Impact of harvest date on aroma compound composition of Merlot and Cabernet-Sauvignon must and wine in a context of climate change: a focus on cooked fruit molecular markers

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ABSTRACT

Choice of harvest date can modulate the aroma intensity of herbaceous/vegetal, fresh fruit and cooked fruit nuances, thus helping the winemaker to produce wine of different styles and balance. Relationships between the sensory attributes and aroma compounds of Merlot and Cabernet-Sauvignon grapes that were harvested sequentially and the resulting wines were evaluated for two vintages: 2012 and 2014. The fine-tuning of the harvest date modulated the aromas of the young wine and impacted the intensity of the cooked fruit aromas for both Merlot and Cabernet-Sauvignon. No correlation was observed between the must and wine in terms of the intensity of the cooked fruit aroma. In order to observe an impact on the intensity of the cooked fruit aroma it was necessary to delay the harvest date of Cabernet-Sauvignon by 4 to 12 days in 2012 and 2014 respectively. This value was 7 days for Merlot wines (2014 vintage). Furanones, lactone and ketones were well correlated with the perceived intensity of the cooked fruit aroma in the young wine. In addition, the highest concentrations of γ-nonalactone, 3-methyl-2,4-nonanedione, massoia lactone and furaneol were detected in Merlot wines made using late-harvested grapes. At the measured concentrations, these compounds can explain the aroma of cooked fruit detected in the red wines. Similar results were obtained for the Cabernet-Sauvignon wines made from grapes from a later harvest. The volatile compounds produced from the lipoxygenase pathway in the grapes were putatively involved in the evolution of the aroma of the red wines from sequential harvest dates, opening up the possibility of managing aroma profiles through harvest date decisions. These findings are important, as the identification of measurable key chemical parameters in grapes could provide grape growers and winemakers objective indicators for predicting final wine style and quality.

KEYWORDS: aroma, wine, ripening, ketones, furanones, lactone, climate change

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INTRODUCTION

Optimal grape maturity is a key parameter which enables winemakers to modulate the sensory profile and quality of a future wine. Depending on grape variety, soil, grape maturity and the winemaking process, the aromatic expression of red wine ranges widely from herbaceous to fruity: reminiscent of green pepper, blackcurrant, blackberry, prune and figs (van Leeuwen et al., 2022). The aromas of wine are a result of the volatile compounds found as free forms in the berry or bonded to glucose, for example (Robinson et al., 2014). Their accumulation is strongly influenced by climatic conditions (micro/mesoclimat) during fruit ripening (Lee et al., 2007).

The winegrower and the winemaker are permanently in search of the optimum sensory equilibrium. « There is no objective point of ideal maturity », Ribèreau-Gayon and Peynaud (1964) rightly pointed out; “the concept of the ripe grape is widely recognized, but maturity is not an absolute criterium...there is no final or limiting physiological state, easy to define, but there are degrees of maturity”. Hence, choice of harvest date is a non-objective decision that is variable in time and space. The way the winegrower defines maturity depends on the variety used and varies over time, and the intended wine-style can change. This definition also evolves with increasing scientific knowledge.

Historically, maturity has been defined by the sugar to acid ratio - more recently referred to as technological maturity. Other types of maturity have been defined, as reviewed by van Leeuwen et al. (2022): physiological maturity, phenolic maturity and aromatic maturity. These different types of maturity depend on the climatic conditions in relation to water status (van Leeuwen et al., 2009), temperature (van Leeuwen et al., 2020) and the intensity of sun exposure of the grapes (Joscelyne et al., 2007). Assessing maturity by berry tasting is crucial for the technical staff to be able to validate their choice of harvest date and trigger the harvest.

The style of the wine produced by the winemaker will depend on their definition of maturity. This is due to different accumulation kinetics of primary and secondary metabolites from the onset of ripening to maturity. Optimal phenolic maturity, for example, is known to be delayed compared to technological maturity (Raja et al., 2017). A study conducted by Antalick et al. (2021) on irrigated Shiraz and Cabernet-Sauvignon (Australia), demonstrated that once berry sugar accumulation has reached a plateau (expressed as a concentration; i.e., mg/berry), the evolution of the aromatic maturity of the wine is not correlated with the technological maturity of the berries.

For these reasons, defining the optimum grape maturity necessary for obtaining a balanced wine in terms of polyphenols, aromas and balance freshness/alcohol is challenging for winegrowers and winemakers, particularly in the context of climate change. Indeed, forecast increases in temperatures are predicted to cause a general advancement of grapevine phenological stages (Duchêne and Schneider, 2005; Petie and Sadras, 2008), with maturation occurring during hotter periods of the year (Duchêne et al., 2010). In addition, growers have increased the interval between veraison and harvest (the so-called “hang-time”) (van Leeuwen and Darriet, 2016); in the last decade in Bordeaux, Merlot grapes were harvested late (in terms of number of days after mid-veraison) compared to the 1980s and 1990s (Geny et al., 2011).

As a consequence of increasing temperatures, high sugar to acid ratios are more often reached before optimum colour development and can cause ‘unbalanced fruit’ notes (Mira de Orduña, 2010; Sadras and Moran, 2012), which are associated with over-ripe fruit aromas, such as prune and dried figs (Allamy, 2015). Rarely detected in Bordeaux red wines before the 2000s, dried and cooked fruit nuances reminiscent of cooked plum and dried figs have become increasingly frequent in young wines, especially those made from Merlot grapes. This effect on the fruity aroma of wine is impacting consumer preferences. Indeed, a recent study has highlighted the impact of alcohol level associated with the increasing aromatic ripeness of Merlot grapes on French consumer preferences and willingness to pay (Tempere et al., 2019).

