Aroma Fingerprint Characterisation of La Mancha Red Wines

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In the present study, Rojal, Moravia Dulce and Tortosí wines were elaborated across four harvests (2006 to 2009) from minority red grape varieties cultivated in the La Mancha region of Spain. Wines were studied by instrumental and sensory analysis to determine the influence of grape variety on the aroma of the wine. Aroma compounds were isolated by solid phase extraction (SPE) to later be analysed using gas chromatography–mass spectrometry (GC/MS). The odour activity values (OAVs) for the different compounds were classified into seven odorant series that describe the aroma profile of these wines (1: fruity, 2: floral, 3: green/fresh, 4: sweet, 5: spice, 6: fatty, and 7: other odours). The total intensities of every aromatic series were calculated as sum of the OAV of each one of the compounds assigned to this series. All wines showed the same sequence, with the highest aroma contribution being those of the sweet and fruity series, followed by fatty. The sensory profile of Rojal, Moravia Dulce and Tortosí wines was evaluated by experienced wine tasters using a non-structured scale. The panellists founded several differences between their sensory profiles. This study provides a complete aromatic characterisation of these wines.

INTRODUCTION

The aroma and flavour of wine are among the main characteristics that define the differences among the vast array of wines and wine styles produced throughout the world (Swiegers *et al.*, 2005). Knowledge of the volatile composition of a wine is of great interest, since these compounds are highly related to beverage flavour. Thus, several studies have been carried out to associate the wine volatiles with the grape variety (Versini *et al.*, 1994; García-Carpintero *et al.*, 2011a, 2011b), climatic conditions (Falcão *et al.*, 2007; Louw *et al.*, 2009; Sánchez-Palomo *et al.*, 2010) and various winemaking practices (Aznar *et al.*, 2003).

The aroma of wines is the result of the contribution of some hundreds of volatile compounds and it is an important factor to consider in their sensorial quality. Furthermore, studies on the identification of impact odorants associated with a particular varietal aroma have also been reported (Rocha et al., 2004; Noguerol-Pato et al., 2009). The odour of one volatile compound is described in terms of one or several descriptors agreed upon by experts (Etiévant, 1991; Guth, 1997; Ferreira et al., 2001). In addition, several authors have used odorant series to describe the aroma of wine (Brugirard et al., 1991). Grouping the volatile compounds with a similar descriptor in odorant series, an odorant profile can be established for each wine and the contribution of each compound to each series can be determined. This procedure makes it possible to relate the quantitative information obtained from the chemical analysis to the sensorial

perceptions with a view to obtaining an odorant profile for the wine (Peinado *et al.*, 2004, 2006).

To understand the complete aroma composition of a wine it is necessary to obtain some information regarding both the volatile composition and sensory properties (Francis & Newton, 2005). Sensory analysis involves the detection and description of qualitative and quantitative sensory components of a product by a trained panel of judges (Meilgard *et al.*, 1999). Quantitative descriptive analysis (Stone & Sidel, 1998) is one of the most comprehensive and informative tools used in sensory analysis. Several authors have studied the aromatic profiles of wines of many varieties using descriptive analysis (De la Presa-Owens & Noble, 1995; Parr *et al.*, 2007; Sánchez-Palomo *et al.*, 2007; Tao *et al.*, 2008; Campo *et al.*, 2010).

Moravia Dulce, Rojal and Tortosí are minority grape varieties cultivated in the La Mancha region of Spain in small areas with special climatologic conditions (warm summers, cold winters and low rain) that could influence the aroma composition of grapes cultivated in a restrained area. The knowledge of the aromatic composition of these varieties can provide opportunities for the adaptation of the characteristics of these minority grape varieties to new winemaking procedures ruled by consumer preferences. The objective of this paper is to report the results of the first study of the odour activity values and aroma series of the wines produced with cv. Rojal, Moravia Dulce and Tortosí grape varieties from the La Mancha region over four

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consecutive vintages. The second objective is to define the sensory characteristics of these monovarietal wines using quantitative descriptive sensory analysis and establish the sensory profile of these wines.

MATERIAL AND METHODS **Wine samples**

Red *Vitis vinifera cv.* Moravia Dulce, Rojal and Tortosí grapes were obtained from the vineyards of La Mancha in the central south-eastern region of Spain. The grapes were harvested at their optimal stage of ripeness and in healthy conditions over four consecutive vintages (2006 to 2009).

Wines were elaborated from two batches of grapes (25 kg each) in 25 L vats with skin maceration until the end of the alcoholic fermentation. Winemaking conditions included the addition of 100 mg/L of SO₂, as K₂S₂O₇ (Sánchez-Palomo et al., 2006), after stemming and crushing, inoculation with Saccharomyces cerevisiae selected yeasts (UCLM S325, Fould-Springer), and the fermentation temperature maintained at 24 °C. Manual punching down was done twice a day. The separation of the wine from the solids was performed when relative density reached a constant value. Subsequently, malolactic fermentation was induced by inoculation with Oenococcus oeni lactic acid bacteria (Lactobacter SP1; Laffort). This second fermentation ended in two to three weeks, as confirmed by TLC (thin layer chromatography); the wines were then racked. After one month, the wines were racked again, filtered through 1.2 µm membranes (Millipore, Bedford, MA, USA), bottled, and stored in a room with a constant temperature of between 16 and 18 °C.

