# CHANGES OF FREE AND GLYCOSIDICALLY BOUND MONOTERPENES AND AROMATIC ALCOHOLS IN MOSCATUEL AND RUBY SEEDLESS TABLE GRAPES DURING DEVELOPMENT

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## Abstract

**Aims**: The evolution of free and bound aromatic compounds (monoterpenes and aromatic alcohols) during ripening was determined in two cultivars of *Vitis vinifera* : Moscatuel (muscat aroma) and Ruby Seedless (neutral aroma). The aim was to identify the aromatic compounds in both varieties and to understand the differences in their aromatic characteristics.

**Methods and results**: The aromatic compounds were measured at six different maturity stages by gas chromatography and mass spectrometry. The most abundant compounds detected in Moscatuel grapes were linalool, geraniol, citronellol, nerol, citral, α-terpineol, linalool oxide I, linalool oxide II, rose oxides, benzyl alcohol and 2-phenylethanol. In general, the concentration of the main terpene compounds increased during grape development. The most abundant compounds detected in Ruby Seedless grapes were benzyl alcohol and 2-phenylethanol at concentrations higher than those observed in Moscatuel. The concentrations of the bound compounds were higher than those of the free forms, except for geraniol, nerol and citronellol in Ruby Seedless. Based on the Odour Activity Values (OAVs), linalool was the most odour-active odorant at the end of maturation in Moscatuel grapes. Other monoterpenes potentially contributing to the muscat aroma (OAV > 1) of this variety were rose oxide, citral and geraniol. In Ruby Seedless none of the studied compounds showed OAV > 1.

**Conclusion**: Several differences between Moscatuel and Ruby Seedless were observed in the monoterpene and alcohol profile and their contribution to the aroma.

**Significance and impact of the study**: This study characterizes and compares two non-previously described varieties of table grape, very appreciated by consumers, with different aromatic characteristics.

**Key words**: aroma, grape, terpenes, glycosides, gas chromatography

## Résumé

**Objectif**: L'évolution des composés aromatiques libres et glycosylés (monoterpènes et alcools aromatiques) pendant la maturation a été déterminée dans deux variétés de *Vitis vinifera* : Moscatuel (avec l'arôme muscat) et Ruby Seedless (avec l'arôme neutre). L'objectif principal de cette étude était d'identifier les composés responsables du profil aromatique dans les deux variétés et de comprendre les différences dans leurs caractéristiques aromatiques.

**Méthodes et résultats**: Les composés aromatiques ont été mesurés à six différents stades de maturité par chromatographie en phase gazeuse et spectrométrie de masse. Les composés les plus abondants détectés dans les baies de Moscatuel étaient le linalol, le géraniol, le citronellol, le nérol, le citral, l'α-terpinéol, l'oxyde de linalol I, l'oxyde de linalol II, l'oxyde de rose, l'alcool benzylique et le 2-phényléthanol. En général, la concentration des principaux composés terpéniques a augmenté au cours du développement de la baie. Dans les baies de Ruby Seedless, les composés les plus abondants étaient l'alcool benzylique et le 2-phényléthanol, avec des concentrations supérieures à celles observées dans la variété Moscatuel. Dans les deux variétés, les concentrations des composés glycosylés étaient plus élevées que ceux des formes libres, à l'exception du géraniol, nérol et citronellol dans Ruby Seedless. À la fin de la maturation du raisin et en fonction de valeurs obtenues du Nombre d'Unités d'Odeur (NUO), le linalol est le composé qui a contribué le plus à l'arôme de la variété Moscatuel. De même, l'oxyde de rose, le citral et le géraniol contribuent potentiellement à l'arôme muscat (NUO > 1) de cette variété. Pour ce qui est de Ruby Seedless, aucun des composés étudiés n'a montré un NUO > 1.

**Conclusion**: On a trouvé de nombreuses différences dans les profils de monoterpènes et d'alcools et leur contribution à l'arôme entre Moscatuel et Ruby Seedless.

**Signification et impact de l'étude**: Cette étude caractérise et compare deux variétés de raisin de table non-décrites précédemment et très appréciées par les consommateurs, avec des caractéristiques aromatiques différentes.

**Mots-clés**: arôme, raisin, terpènes, glycosides, chromatographie en phase gazeuse

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## INTRODUCTION

Table grape is a fruit crop of major economic importance with about 124704 tons produced in the province of Murcia (southeast Spain) annually (C.A.R.M., 2005). The improvement of qualitative features such as aroma (especially the « Muscat » flavour) is one of the main breeding objectives for this crop worldwide. The main molecules identified as responsible for this « Muscat » flavour are monoterpenes, biologically active molecules that belong to the structurally diverse group of isoprenoids. At least 50 molecules belonging to this family have been identified in grapes and wines (Mateo and Jiménez, 2000). Monoterpenols, particularly, have been shown to contribute to the characteristic varietal aroma of grapes of *Vitis vinifera* cultivars (Ebeler, 2001). For example, the terpenic alcohols (linalool, nerol, geraniol, α-terpineol and citronellol) are known to be the main components responsible for the aroma of Muscat grapes (Ribéreau-Gayon *et al*., 1975). Highly aromatic varieties of the Muscat group or Gewurztraminer are characterised by high monoterpenol concentrations (Gunata *et al*., 1985a, Dimitriadis and Williams, 1984), whereas intermediate or low levels are found in less aromatic or neutral varieties, respectively (Mateo and Jiménez, 2000). Other compounds found to be of great aromatic importance such as hydrocarbons, norisoprenoids and some alcohols have also been found in grapes and wines. These aromatic compounds are distributed in the pulp and skin of the berry, with the highest concentration in the latter part (Wilson *et al*., 1986, Gunata *et al*., 1985b). A significant portion of these compounds is present as non-volatile glycosides that can be hydrolyzed (enzymatically or chemically) to the free forms, whence they can contribute to the grape aroma. Still, the bound glycosides forms are generally more abundant than the free ones (Gunata *et al*., 1985a; Dimitriadis and Williams, 1984).

