MANAGEMENT OF GRAPE MASS LOSS IN THE WITHERING PROCESS FOR THE FINAL WINE AROMA: THE AMARONE CASE

Bellincontro A.[a], D'Onofrio C.[b], Accordini D.[c], Tosi E.[d], Mencarelli F[a]*.

[a] Department for Innovation in Biological, Agro-food and Forest Systems (DIBAF) - Postharvest Laboratory, University of Tuscia, Viterbo, Via San Camillo de Lellis snc, 01100 Viterbo, Italy

[b] Department of Agriculture, Food and Environment Science , University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy

[c] Cantina Valpolicella Negrar sca, via Ca' Salgari 2, 37024 Negrar, Verona, Italy

[d] Experimental Centre in Viticulture, Agriculture Service, Provincia di Verona, 37029 San Pietro in Cariano, Verona, Italy

Introduction

In the panorama of dry wines, Amarone and Sfurzat are the only wines where a postharvest treatment of dehydration is applied before vinification. Between the two, Amarone wine is the most known and represents, economically speaking, one of the most important bottles in the panorama of Italian wines (6500 ha produce 13 millions of 750 mL bottles with an average commercial price of 62$/bottle) (Hai una biblio per questo riferimento?).

Special technologies are used to induce water loss from the berries as described by Accordini (2013). The event of water loss in the berries of wine grape has been detailed by Mencarelli & Tonutti (2013). The Authors have distinguished three different processes of water loss: 1) dehydration, when the process is well controlled in terms of temperature, relative humidity (RH) and ventilation; 2) drying, when the process is under uncontrolled conditions such as sun-drying; and 3) withering, when the process occurs in a naturally ventilated room with or without a partial control of temperature and RH. Tonutti & Bonghi (2013) have well described biochemical and molecular change occurring in wine grapes during dehydration, drying or withering process. Since the postharvest berry mass loss (m.l.) is for the 95% a water loss, it causes a water stress that affects polyphenol metabolism whatever the technique used (Figueiredo-González, Cancho-Grande & Simal-Gándara, 2013). The amount of water loss, due to the thermohygrometric and ventilation conditions, influences the concentration of the phenolic metabolites produced (Mencarelli & Bellincontro, 2013; Rolle, Giacosa, Río Segade, Ferrarini, Torchio & Gerbi, 2013; Panceri, Gomes, De Gois, Borge & Bordignon-Luiz, 2013). It has been shown that the process speed and the amount of mass loss influence significantly the changes in the polyphenol gene expression (Bonghi, Rizzini, Gambuti, Moio, Chkaiban, & Tonutti, 2012) and that the dehydration temperature affects the gene expression and polyphenolic metabolites (Mencarelli, Bellincontro, Nicoletti, Cirilli, Muleo & Corradini, 2010; Toffali, Zamboni, Anesi, Stocchero, Pezzotti, Levi, & Guzzo, 2011; Cirilli et al, 2012; Marquez, Serratosa, Lopez-Toledano & Merida, 2012). Beyond polyphenols, also Volatile Organic Compounds (VOCs) are greatly influenced by postharvest water stress of wine grape (Franco, Peinado, Medina & Moreno, 2004; Costantini, Bellincontro, De Santis, Botondi & Mencarelli, 2006; Chkaiban, Botondi, Bellincontro, De Santis, Kefalas & Mencarelli, 2007; Santonico, Bellincontro, De Santis, Di Natale & Mencarelli 2011; Noguerol-Pato, González-Álvarez, González-Barreiro, Cancho-Grande & Simal-Gándara, 2013). During postharvest dehydration, berry VOCs increase due to water loss (concentration effect) but some classes of volatiles increase more than expected, as shown by Nogerol-Pato et al. (2013) and Centioni, Tiberi, Pietromarchi, Bellincontro & Mencarelli (2014). If the dehydration process technically controls water loss, the metabolic change can be managed to obtain a final product with the desired characteristics.

Amarone wine is produced from three main red varieties: Corvina, Corvinone, and Rondinella. In Valpolicella, the grape's ability to shrivel is the most decisive factor in the choice of the variety (Accordini, 2013). This characteristic mainly depends on genetics and on the production techniques which tend to reduce yields while encouraging ripening. The grape's physiological suitability to dehydration is mainly based on the berry structure. The berries must be highly resistant to pedicel separation and have a thin and elastic skin that is not subject to cracking or to common rot. At the same time, they must have low resistance to dehydration; this strongly depends both on genetics and canopy management (Muganu et al, 2011). Corvina is the main variety for the production of Amarone. Thus, it has been extensively studied, in relationship to postharvest withering, for resveratrol content (Versari et al, 2001), molecular analysis (Zamboni et al, 2008), skin wall modification (Zoccatelli et al, 2013), mechanical properties and polyphenol composition (Rolle et al, 2013). Barbanti, Mora, Ferrarini, Tornielli & Cipriani (2008) have studied the withering kinetics of grapes used to produce Amarone and Recioto, and Ferrarini (2014) has reported an extensive comparison of withering kinetics in different thermohygrometric conditions of the three main varieties of Amarone and its effects on acidity, sugars, and colour. He has invented the term "Amarone sweet spot" to indicate the point where composition and metabolic evolution of dehydrated grapes have reached the equilibrium to optimize the quality of Amarone wine. It is not easy to define this spot because it requires a continuous analytical process which could be done, commercially, only with non-destructive sensors because the metabolic process is very dynamic and changes year by year.

The knowledge of VOC changes in wines, coming from different percentage of m.l. of grape and from different varieties, during withering in commercial facility, is very important to program the quality of the final wine. How much should the m.l. between two varieties be? How many grapes of one variety with that percentage of m.l. are necessary in order to have a particular aroma? To answer these questions we have carried out a study on the grape withering of the three main varieties Corvina, Corvinone, and Rondinella, by sampling berries at harvest and at 20, 30% of m.l. and analysing both the VOCs of grapes and of the obtained wines. In particular, in this paper we report data on the wine VOCs focusing on the pattern of specific compounds which decreased or increased in the wines coming from grapes withered at 20 and 30% in order to provide useful information to winemakers.

Materials and Methods

Grapes and withering process

Red wine grape varieties Corvina, Corvinone and Rondinella (*Vitis vinifera* L.) were harvested with a sugar content of 170-190 g $L^{-1}$and titratable acidity of 7-7.3 g L^{-1}, from the vineyards of Cantina Valpolicella di Negrar in the Valpolicella area, close to Verona (Italy). Bunches without any visible presence of fungi and wounds were carefully harvested, and placed in perforated plastic boxes (40cm x 60cm x 12cm) in two layers for a total of about 8 kg. For each variety, 100 plastic boxes were collected and placed inside a large withering facility, located in Cantina Valpolicella di Negrar (Verona) as described by Accordini (2013). The system consisted essentially of dehumidifiers running at ambient temperature with 10–15 air changes per hour. The dehumidifiers have a humid air inlet turned towards the dehydration loft and an opening at the top that directs the air above the trays on which the grapes are lain to dehydrate. There are two other openings on the sides, from one the air at ambient temperature enters, while the other releases the hot air resulting from the cooling of the hot plate inside the dehumidifier. This hot air is released outside so that the temperature of the loft does not increase. Inside the dehydration loft there are large fans (120 cm diameter) moving to distribute the dry air over all the grapes. When this system is properly managed, in cool and sunny days the external air is only circulated in the dehydration loft, while in wet and rainy days, when humidity is close to saturation, the humidity levels are kept between 60 and 70% within the dehydration loft.