Reagents and standards

Dichloromethane and methanol were purchased from Merck (Darmstadt, Germany). Ammonium sulphate and anhydrous sodium sulphate were from Panreac (Barcelona, Spain). Pure water was obtained from a Milli-Q purification system (Millipore, U.S.A.). LiChrolut EN resins were purchased from Merck (Darmstadt, Germany). The chemical standards were supplied by Sigma (St. Louis, MO, USA), Aldrich (Gillingham, UK), Firmenich (Geneva, Switzerland), Panreac (Barcelona, Spain), Merck (Darmstadt, Germany), Fluka (Buchs, Switzerland) and Lancaster (Strasbourg, France).

Standard chemical analysis of musts and wines

Total acidity, °Brix, ethanol, pH, volatile acidity, total and free SO₂ were analysed (O.I.V. International Oenological Codex, 2006). The results are shown in Table 1.

Analysis of major volatiles

Major volatile compounds were analysed by direct injection (Sánchez-Palomo *et al.*, 2006) of a HP-5890 GC with a FID detector, using a CP-Wax-57 capillary column (50 m x 0.25 mm i.d.; 0.25 μ m film thickness). The oven temperature programme was: 40 °C (5 min.) – 4 °C/min – 120 °C. The injector and detector temperatures were 250 and 280 °C respectively. One micro-litre (1 μ L) of tested wine was injected in split mode, at a split ratio of 1:15. The carrier gas was He (0.7 mL/min).

Extraction of minor volatiles

The aroma compounds were separated by adsorption/ desorption on preconditioned polypropylene-divinylbenzene cartridges (Sánchez-Palomo *et al.*, 2006) (LiChrolut EN, Merck), 0.5 g of phase. One hundred millilitres of wine with added 40 μ L of 4-nonanol, as an internal standard, was passed through the LiChrolut EN column at a flow rate of 1 ml/min. The column was rinsed with 50 mL of pure water to eliminate sugars and other low-molecular-weight polar compounds.

The free fraction was eluted with 10 ml of dichloromethane. All dichloromethane extracts were cooled to -20 °C to separate the frozen water from the organic phase by decantation, and then dried over anhydrous sodium sulphate. Using a nitrogen stream, the organic phase was concentrated to a final volume of 200 μ L.

TABLE 1

| General | composition of | f Morav | ia Dulce | , Rojal | and | Tortosí | musts | and | wines |
|---------|----------------|---------|----------|---------|-----|---------|-------|-----|-------|
|---------|----------------|---------|----------|---------|-----|---------|-------|-----|-------|

| | | Morav | ia Dulce | | | R | ojal | | | То | rtosí | |
|---------------------------------------|------|-------|----------|------|------|------|------|------|------|------|-------|------|
| Composition ranges | 2006 | 2007 | 2008 | 2009 | 2006 | 2007 | 2008 | 2009 | 2006 | 2007 | 2008 | 2009 |
| Must composition | | | | | | | | | | | | |
| Titratable acidity ^a (g/L) | 4.53 | 5.17 | 4.69 | 5.07 | 5.94 | 5.71 | 5.28 | 5.23 | 4.93 | 4.87 | 5.28 | 5.64 |
| pH | 3.38 | 3.49 | 3.35 | 3.31 | 3.37 | 3.33 | 3.24 | 3.36 | 3.51 | 3.43 | 3.37 | 3.34 |
| °Brix | 23.4 | 21.6 | 22.7 | 21.9 | 20.4 | 20.8 | 22.6 | 22.8 | 22.8 | 23.1 | 21.9 | 21.1 |
| Wine composition | | | | | | | | | | | | |
| Ethanol (%, v/v) | 12.1 | 11.8 | 12.3 | 11.9 | 11.4 | 11.6 | 12.1 | 12.0 | 11.8 | 12.5 | 12.3 | 11.7 |
| Titratable acidity ^a (g/L) | 4.55 | 5.21 | 4.48 | 5.35 | 6.12 | 5.81 | 5.15 | 5.10 | 4.94 | 4.76 | 5.19 | 5.58 |
| pH | 3.37 | 3.51 | 3.30 | 3.24 | 3.31 | 3.35 | 3.30 | 3.32 | 3.43 | 3.39 | 3.30 | 3.21 |
| Volatile acidity ^b (g/L) | 0.20 | 0.20 | 0.21 | 0.22 | 0.20 | 0.23 | 0.21 | 0.22 | 0.21 | 0.22 | 0.20 | 0.20 |
| Free SO ₂ (mg/L) | 18.7 | 15.6 | 12.8 | 16.8 | 21.2 | 18.6 | 15.4 | 14.9 | 20.6 | 18.3 | 16.3 | 13.5 |
| Total SO ₂ (mg/L) | 34.8 | 41.5 | 31.2 | 40.2 | 28.4 | 40.2 | 35.8 | 37.1 | 29.3 | 34.8 | 35.8 | 29.6 |

^a as tartaric acid; ^b as acetic acid

Gas chromatography-mass spectrometry (GC-MS) analysis

An Agilent Gas Chromatograph model 6890 N, coupled to a Mass Selective Detector model 5973 *inert* equipped with a BP-21, polyethylene glycol TPA-treated capillary column (60 m x 0.25 mm i.d.; 0.25 μ m film thickness), was used. Operating conditions were as follows. Oven temperature program was: 70°C (5 min) – 1 °C/min – 95 °C (10 min) – 2 °C/min – 200°C (40 min). Injector and transfer line temperatures were 250°C and 280°C respectively. Mass detector conditions were: electron impact (EI) mode at 70 eV; source temperature: 178 °C; scanning rate: 1 scan/s; mass acquisition: 40 to 450 amu. One micro-litre (1 μ L) was injected in splitless mode (0.5 min). The carrier gas was helium (1 mL/min).

