CONTRIBUTION OF GRAPE SKIN AND FERMENTATION MICROORGANISMS TO THE DEVELOPMENT OF RED- AND BLACK-BERRY AROMA IN MERLOT WINES

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Abstract

Aim: The aim of this study was to elucidate how an initially neutral Merlot must resulted in a wine with characteristic aromas of red- and black-berry fruit, focusing on the respective contributions of yeast metabolism together with grape juice, pulp, and skins.

Methods and Results: Sensory analyses were performed on Merlot grape skin maceration models, based on observations in the winery. Initial findings revealed that strong fruity nuances appeared during pre-fermentation maceration. In the maceration models used, the development of aroma of red- and black-berry fruit systematically paralleled the growth of the yeast population. The respective roles of grape skins and yeasts were investigated throughout the alcoholic fermentation of model musts with addition of Merlot skins or Merlot skin extract in ethanol. The aromatic nuances revealed by alcoholic fermentation in a must alone had no specific white-, rosé-, or red-wine character. In contrast, wines made by microvinification with grape skins and/or grape skin extract in ethanol had a clear, intense aroma of red- and black-berry. Microvinification with both Merlot skin extract and grape skins revealed the most intense fruity character.

Conclusions: Inodorous skin constituents produced a specific aroma of red- and black-berry fruit after alcoholic fermentation by yeast. The physical presence of grape skins during fermentation enhanced the intensity of the fruity nuances obtained.

Significance and impact of the study: The study established, for the first time, the existence of inodorous constituents in Merlot grape skins, extractible by ethanol and transformed by yeasts to produce a specific aroma of red- and black-berry fruit in the finished wines**.**

Keywords: red wines, grape skins, red- and black-berry aroma, aroma origins, precursors

Résumé

But: Ce travail a été effectué pour tâcher de comprendre comment un moût issu du cépage Merlot et initialement neutre pouvait donner un vin avec des arômes caractéristiques de fruits rouges et noirs.

Méthodes et Résultats: Des analyses sensorielles ont été effectuées sur des macérations de pellicules de Merlot réalisées en fonction d'observations au champ. Nous avons, dans un premier temps, montré que des notes fruitées intenses apparaissaient lors de la macération préfermentaire. Dans les milieux de macération utilisés, le développement des arômes de type fruits rouges et fruits noirs était lié à l'augmentation de la population levurienne. Les rôles des constituants pelliculaires ainsi que des levures ont été étudiés par le biais de la fermentation alcoolique de moûts modèles supplémentés en pellicules de Merlot ou en extrait éthanolique de ces pellicules. Les nuances aromatiques révélées par la fermentation alcoolique du moût seul n'étaient en rien similaires à celles des vins qu'ils soient blancs, rosés ou rouges. Au contraire, les vins obtenus lors des microvinifications en présence de pellicules ou d'extraits pelliculaires présentaient des notes claires et intenses de fruits rouges et noirs. Les microvinifications réalisées avec à la fois pellicules et extraits pelliculaires ont révélé les notes fruitées les plus intenses.

Conclusions: Des constituants inodores des pellicules conduisent à des notes spécifiques de fruits rouges et noirs après la fermentation alcoolique. La présence physique de pellicules lors de la fermentation augmente nettement l'intensité des notes fruitées obtenues.

Impact de l'étude: Ce travail montre pour la première fois l'existence de constituants pelliculaires inodores et extractibles par l'éthanol qui sont transformés par les levures pour conduire à des arômes spécifiques de fruits rouges et noirs dans le vin obtenu.