The plastic boxes were placed on a scale to monitor continuously the mass loss (m.l.). The tests was run until the grapes reached the 50% of mass loss but sampling for chemical analyses and vinification were done only at 20 and 30% m.l. About 200 kg of grapes for each variety were removed after the withering process, at 20 and 30% m.l. and immediately processed for wine production. In total we have 3 samples for each variety, including the wine made from fresh harvested grape.

Vinification

Grapes were manually emptied from the plastic boxes on the grape-sorting tables in order to eliminate rotted berries. Grapes were then crushed and destemmed. 5g hL^{-1} of SO_2 was added.

Fermentation started with addition of commercial yeast (Premium Zinfandel, Enologica Vason, VR, Italy) after rehydration, in three stainless steel containers for each variety and each weight loss. These yeasts resist to high sugar content and can produce high content of glycerol, low acetic acid, and high alcohol content (18%) with low degradation of malic acid. Temperature was controlled between 25 and 30°C. After 24 h from the beginning of the fermentation, malolactic bacteria (ELIOS 1- Lallemand-Italia, VR, Italy) were inoculated. During fermentation and contemporary maceration, cap management consisted of two daily punch down (morning and afternoon, 1 min each). Fermentation ended when sugars were below 2 g L^{-1} for the wine at harvest and between 3.5 and 7.0 for the wine from withered grape.

After racking, the pomace was pressed and resulted in wine added to the main wine.

Rackings were done after 24/48 h from pressing and until complete wine clarity 2-4 g $L^{-1}$of SO_2 were added after the 1st racking. Wine was placed in glass container at cold temperature for tartaric stabilization.

In mid-January, after analytical controls, SO_2 adjustment was performed, and after filtering through 1 μ filter cartridge, the wines were bottled in 750 mL bottles, capped with a plastic air-tight stopper.

Chemical analyses

Wine analyses at the beginning (harvest) and after 20 and 30% m.l. were performed by a calibrated Winescan FT 120 (Foss, Hillerod, DK) to measure sugars, acidity, pH, volatile acidity, total polyphenols and total anthocyanins.

For each variety, 10 bottles of musts before the wine process and 10 bottles of wine at each sampling were collected from the stainless steel tank. For VOCs analysis, three bottles of wine were used.

Aroma compounds were extracted from wines by Solid Phase Extraction (SPE) according to the protocol described by Di Stefano (1991) and Gianotti & Di Stefano (1991). Gas chromatographic analysis was carried out using an Agilent 7890A gas-chromatograph (Agilent Technology, Santa Clara, Ca, USA) and an Agilent 5975C quadrupole Mass Spectrometer (MS). The carrier gas was helium at a constant flow rate of 1 mL min^{-1}. The capillary column was a HP-Innowax (30 m length, 0.25 mm i.d., 0.25 mm film thickness) from Agilent. The temperature program of the column oven started at 30 °C, then increased 30 °C min^{-1} to 60 °C, 2 °C min^{-1} to 190 °C, and 5 °C min^{-1} to 230 °C. The MS detector scanned within a mass range of m z^{-1} 30-450.

Compounds were identified by a combination of matching retention indices with library matches (Nist 08) and authentic standards, which were available for the compounds of interest. The quantification was carried out comparing the peak area of each compound to that of the internal standard.

Sensorial analysis

The sensorial analysis was done by a team of 20 producers and wine makers. They were not professional panelists but operators who knew very well the Amarone characteristics. Tasting was performed in Cantina Valpolicella di Negrar. They were asked to evaluate the main aromatic notes of the wines.

Statistical analysis

Statistical analysis was performed using the SPSS software package, version 17.0 for Windows (SPSS Inc., Chicago, IL, USA). The Tukey B test for $p < 0.05$ was used in order to establish statistical differences by one-way analysis of variance (ANOVA).

Results and Discussion

Figure 1 reports the temperature pattern and relative humidity during grape withering. In the first 40 days, the temperature inside the withering room followed perfectly the outside temperature reaching about 12°C, meaning that the system worked with open windows and fans sucking air from outside to inside. From day 40 to day 65, the outside temperature was higher than inside meaning that the cooling system was working inside to cool grapes. Viceversa, until the end of the withering period, the temperature inside was maintained constantly higher than outside. 10% and 20% m.l. were reached at the same time by the grapes of all the three varieties, while 30% m.l. was achieved on day 50, 64 and 72, in Rondinella, Corvinone and Corvina respectively with rates of mass loss of 0.60, 0.47, and 0.42 %/day (Mencarelli & Bellincontro, 2013). At 50% m.l., the variability was similar: 120, 132 and 140 days in Rondinella, Corvinone and Corvina, respectively. As explained in M&M only samples from 20 and 30% m.l. were analysed and used for vinification because these are the used m.l. for Amarone wine.

Sugar content reached 230-240 L^{-1} at 20% m.l. in the grapes of the three varieties and 280-290 L^{-1} at 30% m.l. Titratable acidity started from 7.0, 7.3, 7.3 g L^{-1}, in Rondinella, Corvinone and Corvina, respectively, and rose to 7.5 g/L at 30% m.l. in all the varieties. Rondinella wine showed the highest acidity in the wine produced at harvest (**Table 1**); successively the acidity decreased, in all the varieties, at 20% m.l. to increase again in the last sampling (30% m.l.) where Corvinone wine exhibited the highest acidity. This pattern of acidity is well-known during grape dehydration. The water stress induced by grape dehydration has a strong effect on grape metabolism and malic acid is consumed at a higher rate initially, while successively a concentration effect occurs. pH values increased in all the three varieties during withering without the changes showed by acidity at 20% m.l. (**Table 1**). This pH behaviour is not anomalous in grape dehydration and is due to the great release of cations such as K^+ which, above all at low temperature as it is our case, salifies giving the typical buffer effect of weak acids and their salts as it is the case of grape must.