Retention index, Wiley mass-spectral library and pure volatile compounds were used for identification, confirmation and preparation of standard solutions of volatile compounds. The relative response areas for each of the volatile compounds to the internal standard were calculated and interpolated in the corresponding calibration graphs. For the calibration, standard solutions were prepared in 12% v/v ethanol with 5 g/L tartaric acid and the corresponding internal standard in the same concentration as in the samples. Calibration curves were drawn for each standard at eight different concentration levels. The measurements of all standards were performed in triplicate.

When the authentic standard were not available, the identification was based on a comparison with the spectral data in the Wiley A Library and the chromatographic dates of the literature, semi-quantitative analysis of these compounds were made assuming response factor equal to one.

Sensory descriptive analysis

The sensory profiles of the studied wines were generated by a panel of 15 trained judges between the ages of 26 and 45, staff members of the University of Castilla-La Mancha, Spain, who were experienced in food and beverage sensory evaluation using quantitative descriptive analysis (Meilgaard *et al.*, 1999; Stone & Sidel, 2004). The same panel members

TABLE 2

Mean scores of sensory aroma profile of Moravia Dulce, Tortosí and Rojal wines elaborated over four consecutive vintages.

| | | Morav | ia Dulc | e | | R | ojal | | | To | rtosí | |
|------------------|------|-------|---------|------|------|------|------|------|------|------|-------|------|
| Attributes | 2006 | 2007 | 2008 | 2009 | 2006 | 2007 | 2008 | 2009 | 2006 | 2007 | 2008 | 2009 |
| Red fruit | 6.28 | 5.54 | 5.3 | 5.76 | 6.58 | 6.64 | 7.75 | 7.98 | 6.58 | 6.64 | 7.75 | 7.98 |
| Fresh | 5.50 | 5.15 | 4.75 | 5.15 | 5.60 | 5.26 | 5.02 | 5.45 | 5.60 | 5.26 | 5.02 | 5.45 |
| Clove | 3.33 | 3.45 | 3.90 | 3.79 | 4.23 | 4.35 | 4.80 | 3.79 | 4.23 | 4.35 | 4.80 | 3.79 |
| Pepper | 4.05 | 3.7 | 4.36 | 3.98 | 1.30 | 1.25 | 1.57 | 1.29 | 1.30 | 1.25 | 1.57 | 1.29 |
| Leather/ tobacco | 3.26 | 2.99 | 3.77 | 3.13 | 3.36 | 3.42 | 2.27 | 2.30 | 3.36 | 3.42 | 2.27 | 2.30 |
| Sweet | 5.19 | 4.85 | 6.29 | 4.63 | 2.21 | 2.83 | 2.36 | 2.64 | 2.21 | 2.83 | 2.36 | 2.64 |
| Fresh fruit | 2.89 | 3.46 | 3.48 | 2.88 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Floral | 0.00 | 0.00 | 0.00 | 0.00 | 2.49 | 1.99 | 2.15 | 2.16 | 0.00 | 0.00 | 0.00 | 0.00 |
| Cassis | 0.00 | 0.00 | 0.00 | 0.00 | 2.49 | 2.26 | 2.19 | 2.56 | 0.00 | 0.00 | 0.00 | 0.00 |
| Lychee | 0.00 | 0.00 | 0.00 | 0.00 | 2.98 | 3.05 | 2.87 | 2.75 | 0.00 | 0.00 | 0.00 | 0.00 |
| Coffee | 0.00 | 0.00 | 0.00 | 0.00 | 4.25 | 3.95 | 4.32 | 3.89 | 0.00 | 0.00 | 0.00 | 0.00 |
| Liquorice | 3.49 | 2.98 | 3.15 | 3.89 | 3.49 | 2.94 | 4.03 | 4.01 | 3.49 | 2.94 | 4.03 | 4.01 |

were used for all the evaluation processes.

Physical-chemical standards were used to define attributes (Noble *et al.*, 1984; Petka *et al.*, 2006). The panellists used a 10 cm unstructured scale to rate the intensity of each attribute. The left-hand end of the scale was "attribute not perceptible", and the right-hand end was "attribute strongly perceptible."

The sensory evaluation of the wines was carried out in every vintage at three months post-bottling. Samples of red wine (20 ml) were presented in standard wine-testing glasses according to standard 3591 (ISO 3591, 1997), covered with a watch-glass to minimise the amount of volatile components escaping. Samples were presented following a randomised block design and three-digit random numbers were used to code each sample. The temperature of the wines was maintained at 15 ± 1 °C, and the evaluations were made in individual booths under white light in a standard sensoryanalysis chamber (ISO 8589, 1998). Wines were sniffed. According to the total number of wines (two batches * two replicates), three wines were sniffed and tasted in each session (a total of four sessions each year). The data was collected using the descriptive ballot consensually generated by the panel. Overall, each judge evaluated each wine with two repetitions.