Mots clés: vin rouge, pellicules, arômes de fruits rouges et noirs, origines des arômes, précurseurs

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INTRODUCTION

Wine is produced from numerous grape varieties with a wide range of aromatic expressions. Paradoxically, wines with very rich, complex aromas are mainly obtained from grapes and musts with little noticeable aroma (Delfini *et al*., 2001 ; Escudero *et al*., 2004 ; Lee and Noble, 2006). The only exception is « floral » varieties, the most famous being Muscat first studied by Ribéreau-Gayon *et al*. (1975). Its unfermented must has a specific floral character, directly correlated with its high terpenic content, which is relatively unaffected by fermentation (Cordonnier and Bayonove, 1974 ; Günata *et al*., 1985). In contrast, the varietal character of Sauvignon blanc and Verdejo grapes is attributed to some polyfunctional thiols, already present in tiny amounts in grapes, but mainly released from S-cysteine conjugates during fermentation by yeast (Campo *et al*., 2005 ; Tominaga *et al*., 1996 ; Tominaga *et al*., 1998). White wine aromas are thus known to be mainly revealed by the fermentation of odourless grape juice.

Varietal aroma in red wine is a much more complex issue. Red wine exhibits highly specific fruity characteristics, described as red-berry and black-berry fruit, that are not detected in white wines (Pineau *et al*., 2010). In the Bordeaux region, Cabernet-Sauvignon wines often have a strong blackcurrant aroma, while Merlot wines are generally characterized by caramel and cherry. Kotseridis and Baumes (2000) identified both furaneol®and homofuraneol® as potentially responsible for the caramel aroma of Bordeaux wines. Recently, Pineau *et al*. (2009) demonstrated that a combination of fifteen ethyl esters and alkyl acetates was responsible for the overall berry fruit-like aroma of red Bordeaux wines.

Empirical observations in the winery indicate that the red- and black-berry characteristics of red wines result from aromatic development throughout alcoholic fermentation. The intensity of these aromas may, moreover, be enhanced by pre-fermentation maceration (Girard *et al*., 1997 ; Girard *et al*., 2001). The production of these typical red- and black-berry aromas occurs during the entire vinification process, which mainly differs from white-winemaking by the presence of grape skins throughout the process. Moreover, skin contact has clearly been shown to enhance the varietal character of some white wines. In Muscat grapes, Wilson *et al*. (1986) showed that monoterpenes were present as both free volatile form in grape pulp and glycoside-bound inodorous form mainly in grape skins. Traditionally, Muscat wine is made by fermenting only the juice obtained by direct crushing and pressing of harvested grapes. However, many studies showed an increase in the terpene content of the wine following pre-fermentation maceration (Cabaroglu and Canbas, 2002 ; Guilloux-Benatier *et al*., 1998 ; Ho *et al*., 1999 ; Schmidt and Noble, 1983). The presence of grape skins throughout the red wine vinification process may thus be an important factor in the aromatic differences between red and white wines.

Common tasting profiles, even among wines obtained from red grapes, feature different expressions, depending on the vinification process. Rosés, made from directly pressed grapes, are often quite simple wines described as fresh and slightly fruity. Clairet wines, produced in the Bordeaux region from grape juice following a short pre-fermentation skin contact, have more intense aromas, but not the complexity of a red wine. « Nouveau » wines, obtained by carbonic maceration of red grapes, are strongly characterized by banana and « boiled-sweet « aromas. Red-wine production, characterized by skin contact throughout vinification, results in very strong aroma reminiscent of red- and black-berry fruits ranging from fresh to jammy (Pineau *et al*., 2009).

Therefore, the main goal of our study was to elucidate how an initially neutral must such as a Merlot must would result in red wine with characteristic fruity aroma of red- and black-berries. Achieving this objective was made possible through focus on the respective contributions of Merlot grape juice, pulp, and skins. The specific role of yeast metabolism was also investigated.

MATERIALS AND METHODS

1. Chemicals and solvents

Analytical grade chemicals and solvents were used. Solvents were purchased from VWR (Fontenay-sous-bois, France) and all other chemicals were purchased from SIGMA-ALDRICH (St Louis, MO, USA).

2. Grapes

The red grapes used were Merlot from the 2006 harvest. They were sourced from the Château Luchey-Halde vineyard in Pessac-Léognan (Bordeaux, France) and manually harvested at technological maturity (sugar content : 176 g/L - total nitrogen content : 130 mg/L - pH : 3.35) from homogenous plots. The grapes were immediately frozen or used for pre-fermentation macerations or vinifications.