Grape ripening is a physiological process that starts at véraison and lasts until the fruit is fully ripe, when maturity is reached. It is a very important period that influences the composition of the grapes. Hence, knowledge of the varietal volatile composition is important to evaluate the aroma potential of the grapes and determine the time needed for grapes to reach maximum potential. The region of Murcia has a large agricultural sector dedicated to table grapes with a cultivated area ~ 6000 ha (C.A.R.M., 2005). Moscatuel is a highly appreciated variety because of its intense muscat aroma. On the other hand, Ruby Seedless is a red variety with a neutral aroma highly valued by the consumers because of its crispy texture and the absence of seeds. The aroma characterization of these two varieties has not previously been reported. In addition to identifying the aromatic compounds in both varieties, the aim of this work was to compare the evolution of these compounds during ripening in muscat and neutral varieties in order to understand the differences in their aromatic characteristics.

## MATERIALS AND METHODS

### 1. Sampling

*Vitis vinifera* L. cvs. Moscatuel and Ruby Seedless grapes from an experimental vineyard at the Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA) located in Torre Pacheco (Murcia, SE Spain) were sampled weekly in June, July and August 2008. The Moscatuel and Ruby Seedless vineyards were planted in 1992 and the morphological identity of the grape berries was assessed using 48 morphological descriptors (O.I.V., 1984). The vines were trained with a single trunk, supported on overhead tendone trellis (parral) and pruned to 4 canes with 10-12 nodes. Five vines of each variety were selected and berries were picked weekly from one bunch per vine (200 berries per variety). For each sampling, berries were classified into six stages of maturity according to Fenoll *et al*. (2009). For the first maturity stage (stage 1), green hard berries (pre-véraison) were considered. For the second to the sixth maturity stage, berry density was used as maturity criterion since this parameter increases after véraison in ripening berries. Therefore the subsequent stages of maturity were berries with a density of less than 60 g/L (stage 2), berries with a density of 60-80 g/L (stage 3), 80-100 g/L (stage 4), 100-120 g/L (stage 5), and 120-140 g/L (only reached by Moscatuel) (stage 6). Berries of each variety at each stage of maturity were collected and divided into three subsamples to be analysed separately. A portion of each subsample was squeezed to measure total acidity by titration with NaOH and total soluble solids (TSS) using a hand-held refractometer. The maturity index was calculated as TSS/titratable acidity. A second portion of 200 g was immediately frozen in liquid nitrogen and kept at -80 ºC for later determination of the aromatic compounds. Finally, the water content of berries at the different maturity stages was determined by difference between fresh and dry weight after drying in an oven at 60 ºC to constant weight. Water content values were used to calculate the concentrations of aromatic compounds in µg/L in order to determine their Odour Activity Values (OAVs).

### 2. Standards and solvents

Nerol, geraniol, α-terpineol, rose oxides, linalool oxide I and II (*trans*-furan linalool oxide and *cis*-furan linalool oxide, respectively), eugenol and sucrose were purchased from Fluka (Buchs, Switzerland); 2-octanol, glucose, and tartaric, malic, citric, succinic and ascorbic acids were purchased from Sigma-Aldrich (St Louis, USA); fructose and sulphuric acid were purchased from Merck KGaA

**Table 1. Retention Index (RI), Retention Time (RT, min), Target (T), Qualifier Ions ($Q_1$, $Q_2$ and $Q_3$, m/z) and Abundance Ratios (%) of Qualifier Ion/Target Ion ($Q_1$/T and $Q_2$/T)* of the studied compounds.**

| | RI | | RT | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Compounds | HP5MSI | DBWAX | HP5MSI | DBWAX | T | $Q_1$ | $Q_2$ | $Q_3$ | $Q_1$/T | $Q_2$/T |
| Rose oxide I | 1114 | 1381 | 10.84 | 19.85 | 139 | 69 | 83 | 154 | 40.2 | 22.3 |
| Rose oxide II | 1133 | 1397 | 11.50 | 20.63 | 139 | 69 | 83 | 154 | 39.8 | 23.3 |
| Linalool oxide I | 1070 | 1468 | 9.39 | 24.19 | 59 | 94 | 93 | 111 | 61.2 | 48.2 |
| Linalool oxide II | 1088 | 1496 | 9.98 | 25.60 | 59 | 94 | 93 | 68 | 63.0 | 47.4 |
| Linalool | 1101 | 1565 | 10.40 | 29.10 | 71 | 93 | 80 | 121 | 86.3 | 35.0 |
| α-Terpineol | 1202 | 1718 | 14.12 | 36.36 | 59 | 93 | 121 | 136 | 87.9 | 78.8 |
| Citral | 1284 | 1756 | 17.62 | 37.99 | 69 | 84 | 94 | 137 | 30.8 | 19.7 |
| Citronellol | 1241 | 1782 | 15.75 | 39.20 | 69 | 67 | 82 | 95 | 63.5 | 53.2 |
| Nerol | 1241 | 1822 | 15.75 | 40.86 | 69 | 93 | 68 | 67 | 60.3 | 26.4 |
| Geraniol | 1268 | 1870 | 16.91 | 42.82 | 69 | 93 | 68 | 67 | 27.3 | 19.8 |
| Benzyl alcohol | 1024 | 1917 | 7.97 | 44.65 | 79 | 108 | 107 | 77 | 94.8 | 66.0 |
| 2-Phenylethanol | 1116 | 1954 | 10.91 | 46.01 | 91 | 92 | 122 | 65 | 57.6 | 26.8 |
| Eugenol | 1366 | 2215 | 21.39 | 55.26 | 164 | 149 | 131 | 103 | 35.2 | 27.0 |
| *Internal standards* | | | | | | | | | | |
| 2-Octanol | 986 | 1440 | 6.89 | 22.79 | 45 | 55 | 97 | 84 | 26.10 | 12.5 |
| Thymol | 1305 | 2226 | 18.55 | 55.66 | 135 | 150 | 91 | 136 | 30.12 | 14.5 |

*Q/T (%) ratios are the results of abundance values of the qualifier ion ($Q_1$, $Q_2$) divided by the abundance of the target ion (T) x 100.