Odour activity values

To evaluate the contribution of a chemical compound to the aroma of a wine, the odour activity value (OAV) is determined. OAV is a measure of the importance of a specific compound to the odour of a sample. It is calculated as the ratio between the concentration of an individual compound and the perception threshold found in the literature (Francis & Newton, 2005; Vilanova *et al.*, 2009).

Statistical analysis

All statistical analyses were performed using the SPSS version 19.0 for Windows statistical package. Analysis of variance (ANOVA) was performed using the general linear model procedure to determine significant differences among the means of chemical data. The Student-Newman-Keuls

test was conducted when the samples exhibited significance between them, with the level of significance set at P < 0.05. PCA employs a mathematical procedure that transforms a set of possibly correlated response variables into a new set of non-correlated variables called principal components (Cozzolino *et al.*, 2009). Principal component analysis (PCA) was performed on the data of the aroma descriptions to find the dominant aroma terms of the wines.

RESULTS AND DISCUSSION

Quantitative descriptive analysis of wines

Table 2 provides the average scores of the olfactory attribute intensities of Moravia Dulce, Rojal and Tortosí wines over four consecutive vintages. The sensory panel found several differences between the aroma profiles of the studied wines from the La Mancha region. Moravia Dulce wines was characterised by red fruit, fresh, clove, pepper, leather, tobacco, sweet, fresh fruit and liquorice attributes. Rojal wines were described by the tasters as red fruit, fresh, clove, pepper, leather, tobacco, sweet and fresh fruit aromas, with a lower intensity of all the attributes than Moravia Dulce, except for fresh fruit and fresh. However, the Tortosí wines presented a different sensory profile, with higher red fruit notes than the others wines, and with fresh, clove, leather, tobacco, floral, pepper, cassis, lychee, coffee and liquorice notes.

The correlation matrix generated from the mean rating of each wine across the 12 attributes was analysed by PCA with rotation. Principal component analysis (PCA) was applied to all aroma term data to obtain a more simplified view of the total aroma characters of the sample wines. The first two principal components represented 99.5% of the total variance, so those PCs after PC 3 made little contribution to the total variance (< 1%). Fig. 1 shows the principal component bi-plot, illustrating the simultaneous projection of the 12 wines and the 12 descriptors.

The studied wines are clearly separated in the consensus space. Rojal wines are situated on the positive x axis along the first principal component according to the intensity of coffee, red fruit, clove and liquorice. Moravia Dulce and Tortosí wines – situated on the negative x axis – are separated from the Rojal wines in relation to the intensity of pepper, sweet, fresh fruit and fresh. Dimension 2 was separated clearly between the Tortosí wines, situated on the negative y axis, and Moravia Dulce and Rojal, on the positive y axis. Wines made with the Tortosí grape variety presented floral, cassis, lychee and fresh aroma descriptors, although the aroma of Moravia Dulce and Tortosí wines was characterised by red fruit, tobacco, leather, pepper, coffee and sweet notes. Also, we could observe that there was no great variability attributable to climatic conditions in each vintage, as the samples from the same grape variety and different vintages are situated close together, so that the wines from Rojal, Moravia Dulce and Tortosí always present similar sensory characteristics, independent of weather conditions.

Odour activity values

Table 3 shows the odour descriptors and the odour threshold of wine aroma compounds obtained by the bibliographic references (Etiévant, 1991; Guth, 1997; Ferreira *et al.*, 2000).

As a preliminary step to achieve the identification of potentially the most important wine odorants of Moravia Dulce, Rojal and Tortosí wines, the odour activity values (OAV) were determined, *i.e.* the ratio between





Distribution in the consensus space; the wine samples are written in bold and the sensory attributes in normal font. Axis "x" represents Dimension 1 and axis "y" represents Dimension 2.

the concentration of each volatile compound and the corresponding odour threshold. Table 4 lists the OAV values for the 32 aroma compounds with OAV > 0.1 in the Moravía Dulce, Rojal and Tortosí wines over the four consecutive years, showing the odorant series of these compounds. On the basis of their odour description and threshold, the most powerful odorants of Moravía Dulce, Rojal and Tortosí wines could be established tentatively. Compounds that exhibited OAVs > 1 were considered to contribute individually to the wine aroma and were designated would-be impact odorants. As can be seen in Table 4, beta-damascenone, ethyl caprilate,

isovaleric acid, ethyl caproate, guaiacol, 4-vinylguaiacol,

isoamyl acetate, 3-methyl-1-butanol, ethyl acetate, butyric acid, 2-phenylethyl alcohol, hexanoic acid, octanoic acid ethyl butyrate and 3-(methylthio-1-propanol) exhibited odour activity (OAV > 1) for all monovarietal wines and for all vintages studied. From a theoretical point of view, the remaining compounds did not contributed directly to the aroma profile (OAV < 1), although some authors believe that they can enhance some notes already present because of synergistic effects with other odorant compounds.