A significant progressive rise was observed in glycerol, achieving similar values in the three varieties, in the wine from 30% m.l. grapes (**Table 1**). As expected in these types of wine, volatile acidity enhanced from around 0.45 mg L^{-1} up to about 0.65 mg L^{-1} in all the samples, without significant differences. A great rise in the content of total polyphenols and total anthocyanins was noted in wines from 20 and 30% m.l. grapes (**Table 1**). Rondinella and Corvinone wines showed the initial highest and lowest content of polyphenols, 1595 mg L^{-1} and 1024 mg L^{-1}, respectively; at 20% m.l., the rise was null in Corvina wine, while in Corvinone and Rondinella it was detected an increase of the 30% and 70% of total polyphenols. In wines from 30% m.l. grapes, the total polyphenols grew to the 25%, 80%, and 91% in Corvina, Corvinone, and Rondinella, respectively. Also the total anthocyanins rose significantly: from 20 and 30% m.l. to 47% and 62% in Corvina, to 55% and 63% in Corvinone, and to 39% and 54% in Rondinella. It is well-known, generally speaking, that water loss during drying, dehydration or withering takes to a solutes concentration. Moreover, depending on the rate and amount of water loss, some important metabolites can behave in relation to the concentration process because the oxidation process can occur in combination with water loss (Serratosa, Lopez-Toledano, Merida & Medina, 2008) or new synthesis of phenolic compounds (Cirilli et al, 2012). In our case, the very long process of water loss due to low temperature and high relative humidity, affects the metabolism of the berry i.e. a longer time of dehydration induces a senescence syndrome and not only water stress (Zoccatelli et al, 2013). Polyphenols are very well known as “plastic” metabolites, very reactive to biotic and abiotic stress. Even in berry dehydration there was a significant increase of specific phenols (Mencarelli et al, 2010; Bonghi et al, 2012; De Sanctis, Silvestrini, Luneia, Botondi, Bellincontro & Mencarelli, 2012; Rolle et al, 2013) depending on the amount of water loss and dehydration temperature in well-controlled conditions of water loss. Figueiredo-González, Cancho-Grande & Simal-Gándara (2013) did not find a similar increase but they worked in a non-specified environmental condition with a very high percentage of water loss (62%) in a very long time (82 days), meaning a sugar increase of 0.3°Brix day^{-1} and weight loss rate of 0.8% day^{-1}; thus, a process very low and, overall, a great water loss. In this condition, it is expected a significant loss of polyphenols due to the oxidation process and to senescence metabolism.

Our data showed a higher rise than the one expected, so it is conceivable that the low temperature of withering delayed water loss stress without provoking immediate cell death but triggering a grape tissue response which is exhibited by a higher synthesis in polyphenols. Ferrarini (2014) reported a similar increase in polyphenols during the withering of Corvina, Corvinone and Rondinella, which had also a higher extractability due to cell wall degradation (Zoccatelli et al, 2013). Rolle et al. (2013) found an increase in total flavonoids of skin and seeds, proanthocyanidins, and low-weight flavanols of seeds, in Corvina grapes dehydrated in controlled conditions. Also Panceri et al. (2013) found a great increase in polyphenols and anthocyanins in Carbernet Sauvignon and Merlot during dehydration under well-controlled condition.

In the aroma formation of Amarone wine, beyond the VOCs coming from genetic characteristics and fermentation process, a significant contribution to wine aroma comes from the withering/dehydration process, as generally occurs in regular dry wine.. As it has been well emphasized by Mencarelli and Bellincontro (2013), Tonutti and Bonghi (2013), and D'Onofrio (2013), the physiology of the berry cell is greatly affected by the water loss occurring during withering/dehydration and by the technology process. In our case, the pool of VOCs changes during withering: most of them decrease; few compounds do not change and few ones increase. Not all of them are significant for the final aroma of the wine, since some have low concentration or a very low perception threshold, but they all contribute to what is called "bouquet" of wine.

Table 2 reports the concentration of main classes of compounds. More than 200 VOCs were detected, grouped in aliphatic alcohols, benzenoids, monoterpenes, nor-isoprenoids, acids, and esters. Aliphatic alcohols were higher in Corvinone and Rondinella wines at harvest; during withering, they did not change in Corvina wine while in Corvinone and Rondinella decreased significantly, and, in the end, the concentration was higher in Corvina than in the other two wines. Benzenoids concentration was the highest in Rondinella and Corvinone at harvest and decreased progressively during withering, reaching 13721, 14990, and 17937 μg L^{-1} respectively in Corvinone, Corvina and Rondinella. The monoterpene content was low compared to the other class of compounds (**Table 2**) and dropped significantly during withering especially at 30% m.l. Nor-isoprenoids had similar concentrations: there was a significant increase (2-3 folds) at 20% m.l., in all the varieties. Acids increased significantly during withering in the wines of the three varieties and, especially, in Rondinella. As expected, also ester concentration declined significantly in the wines coming from withered grapes, from 10-12000 μg L^{-1} to 4-5500 μg L^{-1} in wines from 30% m.l. grapes; most of these esters come from the fermentation process and give fruity notes to wines. 65% of total acids consisted of 2,6 dimetyl 6-hydroxy-2,7-octadienoic acid followed by hexadecanoic and nonanoic acids in the wines of the three varieties but the highest concentration was in Rondinella wine. If the decrease of most of the classes of compounds (benzenoids, monoterpenes, nor-isoprenoids) is due to the oxidative process which occurs during the postharvest withering, the increase of acids in the wines from dehydrated grapes, above all at 30% m.l., is probably due to higher extractability as consequence of the degradation of the cell membrane and cell wall at intense water loss,. Most of them are fatty acids and their contribution to aroma is very low. If in high concentration, give generally negative aroma descriptors in wine (Bakker & Clarke, 2012).

By analysing the concentration of single compounds for each varietal wine, Rondinella wine, at 30% weight loss, showed the highest number of compounds with the highest concentration (25), while Corvina and Corvinone, respectively, had only 13 and 5 compounds with the highest concentration. Thus, Rondinella wine, at 30% m.l., had the highest concentration of VOCs. As it is known, this does not mean that Rondinella has the strongest aroma, because there is no strict correlation between concentration and perception. **Table 3** reports the list of the main compounds of each variety which decreased in concentration during withering. In Corvinone, the loss of compounds occurred already in wines from 20% m.l. grapes. Among esters, ethyl(S)-(-)-lactate (ethereal, buttery, fruity), which was in the highest concentration, and diethyl malate (apple), ethyl 2 and 3 methylbutanoate (fruity, herbaceous) and 4-ethoxy ethyl benzoate (fruity) decreased of about the 80% in the wine from 20% m.l. grapes. Among alcohols, the greatest loss was that of 2-phenylethanol, the most concentrated alcohol, followed by isoamyl alcohol and guaiacol: the first two were below the perception threshold, and the third one was much higher, as reported by Ferreira, Lopez & Cacho (2000). Since guaiacol aromatic nuance (smoke, sweet, medicine) is one of the most common markers in passito red wine, its high concentration is expected. Among nor-isoprenoid, damascenone was the highest in concentration and lost almost 50% already in 20% m.l. wines, remaining much higher than the perception threshold. In contrast, esters such as methylhexanoate, dimethylsuccinate, and ethylvanillate, all characterized by fruity and sweet aroma nuances, increased at 20% m.l. and then decreased slightly or remained constant (**Figure 2C, 2D**). Also specific aromatic compounds as nerol, nonanoate and benzyl alcohol increased (4-6 folds), reaching a concentration which contributed significantly to the aroma of final wines (**Figure 2C** and **2D**).