For a long time, the evolution of aroma compounds in the grapes of non-aromatic grapevine varieties between fruit-set and full ripeness was not well explored, with most studies based on the accumulation of sugars, acids and phenolics (Bonada and Sadras, 2015; Ribèreau-Gayon, 2006). However, preliminary studies reported the accumulation of fruity esters and C6 compounds (alcohols and carbonyls) (Gómez et al., 1995), while others investigated the distribution of C15-norisoprenoid in Cabernet-Sauvignon grapes, such as β-damascenone, TDN and vitispirane (Lee et al., 2007), and of terpenes and benzene derivatives, such as 2-phenylethanol and 2-phenylethanal (Kalua and Boss, 2009). According to the latter author, alcohol to aldehyde ratios could be used for predicting the timing of harvest for enhanced grape and wine aroma.

The aroma of must and red wines made with unripe grapes is systematically marked by grassy, green or capsicum flavours irrespective of grape variety; i.e., the grapes release these flavours directly into the wine. This phenomenon has been particularly well described for non-aromatic wine grape cultivars. Today, it is recognised that 3-isobutyl-2-methoxy pyrazine (IBMP) is responsible for the green pepper odour of Cabernet-Sauvignon grapes and wines (Roujou de Boubee et al., 2000). This compound is present at high levels in green berries and decreases during maturation. IBMP degradation is slowed down when climatic conditions are cool and rainy during the ripening period (Falcão et al., 2007) or under low sunlight exposure (Dunlevy et al., 2013). The C6 alcohols and their derivatives, which come from fatty acid precursors via the lipoxygenase pathway, have also been implicated in the sensory attributes ‘herbaceous’ and ‘green’ (Ribèreau-Gayon, 2006).
Red wines made with overripe or dried grapes share cooked/dried fruit nuances, as reported in several grape cultivars, including Garnacha Tintorera (González-Álvaro et al., 2014), Merlot, Cabernet-Sauvignon (Allamy et al., 2018), and Rondinella (Bellincontro et al., 2016).

Despite a great interest in understanding the aromas of must, as well as the impact of agronomic practices on their molecular basis (Alem et al., 2019; Robinson et al., 2014), until very recently, few studies have addressed the molecular markers of cooked/dried-fruit nuances (reminiscent of dried figs or prunes) found in Merlot and Cabernet-Sauvignon grapes and wines. However, recent studies have highlighted the sensory impact of two new compounds reported in musts from red grapes marked by cooked and dried-fruit flavors: (-)-massoia lactone, reminiscent of coconut and dried figs (Pons et al., 2017), and (Z)-1,5-octadien-3-one, reminiscent of geraniums. The latter aldehyde has recently been reported in must, in which it contributes to the dried fig nuance when present in a specific range of concentrations (Allamy et al., 2018). More recently, Allamy et al. (2018) emphasised the contribution of a group of furanones and lactones to the dried fruit flavors of wines. γ-Nonalactone, reminiscent of coconut and cooked peaches, is also associated with berry shrivelling, which often occurs when grapes are overripe, or when the grapes are infected by U. necator (Pons et al., 2018). The compounds responsible for cooked fruit and caramel notes in wines were identified by the same group of authors as furanone (2,5-dimethyl-4-hydroxy-3(2H)-furanone) and homofuraneol (corresponding to keto-enol equilibrium structures of 5-ethyl-4-hydroxy-2-methylfuran-3(2H)-one and 2-ethyl-4-hydroxy-5-methylfuran-3(2H)-one). Sensory approaches demonstrated that the perceptive interaction between these latter compounds can occur, providing the specific “dried fruit” aromas detected in young red wines. The concentration of these compounds depends on the vintage and the presence of light during the ripening period; the sensory impact of two new compounds reported in musts for Cabernet-Sauvignon grape varieties was also investigated.

### MATERIALS AND METHODS

#### 1. Chemicals and reference compounds

Dichloromethane (CH₂Cl₂) was supplied by Prolabo (Fontenay sous Bois, France). Absolute ethanol (≥ 99.9 %) was obtained from Merck (Darmstadt, Germany). 3-octanol (99 %), ethyl maltol (99 %), furanone (99 %), homofuraneol (97 %) (R/S)-γ-nonalactone (98 %), methional (97 %), phenylacetaldehyde (≥ 95 %) and ethylenediaminetetraacetic acid (EDTA, ≥ 99 %) were supplied by Sigma-Aldrich (Saint-Quentin Fallavier, France). 3-methyl-2,4-nonanediol (99 %) was from Chemos GmbH (Germany). (Z)-1,5-octadien-3-one solution in pentane was a gift from Nestlé® (Lausanne, Switzerland) and (R)-C10 massoia lactone was a gift from Robertet (Grasse, France). Unless otherwise specified, ultrapure water was used in this study (Milli-Q, Millipore, Bedford, MA, USA).

#### 2. Origins of grapes and wines

Vitis Vinifera L. cv. Merlot and Cabernet-Sauvignon grape varieties were obtained from the same winery (Pauillac appellation) in the Bordeaux region from two different parcels (their characteristics are shown in Table SM1). The wines were dry-farmed. The same viticultural practices (thinning, foliar treatments, soil management, etc) were applied to both parcels. The vintages selected for this study were 2012 and 2014, with contrasting rainfall and average temperature during the growing season (Table SM2). The harvest dates of each vintage were determined by the technical staff of the winery (referred to as D in this study). In both vintages, the grapes were manually harvested on three dates in 2012 and four dates in 2014: at 55 (8/10), 59 (D, 12/10), 63 (16/10) days after mid-veraison for Cabernet-Sauvignon in 2012 (Table SM3); at 44 (18/09), 50 (D, 24/09), 54 (28/09), 62 (06/10) days after mid-veraison for Merlot; and at 57 (07/10), 60 (D, 10/10), 62 (12/10), 67 (17/10) days after mid-veraison for Cabernet-Sauvignon in 2014 (Table SM5).

