Volatiles in *Vitis vinifera* L. cv Barbera during ripening as influenced by growing location.

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ABSTRACT
Volatile composition of grapes is essential to wine quality. Wine olfactory complexity depends not only on winemaking techniques, but also on the grapevine varietal aroma that, in turn, is largely influenced by soil and climatic conditions. The aim of this work was to evaluate the influence of growing areas on the evolution of volatile compounds in berries of a non aromatic cultivar, from véraison to harvest. The study was carried out on cv Barbera (AT 84/K 5BB) berries, picked in two different areas of Piedmont region, North-Western Italy, Langhe area and Roero area (Tanaro river orographic right and left, respectively). Grapes were sampled in 2010 in three field replicates of 25 vines each. 200 berries were collected from each field replicate and from both sides of the canopy. Varietal and pre-fermetative volatiles were analyzed by stir-bar sorptive extraction gas chromatography-mass spectrometry technique (SBSE-GC/MS). Several alcohols, aldehydes, esters, benzenoids, monoterpenes, sesquiterpenes and norisoprenoids were identified and quantified. The results showed that the cultivation area influenced the accumulation kinetics of volatiles, their concentrations and profiles. These aspects are important to explain the different performances of a cultivar in various growing areas and to deepen knowledge about the "suitability" of a terroir for a specific variety. Therefore, the terroir, affecting the aromatic complexity of grapes, can potentially diversify monovarietal wines.

Keywords : SBSE-GC/MS, terroir, varietal and pre-fermentative volatiles

1 INTRODUCTION

Wine olfactory complexity is influenced by vinification techniques and yeast strains used during wine-making but it mostly depends on the varietal characteristics of grapes, their maturity and health at harvest. In Europe, wine classification is largely based on geographical areas of grapevine cultivation. In Italy, in many cases, the name of grape varieties is linked to the geographical area they originate from (e.g. Barbera d'Asti, Aglianico del Vulture etc.), to highlight the link between growing areas and varieties.

Vitis vinifera cv Barbera is one of the most important red-grape variety grown in Italy; at present more than twenty clones have been selected and registered in Italy**.** In Piedmont (North-Western Italy) Barbera is the base cultivar for the production of some red VQPRD wines, such as "Barbera d'Alba" DOC, "Barbera del Monferrato" and "Barbera d'Asti" DOCG, the first from the Cuneo province, the latter two from the Asti province. In the Cuneo province, there are two main viticulture districts, 'Langhe' and 'Roero', respectively on the orographic right and left of the Tanaro river; in these areas Barbera is grown for the production of different wines, such as "Barbera d'Alba" and "Barbera d'Alba Superiore" DOC wines. The 'Langhe' district is generally characterized by calcareous-clay soils, whereas the 'Roero' soils are medium-textured with the presence of fossil sand.

Environmental factors, such as topographical, agro-pedological and climatic conditions, usually described by the French term “terroir”, influence grape and wine quality and composition [1]. The influence of environment and cultural practices, such as leaf removal, cover crops and water deficit on volatile composition of grapes and wines has been investigated [2-7]. The increase of the aromatic potential in water deficit conditions may depend on the higher cluster exposure due to reduced vine vigor [8] rather than to a direct effect. Sunlight exposure may activate the deoxyxylulose-5-phosphate pathway that produces monoterpenes and other isoprenoid compounds such as C13–norisoprenoids [9]. Several studies have reported that sunlight exposure increases the concentration of volatile terpenes, norisoprenoids, such as TDN, vitispiranes and actinidols in exposed berries [10] respect to shaded fruit [2]. Besides, water deficit positively influences concentrations of TDN and β-damascenone, but not that of β-ionone [11]. The aim of this work was to study the evolution of varietal and pre-fermentative volatiles, from véraison to harvest, in two Barbera vineyards located in the Roero (BR) and Langhe (BL) districts, to understand the influence exerted by growing locations on the accumulation trend and on concentrations and profiles of some classes of volatiles. This study was carried out on clone ‘AT 84’, characterized by medium vigor and yield (2.7 kg/vine), good sugar accumulation (20.5 %) and moderate acidity (pH 3.11; 8.45 and 4.40 g/L of tartaric and malic acid concentrations, respectively) [12].