It is difficult to predict the overall aroma impact of these wines from the sheer size of the data. To estimate overall wine aroma, the odour descriptors were grouped in different

TABLE 3

Odour descriptors, odorant series and odour thresholds (μ g/L) of the aroma compounds in the studied wines.

| Compounds | Odour descriptors | Odorant series* | Odour threshold |
|---------------------------|--------------------------|------------------------|----------------------|
| Ethyl acetate | fruity, solvent | 1.6 | 7 500 ^a |
| Ethyl butanoate | fruity | 1 | 20 ^a |
| Isoamyl acetate | banana | 1 | 30° |
| Methanol | chemical, medicinal | 6 | 668 000 ^b |
| 1-Propanol | ripe fruit, alcohol | 1.6 | 830 000 ^b |
| Isobutanol | oily, bitter, green | 3.6 | 40 000 ^b |
| 3-Methyl-1-butanol | burnt, alcohol | 4.6 | 30 000 ^a |
| Ethyl hexanoate | green apple | 1 | 14 ^b |
| Ethyl lactate | acid, medicine | 6 | 154 636° |
| 1-Hexanol | flower, green, cut grass | 2.3 | 8 000 ^a |
| (Z)-3-Hexen-1-ol | green, cut grass | 3 | 400 ^a |
| Ethyl octanoate | sweet, fruity | 1.2.4 | 5 ^b |
| Linalool | floral | 2 | 15 ^a |
| Isobutanoic acid | rancid, butter, cheese | 6 | 2 300b |
| Butyric acid | rancid, cheese, sweat | 6 | 173 ^b |
| Ethyl decanoate | sweet/fruity | 1.4 | 200° |
| Isovaleric acid | sweet, acid, rancid | 4.6 | 33° |
| 3-(Methylthio)-1-propanol | cooked vegetable | 7 | 1 000 ^a |
| 2-Phenylethyl acetate | floral | 2 | 250 ^a |
| β-Damascenone | sweet, fruity | 1.4 | 0.05 ^a |
| Hexanoic acid | sweat | 6 | 420 ^b |
| Geraniol | roses, geranium | 2 | 30 ^a |
| Guaiacol | medicine, sweet, smoke | 4.6 | 10 ^c |
| 2-Phenylethyl alcohol | floral, roses | 2 | 10 000 ^a |
| Octanoic acid | sweat, cheese | 6 | 500 ^c |
| Eugenol | spices, clove, honey | 4.5 | 6° |
| 4-Vinylguaiacol | spices/curry | 5 | 40^{a} |
| Decanoic acid | rancid fat | 6 | 1 000 ^b |
| Isoeugenol | clove | 5 | 6 ^b |
| Benzoic acid | chemical | 6 | 1 000 ^b |
| Vanillin | vanillin | 5 | 60 ^b |
| Ethyl vanillate | sweet, honey, vanillin | 4.5 | 990 ^b |
| Acetovanillone | sweet spices | 5 | 1 000 ^b |

*1 =fruity; 2 =floral; 3 =green. fresh; 4 =sweet; 5 =spicy; 6 =fatty; 7 =others.

^a Guth (1997). Thresholds were calculated in a 10% water/ethanol solution.

^b Etiévant (1991). Thresholds were calculated in wine.

^c Ferreira *et al.* (2000). Thresholds were calculated in a 11% water/ethanol solution containing 7 g/L glycerol and 5 g/L tartaric acid. pH adjusted to 3.4 with 1 M NaOH.