Italia table grapes were used as a control of a white grape variety for the microvinification experiments. They were selected for being commercially available at the time when the experiments were conducted.

a) Musts

Model must (pH 3.3), prepared according to Marullo *et al.* (2004), contained the following components (expressed in g/L) : glucose (105), fructose (105), tartaric acid (3), citric acid (0.3), L-malic acid (0.3), $MgSO_4$ (0.2),

Table 1 - Laboratory maceration experiments using Merlot skins: solution used, inhibitor added and maceration time.

Test	Solution used[1]	Inhibitor added[2]	Code: maceration time (days)
1	1	-	$1A_1$: 8 $1A_2$: 15 $1A_3$: 21
	2	-	$1B_1$: 8 $1B_2$: 15 $1B_3$: 21
2	1	100 mg/l pimaricin	$2A_1$: 8 $2A_2$: 8 $2A_3$: 8
	1	-	$2B_1$: 8 $2B_2$: 8 $2B_3$: 8
3	1	100 mg/l pimaricin 12.5 mg/l penicillin 100 mg/l chloramphenicol	3A: 10
	1	100 mg/l pimaricin	3B: 10
	1	12.5 mg/l penicillin 100 mg/l chloramphenicol	3C: 10
	1	-	3D: 10

[1] Solution 1: distilled water spiked with 4 g/L tartaric acid, pH adjusted to 3.5 with KOH (10N); solution 2: dilute alcohol solution obtained by adding 12% ethanol and 4 g/L tartaric acid to distilled water, pH adjusted to 3.5 with KOH (10N). [2] Pimaricin: specific inhibitor of yeast growth; penicillin: specific inhibitor of acetic acid bacteria growth; chloramphenicol: specific inhibitor of bacterial growth.

and KH_2PO_4 (2). Total nitrogen was adjusted to 190 mg/L with 0.3 g/L $(NH_4)_2SO_4$ and 6.44 mL/L amino acid solution containing (in g/L): tyrosine (1.4), tryptophan (13.7), isoleucine (2.5), aspartic acid (3.4), glutamic acid (9.2), arginine (28.6), leucine (3.7), threonine (5.8), glycine (1.4), glutamine (38.6), alanine (11.1), valine (3.4), methionine (2.4), phenylalanine (2.9), serine (6), histidine (2.5), lysine (1.3), cysteine (1), $NaHCO_3$ (20), and proline (46.8) in buffer solution ($NaHCO_3$, 2 %: 20 g/L). Mineral salts were then added (mg/L): $MnSO_4.H_2O$ (4), $ZnSO_4.7H_2O$ (4), $CuSO_4.5H_2O$ (1), KI (1), $CoCl_2.6H_2O$ (0.4), $(NH_4)_6Mo_7O_{24}.4H_2O$ (1), and H_3BO_3 (1). Various vitamins were also introduced (mg/L): mesoinositol (300), biotin (0.04), thiamine (1), pyridoxine (1), nicotinic acid (1), pantothenic acid (1), and p-amino benzoic acid (1). The following fatty acids were added (mg/L): palmitic acid (1), palmitoleic acid (0.2), stearic acid (3), oleic acid (0.5), linoleic acid (0.5), and linolenic acid (0.2). The medium was sterilized by filtration (cellulose nitrate membrane, 0.45 µm, Millipore, France), supplemented with sulphur dioxide (6 g/hL), according to standard winemaking practice, and then inoculated with yeast. Finally, a sterile solution of anaerobic factors was added to obtain 0.5 mL/L Tween 80, 15 mg/L ergosterol and 5 mg/L sodium oleate.

Merlot must was made by destemming, crushing, and pressing the grapes. For laboratory experiments, the grape must was supplemented with 17 g/L glucose, 17 g/L fructose, 20 mg/L ammonium sulphate, and 1.61 mg/L amino acid solution to adjust the sugar and nitrogen content to that of the model must described above. In contrast, musts fermented under winery conditions were not supplemented.

b) Skins

Frozen Merlot and Italia grapes were defrosted and washed using distilled water. Grape skins were separated from pulps and seeds by manually pressing each berry, and were then washed and drained. On average, 200 and 120 g skins were obtained per kg of Merlot and Italia grapes, respectively.