#### 3. Semi-industrial to small scale vinification

In 2012, each batch of Cabernet-Sauvignon grapes from each of the different harvests (see aforementioned dates) were fermented in 12 L stainless steel tanks at a controlled temperature set at 24 °C. The musts were then inoculated with 20 g/L of the F33® commercial S. cerevisiae yeast strain (Laffort, 33270 Floirac, France), which had been rehydrated in water at 37 °C for an hour. To avoid sluggish alcoholic fermentation, yeast assimilable nitrogen (YAN) concentration was adjusted to 200 mg/L before each trial using ammonium sulfate (Laffort, 33270 Floirac, France). The fermentations were monitored daily by electronic densimeter in order to compare the fermentation kinetics modalities and to prevent sluggish fermentation. The fermentations lasted for 13 ± 1 days on average. The end of fermentation was reached when the reducing sugar level was below 2 g/L. The wines were fermented in 12 hL stainless steel tanks at a controlled temperature set at 24 °C. The musts were then inoculated with 20 g/hL of the F33® commercial S. cerevisiae yeast strain (Laffort, 33270 Floirac, France), which had been rehydrated in water at 37 °C for an hour. To avoid sluggish alcoholic fermentation, yeast assimilable nitrogen (YAN) concentration was adjusted to 200 mg/L before each trial using ammonium sulfate (Laffort, 33270 Floirac, France). The fermentations were monitored daily by electronic densimeter in order to compare the fermentation kinetics modalities and to prevent sluggish fermentation. The fermentations lasted for 13 ± 1 days on average. The end of fermentation was reached when the reducing sugar level was below 2 g/L. The wines were stored in 3-year-old oak barrels after separation from the skins (225 L). Spontaneous malolactic fermentations were conducted in the barrels. Once achieved, SO₂ was added at 50 mg/L. One month later, samples of wine were taken from the tops of the barrels and transferred to bottles flushed with nitrogen and sealed with a natural cork stopper. Due to the age of the barrels and the short contact time with oak wood, the impact of oak on the composition of the wine was considered negligible. The samples were analysed 3 months after bottling. In this experiment, which was conducted in real conditions at industrial scale, no biological replicates were possible.
In 2014, the experiments were conducted on parcels planted with Merlot and Cabernet-Sauvignon. The grapes were harvested and vinified in the laboratory in 10 L stainless steel tanks following the same protocol as that described previously for the 2012 experiments, but without oak wood contact. The winemaking protocol was also slightly different in terms of malolactic fermentation management: the wines were inoculated with Lactoenos® bacterial strains (Laffort, Floirac, 10 g/L). The experiments were conducted in duplicate. Must samples were collected just after crushing the grapes once the tank had been filled. Before chemical analysis, the must and wine samples were centrifuged at 3000 rpm for 10 min to remove the lees. The wines were then stored at a constant temperature of 10 °C for future wine sensory and chemical evaluation.

4. Aroma compound analysis

γ-Lactones, (Z)-1,5-octadien-3-one and 3-methyl-2,4-nonanediol (MND) in musts and wines were analysed by HS-SPME-GC-MS, as described by Allamy et al. (2017), (2018) and Pons et al. (2018) respectively. Briefly, for lactone and MND, MQ water (9 mL) and wine (1 mL) were placed in a 20-mL amber vial containing 5 g ammonium sulfate, 3 μL EDTA solution (60 g/L, H2O) and 10 μL each of internal standards 1 (IS1) and 2 (IS2): 100 mg/L 3-octanol and 2-octanol respectively in EtOH. Samples were extracted using a 65 μm polydimethylsiloxane-divinylbenzene (PDMS/DVB, Supelco, 38297 Saint-Quentin-Fallavier, France) fiber under the following conditions: extraction temperature: 50 °C, incubation time: 5 min; extraction time, 25 min and agitation speed 500 rpm.

For (Z)-1,5-octadien-3-one, 6 g of ammonium sulfate, 10 μL of octan-2-ol (IS, 100 mg/L), 5 mL of aqueous solution (5 g/L tartaric acid with ultrapure water (Milli-Q; Millipore, Bedford, MA, USA) adjusted to pH 1 (37 % HCl), and 5 mL of must were added successively to a 20 mL amber vial. Samples were extracted using a fiber coated with divinylbenzene / carbboxen / polydimethylsiloxane (DVB/CARB/PDMS; Supelco, 38297 Saint-Quentin-Fallavier, France) under the same conditions.

The fiber was desorbed in the GC (3400 Cx, Agilent Technologies, France) injector (PTV, 240 °C) and separated on a polar BP20 column (SGE, France, polyethylene glycol, 50 m x 0.22 mm i.d., x 0.25 μm film thickness). The calibration curves were prepared for each analyte using seven concentration points and three replicate solutions per point in red wines. Calibration curves were obtained by linear regression, plotting the response ratio against concentration ratio. For 2012 and 2014 experiments, each sample was analysed in triplicate.

5. Oenological parameters

The must and wines samples were analysed for basic parameters of maturity and quality. Reducing sugar, pH, malic acid, titratable acidity, alcohol level and volatile acidity were measured according to Allamy et al. (2018).