(Darmstadt, Germany); and linalool, citronellol, benzyl alcohol, 2-phenylethanol, citral and thymol were purchased from Acros Organics (Geel, Belgium). All solvents used in this study were of high purity grade and were supplied by Scharlau (Barcelona, Spain).

### 3. Volatile analysis

The analysis of free and glycosidically bound aromatic compounds was carried out according to the method described by Di Stefano (1991) with some modifications. Two hundred grams of berries were deseeded and ground under liquid nitrogen using a Dangoumau ball grinder. Fifty grams of ground berries were suspended with a Polytron PT2000 homogenizer (Kinematica AG, Lucerne, Switzerland) in 100 ml of pure water containing 0.5 g of D-gluconic acid lactone (Sigma) to inhibit grape , β-glucosidase activity. Five microliters of 2-octanol (0.4 g/L) and 5 µL of thymol (0.4 g/L) were added as internal standards. After stirring for 15 minutes at 4 ºC, the mixture was centrifuged (9000 g; 20 minutes; 4 ºC) in an Eppendorf 5810R centrifuge (Hamburg, Germany). The supernatant was filtered through glass wool, stirred in the presence of 1 g of polyvinylpolypyrrolidone (Sigma) to eliminate the high levels of phenolic compounds capable of inhibiting glycosidase activities, and filtered again through glass wool. The clear filtrate was then passed through the SPE column containing 0.5 g of $C_{18}$ Varian (Lake Forest, USA) already activated with 10 ml of methanol and 20 ml of water at a flow rate of 1 ml/min. The column was rinsed with 50 ml of Milli Q grade water (Millipore, Bedford, MA, USA) to eliminate sugars, acids, and other low molecular weight polar compounds. The free fraction was eluted with 100 ml of dichloromethane. The extract was water dried over $Na_2SO_4$, and the solvent was concentrated to 2 ml by distillation through a Vigreaux column at 35 ºC.

The bound fraction was eluted with 50 ml of methanol and the extract was concentrated to 1 ml under vacuum with a Büchi model R-205 rotavapor (Flawil, Switzerland) at 35 ºC. The extract was then transferred into a small tube and concentrated to dryness at 40 ºC under a stream of nitrogen. The dried glycosidic extract was dissolved in 1 ml of citrate-phosphate buffer (0.2 M, pH 5). The mixture was washed five times with 1.5 mL of dichloromethane to eliminate possible traces of free volatiles. Enzymatic hydrolysis was carried out using

**Table 2. Changes in total soluble solid (TSS, ºBrix), pH, titratable acidity (g tartaric/L), maturity index, sugars (glucose, fructose and sucrose, mg/g grape) and organic acids (tartaric acid, malic acid and citric acid, mg/g grape) in developing Moscatuel and Ruby Seedless grapes.**

| | Moscatuel | | | | | | Ruby Seedless | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-véraison | <60 g/L | 60-80 g/L | 80-100 g/L | 100-120 g/L | 120-140 g/L | Pre-véraison | <60 g/L | 60-80 g/L | 80-100 g/L | 100-120 g/L |
| TSS | 7.8 ± 0.0 | 8.3 ± 0.0 | 11.4 ± 0.0 | 13.5 ± 0.0 | 16.6 ± 0.0 | 18.5 ± 0.0 | 6.5 ± 0.0 | 8.2 ± 0.0 | 11.4 ± 0.0 | 13.1 ± 0.0 | 17.5 ± 0.0 |
| Acidity | 34.8 ± 0.1 | 31.5 ± 0.3 | 15.5 ± 0.1 | 13.2 ± 0.1 | 8.8 ± 0.1 | 8.6 ± 0.1 | 36.3 ± 0.1 | 25.7 ± 0.2 | 6.7 ± 0.1 | 5.9 ± 0.0 | 4.4 ± 0.0 |
| Maturity Index | 0.22 | 0.26 | 0.74 | 1.02 | 1.90 | 2.16 | 0.18 | 0.32 | 1.70 | 2.22 | 3.98 |
| Glucose | 25.6 ± 0.7 | 41.8 ± 2.6 | 53.7 ± 3.3 | 63.1 ± 2.1 | 69.4 ± 1.7 | 77.5 ± 1.9 | 27.6 ± 0.7 | 58.9 ± 0.1 | 82.9 ± 2.5 | 92.3 ± 6.5 | 87.3 ± 6.2 |
| Fructose | 14.2 ± 0.5 | 31.6 ± 2.2 | 48.4 ± 2.8 | 57.5 ± 2.1 | 63.7 ± 1.9 | 75.2 ± 2.6 | 22.0 ± 0.7 | 61.6 ± 0.4 | 86.7 ± 2.8 | 96.9 ± 1.5 | 85.7 ± 5.3 |
| Sucrose | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | 0.8 ± 0.2 | 0.7 ± 0.3 | 1.7 ± 0.1 | 1.8 ± 0.3 |
| Tartaric acid | 9.8 ± 0.4 | 8.6 ± 0.3 | 6.7 ± 0.5 | 6.9 ± 0.3 | 8.0 ± 0.3 | 7.0 ± 0.0 | 9.4 ± 0.2 | 7.4 ± 0.4 | 7.1 ± 0.6 | 7.4 ± 0.1 | 7.5 ± 0.2 |
| Malic acid | 22.8 ± 1.2 | 17.2 ± 0.8 | 4.8 ± 0.6 | 4.8 ± 0.2 | 5.2 ± 0.2 | 2.6 ± 0.1 | 8.3 ± 0.1 | 1.7 ± 0.0 | 1.6 ± 0.1 | 1.4 ± 0.0 | 1.1 ± 0.1 |
| Citric acid | 0.7 ± 0.0 | 0.7 ± 0.1 | 0.4 ± 0.1 | 0.4 ± 0.0 | 0.4 ± 0.0 | 0.4 ± 0.1 | 0.4 ± 0.0 | 0.3 ± 0.0 | 0.4 ± 0.1 | 0.4 ± 0.0 | 0.4 ± 0.0 |