3. Ethanol extraction of grape skins

Constituents were extracted from 200 g of grape skins using 200 ml of distilled water/ethanol solution (1/1 : v/v). The extraction was performed in a sterile closed Erlenmeyer flask with continuous agitation for 5 days. The extract was then paper-filtered and concentrated using a Rotavapor® with a bath temperature of 20 °C, until the ethanol had been eliminated. The extract obtained was a very thick syrup with fresh grape aromas.

4. Skin macerations

a) Winery conditions

Merlot grapes from two homogeneous plots in the vineyard were destemmed, crushed, put into separate vats (1 and 2), and supplemented with 6 g/hL SO_2. Dry ice was used to reduce the temperature inside the vats to 10-12 °C. This temperature was maintained throughout maceration. Macerations were monitored by tasting and lasted for 4 to 5 days.

b) Laboratory conditions

Two solutions were used: distilled water spiked with 4 g/L tartaric acid (solution 1) and dilute alcohol solution obtained by adding 12 % ethanol and 4 g/L tartaric acid to distilled water (solution 2). Both solutions were adjusted to pH 3.5 with KOH (10 N). The three maceration tests are presented in table 1. Each sample contained 300 ml of solution and 50 g of Merlot grape skins in a sterile, closed Erlenmeyer flask. Maceration took place in a dark room where the temperature was maintained at 11-12 °C.

5. Microvinifications

Active Dry Yeast (10 g/hL, *Saccharomyces cerevisiae*, F10, Sarco SA, France) was used in all vinification experiments to avoid possible aromatic variations induced by different yeast strains. Rojas *et al*. (2003) showed, in particular, that indigenous yeasts may contribute to the expression of a varietal or endemic character in wines. To ensure homogeneity, the non-macerated Merlot must used for both winery and laboratory microvinifications was taken from the same vat. Moreover, the Merlot skins were taken from a grape sample from the same harvest used for rosé and red wine microvinifications in the winery.

a) Winery conditions

The Merlot grapes used for both rosé and red wines, with and without pre-fermentation maceration, were destemmed, crushed, put into two vats (A and B), and supplemented with 6 g/hL SO_2. Vat A was immediately inoculated to make the non-macerated red wine. Vat B was cooled to 10-12 °C using dry ice. A 60 l sample of cooled, non-macerated must was taken from vat B and inoculated immediately to make the non-macerated rosé wine. Pre-fermentation maceration continued in vat B at a constant temperature of around 10 °C for 5 days, after which it was inoculated to make the macerated red wine. A 60 l sample of cooled, macerated, inoculated must from vat B was fermented to make the macerated rosé wine. The vat temperature was maintained at 20-22 °C and 28-30 °C for the rosé and the red wines, respectively, and alcoholic fermentation was monitored by optical density measurements Once fermentation was completed, i. e. when density had dropped to 0.990, the wines were frozen until required for sensory analysis.

b) Laboratory models

The F10 yeast strain was pre-cultured for 24 heures in the model must or in the non-macerated Merlot must diluted with distilled water (1/1 : v/v). Fermentation took place in 375 ml glass bottles containing 300 ml of must inoculated with $3.5*10^6$ yeast cells/mL. Adding cultured yeast in exponential growth phase ensured good fermentation kinetics and prevented indigenous strains from taking over the selected yeast strain during laboratory microvinifications. Cultures were incubated at 20 °C with agitation (50 rpm), and fermentation was monitored by measuring weight loss. When fermentation was completed (stable weight for 48 heures, after 8 to 10 days), the samples were filtered and immediately tasted or frozen prior to tasting. The microvinification samples contained must, either alone or supplemented with grape skins and/or grape skin extract prior to inoculation. The initial composition of each sample is presented in table 2.