2 MATERIALS AND METHODS

2.1 Vineyard description and sampling

The study was carried out in 2010 in two *Vitis vinifera* L. cv Barbera vineyards, located at Castellinaldo, (44°47'0" N 8°2'0" E, 210 m a.s.l., Roero area) and at Alba (44°43'0" N 8°5'0" E, 250 m a.s.l., Langhe area). In both vineyards Barbera vines (clone AT 84) were grafted onto Kober 5BB, trained to the vertical shoot positioned (VSP) system and Guyot pruned. In the Roero vineyard, vines were planted in 1993 in a clay loam soil at a spacing of 2.60 × 0.90 m with N-S rows orientation and North exposure. In the Langhe vineyard, vines were planted in 1996 in a silty clay loam soil at a spacing of 2.30 × 1.00, and row orientation was NW-SE with West exposure.

For each vineyard, three field replicates of 20 vines each were established; fortnightly samplings started at pre-véraison and ended at harvest. 250-300 berries were collected from each field replicate and from both sides of the canopy to overcome the possible effects of different light exposure. Berries were detached from the rachis in groups of 3 to 5 each from the upper, the middle and the bottom part of the cluster. Berries were stored in portable refrigerators and transported to the laboratory; berries were separated from the rachis and a subgroup of 200 berries was weighed and stored at – 20°C until volatile analysis. The remaining berries were crushed and the must total soluble solids were measured with a digital refractometer (ATAGO, PR-32).

2.2 Chemical analysis

Berries were crushed for 2 min without breaking seeds. 10 g of homogenized grapes were diluted with distilled water and 2-heptanol was added as internal standard. 20 mL of the aqueous grape extract were stirred with a PDMS-coated stir bar (Twister®, Gerstel, Mulheim and der Ruhr, Germany) for 6 hours at room temperature. The Twister® was transferred into a thermal desorption unit (TDU, Gerstel, Mulheim and der Ruhr, Germany) for desorption. The desorbed analytes were cryo-focused at 0°C, with CO_2, in a programmed temperature vaporization injector (CIS 4, Gerstel, Germany); after cryo-focalization, analytes were transferred to the GC column (DB-WAX, J&W 122-7032, 30 m × 0,25 µm × 0,25 mm ID). The GC was a 7890A gas chromatograph interfaced with MS 5975C mass spectrometer (Agilent Technologies). The GC oven initial temperature was set at 40°C for 10.00 min, then at 180°C with a ramp of 2.5°C/min. Temperature increased to 200°C at 1°C/min and was maintained for 10 min. The identification of compounds was performed using a NIST and Wiley Libraries (NIST-05a; Wiley7). Volatile compounds were quantified only when they were present in at least two replicates of the three for each sample. The results were expressed as microgram equivalents of internal standard per Kg of fresh berry.

