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Prediction of Muscat aroma in table grape by analysis of rose oxide

Leonor Ruiz-García [a], Pilar Hellín [b], Pilar Flores [b], José Fenoll [b,*]

[a] Departamento de Biotecnología y Protección de Cultivos, Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA), C/Mayor s/n, La Alberca, 30150 Murcia, Spain
[b] Departamento de Calidad Alimentaria, Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA), C/Mayor s/n, La Alberca, 30150 Murcia, Spain

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ABSTRACT

Aroma is an important quality characteristic in Muscat grapes and constitutes a major concern for viticulturist and grapevine breeders. For this reason, Muscat aroma variability was characterised in a segregating progeny and in a collection of table grapes, to assess the usefulness of the presence or absence of rose oxide for predicting Muscat genotypes. Simple tasting and an analysis of free and bound aroma compounds, including rose oxide, linalool oxide, linalool, α-terpineol, citronellol, nerol, geraniol, benzyl alcohol and 2-phenylethanol, were carried out. The association between Muscat score and the compounds considered as active odorants according to their odour activity values was also evaluated. The results obtained pointed to a highly significant correlation between the presence/absence of rose oxide in grapes and the presence/absence of Muscat aroma. Thus, this analysis could be a useful tool for identifying Muscat cultivars in a more objective way than sensory analysis.

1. Introduction

Aroma is a key attribute for consumers and therefore one of the major traits to be taken into account in the selection of table grapes. In particular, Muscat aroma is greatly appreciated for winemaking and in grapes destined for fresh consumption, which is why Muscat germplasm has been extensively used by grape breeders to develop new Muscat cultivars (Crespan & Milani, 2001). Many aroma compounds, such as C_6-alcohols, terpenes, norisoprenoids and benzene compounds, have been identified and quantified in grapes and wines (Hellin, Manso, Flores, & Fenoll, 2010; Petka, Ferreira, Gonzalez-Viñas, & Cacho, 2006; Rapp & Mandery, 1986). These compounds are influenced by numerous factors that include the grape cultivar, soil type, weather conditions and other cultivation factors (Carballeira Lois, Cortés Diéguez, Gil de la Peña, & Fernández Gómez, 2001), and their role in grape aroma is dependent on their concentration, odour thresholds and interaction with other compounds (Ferreira, Ortín, Escudero, López, & Cacho, 2002; Guth, 1997). In addition, these compounds appear either in their free form, directly contributing to aroma, or as bound sugar conjugates, which are non-volatile and make no direct contribution to the aroma of the grape. Nevertheless, these bound sugar conjugates can play an important role in tasting due to the physicochemical changes that occur in the mouth while eating and which lead to the non-volatile compounds being detected by taste-receptor cells in the taste-buds located on the tongue and at the back of the oral cavity (van Ruth & Roozen, 2000).

Several families of compounds are responsible for the primary aroma of grapes, such as methoxypyrazines, C_{13}-norisoprenoids, volatile thiols and dimethyl sulfide in Cabernet, Chardonnay, Sauvignon and Syrah, respectively (Allen, Lacey, Harris, & Brown, 1991; Sefton, Francis, & Williams, 1993; Segurel, Razungles, Riou, Salles, & Baumes, 2004; Segurel, Razungles, Riou, Trigueiro, & Baumes, 2005; Tominaga, Guyot, Peyrot des, & GachonsDubourdieu, 2000). In the aroma of Muscat cultivars monoterpenols play an important role, being a group of flavour compounds with floral and fruity notes. In addition, these compounds could also contribute significantly to the aroma of several other cultivars (Salinas, Zalacain, Pardo, & Alonso, 2004). According to several studies on Muscat cultivars, most monoterpenols showed higher concentrations in the bound than in the free fraction and can easily isomerise and oxidise to form oxides and aldehydes (Dimitriadis & Williams, 1984; Günata, Bayonove, Baumes, & Cordonnier, 1985a).

Citronellol, geraniol, linalool, nerol, and α-terpineol are known to be the main components responsible for the aroma of Muscat grape (Ribéreau-Gayon, Boidron, & Terrier, 1975). In addition, recent works have found that rose oxide in 'Muscat Hamburg' (Fenoll, Manso, Hellin, Ruiz, & Flores, 2009) and 'Moscatuel' (Fenoll, Martinez, Hellin, & Flores, 2012) contribute to the Muscat aroma. Rose oxide is a cyclic monoterpenoid ether that can be detected in many fruits and essential oil-producing plants (Kreis & Mosandl, 1994; Naves, Lamparsky, & Ochsner, 1961; Von Sydow & Karlsson, 1971). Although rose oxide is a minor compound in rose and geranium oils, it contributes substantially to the unique bloomy-green

* Corresponding author. Tel.: +34 968366798; fax: +34 968366792.
E-mail address: jose.fenoll@carm.es (J. Fenoll).

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top notes of these essential oils. Moreover, rose oxide is responsible for the lychee notes of certain Gewürztraminer wines (Guth, 1997). Wüst, Beck, Dietrich, and Mosandl (1996), and Wüst, Rexroth, Beck, and Mosandl (1998) showed that the plants are able to convert citronellyl glucosides into the corresponding rose oxides by an enzymatic oxidation of citronellol in the allylic position with subsequent cyclisation of the resulting diol. Unlike the other monoterpenes, which are also detected in non-Muscat cultivars although in low concentrations, rose oxide has only been detected in Muscat cultivars of both table and wine grapes (Fenoll et al., 2009, 2012; Girard, Fukumoto, Mazza, Delaquis, & Ewert, 2002).

