of the compound.
[b] Mean olfactometric intensities (0–3 scale, eight judges).

4. Conclusions

This work provide a better knowledge of the volatile composition of Zalema white wines, which could help winemakers to optimize operational conditions (harvest

parameters, juice preparation, fermentation techniques, use of yeasts, bacteria and enzymes, etc.) in order to emphasize one or more aromas in the final wines.

OAV values and GC-O analysis have shown that the main aroma contributors to Zalema white wines are compounds originating from yeast metabolism, mainly fatty acids and their ethyl esters. Other compounds, such as β-damascenone, isoamyl alcohol and its acetate ester, were also determined to be powerful odorants. These odorants are associated with ''fruity'', ''ripe fruit'', ''sweet'' and ''fresh'' odour descriptors, which are closely related to the aroma of Zalema wines described by tasters. They also possess a low terpenic character, and other ''key'' odorants, such as volatile phenols or lactones, that could provide a characteristic aroma, were not found as powerful odorants.

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