Values are mean ± standard error (n = 3). n.d. : not detected

200 µL of a commercial preparation AR-2000 (0.1 g/mL) with glycosidase side activities (Gist Brocades, France). After stirring, the tube was sealed and placed in a water bath at 40 ºC for 16 heures After the addition of 5ÕL of 2-octanol (0.4 g/L) and 5ÕL of thymol (0.4 g/L) as internal standards, the mixture was extracted five times with 0.4 mL of dichloromethane. The extract was dried over $Na_2SO_4$ and stored at -20 ºC until analysis. All analyses were performed in triplicate.

## 4. Gas chromatography and mass spectrometry

Final extracts were analysed by gas chromatography with a flame ionization detector (FID) and volatile compounds were confirmed by a gas chromatograph (GC) equipped with a mass spectrometer. For the former analysis, an Agilent (Waldbronn, Germany) HP 6890 GC equipped with a flame ionization detector and automatic Agilent 7683 split/splitless injector was used. The columns used were HP-5MSI (30 m x 0.25 mm i.d.) with 0.25 Õm film thickness and DB-WAX (30 m x 0.32 mm i.d.) with 1.0 Õm film thickness. Both stationary phases were supplied by Agilent Technologies. Helium was used as the carrier gas (constant pressure eluting, thymol 18.55 minutes for the HP-5MSI column and 55.66 min for the DB-WAX column). A 2 ÕL sample was injected into the GC using « splitless » mode. The injector and detector were operated at 250 and 280 ºC, respectively. The temperature program for the HP-5MSI column was 60 ºC to 240 ºC at 3.0 ºC/min and for the DB-WAX column was 60 ºC to 175 ºC at 2.5 ºC/min, after which it was increased to 195ºC at a rate of 3.5ºC/min followed by a final ramp to 220ºC at a rate of 2.5ºC/min, and held for 15 minutes The total analysis time for the HP-5MSI and the DB-WAX columns was 60.00 and 76.71 min, respectively. The equilibrium time was une minute

Standard solutions (containing rose oxides, linalool oxide I and II, linalool, α-terpineol, citral, citronellol, nerol, geraniol, benzyl alcohol, 2-phenylethanol, eugenol, 2-octanol and thymol) with concentrations of 5 to 250 Õg/L and 0.25 to 12.5 mg/L were injected in the GC/FID to calculate the linearity of the detector response of the studied compounds. The FID response for volatile compounds was linear in the concentration range assayed with determination coefficients higher than 0.998.

For the confirmatory analysis, an Agilent HP 6890 GC equipped with a 5973N mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) was operated in electron impact ionization mode with an ionizing energy of 70 eV, scanning from m/z 40 to 300 at 3.21 scans per s. The ion source temperature was 230 ºC and the quadrupole temperature was 150 ºC. The electron multiplier voltage (EM voltage) was maintained at 1300 V, and a solvent delay of 6.0 min was used. Gas chromatography was performed in the same conditions as used in GC/FID. Analysis was performed with selected ion monitoring (SIM) mode using target (T, base ion) and qualifier ions ($Q_1$, $Q_2$, $Q_3$, and other characteristic ions of lower intensity from primary ionization). Aromatic compounds were identified by retention index and retention times, obtained by using two GC columns with stationary phases of differing polarities and compared with those of known compounds, and by comparison of mass spectra using the NBS75K library (U.S. National Bureau of Standards, 1986) and spectra obtained from the standard. Also, target and qualifier ions and the qualifier-to-target abundance ratios (Q/T %) of pure reference standards were compared with those obtained for the aromatic compounds under study. The target and qualifier abundances were determined by injection of individual chemical standards under the same chromatographic conditions, using full

scan with the mass/charge ratio ranging from m/z 40 to 300. Table 1 lists the compounds along with their retention index, retention times, the target and qualifier ions, and their qualifier-to-target abundance ratios. Retention times had to be within ± 0.une minute of the expected time, and qualifier-to-target ratios had to be within a 10 % range for positive confirmation. Quantitation was achieved by relating the peak area of the analytes to the peak area of the internal standard 2-octanol.

### 5. Sugar and organic acids analysis

Samples of whole cluster were homogenized using a Polytron PT-MR 3100 (Kinematica; Lucerne, Switzerland) in ultrapure water at 50 ºC and successively extracted to a final volume to 50 ml. The extracts were centrifuged at 10000 g for 10 minutes in an Eppendorf centrifuge (Biotech. International, Germany) and the supernatant was recovered, filtered through 0.45 µL and purified on a $C_{18}$ Sep-Pak cartridge. For sugars, samples of 20 µL of extract were analysed using an HPLC system (Hewlett-Packard, Germany) equipped with a quaternary pump and a refraction index detector. Separation was performed on a 305 x 7.8 mm i.d., 9 µm ionic-exchange HC-75 column with ultrapure water as the mobile phase. Organic acids were analysed using a photodiode array detector. The separation was performed on a 250 x 4.6 mm i.d., 5 µm Mediterranea Sea 18 column (Teknokroma, Barcelona, Spain). The reading was taken at 210 nm.