In Corvina wines, compounds such as isopenthyl acetate, ethyl(S)-(-)-lactate, 2 and 3 methyl butanoate, 2-phenylethanol, and 4-ethoxy ethylbenzoate decreased during withering, similar to what observed in Corvinone wines; beyond those, there was a significant loss of 1-hexadecanol, trans-8-OH-linalool, triacetin, and N-(3-methylbutyl) acetamide (*Botrytis cinerea* marker). As in Corvinone wines, benzyl alcohol increased greatly and at higher concentration than in Corvinone wines (**Figure 2A**) already at 20% m.l. and it was the only volatile compound found in both varietal wines. In Corvina wines, alcohol was the main chemical group which showed a significant increase; the main alcohols were isoamyl alcohol, in the highest concentration (10317 μg L^{-1} at 30% of m.l, data not shown), and 1-hexanol, p-cymen-8-ol, 2,3 pinanediol, 3-oxo-ionol and 3-methyl-1-pentanol (Figure **2A** and **2B**). Other increasing compounds were coumaran and damascenone (**Figure 2A** and **2B**).

In Rondinella wine, a significant decrease occurred for ethyl(S)-(-)-lactate, 2-

phenylethanol, in the highest concentration (at the same level as in Corvinone wine), and for triacetin and 4-ethoxy ethylbenzoate (**Table 3**). Hexanol and nonanoate increased greatly at 30% m.l. but also methyl vanillate, damascenone, 3-oxo-ionol, eugenol, p-cymen-8-ol, 2,3 pinanediol, coumaran and raspberry keton rose significantly (**Figure 2E** and **2F**). 11, 9, and 8 VOC markers were identified, respectively, for Rondinella, Corvina, and Corvinone. There are 6 compounds in common between Rondinella and Corvina, only one, benzyl alcohol, between Corvina and Corvinone while there is no compound at all in common between Corvinone and Rondinella.

The markers characterizing Corvinone wine are mainly esters such as methylhexanoate (fruity), ethylvanillate (smoky), dimethylsuccinate (fruity) and acids such as cinnamate (cinnamon), hexanoate (sweat), and nonanoate (waxy, fatty). As reported above, esters are mainly produced during the fermentation process and are lost during aging. Also the esters produced during grape withering are easy lost depending on the dehydration temperature (Santonico et al, 2010). As regards, the monoterpene alcohol, nerol (rose), monoterpene biosynthesis occurs during grape ripening, starting from berry set. These compounds are mainly found in the berry skin. Thus, concentrations of geraniol and nerol, which characterize the aroma of young wines, can rapidly decrease after 2/3 years of bottle ageing and no longer contribute in the same way to wine aroma (Darriet, Thibon & Dubourdieu, 2012). In our case, the significant increase of nerol in Corvinone wine is due to the stress effect as consequence of water loss since monoterpenes act as plant volatile messengers (Loreto & Schnitzler, 2010). Moreover, the increase of fatty acids and their esters such as nonanoate and methylhexanoate in wines coming from greater mass loss of berries (20 and 30% m.l.), might be the result of berry cell wall degradation during the withering process (Zoccatelli et al, 2013) but also of the yeast degradation during fermentation. Finally, dimethyl succinate is an ester which has been found in high concentration in Madeira wines during aging (Câmara, Alves & Marques, 2006).

On one hand, in Corvina wines, there was a reduction of the compounds similar to the ones described for Corvinone. On the other hand, balsamic compounds, such as p-cymen-8-ol (balsamic), 2,3 pinanediol (resin), and all the compounds deriving by water stress and carotenoid degradation during withering, such as damascenone (apple compote) and 3-oxo-ionol (tobacco), and, coumaran (green tea nuance) increased.

Sensorial analysis with the main aromatic nuances identified by the panelists is reported in **Table 4**. Matching the aromatic nuances of the single wine, in the ratio 1:1, from 20 and 30% m.l. grapes, revealed a great potential complexity for Rondinella 20% and 30%, Rondinella 20% and Corvina 30%, Rondinella 30% and Corvina 20%, Corvinone 20% and Corvina 30%, Rondinella 30% and Corvinone 20%. These results mean that combining two wines coming from lower and higher percentages of mass loss is better than combining wines coming from grapes with the same percentage of mass loss. If we match three wines sorted on the basis of the frequency of the presence of the above wines in the combination (Rondinella 30% = 3; Corvina 30% = 2; Corvinone 20% = 2), the aromatic profile should be the following: fruit compote, balsamic (eucalyptus), green tea, spicy (clove), overripe fruit, medicinal, vanilla, withered rose, smoky, honey. This is just a sum of the aromatic nuances of wine without any aging, but it cannot be the real situation of the combination. However, if we check the aromatic profile of Amarone commercial wines, the same nuances are identified: "in the wines we in fact find aromas belonging to different families, from flowers, fruits and fruit jam aromas, to empyreumatic and balsamic aromas. (http://www.diwinetaste.com/dwt/en2007063.php)".

CONCLUSION

We can conclude that great changes of VOCs take place during dehydration. Wines produced from dehydrated grapes have a complete different aroma if compared to the wines coming from non-dehydrated grapes In the case of Amarone, the wines from the 3 varieties behave similarly in the loss of the main classes of compounds during withering but behave differently in the increase of some compounds which we have considered as markers: Corvina and Rondinella have markers with the aromatic nuances of balsamic, tobacco, apple compote and green tea, while Corvinone has markers with fruity, cinnamon, smoky and waxy nuances. Starting from this information it is possible to decide how much should the mass loss be in order to have special VOCs in the final wine and, overall, it is possible to use a different amount of grape Corvina, Corvinone, and Rondinella, withered at different percentage of mass loss, to obtain different final Amarone. Probably the concentration of some of these compounds is the effect of an asymptomatic presence of *Botrytis cinerea* in the berry (Fedrizzi et al., 2011), as well as of some specific identified compounds such as N-(3-methylbutyl)-acetamide. This is because it is very difficult to control the fungus in the huge commercial facilities used for grape withering in Valpolicella area where we made our tests.

Conflict of interest

The authors declare no conflicts of interest

ACKNOWLEDGMENTS……

REFERENCES

Accordini, D., (2013). Amarone. In F. Mencarelli, & P. Tonutti (Eds.), *Sweet, Reinforced and Fortified Wine: Grape biochemistry, technology and vinification* (pp. 189-203). Wiley and Blackwell Ltd.

Bakker, J., & Clarke, R. J. (2012). Volatile components. In Wine Flavour Chemistry, 2nd ed.; John Wiley & Sons, Ltd: Chichester, UK, 2012; pp. 155–238.

Barbanti, D., Mora, B., Ferrarini, R., Tornielli, G. B., & Cipriani, M. (2008). Effect of various thermo-hygrometric conditions on the withering kinetics of grapes used for the production of “Amarone” and “Recioto” wines. *Journal of Food Engineering, 85*(3), 350-358.

Bonghi, C., Rizzini, F. M., Gambuti, A., Moio, L., Chkaiban, L., & Tonutti, P. (2012). Phenol compound metabolism and gene expression in the skin of winegrape (Vitis vinifera L.) berries subjected to partial postharvest dehydration. *Postharvest Biology and Technology*, *67*, 102–109.

Câmara, J. S., Alves, M. A., & Marques, J. C. (2006). Changes in volatile composition of Madeira wines during their oxidative ageing. *Analytica Chimica Acta*, 563(1–2), 188–197.