6. Sensory analysis

A sensory analysis was conducted on the must and wine samples, which comprised a blend of the two replicates. This took place in a tasting room at the oenology research unit of the Institute des Sciences de la Vigne et de Vin (ISVV, 33883 Villenave d’Ornon, France) containing 10 individual booths, according to Allamy et al. (2018). All of the tastings were carried out in black glasses corresponding to AFNOR (ISO 3591) standards filled with 30 mL of solution. In all the experiments, the glasses were labelled with 3-digit random codes and presented to the panelists in random order. The panel consisted of 12 judges, who were all wine professionals from the staff of the ISVV research unit with extensive experience in wine tasting and aroma recognition. There were eight female and four male judges, ranging from 24 to 45 years old. They were selected for their experience in assessing the aromas of musts and young red Bordeaux wines. They were asked to evaluate the intensity of the vegetative/herbaceous, fresh fruit and cooked fruit characters based on a 0 to 10 non-structured scale.

7. Statistical analysis

The application conditions of ANOVA, homogeneity of variance (Levene’s test) and normality of residuals (Shapiro-Wilk test) were verified using R software (R foundation for Statistical Computing, England). When these conditions were not reached, a bilateral nonparametric, Kruskal-Wallis test was applied (multiple pairwise comparisons following the Steem-Dwass-Critchlow-Flogner procedure). The risk α was set to 5 %. For the sensory analysis, the statistical analysis was conducted on centered data. Principal component analysis (PCA) was carried out with the SPAD8 (Cohers, France) software to explore the relationship between the different variables. The relationship between the variables were evaluated by Person correlation. Beforehand, the normality of the distribution had been evaluated using the previously described procedure.

RESULTS

1. Climatic conditions

Rainfall and minimum and maximum temperatures for each decade from July to October 2012 and 2014 are shown in Table SM2. Between the onset of ripening and full ripeness, the main differences temperature and rainfall were recorded.
In 2012, the summer was relatively hot until mid-September, with 22 days of maximum temperatures above 30 °C. In addition, this vintage was dry, with 11.5 mm rainfall from the start of August to mid-September. From mid-September to mid-October, total rainfall was relatively high compared to the average (1995-2011). Conversely, the summer of 2014 was cooler, with only 6 days of maximum temperature above 30 °C, of which 5 days in September, and it was dryer during the final stage of maturation. Both vintages had similar total rainfall between August and September (68.5 and 69.0 mm in 2012 and 2014 respectively), but the distribution was reversed. During the ripening period of 2014, 74 % of total rainfall fell in August, compared to only 12 % in 2012. In addition, 2 weeks before optimum maturity (date D), Cabernet-Sauvignon received 27.5 mm of rain in 2012 and 47.0 mm in 2014, whereas the average temperature was lower during the same period in 2012 compared to 2014.

According to Boss et al. (2018), the profile of volatile compounds in wine may be influenced by changes in grape composition in the last 2 to 4 weeks of berry development, suggesting a stronger impact of the climatic conditions during this period. For this reason, Figure 1 focuses particularly on the climatic conditions close to the harvest date. As can be seen in Figure 1A for the 2012 vintage, the harvest period (second week of October) was relatively wet. The climatic conditions were cooler and more humid compared to the earlier part of the maturation phase. Conversely, in 2014, the weather during the last part of the maturation was quite hot and dry compared to 2012, with maximum temperatures higher than 30 °C being recorded a few days before the first harvest date of Merlot grapes (Figure 1B). Sixty-four mm of rain fell during the subsequent harvest dates of Cabernet-Sauvignon (6 October to 17 October), which prevented the trial from being carried out beyond DCS+7.

2. Impact of harvest date on must and wine composition and aromas produced from Cabernet-Sauvignon grapes in 2012.

In the 2012 vintage, the impact of harvest date on must and wine composition and the aromas of Cabernet-Sauvignon was investigated for a short interval between early and late harvest (D ± 4 days). This experiment was conducted in real winemaking conditions and using a large volume (12 hl).

2.1 Evaluation of maturity based on oenological parameters.

The impacts of the harvest date on the main oenological parameters of must and wine are given in Tables SM3 and SM4 respectively. Sugar and malic acid concentrations were significantly modified (p < 0.05) within the 8-day interval between the first and last harvest date, whereas the other parameters, such as pH and total acidity, which are usually monitored in order to determine optimal harvest date, were not significantly modified. Finally, and surprisingly, the main oenological parameters monitored in the wines were not significantly modified by the short interval in harvest date.

2.2 Sensory and analytical characterisation of the wines.

The first objective of the study was to investigate the impact of harvest date on wine aroma. To do so, three main descriptors were used to describe the aroma of the wines: herbaceous/vegetative, fresh fruit and cooked fruit. In Figure 2, it can be observed that the experts perceived significant differences in the aroma of the wines produced from the Cabernet-Sauvignon grapes grown in the same plot but harvested at different dates. For this vintage and in our experimental conditions, there were no significant differences in the intensity of the herbaceous/vegetal and fresh fruity aromas within the harvest date range. Conversely, it was shown that within the four-day interval the intensity of the cooked fruit aroma of the red wine was significantly modified, while its main oenological parameters were not significantly modified (Table SM4).
Given the specific maturation conditions of 2012 in Pauillac (i.e., a dry and warm onset of the ripening period followed by a rainy and cool end of maturation) the evolution of the aroma of the wine tended to be very quick.

In the 2012 vintage, of the seven selected markers, only phenylacetaldehyde and massoia lactone were significantly modified in the grape must during maturation, although the amplitude of their concentration was very low. In the wines, the results contrasted more highly, with the harvest date strongly impacting the concentrations of MND, γ-nonalactone, massoia lactone, furaneol and homofuraneol. In addition, of these compounds, only MND showed concentration levels (maximum 42.1 ng/L) higher than its individual odour detection threshold in model solution (16 ng/L). However, these levels are not high enough to make this compound a strong contributor to the cooked fruit aroma of the wine (ODT\textsubscript{w} = 62 ng/L). As the late harvest increased in lactone and furanone levels, perceptual interaction with other lactones (Jarauta \textit{et al.}, 2006), as well as furanones (Allamy \textit{et al.}, 2018), may explain the increase in intensity of the cooked fruit nuances.