## RESULTS AND DISCUSSION

### 1 Changes in classic parameters during berry development

The evolution of TSS, titratable acidity, maturity index, sugars and organic acids of the Moscatuel and Ruby Seedless grapes during the different stages of berry maturation are shown in table 2. As expected, TSS and the maturity index increased while titratable acidity decreased during maturation. The sharpest decrease in titratable acidity was observed between stages 2 and 3. However, the greatest increase in the maturity index was observed between stages 2 and 3. Glucose and fructose

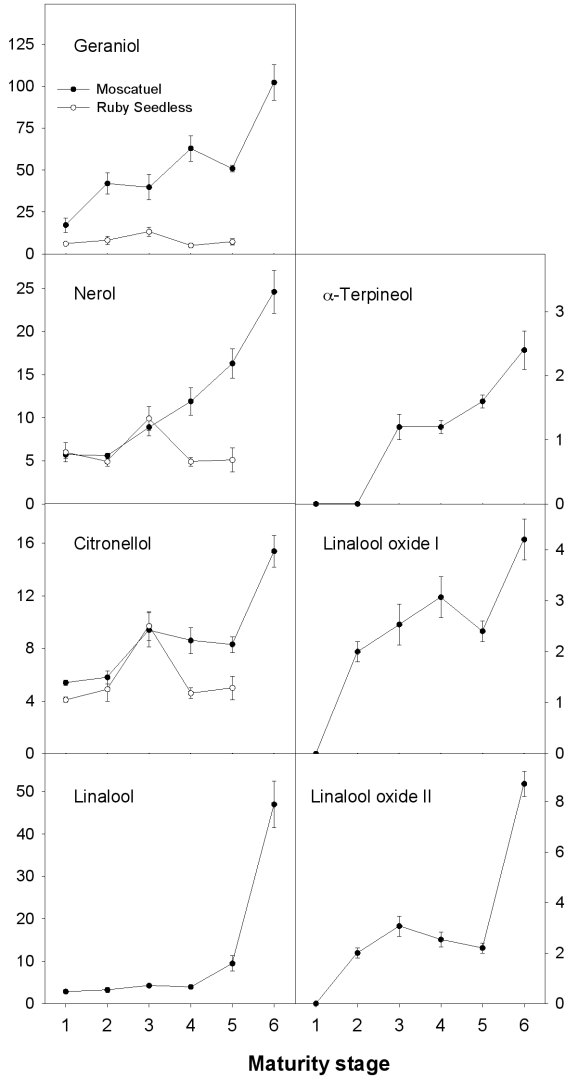


**Figure 1. Evolution of free monoterpenes (µg/kg) in developing Moscatuel and Ruby Seedless grapes.**
Stages of maturity: 1) green hard berries (pre-véraison), 2) berries with a density of less than 60 g/L, 3) berries with a density of 60-80 g/L, 4) 80-100 g/L, 5) 100-120 g/L, and 6) 120-140 g/L. Values are mean ± standard error (n = 3).

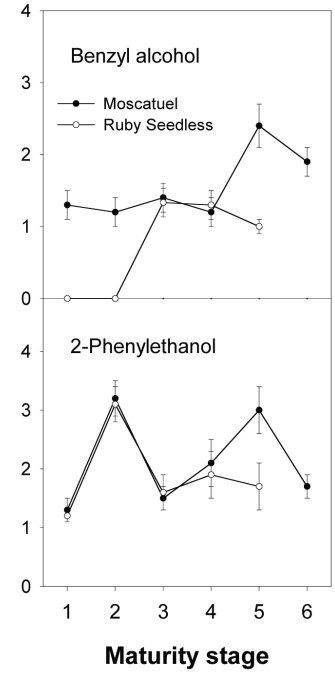


**Figure 2. Evolution of free aromatic alcohols (µg/kg) in developing Moscatuel and Ruby Seedless grapes.**
Stages of maturity: 1) green hard berries (pre-véraison), 2) berries with a density of less than 60 g/L, 3) berries with a density of 60-80 g/L, 4) 80-100 g/L, 5) 100-120 g/L, and 6) 120-140 g/L. Values are mean ± standard error (n = 3).