Centioni, L., Tiberi, D., Pietromarchi, P., Bellincontro, A., & Mencarelli, F. (2014). Effect of Postharvest Dehydration on Content of Volatile Organic Compounds in the Epicarp of the Cesanese Grape Berry. *American Journal of Enology and Viticulture, 65*, 333-340.

Chkaiban, L., Botondi, R., Bellincontro, A., De Santis, D., Kefalas, P., & Mencarelli, F. (2007). Influence of postharvest water stress on lipoxygenase and alcohol dehydrogenase activities, and on the composition of some volatile compounds of Gewürztraminer grapes dehydrated under controlled and uncontrolled thermohygrometric conditions. *Australian Journal of Grape and Wine Research*, *13*, 142–149.

Cirilli, M., Bellincontro, A., De Santis, D., Botondi, R., Colao, M. C., Muleo, R., et al. (2012). Temperature and water loss affect ADH activity and gene expression in grape berry during postharvest dehydration. *Food Chemistry*, *132*, 447–454.

Costantini, V., Bellincontro, A., De Santis, D., Botondi, R., & Mencarelli, F. (2006). Metabolic changes of Malvasia grapes for wine production during postharvest drying. *Journal of Agricultural and Food Chemistry, 54*, 3334–3340.

Darriet, P., Thibon, C., & Doboudieu, D. (2012). Aroma and aroma precursors in grape berry. In H. Gerós, M. M. Chaves, and S. Derlot (Eds.), The Biochemistry of Grape Berry (pp. 111-136). Bentham Science Publishers.

D’Onofrio, C., (2013). Change in volatile compounds. In F. Mencarelli, & P. Tonutti (Eds.), *Sweet, Reinforced and Fortified Wine: Grape biochemistry, technology and vinification* (pp. 91-103). Wiley and Blackwell Ltd..

De Sanctis, F., Silvestrini, M. G., Luneia, R., Botondi, R., Bellincontro, A., & Mencarelli, F. (2012). Postharvest dehydration of wine white grapes to increase genistein, daidzein and the main carotenoids. *Food Chemistry, 135*, 1619–1625.

Di Stefano, R. (1991). Proposal for a method of sample preparation for the determination of free and glycoside terpenes of grapes and wines. *Bulletin de l'OIV, 64*, 219-222.

Fedrizzi, B., Tosi, E., Simonato, B., Finato, F., Cipriani, M., Caramia, G., et al. (2011). Changes in wine aroma composition according to botrytised berry percentage: a preliminary study on Amarone wine. *Food Technology Biotechnology, 49*, 529–535.

Ferrarini, R. (2014). L’effetto “Appassimento” su Corvina, Corvinone e Rondinella. *L’Enologo, 4*, 26-34.

Ferreira, V., Lopez, R., & Cacho, J. F. (2000). Quantitative determination of the odorants of young red wines from different grape varieties. *Journal of the Science of Food and Agriculture*, *80*, 1659-1667.

Figueiredo-González, M., Cancho-Grande, B., & Simal-Gándara, J. (2013). Evolution of colour and phenolic compounds during Garnacha Tintorera grape raisining. *Food Chemistry*, *141*, 3230-40.

Figueiredo-González, M., Cancho-Grande, B., & Simal-Gándara, J. (2013). Evolution of colour and phenolic compounds during Garnacha Tintorera grape raisining, *Food Chemistry*, *141*, 3230-40.

Franco, M., Peinado, R. A., Medina, M., & Moreno, J. (2004). Off-vine grape dryinf effect on volatile compounds and aromatic series in must from Pedro Ximenez grape variety. *Journal of Agricultural and Food Chemistry*, *52*, 3905-3910.

Gianotti, S., & Di Stefano, R. (1991). Metodo per la determinazione dei composti volatili di fermentazione. *L'Enotecnico, 10*, 61-64.

Loreto, F. & Schnitzler, J. P. (2010). Abiotic stresses and induced BVOCs, *Trends in Plant Science*, *15*, 154–166.

Marquez, A., Serratosa, M. P., Lopez-Toledano, A., & Merida, J. (2012). Colour and phenolic compounds in sweet red wines from Merlot and Tempranillo grapes chamber-dried under controlled conditions. *Food Chemistry*, *130*, 111–120

Mencarelli, F., & Bellincontro, A. (2013). In F. Mencarelli, & P. Tonutti (Eds.), *Sweet, Reinforced and Fortified Wine: Grape biochemistry, technology and vinification* (pp. 51-74). Wiley and Blackwell Ltd..

Mencarelli, F., Bellincontro, A., Nicoletti, I., Cirilli, M., Muleo, R., & Corradini, D. (2010). Chemical and biochemical change of healthy phenolic fractions in winegrape by means of postharvest dehydration. *Journal of Agricultural and Food Chemistry*, *58*, 7557–7564.

Muganu, M., Bellincontro, A., Barnaba, F. E., Paolocci, M., Bignami, C., Gambellini, G., et al. (2011). Influence of bunch position in the canopy on berry epicuticular wax during ripening and on weight loss postharvest dehydration. *American Journal of Enology and Viticulture*, *62*, 91–98.

Noguerol-Pato, R., González-Álvarez, M., González-Barreiro, C., Cancho-Grande, B., & Simal-Gándara, J. (2013). Evolution of the aromatic profile in Garnacha Tintorera grapes during raisining and comparison with that of the naturally sweet wine obtained. *Food Chemistry, 139*, 1052-1061.

Panceri, C. P., Gomes, T. M., De Gois, J. S., Borge, D. L. G., & Bordignon-Luiz, M. T. (2013). Effect of dehydration process on mineral content, phenolic compounds and antioxidant activity of Cabernet Sauvignon and Merlot grapes. *Food Research International 54*, 1343-1350.

Rolle, L., Giacosa, S., Río Segade, S., Ferrarini, R., Torchio, F., & Gerbi, V. (2013). Influence of different thermohygrometric conditions on changes in instrumental texture properties and phenolic composition during postharvest withering of ”corvina” winegrapes (Vitis vinifera L.). *Drying Technology 31*, 549-564.

Santonico, M., Bellincontro, A., De Santis, D., Di Natale, C., & Mencarelli, F. (2010). Electronic nose to study postharvest dehydration of wine grapes. *Food Chemistry*, *121*, 789–796.

Serratosa, M. P., Lopez-Toledano, A., Merida, J., & Medina, M. (2008). Changes in color and phenolic compounds during the raisining of grape cv. Pedro Ximenez. *Journal of Agricultural and Food Chemistry*, *56* (8), 2810–2816.

Toffali, K., Zamboni, A., Anesi, A., Stocchero, M., Pezzotti, M., Levi, M., & Guzzo, F. (2011). Novel aspects of grape berry ripening and post-harvest withering revealed by untargeted LC-ESI-MS metabolomics analysis. *Metabolomics*, *7*, 424–436.

Tonutti, P., & Bonghi, C. (2013). Biochemistry and physiology of dehydrating berries. In F. Mencarelli, & P. Tonutti (Eds.), *Sweet, Reinforced and Fortified Wine: Grape biochemistry, technology and vinification* (pp. 77-89). Wiley and Blackwell Ltd..