2.3 Global approach
In order to determine the overall impact of harvest dates on grape and wine chemical composition, a PCA was conducted on the main parameters selected for this study to investigate the relationship between the classical analytical oenological parameters of the must, the aroma compounds and the sensory analysis results obtained for the wines (Figure 3). The fine analytical parameters obtained from the musts were not included due to the low impact of harvest date on their evolution in 2012 (Table 1).

The selection of the two principal components explain 94.65 % of the total variance, of which Axis 1 explains 71.60 % of the variance and Axis 2 explains 23.05 %. This means that the data are mainly structured around Axis 1, according to the maturity level of the grapes and the delay in harvest date compared to that selected by the technical staff.

### TABLE 1. Impact of harvest date on the composition and distribution of aroma compounds in Cabernet-Sauvignon musts and wines for the 2012 vintage.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>ODT(\textsuperscript{1)})</th>
<th>Must min-max</th>
<th>(p)</th>
<th>ODT(\textsuperscript{2)})</th>
<th>Wine min-max</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MND(\textsuperscript{3)})</td>
<td>62</td>
<td>17.3-25.2</td>
<td>ns</td>
<td>16</td>
<td>21.6-42.1</td>
<td>**</td>
</tr>
<tr>
<td>Methional(\textsuperscript{4)})</td>
<td>nd</td>
<td>tr</td>
<td></td>
<td>2.4</td>
<td>1.5-1.7</td>
<td>n.s.</td>
</tr>
<tr>
<td>Phenylacetaldehyde(\textsuperscript{4)})</td>
<td>6.4</td>
<td>3.4-7.1</td>
<td>*</td>
<td>30</td>
<td>7.9-9.9</td>
<td>n.s.</td>
</tr>
<tr>
<td>γ-Nonalactone(\textsuperscript{4)})</td>
<td>0.9</td>
<td>0.7-0.9</td>
<td>ns</td>
<td>27</td>
<td>3.9-9.9</td>
<td>*</td>
</tr>
<tr>
<td>Massoia lactone(\textsuperscript{4)})</td>
<td>10</td>
<td>tr– 0.4</td>
<td>*</td>
<td>11</td>
<td>5.8-9.9</td>
<td>*</td>
</tr>
<tr>
<td>Furaneol(\textsuperscript{4)})</td>
<td>10.5</td>
<td>3.3-5.2</td>
<td>ns</td>
<td>49.0</td>
<td>8.0-37.4</td>
<td>***</td>
</tr>
<tr>
<td>Homofuraneol(\textsuperscript{4)})</td>
<td>5.5</td>
<td>1.9-2.4</td>
<td>ns</td>
<td>26.5</td>
<td>2.5-8.1</td>
<td>*</td>
</tr>
</tbody>
</table>

\(1\) Odour detection threshold in model must and in model wine\(\textsuperscript{2)}\) according to Allamy \textit{et al.} (2018). Concentrations expressed in ng/L\(\textsuperscript{3)}\) and µg/L\(\textsuperscript{4)}\) tr = trace. ns = non significant, * \(p < 0.05\), ** \(p < 0.01\), *** \(p < 0.001\).
In the diagram, the fresh fruit and cooked fruit descriptors are in opposite directions to each other, whereas herbaceous/vegetal was almost orthogonal to the other descriptors and molecular markers. This representation of the results emphasises the strong impact of maturity level on the selected descriptor and aroma compounds.

3. Impact of harvest date on must and wine composition and aromas of wine produced from Merlot and Cabernet-Sauvignon grapes in 2014.

Sampling was performed within an 18- and 10-day time frame for Merlot and Cabernet-Sauvignon respectively (four sampling dates). Conversely to the previous experiment conducted in 2012, the sensory analysis was carried out on the musts and wines and the primary and secondary metabolites were also quantified.

3.1 Impacts on major oenological parameters.

In Merlot and Cabernet-Sauvignon musts, sugar accumulation was significantly modified as a result of the late sampling date (Table SM5). Regarding the other parameters, significant but inconsistent differences were found during the ripening period, probably due to the rainfall during the later harvest dates (Figure 1). Similar observations were made in the experiment conducted on Cabernet-Sauvignon. In the wines, alcohol significantly increased with the delay in harvest (Table SM6). A 0.89 % vol. increase was found between D-6 and D+12 for Merlot and a 0.77 % vol. increase between D-3 and D+7 for Cabernet-Sauvignon.

3.2 Sensory and analytical characterisation of the wines.

The results of the sensory analysis of the musts and wines according to the harvest date are shown in Figure 4. Regarding the Merlot must samples, the harvest date significantly modified (p < 0.05) the intensity of the fresh fruit and herbaceous/vegetative character, whereas the cooked fruit intensity was not impacted. Harvest date D resulted in the highest intensity of the herbaceous/vegetative character, whereas harvest D-3 showed the highest score in terms of fresh fruit aroma. These results are not consistent with those of the sensory analyses conducted on the produced wines, which showed significant differences in terms of the herbaceous/vegetal and cooked fruit aromas of the young wine from the delayed harvested. In the experimental conditions of 2014, a 12-day delay after harvest date D induced a significant increase in the perceived cooked fruit aroma of the wine.