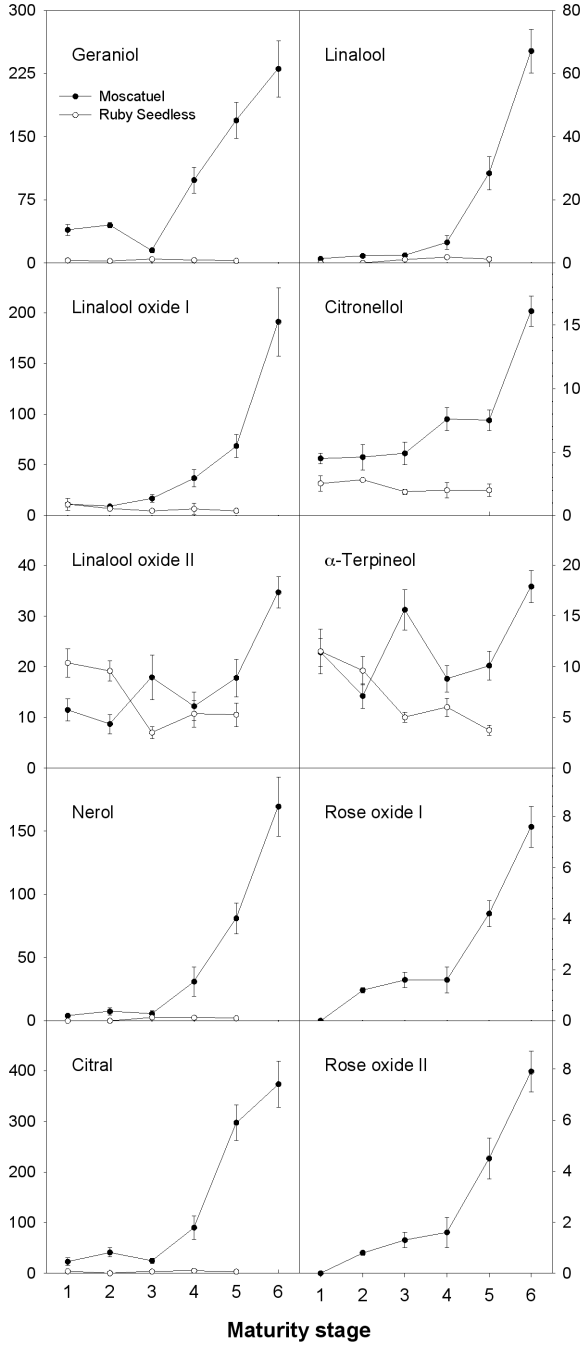


**Figure 3. Evolution of glycosidically bound monoterpenes (µg/kg) in developing Moscatuel and Ruby Seedless grapes.** Stages of maturity: 1) green hard berries (pre-véraison), 2) berries with a density of less than 60 g/L, 3) berries with a density of 60-80 g/L, 4) 80-100 g/L, 5) 100-120 g/L, and 6) 120-140 g/L. Values are mean ± standard error (n = 3).

were the predominant sugars in both varieties and sucrose was present at trace levels in Ruby Seedless but not in Moscatuel, which agrees with the finding of Liu *et al.* (2006). In both cultivars, the concentration of sugars increased with berry ripening and, at the end of ripening, Ruby Seedless presented higher concentrations than Moscatuel. In Moscatuel, glucose values were always higher than those of fructose while no significant differences were found between the two sugars during the ripening of Ruby Seedless. In all stages of ripening, total sugars were higher in Ruby Seedless berries. The differences in sugar concentration between the two varieties might be associated with the activity of the enzymes involved in the sugar metabolism in grape berries (Varandas *et al.*, 2004). As regards organic acids, three main compounds were identified: tartaric, malic and citric acids. Tartaric and malic acids were found in greater proportions in both varieties and their concentration decreased during berry ripening. Ascorbic and succinic acids were not determined in either variety. A decrease in the two main acids (tartaric and malic) in grapes has been reported by many authors (Asrey *et al.*, 2008). The tartaric acid content was significantly higher than that of malic acid throughout the ripening of Ruby Seedless berries and from the third stage to the end of ripening in Moscatuel.

## 2. Free volatile compounds

A total of nine free volatile compounds (monoterpenes and aromatic alcohols) were identified in Moscatuel and five in Ruby Seedless (Figures 1 and 2). At the first maturity stage (pre-véraison), geraniol was the most abundant compound detected in both varieties, followed by nerol and citronellol. At this stage, the sum of geraniol, nerol and citronellol represented about 84 % and 83 % of total free volatile aromatic compounds in Moscatuel and Ruby Seedless, respectively. During the last maturity stage, geraniol was the most abundant compound in both varieties, although Moscatuel showed concentrations 14-fold higher than Ruby Seedless. Geraniol is one of the main compounds contributing to the distinctive floral character of Muscat varieties (Lund and Bohlmann, 2006). A high concentration of free geraniol in green berries, as observed here in Moscatuel, suggests a significant role for this compound, derived from geranyl diphosphate (GPP), in the biosynthesis of other terpenes during ripening (Hidalgo Togores, 2002). As regards nerol, its evolution during ripening depended on the variety. In Moscatuel, its concentration increased substantially during maturation while in Ruby Seedless it stayed almost constant throughout berry development. Citronellol was present in Moscatuel and Ruby Seedless throughout the maturation period. This contrasts with other Muscat varieties, such as Muscat of Alexandria, in which citronellol is not detected as free terpenol at any stage of the berry development (Wilson *et al.*, 1984). Although nerol and citronellol concentrations were similar for both varieties at the green maturation stage, both values were higher in Moscatuel at the end of maturation (5 and 3-fold higher, respectively). Besides geraniol, nerol and citronellol, other terpene alcohols that impart a floral character to berries are linalool and α-terpineol (Ribéreau-

Gayon *et al*., 1975). In Moscatuel, the linalool concentration increased throughout the maturation period, as previously reported for Muscat Hamburg and Muscat of Alexandria grapes (Wilson *et al*., 1984, Fenoll *et al*., 2009), and in the last stage it reached a level that exceeded the concentration of all other aromatic compounds except geraniol. However, in Ruby Seedless linalool was not detected as free terpenol at any stage of berry development. Similarly, the α-terpineol concentration increased during ripening in Moscatuel, while this compound was not detected as free terpenol in Ruby Seedless at any stage of berry development. Nerol, citronellol, linalool and α-terpineol, even though their concentrations are usually lower than that of geraniol, are considered the principal compounds responsible for the muscat aroma in grape varieties (Mateo and Jiménez, 2000). Finally, the high oxidation state monoterpenes, linalool oxide I and linalool oxide II, were only detected in Moscatuel, where they increased throughout berry development.