Versari, A., Parpinello, G. P., Tornielli, G. B., Ferrarini, R., & Gulivo. C. (2001). Stilbene compounds and stilbene synthase expression during ripening, wilting, and UV treatment in grape cv. Corvina. *Journal of Agricultural and Food Chemistry, 49*, 5531-5536.

Zamboni, A., Minoia, L., Ferrarini, A., Tornielli, G.B., Zago, E., Delledonne, et al. (2008) Molecular analysis of post-harvest withering in grape by AFLP transcriptional profiling. *Journal of Experimental Botany 59*, 4145–4159.

Zoccatelli, G., Zenoni, S., Savoi, S., Dal Santo, S., Tononi, P., Zandonà V., et al. (2013). Skin pectin metabolism during the postharvest dehydration of berries from three distinct grapevine cultivars. *Australian Journal of Grape and Wine Research*, *19*, 171-179.

FIGURE AND TABLE CAPTION

Figure 1. Pattern of temperature (°C) and relative humidity (%) outside and inside the dehydration room during the grape withering. Daily monitoring of environmental conditions was carried out by sensors installed on the equipment for withering management (Sordato, Verona, Italy). Vertical dotted lines indicate the days when the indicated weight loss was reached. The box and the oval outline enclosing days of dehydration indicate the variability to reach respectively 30 and 50% of weight loss among the three varieties.

Figure 2. Trend of VOC markers measured in Corvina (**A** and **B**), Corvinone (**C** and **D**), and Rondinella (**E** and **F**) wines produced at harvest (0), 20% and 30% of grape m.l., respectively. Data are expressed in μg L^{-1} and are the average (±SD) of three analyses carried out on three separate bottles. Different letters represent significant statistical differences per $p < 0.05$.

Table 1. Main enological parameters measured in Corvina, Corvinone, and Rondinella wines produced at harvest (0), 20%, and 30% of grape m.l., respectively. Data are the average (±SD) of three analyses carried out on three separate bottles. Different letters represent significant statistical differences per $p < 0.05$.

Table 2. Main categories of volatile compounds (Aliphatic alcohols, Benzenoids, Monoterpenes, Nor-isoprenoids, Acids, and Esters) measured in Corvina, Corvinone, and Rondinella wines produced at harvest (0), 20%, and 30% of grape m.l., respectively. Data are the average (±SD) of three analyses carried out on three separate bottles. Different letters represent significant statistical differences per $p < 0.05$.

Table 3. Main VOC markers detected in Corvina, Corvinone, and Rondinella wines produced at harvest (0), 20%, and 30% of grape m.l., respectively. Principal descriptors of aromatic impact are also reported for each volatile. Data are expressed in $\mu g\ L^{-1}$ and are the average (±SD) of three analyses carried out on three separate bottles. Different letters represent significant statistical differences per $p < 0.05$.

Table 4. Table of aromatic nuances appreciable in Corvina, Corvina, and Rondinella wines produced at harvest (0), 20%, and 30% of grape m.l., respectively. Characteristic flavors are referred to each single condition of dehydration of the three grape varieties and matching them as combined effect.