3. RESULTS AND DISCUSSION

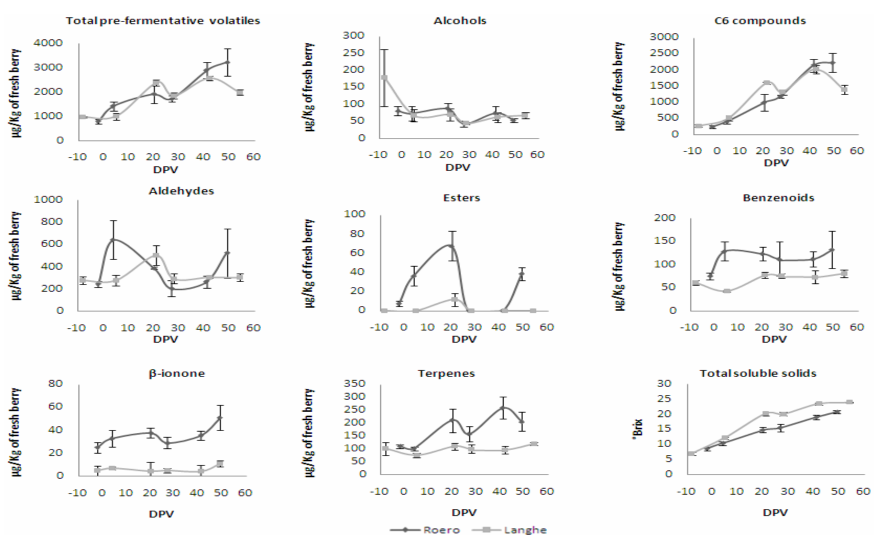

Figure 1 : Accumulation trend of the main classes of varietal and pre-fermentative volatiles in Barbera grapes from Roero and Langhe areas in Piedmont (North-West Italy). Average values of three replicates ± standard errors. (DPV = Days Post Véraison).

The volatiles isolated and quantified with the SBSE-GC/MS technique during ripening of cv Barbera in two growing areas, Roero and Langhe, were grouped in 8 chemical classes (alcohols, aldehydes, esters, benzenoids, monoterpenes, sesquiterpenes and norisoprenoids) according to their chemical similarity and biosynthetic pathways (Fig. 1). In both Barbera growing areas the accumulation of volatiles started before véraison; these results confirm the activation of volatile biosynthetic pathways already in this period, as observed by Kalua and Boss [13]. In BR the accumulation trend continued until harvest, whereas in BL the maximum accumulation of volatiles was reached at 42 DPV with a successive decrease until harvest (Fig. 1). Also soluble solids (Fig. 1) increased until harvest in BR, whereas in BL they reached the peak of maximum accumulation at 42 DPV, followed by a plateau phase, suggesting a possible correlation between the accumulation of sugars and that of volatiles; the differences detected between the two locations confirm the environmental control of berry ripening [14], and the possible influence of microclimate within the canopy on berry composition [15, 16]. Previous studies have shown that an increase in wine secondary metabolite contents, including volatiles, was tied to an increase in must soluble solids [8]. Thus, for the same variety, dates of harvest that maximize the aromatic expression in grapes, should be decided according to the environmental conditions, because in different grape-growing areas the accumulation trend of primary and secondary metabolites proceeds differently. The differences detected in total volatile concentrations in grapes from the two growing areas may depend on the different rainfall levels measured between 40 DPV and harvest: 26 mm in BR and 62 mm in BL. In general, from budbreak to harvest, BR received much less rainfall (447 mm) than BL (520 mm), which resulted in a higher volatile concentration in grapes from BR respect to BL. However, the correlation between water availability and aroma potential at harvest is not very clear, being profoundly influenced by the climatic conditions of the growing areas [17, 18].

Alcohols and aldehydes, other than C6 compounds (Fig. 1), showed similar patterns of accumulation kinetics during ripening in grapes from the two growing areas, suggesting that the β-oxidation of fatty acids is probably controlled more by the genotype than by the growing area itself. BR showed a higher concentration of benzenoids during berry ripening compared to BL (Fig. 1). The lower water availability from rainfall in the Roero area probably influenced benzenoid accumulation. Indeed, as water deficit increased anthocyanin biosynthesis[19], benzenoids deriving from the phenylpropanoid pathway, many of them being intermediate products, might probably take advantage from weak water stress conditions.

Differences between BR and BL were also found for esters in terms of accumulation kinetics (Fig. 1) and qualitative profile; among esters, hexyl-acetate was not detected in BL grapes (data not shown). The presence of hexyl-acetate exclusively in BR grapes and in only two stages of ripening (-2 and 4 DPV, data not shown), suggests that the activity of alcohol acetyl transferase might be under environmental control and, in agreement with Kalua and Boss[13], the activity of this enzyme appeared irrelevant in post-véraison. In general, esters in BR grapes increased until 20 DPV and decreased thereafter.