As regards aromatic alcohols, benzyl alcohol and 2-phenylethanol were found in both varieties. The levels of benzyl alcohol and 2-phenylethanol are usually high in non-Muscat grape varieties, in which terpenols are less abundant (Voirin *et al*., 1992a, Selli *et al*., 2003, Dieguez *et al*., 2003, Rocha *et al*., 2000). However, the concentration of these compounds was low and fluctuated during ripening in both Ruby Seedless and Moscatuel.

### 3. Glycosidically bound compounds

The method for analysing glycosidically bound compounds involved enzymatic hydrolysis. Acid hydrolysis was not used because it can induce changes in the terpenoid composition (Gunata *et al*., 1985a). At present, the exact aromatic role of glycosidically bound compounds is not clear. According to some authors, they play an important role as aroma precursors in the biosynthesis of terpenoids, since their aglycone groups have odour properties (Marais, 1983). In contrast, other authors suggest that they are a transport and/or an accumulation form during cell biosynthesis (Loomis and Croteau, 1980).

A total of 13 and 11 bound volatile compounds (monoterpenes and aromatic alcohols) were identified during ripening in Moscatuel and Ruby Seedless, respectively (Figures 3 and 4). In general, the levels of aromatic compounds were much higher in the bound fraction than in the free fraction, except for geraniol, nerol and citronellol in Ruby Seedless (Figure 3). In addition, some compounds that were not detected in the free form were found in the bound fraction. For example, glycosides of citral, rose oxide I and rose oxide II were detected in Moscatuel, while glycosides of citral, linalool, α-terpineol, linalool oxide I and linalool oxide II were detected in Ruby Seedless.

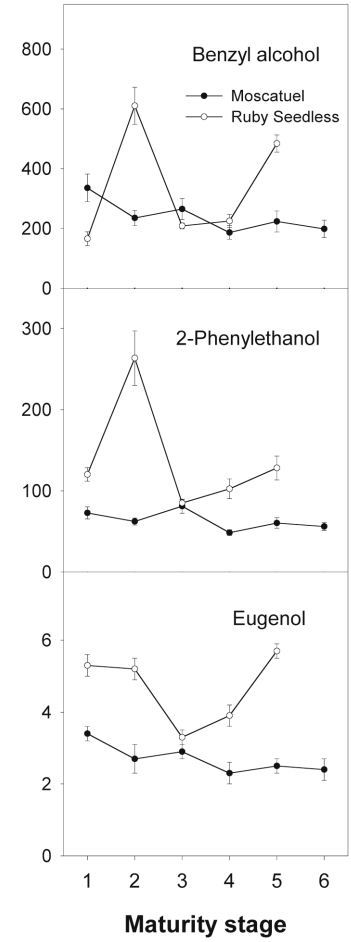


**Figure 4. Evolution of glycosidically bound aromatic alcohols (µg/kg) in developing Moscatuel and Ruby Seedless grapes.**

Stages of maturity: 1) green hard berries (pre-véraison), 2) berries with a density of less than 60 g/L, 3) berries with a density of 60-80 g/L, 4) 80-100 g/L, 5) 100-120 g/L, and 6) 120-140 g/L. Values are mean ± standard error (n = 3).

In the green stage of ripening, the geranyl derivative was the most abundant glycosidically bound terpene detected in Moscatuel. Its concentration initially decreased from the second to the third stage of maturation before increasing towards the last stage. A similar evolution was observed for nerol and citral glycosides. Linalool and citronellol glycosides increased during berry development. At the mature stage, citral glycoside was the most abundant compound in this variety. In Ruby Seedless, concentrations of geraniol, nerol, citral, linalool, linalool oxide I and citronellol glycosides were low and fluctuated during ripening. The concentrations of α-terpineol glycoside in Moscatuel varied between 7.1 and 17.9 µg/kg but decreased in Ruby Seedless during ripening.

Linalool oxide I and II and rose oxide I and II were also identified in Moscatuel, where they increased during ripening. Rose oxide is synthesized from citronellol (Girard *et al*., 2002) and seems to be associated with aroma

in Muscat grapes (Fenoll *et al*., 2009). In Ruby Seedless, linalool oxide I and II were also identified, the latter being the most abundant glycosidically bound terpene detected in this variety. However, their concentrations slightly decreased during ripening.

As regards aromatic alcohols, benzyl alcohol, 2-phenylethanol and eugenol were identified in the glycosidically bound fraction in both Moscatuel and Ruby Seedless (Figure 4). Voirin *et al*. (1992b) indicated that the presence of aromatic alcohols is associated with neutral cultivars. Accordingly, Ruby Seedless showed higher aromatic alcohol values than those of Moscatuel during almost the whole maturation period. 2-Phenylethanol is a derivative of the phenylpropanoid metabolism in grapes that has a floral, rose-like aroma. As for benzyl alcohol, it has been described as a floral aroma component (Lamikanra, 1987, Lamikanra *et al*., 1996, Ramey *et al*., 1986). During all maturation stages, bound benzyl alcohol was more abundant than bound 2-phenylethanol and their concentrations fluctuated during ripening. Finally, eugenol glycoside was detected at low concentrations in both Moscatuel and Ruby Seedless, these levels remained constantly low for Moscatuel but fluctuated and increased toward the end of maturation for Ruby Seedless.

### 4. Odour activity values

The OAVs of aromatic compounds were calculated to estimate their sensory contribution to the general flavour. OAVs were calculated using the equation OAV = c/t, where c is the total concentration of each compound in the grape sample and t is the odour threshold value of the compound in water (Schieberle, 1991). In this work, t values were taken from information available in the literature (Karagiannis *et al*., 2000 ; Ohloff, 1978, Buttery *et al*., 1988 ; Buttery *et al*., 1990 ; Takeoka *et al*., 1990). In agreement with other authors, only compounds with OAVs > 1 were considered as active odorants (Guth, 1997).