6. Microorganism counts

The yeast cell count was determined using the method described by Zott *et al.* (2008). Briefly, the samples were plated on YPG-based medium prepared by adding yeast extract (10 g/L), tryptone (pancreatic digest of casein, 10 g/L), glucose (20 g/L) and agar (30 g/L) to distilled water, and adjusting pH to 4.5 with orthophosphoric acid.

Table 2 - Laboratory microvinification tests: must used and elements added prior to inoculation and fermentation.

Test	Must used (250 ml)	Skin-to-juice ratio*				Other additions
		Merlot skins	Merlot skin extract	Italia skins	Italia skin extract	
4A	model must					
4B	Merlot must					
4C	model must	2.5:1				
4D	Merlot must	2:1				
4E	model must			2.5:1		
4F	Merlot must			2:1		
4G	model must					Merlot skins extracted in ethanol then washed (skin-to-juice ratio = 2.5:1)
4H	Merlot must		2:1			
4I	Merlot must				2:1	
4J	Merlot must		2:1	2:1		
4K	model must		2.5:1			Merlot skins extracted in ethanol then washed (skin-to-juice ratio = 2.5:1)

* Skin-to-juice ratio based on that of Merlot grapes, where skins represent approximately 20% of total weight. A ratio of 1:1 indicates that the grape skins added to the must represented 20% of the total weight of the sample or that the grape skin extract added corresponded to an initial quantity of grape skins equivalent to 20% of the total weight.

Sterilized YPG-based medium was supplemented with 0.15 g/L biphenyl and 0.1 g/L chloramphenicol to inhibit mould and bacterial growth.

Acetic and lactic acid bacteria were counted on a specific medium prepared by adding grape juice (500 ml/L), yeast extract (5 g/L), Tween 80 (1 ml/L), and agar (30 g/L) to distilled water. The pH was adjusted to 4.5 with orthophosphoric acid and the medium was sterilized. For specific lactic acid bacteria counts, 0.1 g/L pimaricin and 12.5 mg/L penicillin were also added to the medium.

Four successive 10-fold dilutions of macerated and non-macerated must samples were plated (100 µL) in duplicate. Cell counts are given as CFU (Colony Forming Unit)/ml and were calculated by averaging the CFUs of the two plates corresponding to the lowest dilution where single colonies were observed.

7. Sensory evaluations

The sensory evaluations were performed under controlled room temperature (20 °C), in individual booths, using black Afnor (Association Française des Normes) glasses containing about 40 ml liquid and covered with petri dishes.

a) Panels

There were two panels of assessors. Panel 1 consisted of 14 trained and experienced judges – winemakers and researchers - all members of research laboratories at the Bordeaux Faculty of Oenology (France). During regular tastings of wine samples, they showed a good aptitude for recognising specific red- and black-berry aromas, described as « redcurrant - raspberry - strawberry » and « blackcurrant - blackberry - cherry », respectively. Fruit liqueurs were used as reference samples for these descriptors.

The three assessors on panel 2 were selected as representative members of panel 1, following the tasting described above. All three were researchers working specifically on fruity aromas in wine and were thus considered experts in this field.

b) Tasting winery must and wine

For the must tasting, the assessors on panel 1 were asked to describe the dominant aromas they perceived and to rate them on a 6-point scale labelled very light, light, medium, strong, intense, and very intense. Only descriptors given by a majority of 9 judges out of 12 were retained.

For the wine tasting, the assessors on panel 1 were asked to rate the intensity of the overall red- and black-berry aroma they perceived in the wines on a 10-point scale ranging from 0 = absent to 10 = very intense. Significant differences among wines and in the overall rating by the assessors were statistically determined using one-way analysis of variance (« wine treatment » used as single factor and aall pair-wise comparisons adjusted for multiple testing using Tukey's Honest Significant Difference at $\alpha = 0.05$) and Kruskal-Wallis tests, respectively.

c) Tasting must and wine from laboratory microvinification experiments

The assessors on panel 2 were asked to describe the dominant aromas in both musts and wines and to rate the intensity of each aroma perceived on a 6-point scale labelled very light, light, medium, strong, intense, and very intense. Assessors were asked to focus on the red- and/or black-berry fruit characteristics of each sample, but were free to use their own descriptors. Responses were compared and only descriptors common to all three assessors were retained.