| Odour activity values for arc | ima con | spunodu | in Mora | via Dulc | e, Rojal a | חוח זחות | | | | | ~ | 0 | | | | | | |
|---|-----------|-----------|-------------|------------|-------------------|------------|------------|-------------|------------|---------|---------------------|--------|------|------|-------|-------|---------------------|--------|
| | | | Morav | ia Dulce | 0 | | | | R | ijal | | | | | Torte |)SÍ | | |
| Compounds | 2006 | 2007 | 2008 | 2009 | Mean | SD | 2006 | 2007 | 2008 | 2009 | Mean | SD | 2006 | 2007 | 2008 | 2009 | Mean | SD |
| β-damascenone | 20.4 | 72.4 | 81.2 | 102 | 69.0 ^a | (34.7) | 0.00 | 9.60 | 5.98 | 2.80 | 4.60^{b} | (4.14) | 0.00 | 1.05 | 25.8 | 15.01 | 10.5^{b} | (12.3) |
| Ethyl octanoate | 64.0 | 65.2 | 64.6 | 70.0 | 66.0^{a} | (2.74) | 74.0 | 90.0 | 85.4 | 86.5 | 84.0^{a} | (6.93) | 42.0 | 98.2 | 104 | 100 | 86.1 ^a | (29.5) |
| Isovaleric acid | 48.4 | 54.9 | 57.0 | 66.0 | 56.6 ^a | (7.27) | 56.7 | 72.1 | 62.8 | 63.9 | 63.9ª | (6.33) | 50.9 | 52.8 | 71.2 | 54.0 | 57.2ª | (9.40) |
| Ethyl hexanoate | 18.5 | 23.0 | 27.1 | 34.2 | 25.7 ^a | (6.67) | 25.0 | 30.7 | 27.8 | 26.5 | 27.5 ^a | (2.42) | 15.0 | 34.9 | 35.7 | 35.6 | 30.3ª | (10.2) |
| Guaiacol | 11.7 | 16.8 | 15.8 | 17.3 | 15.4^{a} | (2.54) | 4.17 | 6.61 | 5.80 | 6.45 | 5.76 ^b | (1.11) | 3.50 | 4.35 | 5.50 | 4.65 | 4.16^{b} | (0.31) |
| 4-Vinylguaiacol | 7.18 | 8.12 | 8.03 | 11.8 | 8.78ª | (2.06) | 0.31 | 1.16 | 0.45 | 0.74 | 0.98^{b} | (0.15) | 2.70 | 4.22 | 4.25 | 4.02 | 3.80° | (0.74) |
| Isoamyl acetate | 1.33 | 5.75 | 9.91 | 15.0 | 8.00 ^a | (5.84) | 1.00 | 17.6 | 9.97 | 3.42 | 8.00 ^a | (7.44) | 1.33 | 15.6 | 32.0 | 28.4 | 19.3ª | (13.9) |
| 3-Methyl-1-butanol | 6.50 | 6.57 | 6.5 | 6.83 | 6.60 ^a | (0.16) | 6.17 | 6.63 | 6.25 | 6.41 | 6.37^{a} | (0.20) | 5.87 | 5.99 | 6.43 | 6.39 | 6.17 ^a | (0.28) |
| Ethyl acetate | 3.17 | 4.59 | 4.09 | 5.41 | 4.32 ^a | (0.94) | 3.31 | 4.92 | 4.91 | 4.90 | 4.51 ^a | (0.80) | 3.39 | 4.32 | 4.47 | 4.29 | 4.12 ^a | (0.49) |
| Butyric acid | 3.64 | 4.15 | 4.05 | 5.03 | 4.22 ^a | (0.58) | 5.32 | 8.27 | 7.29 | 6.15 | 6.76^{b} | (1.29) | 5.20 | 6.78 | 6.99 | 6.89 | 6.47 ^b | (0.85) |
| 2-Phenylethyl alcohol | 3.19 | 3.77 | 3.85 | 4.24 | $3.76^{\rm a,b}$ | (0.43) | 2.51 | 3.45 | 3.03 | 3.15 | 3.04^{a} | (0.39) | 1.33 | 15.6 | 32.0 | 28.4 | 4.27 ^b | (0.57) |
| Hexanoic acid | 2.95 | 3.39 | 3.57 | 4.02 | 3.48^{a} | (0.44) | 3.76 | 6.24 | 5.74 | 5.87 | 5.40^{b} | (1.12) | 5.50 | 5.55 | 5.57 | 5.56 | 5.55 ^b | (0.03) |
| Octanoic acid | 3.22 | 3.40 | 3.21 | 3.52 | 3.34^{a} | (0.15) | 3.96 | 4.96 | 3.98 | 4.21 | 4.28^{b} | (0.47) | 0.05 | 0.06 | 0.08 | 0.05 | 4.94° | (0.52) |
| Ethyl butanoate | 3.00 | 2.78 | 2.70 | 3.50 | 3.00 ^a | (0.36) | 3.00 | 3.50 | 3.02 | 3.00 | 3.13^{a} | (0.25) | 3.50 | 4.35 | 5.50 | 4.65 | 4.50^{b} | (0.83) |
| 3-(Methylthio)-1-propanol | 0.59 | 1.36 | 1.32 | 2.21 | 1.37^{a} | (0.66) | 2.51 | 2.68 | 2.45 | 2.65 | 2.57 ^b | (0.11) | 1.96 | 2.45 | 3.67 | 3.07 | 2.79 ^b | (0.74) |
| Vanillin | 1.02 | 1.03 | 1.02 | 1.04 | 1.03 ^a | (0.01) | 0.08 | 0.11 | 0.08 | 0.09 | 0.09 ^b | (0.01) | 0.09 | 0.29 | 0.49 | 0.39 | 0.32° | (0.17) |
| Isobutanol | 1.01 | 1.01 | 1.01 | 1.02 | 1.01 ^a | (0.01) | 0.77 | 1.03 | 06.0 | 0.79 | $0.87^{\rm a}$ | (0.12) | 0.87 | 0.87 | 1.03 | 0.88 | 0.91 ^a | (0.08) |
| Methanol | 0.09 | 0.09 | 0.09 | 0.28 | 0.14^{a} | (0.10) | 0.06 | 0.12 | 0.07 | 0.08 | 0.08^{a} | (0.03) | 0.05 | 0.06 | 0.08 | 0.05 | 0.06^{a} | (0.01) |
| Ethyl lactate | 0.09 | 0.10 | 0.10 | 0.10 | 0.10^{a} | (0.01) | 0.10 | 0.15 | 0.12 | 0.11 | 0.12^{a} | (0.02) | 0.12 | 0.16 | 0.16 | 0.15 | $0.15^{\rm b}$ | (0.02) |
| 1-Hexanol | 0.21 | 0.22 | 0.23 | 0.25 | 0.23^{a} | (0.02) | 0.18 | 0.27 | 0.25 | 0.26 | 0.24^{a} | (0.04) | 0.17 | 0.20 | 0.23 | 0.17 | 0.19^{a} | (0.03) |
| (Z)-3-Hexen-1-ol | 0.25 | 0.40 | 0.41 | 0.41 | 0.37^{a} | (0.08) | 0.37 | 0.52 | 0.44 | 0.47 | 0.45 ^a | (0.06) | 0.05 | 0.08 | 0.13 | 0.11 | 0.09 ^b | (0.04) |
| Linalool | 0.17 | 0.81 | 0.89 | 0.95 | 0.71 ^a | (0.36) | 0.61 | 0.93 | 0.84 | 0.75 | 0.78^{a} | (0.14) | 1.04 | 1.79 | 1.80 | 1.79 | 1.61^{b} | (0.38) |
| Isobutanoic acid | 0.52 | 0.53 | 0.54 | 0.55 | 0.54^{a} | (0.01) | 0.58 | 0.66 | 0.61 | 0.65 | 0.63^{a} | (0.04) | 0.34 | 0.67 | 0.73 | 0.66 | 0.60 ^a | (0.18) |
| Ethyl decanoate | 0.35 | 0.37 | 0.35 | 0.4 | 0.37^{a} | (0.02) | 0.35 | 0.40 | 0.39 | 0.38 | 0.38^{a} | (0.02) | 0.25 | 0.39 | 0.40 | 0.40 | 0.36^{a} | (0.07) |
| 2-Phenylethyl acetate | 0.08 | 0.35 | 0.45 | 0.64 | 0.38^{a} | (0.23) | 0.12 | 0.52 | 0.22 | 0.41 | 0.32^{a} | (0.18) | 0.04 | 0.19 | 0.72 | 0.15 | 0.28ª | (0.30) |
| Geraniol | 0.00 | 0.09 | 0.1 | 0.11 | 0.08^{a} | (0.05) | 0.30 | 0.33 | 0.32 | 0.30 | $0.31^{\rm b}$ | (0.02) | 3.39 | 4.32 | 4.47 | 4.29 | 0.00° | (0.00) |
| Decanoic acid | 0.41 | 0.59 | 0.66 | 0.66 | 0.58^{a} | (0.12) | 0.48 | 0.62 | 0.52 | 0.49 | 0.67^{a} | (0.38) | 0.44 | 0.46 | 0.49 | 0.45 | 0.46^{a} | (0.02) |
| Ethyl vanillate | 0.53 | 0.53 | 0.55 | 0.56 | 0.54^{a} | (0.02) | 0.11 | 0.22 | 0.20 | 0.21 | 0.19^{b} | (0.05) | 0.19 | 0.24 | 0.27 | 0.20 | $0.23^{\rm b}$ | (0.04) |
| Isoeugenol | 0.30 | 0.45 | 0.44 | 0.45 | 0.41^{a} | (0.07) | 2.85 | 4.43 | 4.39 | 4.42 | $0.53^{\rm b}$ | (0.06) | 0.00 | 0.21 | 0.42 | 0.24 | 0.22 ^a | (0.17) |
| Acetovanillone | 0.16 | 0.18 | 0.19 | 0.19 | 0.18^{a} | (0.01) | 0.08 | 0.11 | 0.09 | 0.10 | 0.10^{b} | (0.01) | 0.07 | 0.10 | 0.15 | 0.09 | 0.10^{b} | (0.03) |
| Benzoic acid | 0.10 | 0.13 | 0.14 | 0.14 | 0.13^{a} | (0.02) | 0.15 | 0.18 | 0.16 | 0.18 | $0.17^{\rm b}$ | (0.02) | 0.04 | 0.06 | 0.08 | 0.08 | 0.07° | (0.02) |
| Eugenol | 0.00 | 0.00 | 0.00 | 0.00 | 0.00^{a} | (0.00) | 0.75 | 1.08 | 1.07 | 1.01 | $0.98^{\rm b}$ | (0.15) | 0.05 | 0.06 | 0.08 | 0.07 | 0.07^{a} | (0.01) |
| . ^{a, b, c} Different superscripts in th | ie same r | ow indice | te statisti | cal differ | ences at th | e 0.05 lev | rel accoro | ding to the | e Student- | Newman- | Keuls tes | ït. | | | | | | |