For Ruby Seedless, the OAVs of all the compounds were under the odour threshold during maturation, a result that reflects the neutral aroma of this cultivar (Table 3). Among all the aromatic compounds determined in Moscatuel, only geraniol was detected at levels above its odour threshold (OAVs > 1) throughout the maturation period. In stage 2, rose oxides, linalool and citral reached OAVs > 1. As a consequence, their contribution to the aroma can be considered to start from this maturation stage. Sharp increases in rose oxides, linalool and citral OAVs were observed in maturity stages 5 and 6. Linalool showed the greatest OAV at the end of the maturation period. These results agree with those of other authors who reported a high contribution of linalool to the grape muscat aroma (Mateo and Jiménez, 2000 ; Fenoll *et al*., 2009). Recent studies have reported the relevance of substances at concentrations of at least 20 % of their threshold unit (OAV > 0.2) to the overall aroma (Gómez-Míguez *et al*., 2007). According to this, nerol and citronellol would also contribute to the final aroma since their OAVs were 0.65 and 0.80 at the end of maturation, respectively. These values were lower than those previously reported for Muscat Hamburg grapes, which showed OAVs > 1 for nerol and citronellol at the end of the maturation (Fenoll *et al*., 2009).

## CONCLUSION

In conclusion, the main compounds detected in the free fraction of Moscatuel were linalool, geraniol, citronellol, nerol, α-terpineol, linalool oxide I, linalool oxide II, benzyl alcohol and 2-phenylethanol, while in Ruby Seedless they were geraniol, citronellol, nerol, benzyl alcohol and 2-phenylethanol. As regards the bound fraction, the main compounds detected were geraniol, linalool, citral, nerol, citronellol, α-terpineol, linalool oxide I, linalool oxide II, rose oxide I, rose oxide II, benzyl alcohol and 2-phenylethanol in Moscatuel, and benzyl alcohol and 2-phenylethanol in Ruby Seedless. According to other studies on Muscat varieties, most compounds have higher concentrations in the bound fraction than in the free fraction. However, some varietal differences in the detected compounds and their contribution to the total aromatic composition were observed with regard to previous studies. Based on the OAVs, linalool was the most odour-active odorant at the end of maturation in Moscatuel grapes. Other monoterpenes potentially contributing to the muscat aroma of this variety were rose oxide, citral and geraniol (OAVs > 1), and to a lesser extent nerol and citronellol (OAV > 0.2). In Ruby Seedless none of the studied compounds showed OAV > 1.

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**Table 3. Odour Activity Values (OAVs) of the compounds with the most influence on the aroma of Moscatuel and Ruby Seedless grapes.**

| Compounds | Published odour threshold in water (µg/L) | Moscatuel | | | | | | Ruby Seedless | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pre-véraison | <60 g/L | 60-80 g/L | 80-100 g/L | 100-120 g/L | 120-140 g/L | Pre-véraison | <60 g/L | 60-80 g/L | 80-100 g/L | 100-120 g/L |
| Linalool oxide I | >3000[a] | 0.00 | 0.00 | 0.01 | 0.01 | 0.02 | 0.07 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Linalool oxide II | >3000[a] | 0.00 | 0.00 | 0.01 | 0.00 | 0.01 | 0.01 | 0.01 | 0.01 | 0.00 | 0.00 | 0.00 |
| Rose oxide I (trans) | 0.5[b] | - | 2.40 | 3.20 | 3.20 | 8.40 | 15.20 | - | - | - | - | - |
| Rose oxide II (cis) | 0.5[b] | - | 1.60 | 2.60 | 3.20 | 9.00 | 15.80 | - | - | - | - | - |
| Linalool | 6[c] | 0.68 | 1.01 | 1.10 | 1.73 | 6.31 | 19.02 | 0.00 | 0.00 | 0.18 | 0.30 | 0.17 |
| α-Terpineol | 330[d] | 0.03 | 0.02 | 0.05 | 0.03 | 0.04 | 0.06 | 0.03 | 0.03 | 0.02 | 0.02 | 0.01 |
| Citral | 32[c] | 0.72 | 1.29 | 0.77 | 2.82 | 9.29 | 11.67 | 0.13 | 0.03 | 0.11 | 0.16 | 0.10 |
| Citronellol | 40[b] | 0.25 | 0.26 | 0.36 | 0.41 | 0.40 | 0.79 | 0.17 | 0.19 | 0.29 | 0.17 | 0.18 |
| Nerol | 300[b] | 0.03 | 0.04 | 0.05 | 0.14 | 0.32 | 0.65 | 0.02 | 0.02 | 0.04 | 0.02 | 0.02 |
| Geraniol | 40[d] | 1.41 | 2.17 | 1.37 | 4.03 | 5.50 | 8.32 | 0.22 | 0.26 | 0.44 | 0.21 | 0.24 |
| *Other compounds* | | | | | | | | | | | | |
| Benzyl alcohol | 10000[e] | 0.03 | 0.02 | 0.03 | 0.02 | 0.02 | 0.02 | 0.02 | 0.06 | 0.02 | 0.02 | 0.05 |
| 2-Phenylethanol | 1100[e] | 0.07 | 0.06 | 0.08 | 0.05 | 0.06 | 0.05 | 0.11 | 0.24 | 0.08 | 0.09 | 0.12 |
| Eugenol | 6[c] | 0.57 | 0.45 | 0.48 | 0.38 | 0.42 | 0.40 | 0.88 | 0.87 | 0.55 | 0.65 | 0.95 |

[a]Karagiannis *et al.*, 2000; [b]Ohloff, 1978; [c]Buttery *et al.*, 1990; [d]Takeoka *et al.*, 1990; [e]Buttery *et al.*, 1988.



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