RESULTS AND DISCUSSION

1. Reliability of the sensory evaluation results

This investigation was based on sensory analyses, which can involve interactions between visual and olfactory stimuli. This was avoided by using black glasses for each tasting session. One key finding of Morrot *et al*. (2001) was that when a white wine was coloured red, the sensory descriptors applied to the odour of the wine were consistently those normally used for red rather than white wine.

The assessors' ability to perceive and assess an overall aroma of red- and black-berry fruit was evaluated by applying the Kruskal-Wallis test to the wine tasting results obtained from panel 1. This revealed no difference among the assessors' scoring (H value = 2.49, associated P-value = 1, compared to $\alpha = 0.05$ confidence limit). Three assessors were selected to form panel 2 based on these wine tasting results : their ratings reflected the average of the whole panel, so they were considered representative assessors of the specific fruity aromas under study.

The assessors on panel 2 were also asked to perform description tasks. As shown by Gawel and Godden (2008), the taster repeatability cannot be guaranteed, when assessing wines for quality. Nevertheless, using the combined scores of a small team of tasters (three in that publication) results in more consistent quality assessments. Thus, the three experienced assessors, chosen among the members of a laboratory specialized in wine aroma research, ensured the reliability of the sample descriptions obtained. Moreover, the attributes generated by consumers

or untrained panellists are known to be more ambiguous and repetitive, but less specific, than those proposed by trained assessors (Chollet and Valentin, 2001 ; Chollet and Valentin, 2006 ; Chollet *et al*., 2005). This point was confirmed by comparing the descriptors given by panel 2: all three noted a « fermentation aroma ». When asked to define this term, the assessors agreed that this descriptor covered a blend of « banana », « cider », « cucumber », « fatty acids », and « higher alcohols ». None of them mentioned berry fruit aromas.

2. Fruity character revealed during pre-fermentation maceration

Variations in the aromatic description of two different Merlot musts during pre-fermentation maceration in the winery are presented in table 3. When first put into vats (day 0), the two musts were consistently described as exhibiting light herbaceous characteristics. This was probably due to C6 aldehydes and alcohols, well-known to develop in early stages of the winemaking process (destemming, crushing, maceration, and pressing) (Baumes *et al*., 1988). These compounds, produced by enzymatic cleavage of polyunsaturated linoleic and linolenic fatty acids concentrated in the grape skins, are commonly regarded as pre-fermentation aroma compounds (Cordonnier and Bayonove, 1981 ; Crouzet, 1986 ; Cordonnier, 1989). Ferreira *et al*. (1995) found that large quantities were produced during pre-fermentation skin contact, using Chardonnay.

On the contrary, at the end of maceration (day 5), the musts were characterized by fruity aromas, reminiscent of strawberry, raspberry, redcurrant, and blackberry, varying from fresh to jammy and perceived as intense or very intense. Yeast cell counts in the Merlot must in vat 1 is presented in figure 1. At day 0 of the maceration period, the must had a total yeast population of 6.3 x 10^3 CFU/ml.