aromatic series and every compound was assigned to one or several aromatic series based on a similar odour descriptor used. The method based in the OAV has been used in recent years in studies on wine aroma, such as in the discrimination of wines obtained from different grape varieties (Guth, 1997; Sánchez-Palomo *et al.*, 2010). Nevertheless, as odour threshold is affected by additive, synergic and antagonistic effects of the volatile compounds in a matrix, the identification of the most powerful odorants only on the basic of their OAV values should be considered tentative.

The series used in this work group compounds with similar odour descriptors and represent the main constituents of the aroma profile of the wine: fruity, floral, green/fresh, sweet, spice, fatty and other odours, taking into account their use in previous papers (Gómez-Míguez et al., 2007; Lorenzo et al., 2008; Sánchez-Palomo et al., 2010). Because of the high complexity of olfactory perceptions, some aroma compounds were included in two or more odorant series according to the finding of some authors (Zea et al., 2007). The total intensities for every aromatic series were calculated as the sum of the OAV of each one of the compounds assigned to this series, and the results are graphed in Fig. 2. This procedure makes it possible to relate quantitative information obtained by chemical analysis to sensory perception, providing a single aroma profile. It has recently been used by some authors (Franco et al., 2004; Zea et al., 2007; Lorenzo et al., 2008).

With regard to the aromatic series, all wines showed the same sequence; as a result, the highest aroma contribution was those of the 4 – sweet and 1 – fruity series, followed by 6 -fatty. Moravia Dulce wines showed higher $\sum OAV$ values for these aromatic series in relation to Rojal and Tortosí

wines.