FIGURES AND TABLES

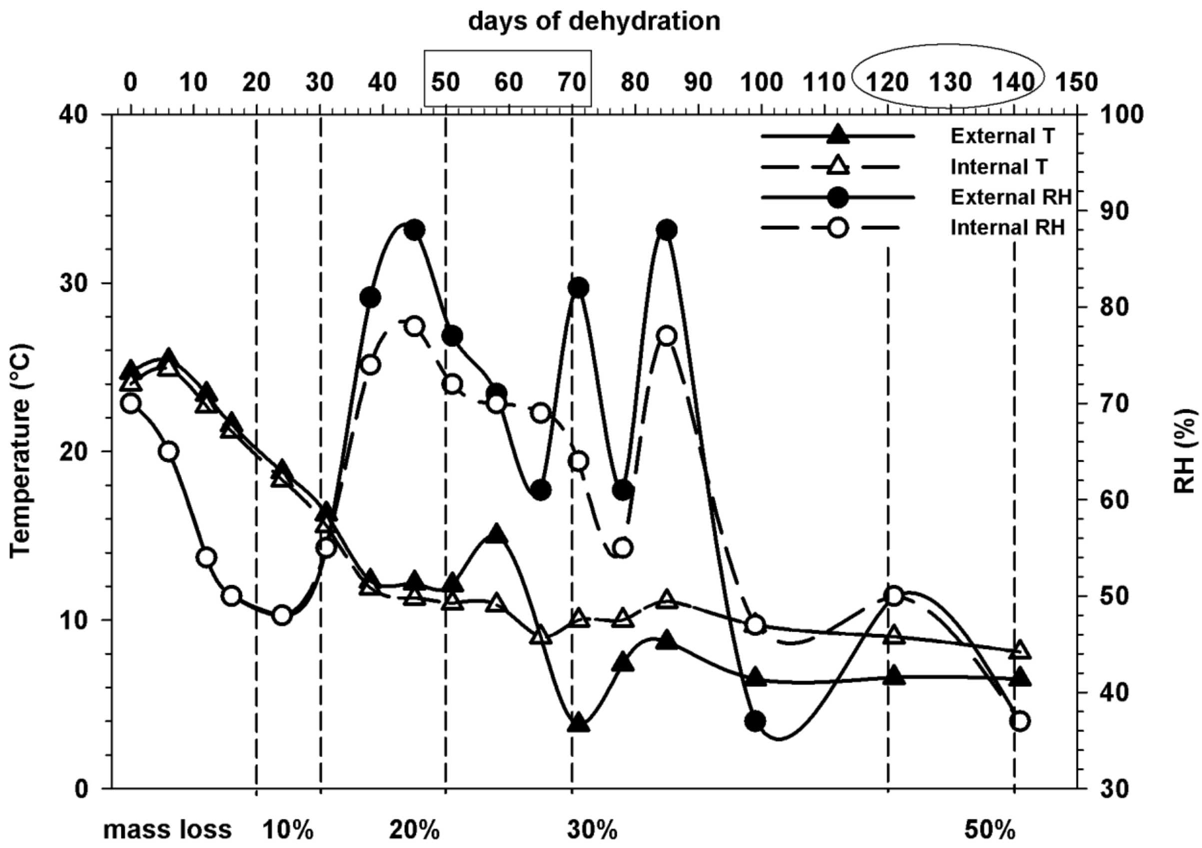

Figure 1

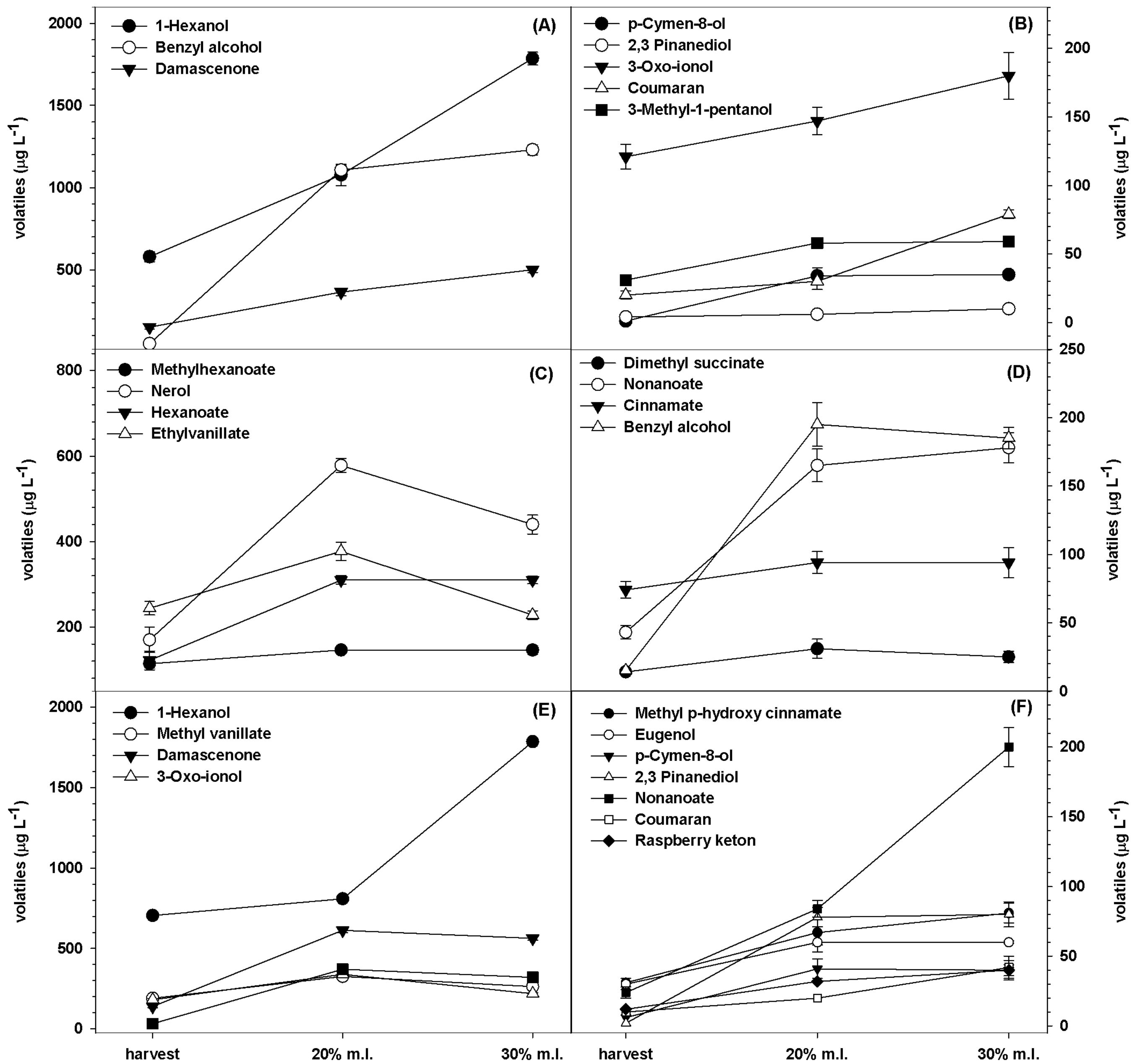

Figure 2 (A, B, C, D, E, F)

	Titratable acidity (g L^{-1})	**pH**	**Glycerol** (mg L^{-1})	**Volatile acidity** (g L^{-1})	**Total polyphenols** (mg L^{-1})	**Total anthocyanins** (mg L^{-1})
Corvina 0	6.30 ± 0.04e	3.13 ± 0.02ef	6.60 ± 0.18d	0.52 ± 0.03bc	1326 ± 24c	125 ± 11f
Corvina 20%	5.46 ± 0.09h	3.31 ± 0.04b	8.10 ± 0.32c	0.48 ± 0.03cd	1376 ± 43c	184 ± 10de
Corvina 30%	6.70 ± 0.10cd	3.42 ± 0.05a	9.40 ± 0.40a	0.62 ± 0.08ab	1652 ± 28b	203 ± 21cd
Corvinone 0	6.50 ± 0.06de	3.20 ± 0.03d	6.00 ± 0.21de	0.48 ± 0.09cd	1024 ± 37d	161 ± 12e
Corvinone 20%	5.72 ± 0.11g	3.34 ± 0.06ab	8.70 ± 0.23b	0.47 ± 0.03cd	1338 ± 37c	249 ± 18b
Corvinone 30%	7.45 ± 0.09a	3.38 ± 0.06a	9.54 ± 0.65a	0.68 ± 0.06a	1840 ± 41a	263 ± 18ab
Rondinella 0	6.90 ± 0.07bc	3.07 ± 0.04f	5.65 ± 0.25e	0.44 ± 0.04d	930 ± 22d	181 ± 20de
Rondinella 20%	5.90 ± 0.10fg	3.23 ± 0.07cd	9.00 ± 0.23ab	0.51 ± 0.06cd	1595 ± 27b	251 ± 19b
Rondinella 30%	6.89 ± 0.06bc	3.30 ± 0.05bc	9.48 ± 0.18a	0.65 ± 0.08a	1779 ± 30a	278 ± 27a

Table 1

	At harvest	20%	30%
Aliphatic alcohols (μL L^{-1})			
Corvina	12941 ± 282def	11971 ± 253fg	12162 ± 102efg
Corvinone	15727 ± 131a	11907 ± 153fg	10299 ± 156h
Rondinella	14003 ± 276bc	13461 ± 151cd	11520 ± 202g
Benzenoids (μL L^{-1})			
Corvina	18178 ± 202cd	19937 ± 387b	14990 ± 290ef
Corvinone	24930 ± 287a	20048 ± 190b	13721 ± 285f
Rondinella	25290 ± 300a	25359 ± 253a	17937 ± 184d
Monoterpenes (μL L^{-1})			
Corvina	336 ± 32bc	311 ± 21c	76 ± 8e
Corvinone	373 ± 43ab	313 ± 17c	74 ± 8e
Rondinella	367 ± 28ab	336 ± 32bc	186 ± 10d
Nor-isoprenoids (μL L^{-1})			
Corvina	326 ± 21c	555 ± 31b	26 ± 3f
Corvinone	560 ± 23b	991 ± 42a	84 ± 6e
Rondinella	294 ± 35c	1019 ± 42a	109 ± 6d
Acids (μL L^{-1})			
Corvina	499 ± 25f	429 ± 23f	1336 ± 76b
Corvinone	650 ± 25e	708 ± 19cd	1367 ± 78b
Rondinella	682 ± 34de	769 ± 27c	1874 ± 93a
Esters (μL L^{-1})			
Corvina	11064 ± 166bc	7575 ± 108e	4055 ± 120i
Corvinone	12001 ± 176a	8067 ± 120de	4980 ± 91h
Rondinella	10682 ± 239c	6551 ± 108f	5523 ± 90gh