Different results were obtained for Cabernet-Sauvignon. In the must, the harvest date did not significantly modify any of the aroma intensities, whereas it significant impacted (p < 0.05) those of the young wine. The choice of harvest date modulated the intensity of the herbaceous/vegetative, fresh fruit and cooked fruit aromas. In this trial, a 7-day delay in the harvest (date D + 7) resulted in a cooked fruit aroma being significantly perceived in the wine.

These observations show the strong influence of fermentation on the cooked fruit nuances in the young wine. Based on these sensory data, a set of molecular markers were analysed in the musts and wines.
Compared to the analyses carried out in 2012, Strecker aldehydes (methional and phenylacetaldehyde) were excluded due to their low concentrations and low variability during the harvest date experiment. Concentrations of \((Z)-1,5\text{-octadien-3-one}\) were measured, because it has been shown to be a powerful aroma compound reminiscent of geranium and dried figs in must (Allamy et al., 2017).

In the must, slight differences in the concentrations of lactones and furanones were found depending on the variety (Table 2). The concentrations of ketones were significantly modified as a result of harvest date for both varieties \((p < 0.001)\). The highest levels of MND (88.6 ng/L) and \((Z)-1,5\text{-octadien-3-one}\) (265.2 ng/L) were observed in Merlot. These values were higher than their individual ODT, suggesting an impact on the aroma of the must. The concentration of \((Z)-1,5\text{-octadien-3-one}\) ranged from 50.5 to 265.2 ng/L in the Merlot musts and from 12.2 to 51.6 ng/L in the Cabernet-Sauvignon musts.

In the wines, the harvest date significantly impacted the level of ketones \((p < 0.05)\), lactones \((p < 0.001)\) and furaneol \((p < 0.01)\), whereas homofuraneol concentrations were not impacted, and \((Z)-1,5\text{-octadien-3-one}\) was not detected due to its reduction by yeast reductase during alcoholic fermentation (Allamy et al., 2017). The impact of delaying harvest on the aroma and aroma compound composition of must and wine from Merlot and Cabernet-Sauvignon was represented in a principal component analysis (PCA). The compositional differences between the must and the wine are linked to level of maturity (Figure 5).
### TABLE 2. Impact of harvest date (D) on the volatile compound composition of Merlot and Cabernet-Sauvignon musts and wines (2014 experiment).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sample</th>
<th>Merlot</th>
<th>Cabernet-Sauvignon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D-6</td>
<td>D</td>
</tr>
<tr>
<td>MND(1)</td>
<td>Must</td>
<td>41.9±4.4</td>
<td>85.6±2.2</td>
</tr>
<tr>
<td></td>
<td>Wine</td>
<td>20.7±1.4</td>
<td>29.7±1.9</td>
</tr>
<tr>
<td>γ-Nonalactone(2)</td>
<td>Must</td>
<td>2.9±0.8</td>
<td>3.0±0.4</td>
</tr>
<tr>
<td></td>
<td>Wine</td>
<td>6.3±0.4</td>
<td>6.4±0.5</td>
</tr>
<tr>
<td>Massoia lactone(2)</td>
<td>Must</td>
<td>8.2±0.3</td>
<td>6.4±0.6</td>
</tr>
<tr>
<td></td>
<td>Wine</td>
<td>3.7±0.6</td>
<td>18.6±1.8</td>
</tr>
<tr>
<td>Furaneol(2)</td>
<td>Must</td>
<td>9.2±0.5</td>
<td>8.7±0.6</td>
</tr>
<tr>
<td></td>
<td>Wine</td>
<td>36.0±2.9</td>
<td>43.3±4.4</td>
</tr>
<tr>
<td>Homofuraneol(2)</td>
<td>Must</td>
<td>4.0±1.6</td>
<td>2.9±0.8</td>
</tr>
<tr>
<td></td>
<td>Wine</td>
<td>5.6±1.0</td>
<td>5.0±0.9</td>
</tr>
<tr>
<td>(Z)-1,5-Octadien-3-one(1)</td>
<td>Must</td>
<td>54.5±3.2</td>
<td>150.5±9.1</td>
</tr>
<tr>
<td></td>
<td>Wine</td>
<td>tr</td>
<td>tr</td>
</tr>
</tbody>
</table>

Concentrations expressed in ng/L(1) and µg/L.(2) ns = non significant, * p < 0.05, ** p < 0.01, *** p < 0.001. tr = trace.

### FIGURE 5. Principal component analysis for Merlot and Cabernet-Sauvignon musts (A) and wines (B) showing the loadings plot distribution based on concentrations of volatile compounds, sugar level and sensory descriptors intensity. The variable harvest date “Days” was added as a supplementary variable corresponding the difference to the control date D (in blue) (refer to Figure 3 for abbreviations).
TABLE 3. Pearson correlation matrix of aroma compound contents and aroma descriptor intensities in Merlot and Cabernet-Sauvignon red wines from sequential harvest dates.