On the other hand, the aromatic series 2 - floral, 3 - greenand 5 - spice were the minor aroma categories. Nevertheless, some of these attributes were characteristics in the sensory profile of the studied wines. Floral notes were found in the sensory profile of Tortosí wines; Moravia Dulce wines presented spice notes like pepper and clove; and green/fresh notes were described in Rojal wines.

The values of the total intensity of the different aromatic series were obtained as the sum of the individual OAVs of each one of the components, without bearing in mind the rest of the compounds present in the matrix of the wine. Nevertheless, when combined, synergy, suppression and matrix effects may alter the intensity of the descriptors, masking the descriptors of some aromatic series and increasing the intensity of others odour descriptors. These results are in agreement with the results obtained in bibliographic references in red wines made from Merlot and Cabernet Sauvignon grape varieties (Gürbüz *et al.*, 2006) and in wines made with the Verdejo grape variety (Sánchez-Palomo *et al.*, 2010).

As odour thresholds are affected by great imprecision, and synergic, additive and antagonistic effects can take place, these values should not be taken as close boundaries, but rather as an approximation of the number of odorants that constitute the odour of such wines. The most potent odorants of each wine are practically the same, but only change in relation to relative order from one sample to another.

The different compounds are those that have a more acute role in the perception of sensory differences between wines. At present, this property can only be verified by means of sensory tests, although an approximation can be obtained by considering the variability in geometric terms



FIGURE 2

The average sum of odour activity values (Σ OAVs) for aromatic series in Moravia Dulce, Rojal and Tortosí wines in four consecutive vintages (2006 to 2009).

of concentration or of concentrations normalised by their threshold (OAV) (López *et al.*, 2003). This approximation is explained in Table 5. This table shows the group of aroma compounds capable of introducing differences in the three varietal wines studied. The value OAVmax/OAVmin was calculated in order to know which compounds are responsible for the increase in the final aroma of the three types of wine studied. Some important conclusions can be drawn from these results.

The components with the greatest capacity to introduce modifications of the aroma composition of the studied wines are isoeugenol, eugenol, β -damascenone, 4-vinylguaiacol, 2-phenylethyl acetate and vanillin. The ratio between the maximum and the minimum OAVs was > 10 in all cases. The

TABLE 5

Determination of OAVmax/OAVmin in the aroma compounds of Moravia Dulce, Rojal and Tortosí wines elaborated over four consecutive vintages.

| Compounds | OAVmax/OAVmin |
|---------------------------|---------------|
| Isoeugenol | 18.0 |
| Eugenol | 15.1 |
| β-Damascenone | 15.0 |
| 4-Vinylguaiacol | 13.1 |
| 2-Phenylethyl acetate | 13.0 |
| Vanillin | 11.0 |
| Geraniol | 4.1 |
| Guaiacol | 3.6 |
| (Z)-3-Hexen-1-ol | 3.3 |
| Ethyl vanillate | 2.9 |
| Benzoic acid | 2.6 |
| Linalool | 2.1 |
| 3-(Methylthio)-1-propanol | 2.0 |
| Isoamyl acetate | 2.0 |
| Methanol | 1.9 |
| Acetovanillone | 1.9 |
| Butyric acid | 1.6 |
| Hexanoic acid | 1.6 |
| Ethyl lactate | 1.5 |
| Ethyl butanoate | 1.5 |
| Octanoic acid | 1.5 |
| 2-Phenylethyl alcohol | 1.4 |
| Decanoic acid | 1.4 |
| Ethyl octanoate | 1.3 |
| 1-Hexanol | 1.3 |
| Isobutanoic acid | 1.2 |
| Ethyl caproate | 1.2 |
| Isobutanol | 1.2 |
| Isovaleric acid | 1.1 |
| Ethyl acetate | 1.1 |
| 3-Methyl-1-butanol | 1.1 |
| Ethyl decanoate | 1.1 |

β-damascenone concentration is related mainly to the grape variety used (Ribereau-Gayon *et al.*, 2000; Flanzy, 2003), with Moravia Dulce wines presenting the highest values of this varietal compound. Although the OAVs of some of these compounds have a low value, if the maximum/minimum OAV ratio is elevated it can be verified that the grape variety employed is very important as a differentiator. A second group is made up of the components with a maximum/minimum OAV ratio of between 2 and 10. This group includes varietal aromas like linalool, geraniol and (Z)-3-hexen-1-ol, and also aromas generated by the yeast's metabolism. The last group is composed of 1-hexanol, and the rest are aromas generated by yeast metabolism. Some of these compounds have high OAVs, but the maximum/minimum OAV ratio is well bellow 2.0, which confirms their secondary importance.

CONCLUSIONS

This work provides better knowledge of the aroma fingerprint of Moravia Dulce, Rojal and Tortosí wines. The repetition of the analysis in four different vintages could be interesting for the winery companies to understand the behaviour of wines from these grape varieties. Clear differences were observed between the aromatic and sensory composition of wines from the three grape varieties. The aroma compounds with the greatest capacity to introduce modifications in the aroma composition of the studied wines were isoeugenol, eugenol, β-damascenone, 4-vinylguaiacol, 2-phenylethyl acetate and vanillin. Sweet, fruity and fatty were the aromatic series that contributed most markedly to the aroma profile of Moravia Dulce, Rojal and Tortosí wines. We can affirm that these minority grape varieties are perfectly adapted to the conditions of the La Mancha region, presenting a sensory aroma profile that is interesting for obtaining quality wines.

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