Table 2

Compound	descriptor	harvest	20%	30%
CORVINA			($\mu L\ L^{-1}$)	
Isopenthyl acetate	*fruity*	442 (±31)	351 (±37)	326 (±17)
Ethyl(S)-(-)-lactate	*Ethereal, buttery, fruity*	3446 (±62)	2134 (±41)	1786 (±31)
Ethyl, 2 and 3 methyl butanoate	*Fruity (pineapple, banana), apple, herbaceous*	260 (±16)	182 (±8)	141 (±11)
1-hexadecanol	*Waxy, floral*	2499 (±111)	1221 (±31)	792 (±67)
2-phenylethanol	*Floral, woody (rose-honey like)*	17679 (±125)	18370 (±97)	13988 (±104)
Trans-8-OH-linalool	*floral*	228 (±11)	169 (±9)	130 (±12)
Diethyl succinate	*Fruity*	3247 (±37)	2024 (±51)	1018 (±21)
Triacetin	*Mild tropical fruit*	1486 (±12)	1195 (±27)	648 (±44)
4-ethoxy, ethylbenzoate	*Fruity (raspberry, guava, fejoa)*	1127 (±72)	533 (±33)	208 (±22)
N-(3-methylbutyl) acetamide	*Mild vinegar*	1727 (±82)	1646 (±62)	611 (±35)
CORVINONE			($\mu L\ L^{-1}$)	
Isopenthyl acetate	*fruity*	480 (±30)	410 (±26)	319 (±18)
Isoamyl alcohol	*Fruity-winery whisky, malt*	12462 (±110)	10165 (±70)	10036 (±85)
Ethyl(S)-(-)-lactate	*Ethereal, buttery, fruity*	3999 (±31)	674 (±25)	659 (±17)
Ethyl, 2 and 3 methyl butanoate	*Fruity (pineapple, banana), apple, herbaceous*	324 (±18)	167 (±17)	138 (±9)
Damascenone	*Fruity, honey*	185 (±11)	102 (±9)	72 (±9)
Guaiacol	*Smoke, sweet , medicine*	1764 (±110)	1058 (±91)	1068 (±40)
2-phenylethanol	*Floral, woody (rose-honey like)*	24150 (±120)	13062 (±65)	13062 (±50)
Diethyl malate	*apple*	1704 (±32)	577 (±21)	560 (±11)
4-ethoxy, ethylbenzoate	*Fruity (raspberry, guava, fejoa)*	1237 (±31)	176 (±11)	159 (±10)
RONDINELLA			($\mu L\ L^{-1}$)	
Isopenthyl acetate	*fruity*	270 (±27)	178 (±31)	100 (±14)
Isoamyl alcohol	*Fruity-winery, whisky, malt*	10870 (±112)	10433 (±69)	10377 (±92)
Ethyl(S)-(-)-lactate	*Ethereal, buttery, fruity*	2953 (±61)	1273 (±35)	233 (±27)
Ethyl, 2 and 3 methyl butanoate	*Fruity (pineapple, banana), apple, herbaceous*	300 (±12)	246 (±7)	170 (±16)
1-hexadecanol	*Waxy, floral*	2261 (±42)	2080 (±12)	1800 (±12)
2-phenylethanol	*Floral, woody (rose-honey like)*	24738 (±133)	24235 (±92)	13988 (±66)
Trans-8-OH-linalool	*floral*	280 (±22)	131 (±13)	134 (±10)
Diethyl succinate	*Fruity*	2744 (±74)	2250 (±107)	1526 (±67)
Triacetin	*Mild tropical fruit*	1740 (±77)	693 (±22)	726 (±17)
4-ethoxy, ethylbenzoate	*Fruity (raspberry, guava, fejoa)*	1312 (±21)	576 (±19)	564 (±14)

Table 3

	Corvina 20%	Corvina 30%	Corvinone 20%	Corvinone 30%	Rondinella 20%	Rondinella 30%
Corvina 20%	*fruity, floral, fruit compote, slight balsamic*	*balsamic, resin, spicy, floral, fruit compote, vanillic, fruity, floral*	*fruity, floral, fruit compote, balsamic, withered rose, smoky, honey, slightly medicinal*	*withered rose, smoky, honey, slightly medicinal, intense tobacco, ginger*	*fruity, tropical fruit, floral, honey, malt, floral, fruit compote, slight balsamic*	*apple compote, balsamic, green tea, spicy, overripe fruit, medicinal, floral, slight balsamic*
Corvina 30%	*balsamic, resin, spicy, floral, fruit compote, vanillic, fruity, floral*	*balsamic, resin, spicy, floral, fruit compote, vanillic*	*balsamic, resin, spicy, floral, fruit compote, vanillic, withered rose, smoky, honey, slightly medicinal*	*balsamic, resin, spicy, floral, fruit compote, vanillic, intense tobacco, ginger*	*fruity, tropical fruit, floral, honey, malt, balsamic, resin, spicy, fruit compote, vanillic*	*apple compote, balsamic, green tea, spicy, overripe fruit, medicinal, vanillic*
Corvinone 20%	*fruity, floral, fruit compote,*	*balsamic, resin, spicy, floral, fruit compote, vanillic,*	*slightly fruity, balsamic, withered rose, smoky, honey, slightly medicinal*	*withered rose, smoky, honey, slightly medicinal, intense tobacco, ginger*	*tropical fruit, floral, honey, malt, balsamic, withered rose, smoky, honey, slightly medicinal*	*apple compote, balsamic, green tea, spicy, overripe fruit, medicinal, withered rose, smoky, honey*
Corvinone 30%	*fruity, floral, fruit compote, balsamic, spicy, intense tobacco, ginger*	*balsamic, resin, spicy, floral, fruit compote, vanillic, intense tobacco, ginger*	*withered rose, smoky, honey, slightly medicinal, intense tobacco, ginger*	*slight tropical fruit, apple compote, balsamic, spicy, intense tobacco, ginger*	*fruity, tropical fruit, floral, honey, malt, apple compote, balsamic, spicy, intense tobacco, ginger*	*apple compote, balsamic, green tea, spicy, overripe fruit, medicinal, intense tobacco, ginger*
Rondinella 20%	*tropical fruit, floral, honey, malt, balsamic, withered rose, smoky, honey, slightly medicinal*	*fruity, tropical fruit, floral, honey, malt, apple compote, balsamic, spicy, intense tobacco, ginger*	*fruity, tropical fruit, floral, honey, malt, floral, fruit compote, slight balsamic*	*fruity, tropical fruit, floral, honey, malt, balsamic, resin, spicy, fruit compote, vanillic*	*fruity, tropical fruit, floral, honey, malt*	*tropical fruit, floral, honey, malt, withered rose, smoky, honey, apple compote, balsamic, green tea, spicy, overripe fruit, medicinal*
Rondinella 30%	*apple compote, balsamic, green tea, spicy, overripe fruit, medicinal, withered rose, smoky, honey,*	*apple compote, balsamic, green tea, spicy, overripe fruit, medicinal, intense tobacco, ginger*	*apple compote, balsamic, green tea, spicy, overripe fruit, medicinal, floral, slight balsamic*	*apple compote, balsamic, green tea, spicy, overripe fruit, medicinal, vanillic,*	*tropical fruit, floral, honey, malt, withered rose, smoky, honey, apple compote, balsamic, green tea, spicy, overripe fruit, medicinal*	*apple compote, balsamic, green tea, spicy, overripe fruit, medicinal*

Table 4