<table>
<thead>
<tr>
<th>Compounds 1</th>
<th>MND</th>
<th>γ-C9</th>
<th>Mlact</th>
<th>Fur</th>
<th>HFur</th>
<th>Veg.</th>
<th>Fresh F.</th>
<th>Cooked F.</th>
<th>Sugar (must)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MND</td>
<td>1</td>
<td>0.897</td>
<td>0.698</td>
<td>0.806</td>
<td>0.304</td>
<td>0.426</td>
<td>0.263</td>
<td>0.855</td>
<td>0.710</td>
</tr>
<tr>
<td>γ-C9</td>
<td>1</td>
<td>0.545</td>
<td>0.907</td>
<td>0.400</td>
<td>0.407</td>
<td>-0.08</td>
<td>0.968</td>
<td>0.895</td>
<td></td>
</tr>
<tr>
<td>Mlact</td>
<td>1</td>
<td>0.595</td>
<td>-0.115</td>
<td>-0.478</td>
<td>-0.178</td>
<td>0.504</td>
<td>0.405</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fur</td>
<td>1</td>
<td>0.299</td>
<td>-0.326</td>
<td>-0.08</td>
<td>0.971</td>
<td>0.829</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HFur</td>
<td>1</td>
<td>0.404</td>
<td>-0.04</td>
<td>0.391</td>
<td>0.456</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veg.</td>
<td>1</td>
<td>0.904</td>
<td>0.478</td>
<td>0.311</td>
<td>0.522</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh F.</td>
<td>1</td>
<td>0.02</td>
<td>0.139</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cooked F.</td>
<td>1</td>
<td>0.864</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sugar (must)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 MND, 3-methyl-2,4-nonanedione; γ-C9, γ-nonalactone; Mlact, massoia lactone; Fur, furaneol; HFur, homofuraneol; Veg., herbaceous/vegetative; Fresh F., fresh fruit; Cooked F., cooked fruit. Values in italics are significant at p < 0.05 or p < 0.01 in bold.

This representation explains 69.11 % of the total variance for must, with 43.93 % and 25.18 % on Axes 1 and 2 respectively (Figure 5A). As can be seen in Figure 5B, PCA explained 78.3 % of the total variance for wine, with 59.12 % and 19.18 % on Axes 1 and 2 respectively. For both varieties, delayed harvest tended to induce the formation of lactone and furanones in the must. However, this chemical evolution did not induce a clear and general modification of the intensity of the cooked fruit aroma perceived in both the Merlot and the Cabernet-Sauvignon samples. In addition, MND tends to be associated with the herbaceous/vegetative character of the must, although they were not significantly correlated (R² = 0.159, p = 0.220; Table SM7).

In the wine (Figure 5B), there was a much closer link between aroma compound composition (including γ-nonalactone, massoia lactone, furaneol and MND), cooked fruit intensity and harvest date; these analytical and sensory parameters evolved in a similar manner. This evolution seems to be linked to the amount of sugar in the must. As previously mentioned, the concentrations of homofuraneol were not significantly modified in this trial (Table 2). This explains the quasi-orthogonal position of this variable compared to others chemical markers of the dried/cooked fruit aroma.

For further investigation, a Pearson correlation analysis was carried out on the overall analytical data and sensory evaluation score of the Merlot and Cabernet-Sauvignon wines (Table 3). The cooked fruit intensity was strongly correlated with MND (R² = 0.855, p < 0.01), γ-nonalactone (R² = 0.968, p < 0.01) and furaneol (R² = 0.971, p < 0.01). In addition, the sugar level in the must was also well correlated with MND (R² = 0.710, p < 0.05), γ-nonalactone (R² = 0.895, p < 0.01), furaneol (R² = 0.829, p < 0.01) and the cooked fruit intensity of the wine (R² = 0.864, p < 0.01).

DISCUSSION

The impact of harvest date on grape and wine composition was evaluated by sensory analysis, as well as by investigating the evolution of primary and secondary metabolites of two major grapevine varieties in two vintages in the Bordeaux area. The results of this study conducted at both industrial and laboratory scale demonstrate that choice of harvest date induces a shift from fresh fruit to cooked fruit aromas. This sensory evolution was systematically detected in red wines, but much more inconsistently in must. The modification of aromatic maturity was evidenced in both vintages, with different rainfall and temperature distributions. Warm and rainy weather occurring close to harvest seemed to have created the ideal conditions for the development of cooked fruit aromas in the wines.

Our results build on previous studies which reported an increase in sensory assessment scores of hotness, bitterness, sweetness and dark fruit as a result of extending Cabernet-Sauvignon fruit maturation in California (Heymann et al., 2013) and in Australia (Bindon et al., 2014). In addition, our results showed no link between the sensory attributes of the berries and those detected in the wines: the sensory attributes of the highest intensity in the must were not perceived as such in the wines. These results are consistent with those obtained for Shiraz by Olarte Mantilla et al. (2015), who found that the sensory attributes of must and wine were not correlated. It was possible to link certain sensory attributes of the berries to those detected in wines, but they were not identical; e.g., “berry seed bitterness” in must vs. “savory spice” in wine.

In the present study, the sensory modifications observed in the wine from later harvest dates were validated by the quantification of aroma compound composition. The changes in the composition of the wine volatiles reflect their evolution...
during grape ripening. The compounds selected in this study were those identified in a previous study in over-ripe and shrunken Merlot and Cabernet-Sauvignon grapes. Most of the compounds involved in the perception of the cooked fruit aroma of the overripe grapes were detected at higher levels in the wines produced from later harvest dates; this was the case for both must and wine for both varieties studied. Concentrations were higher in the wine than in the must. The impact of alcoholic fermentation on the formation of these compounds has previously been reported by Allamy et al. (2018). Preursors of these compounds were not formally identified, but it seems that yeast metabolism plays an important role in revealing the bound volatile fraction produced in the berries when they are harvested late.

In the first experiment conducted at semi-industrial scale, we demonstrated that molecular markers of dried/cooked fruits of red wines are quite closely linked to the maturity of the grapes, and could thus be used as new chemical markers of grape maturity. These results confirm the observation made at laboratory scale that aroma compounds, such as furanones, lactone et ketones, are associated through perceptual interaction with the production of dried fruit nuances (Allamy et al., 2018).