In all stages of ripening, in both BR and BL grapes, the most important class of chemical compounds detected as 'varietal and pre-fermentative' volatiles was that of C6 compounds (Fig. 1) in agreement with Kalua and Boss [13]. The presence of these compounds during the whole period of sampling suggests that enzymes responsible for their formation are active throughout the berry development similarly to what was previously assessed from fruit-set to harvest by Kalua and Boss [13]. BL showed an increase until 40 DPV and a decrease until harvest, suggesting that in fully ripe grapes the concentration of these molecules decreases, as observed by others authors [16, 20]. On the contrary, in BR grapes at harvest the content of these compounds was still high; this likely suggests that these grapes were not fully ripe, yet, in agreement with the still on going accumulation of soluble solids (Fig. 1).

In our work BR and BL showed different accumulation kinetics for β-ionone and terpenes (Fig. 1), with higher concentrations in BR than BL during all stages of ripening. This aspect is very important because these compounds have low sensory thresholds, particularly β-ionone [21], and give important contributions to grape and wine aroma even at very low concentrations. These results could be pivotal to explain the sensory differences among the wines of the two growing locations, which in part derive from the influence of pedo-climatic conditions on the grape genotype. From véraison to harvest, in both Roero and Langhe areas, mean temperatures were the same (18,2 °C) as well as the sum of degree days base 10°C (1615 and 1619 °C, respectively). But, as we did not measure berry temperature, we do not know if the different vineyard exposure has been able to influence berry temperature and, consequently, the accumulation of terpenes and β-ionone, being known that warmer temperatures during berry ripening leaded to a reduction of monoterpene concentration [2]. We can speculate that as in our latitudes West exposure receives more light than North exposure, an effect of light on volatile accumulation is possible, as shown by Vilanova et al. [4]. Furthermore, it is not to exclude a direct effect of rainfall availability on volatiles, as the lower rainfall detected in BR may have favoured higher monoterpene accumulation. In BL, the concentration of terpenes was more or less constant during all stages of ripening, whereas in BR it increased during ripening.

In addition, the differences detected between grapes from the two growing areas may depend on the different soil types, which might influence directly (texture, active limestone) or indirectly (water holding capacity, nutrient availability) berry composition. The two soils showed a similar clay concentration (27% BR and 29% BL), whereas BR had more sand (43%) and BL more silt (57%). The different percentages of sand and silt determining different physical and hydrological properties could influence the volatile accumulation, particularly that of terpenes and β-ionone. Sabon and co workers [22], have found that in wines certain molecules, such as β-ionone, β-damascenone, geraniol, and Z-hexen-2-enol, can be used as indicators of the grapegrowing area.

Sesquiterpenes were found in one date (41 DPV) in BR grapes, and in two dates in BL ones (-8 and 54 DPV); in BR a sesquiterpene (whose more abundant fragment was 41 m/z) was found, whereas in BL grapes also farnesol was identified (data not shown), in both dates. In literature, data regarding the accumulation kinetics of these compounds are not always in agreement [6, 13]. These differences are probably due to the different varietal behavior, pedo-climatic conditions or different reaction to external stimuli mediated by jasmonate signals [23].

4 CONCLUSIONS

Data collected during our experiment have highlighted the influence of environmental conditions on varietal and pre-fermentative volatile accumulation detected by the SBSE-GC/MS technique. BR grapes showed a higher accumulation of monoterpenes, β-ionone and benzenoids respect to BL ones. Alcohols, aldehydes and C6 compounds showed similar patterns of accumulation kinetics and concentrations during ripening in grapes from the two growing areas. No major differences in the profile were detected except for farnesol that was exclusively detected in grapes from BL and hexyl-acetate, exclusively detected in grapes from BR.

The environment, influencing berry volatile concentrations, may thus affect the sensory perception of wines; further studies, including the composition of wines extended for a longer period [8] are underway in our laboratory.

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