The aim of this work was to assess the usefulness of the presence or absence of rose oxide for the prediction of Muscat genotypes in table grape breeding programs. With this purpose, Muscat aroma variability was characterised by precise quantification of the free and bound aroma compounds and by simple tasting in a segregating progeny and in a collection of table grapes.

2. Materials and methods

2.1. Plant material and sampling

Two distinct groups of plants were considered in this study: an F_1 segregating population, which segregates for Muscat flavour and other traits, and a collection of sixteen table grape cultivars, including eight Muscat ('Matilde', 'Superior Seedless', 'Early Muscat', 'Gold', 'Italia', 'Muscat of Alexandria', 'Muscat Hamburg' and 'Moscatuel') and eight non-Muscat ('Aledo', 'Alfonso Lavallée', 'Autumn Seedless', 'Exotic', 'Michele-Palieri', 'Napoleón', 'Ohanes' and 'Ruby Seedless') individuals. All the plants were grown in an experimental vineyard at the Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA) located in Torrepacheco (Murcia, SE Spain).

The F_1 segregating population was derived from controlled crosses between the table grape cultivars 'Ruby Seedless' (non-Muscat female progenitor) and 'Moscatuel' (Muscat male progenitor). Fifty-six and fifty-one individuals of the progeny were evaluated for aroma compounds (monoterpenes and aromatic alcohols) in 2007 and 2008, respectively. For each hybrid we randomly sampled more than 500 g of berries from 3 representative clusters at harvest (ripened clusters). The sixteen table grape cultivars were sampled at harvest in 2009. Three vines were selected and more than 500 g of berries were picked from bunches (three bunches per vine from the three selected vines). Afterwards, berries from each subsample were squeezed to measure total acidity by titration with NaOH and total soluble solids (TSS) using a hand-held refractometer. A second sample of 300 g of sorted berries was immediately frozen in liquid nitrogen, divided in three subsamples to be analysed separately, and kept at −80 °C for later determination of aroma compounds.

2.2. Sensory analysis

The progeny and the collection of table grape cultivars were evaluated for Muscat flavour by tasting each year. The sensory panel was composed of three females and two males, 20–45 years of age, who tasted at least four berries per plant. All members of the panel had extensive grape-tasting experience. In training sessions, panellists scored the intensity of Muscat aroma in grapes using a 3-point scale (0 = non-Muscat flavour, 1 = slight Muscat flavour, 2 = Muscat flavour). Berries were tasted on the day they were picked. 'Ruby Seedless' and 'Moscatuel' were used as reference standards of non-Muscat and Muscat grapes, respectively. The panellists rinsed their mouths with water after each tasting. Analysis of variance was applied considering a significance level of 5%.

2.3. Standards and solvents

Nerol, geraniol, α-terpineol, rose oxide (*cis* + *trans*) and linalool oxide I and II (*cis*-furan linalool oxide and *trans*-furan linalool oxide, respectively) were purchased from Fluka (Buchs, Switzerland); 2-octanol was purchased from Sigma–Aldrich (St Louis, MO); and linalool, citronellol, benzyl alcohol, 2-phenylethanol 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).

2.4. Volatile analysis

Analysis for free and glycosidically linked aroma compounds was carried out according to the method of Di Stefano (1991) with some modifications. Two hundred grams of berries were deseeded and ground under liquid nitrogen using a Dangoumau ball grinder. The ground berries (50 g) were suspended with a Polytron PT2000 homogeniser (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 microlitre of 2-octanol (0.4 g L^{-1}) were added as internal standards. After stirring for 15 min at 4 °C, the mixture was centrifuged (9000*g*, 20 min, 4 °C) with an Eppendorf model 5810R centrifuge (Hamburg, Germany). The supernatant was filtered through glass wool. The filtrate was stirred in the presence of 1 g of polyvinylpolypyrrolidone (Sigma) to eliminate the high levels of phenolic compounds capable of inhibiting glycosidase activities. The mixture was filtered again through glass wool and the clear filtrate was passed through the SPE column containing 0.5 g of C_{18} Varian Inc. (Lake Forest, CA) already activated with 10 mL of methanol and 20 mL of water at a flow rate of 1 mL min^{-1}. The column was rinsed with 50 mL of pure water 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 removed up to 2 mL by distillation through a Vigreux column at 35 °C.

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 °C. The extract was then transferred to a test tube and concentrated to dryness at 40 °C 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 5 times with 1.5 mL of dichloromethane to eliminate possible traces of free volatiles. The enzymatic hydrolysis was carried out using a commercial preparation AR-2000 with glycosidase side activities (Gist Brocades, France). After stirring, the tube was sealed and placed in a water bath at 40 °C for 16 h. After the addition of 5 μL of 2-octanol (0.4 g L^{-1}) as internal standard, the mixture was extracted 5 times with 0.4 mL dichloromethane. The extract was dried over Na_2SO_4 and stored at −20 °C until analysis. All analyses were performed in triplicate.

2.5. Gas chromatography and mass spectrometry

The final extracts were analysed by gas chromatography with a flame ionisation detector (FID) and the volatile compounds were confirmed by gas chromatograph–mass spectrometry. For the former analysis, an Agilent (Waldbronn, Germany) HP 6890 gas chromatograph equipped with a flame ionisation detector and automatic split/splitless injector (Agilent 7683) was used. The columns used were an HP-5MSI (30 m × 0.25 mm i.d. and 0.25 μm film thickness) and a DB-WAX (30 m × 0.32 mm i.d. and 1.0 μm film thickness). Both columns were supplied by Agilent Technologies. Helium was used as the carrier gas (constant pressure eluting, thymol 18.55 min for HP-5MSI column and 55.66 min for DB-WAX