The two ketones, MND and (Z)-1,5-octadien-3-one, were significantly and strongly impacted by the harvest date in both varieties. The magnitude of the concentrations obtained for (Z)-1,5-octadien-3-one in the winemaking conditions were similar to those previously obtained during a dehydration experiment at laboratory scale (Allamy et al., 2018). In this experiment, this unsaturated aldehyde was found at high levels in Merlot musts (~100 ng/L), contributing to nuances of the dried fruit aroma. In addition, this previous work demonstrated the complex sensory contribution of this ketone to the must: at concentrations ranging from 64 to 96 ng/L, it contributed to dried fig nuances, whereas at higher levels it was mostly associated with a geranium aroma. In this context, the perceptual interaction between high MND (85.6 ng/L) and (Z)-1,5-octadien-3-one (150.5 ng/L) levels detected in Merlot must harvested at date D (Table 2) might explain the herbaceous/vegetative character of this sample (Figure 4). Conversely, it should be noted that furaneol - reminiscent of caramel and cooked fruit notes - developed antagonistic effects against (Z)-1,5-octadien-3-one - reminiscent of geranium - at high concentrations (>128 ng/L). This antagonistic phenomenon might explain the lack of sensory modifications during the last stage of maturation, in contrast to the major modification of its aroma compound composition.

Indeed, the MND, furaneol, massoia lactone and γ-nonalactone concentrations in wines produced from Cabernet-Sauvignon in 2012 and 2014 were significantly modified by the harvest date. Similar results were obtained for Merlot for the experiment conducted in 2014. These findings illustrate the impact of alcoholic fermentation on secondary metabolites and, in turn, their impact on the aroma of red wines. We therefore consider that these results illustrate the response of yeast metabolism to changing must composition and must sugar concentration, and may reflect changes in the concentrations of grape derived wine volatile precursors in the fruit. These observations are consistent with those made by Bindon et al. (2013) for Cabernet-Sauvignon and Riesling wines.

The formation mechanisms and possible precursors of these compounds in both the grapes and wine have not been clearly identified. However, some of them may be linked to oxidation mechanisms through the accumulation of reactive oxygen species (ROS) during the end of the ripening period, such as H$_2$O$_2$ and O$_2$-, which are known to activate fruit senescence (Jimenez et al., 2002), while others might be linked to the presence of glycosylated precursors.

The formation mechanisms of (Z)-1,5-octadien-3-one in must are still unknown to date. However, in the literature, (Z)-1,5-octadien-3-one is claimed to be an oxidation by-product of other food products, possibly arising from the oxidation of precursors, such as α-linolenic acid and their n-3 counterparts, eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) in the presence of copper ions or lipoxygenase (Hammer and Schieberle, 2013; Ulrich and Grosch, 1988). In wine, MND has been described as an oxidation product of α and β-hydroxyketones (Peterson et al., 2020); its formation via the oxidation cleavage of unusual fatty acid, called furan fatty acid, has been hypothesised as being the reason for its formation in both grapes and young wine (Pons et al., 2012). These mechanisms, which are associated with the formation of carbonyls, have been shown to exist by the presence of reactive oxygen species accumulation (ROS), which is involved in oxidation mechanisms within the fruit, from the onset of ripening to the mature stage (Pilati et al., 2014), and especially during over-ripening (Muñoz and Munné-Bosch, 2017).

As regards lactones, to the best of our knowledge, the occurrence of massoia lactone has hardly been studied and its precursors are still unknown; conversely, γ-nonalactone has been described in other beverages and food products, such as beer (Tressl et al., 1978) and butter (Yoshinaga et al., 2019), as an end oxidation product of linoleic acid. Recently, 4-oxononanoic acid, which is a direct precursor of γ-nonalactone, has been identified in must (Ferron et al., 2020). This hydroxyacid is biotransformed into γ-nonalactone by S. cerevisiae with a yield of approximately 95 %, and maximum concentrations of 60 µg/L and 27 µg/L in Merlot and Cabernet-Sauvignon musts respectively.

To date, the formation mechanisms of furaneol in grapes and wines have not been thoroughly described, but they have been found to be numerous and complex in food products and beverages (Xiao et al., 2021). Furaneol has been described as being a by-product of the Maillard reaction, resulting from the reaction of glycin and xylose (Blank et al., 1997) or rhamnose and arginine (Haleva-Toledo et al., 1997). Furaneol can be also generated from D-glucose and D-fructose during the roasting of coffee beans (Poisson et al., 2019). These reactions produce the same reaction intermediate, acetylformoin, which needs to be reduced to be converted into furaneol.
This reduction can be carried out chemically or biochemically via the metabolism of *S. cerevisiae*. We hypothesise that the latter mechanism is responsible for its formation during alcoholic fermentation due to its reductive activity. Furaneol has also been identified and detected at high levels in Muscat Bailey grapes in the form of glycosylated precursors (Sasaki et al., 2015). These findings provide an alternative explanation of its formation during alcoholic fermentation, because it is released as a result of yeast β-glucosidase activity (Fernández-Pacheco et al., 2021).

CONCLUSIONS

The findings of the present study show how the late harvest of Merlot and Cabernet-Sauvignon berries can induce a significant modification of the aroma of the wine without, surprisingly, clear modifications to the aroma of the must. This study therefore demonstrates that it is difficult to accurately predict wine aroma through the sensory evaluation of must aroma. In addition, our study shows that a short delay in harvest can induce a significant modification to the sugar concentrations of the must and increase the intensity of the cooked/dried fruit aromas. The molecular basis for these modifications is the increase in the impact of aroma compounds in must and wine, such as furaneol, MND, (Z)-1,5-octadien-3-one, massoia lactone and γ-nonalactone.

Future research is needed to determine if the observations regarding sensory and aroma compound evolution are applicable to varieties growing under different climatic conditions. Finally, these findings may help the wine-producing sector develop tools for preventing cooked/dried fruits aromas in wines, which will be increasingly challenging in the context of climate change.

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REFERENCES