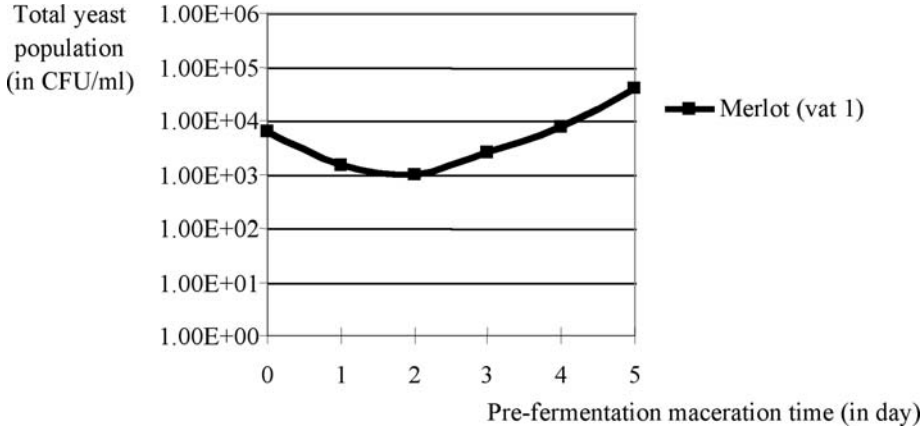

Figure 1 - Total yeast population in a Merlot must during cool pre-fermentation maceration in the winery.

Table 3 - Aroma development in two different Merlot musts (vats 1 and 2) during cool pre-fermentation maceration in the winery.

Vat	Maceration time	Aroma description	Intensity
1	0	herbaceous aroma: hay	light
		earthy aroma: dust	very light
	2 days	herbaceous aroma: fresh grass	light
		fruity aroma: grape juice	very light
	4 days	fruity aroma: strawberry, raspberry	medium
	5 days	fruity aroma: strawberry jam	strong
2	0	herbaceous and soapy aromas	very light
	2 days	neutral aroma	light
		fruity aroma: under-ripe raspberry, redcurrant	light
	4 days	fruity aroma: fresh blackberry	intense
	5 days	fruity aroma: strawberry yoghourt, blackberry jam	very intense
		vinegar aroma	light

Note: sensory assessments were performed by a panel of 14 experienced assessors who were asked to describe the dominant aromas they perceived in the musts. Only descriptors given by a majority of 9 assessors out of 12 were retained. Perceived intensities were evaluated using a 6-point scale (very light, light, medium, strong, intense, and very intense).

There was very little yeast activity during the first two days, as indicated by the decreasing population. In contrast, starting on the third day of maceration, the yeast population gradually increased to approximately ten times its initial value. The pre-fermentation macerations in the winery thus suggested that the fruity aromas and the total yeast population developed simultaneously.

Laboratory experiments modelling pre-fermentation maceration in the winery initially consisted of macerating Merlot skins in distilled water. They showed considerable aroma development, as presented in table 4 (Merlot skins macerated in distilled water). Initially perceived as herbaceous, reminiscent of grape juice, by the 3rd day, the maceration started to reveal raspberry and blackberry aromas. The fruity character increased markedly in intensity on the 6th day, accompanied by gas release. On the 7th and 8th days, very intense cider and banana nuances were also perceived, described by the panel as fermentation aromas. At the end of maceration, analysis of the microorganisms revealed the presence of yeasts and acetic acid bacteria, with populations of 10^3-10^4 CFU/mL and 10-10^2 CFU/mL, respectively, whereas they were absent from the initial distilled water (data not shown). The aromatic development and increase in the total yeast population were very similar to those observed in winery samples. This laboratory model was thus considered representative of pre-fermentation maceration.

Samples macerated with a specific inhibitor of yeast growth (Table 4, Merlot skins macerated in distilled water with 100 mg/l pimaricin) produced only herbaceous aromas, initially described as fresh grapes, then cucumber and hay nuances at the end of maceration time. This aromatic development was similar to findings reported by Delfini *et al*. (2001). Analysis of the microorganisms confirmed the absence of yeasts. In contrast, the acetic acid bacteria population was 10 to 100 times larger than in the models without pimaricin (data not shown). This was attributable to the inhibition of acetic acid bacteria growth by yeasts, widely described since the 1980s (Lonvaud-Funel *et al*., 1988a and b; Wibowo *et al*., 1985). Moreover, Beelman *et al*. (1982) showed that amino acid assimilation by yeast in fermenting must was very rapid, leading to nutritional deficiencies that hinder the growth of acetic acid bacteria.

Table 4 - Daily evolution of the nature and the intensity of the aroma nuances perceived in Merlot skin macerations in two media: distilled water and distilled water supplemented with pimaricin to inhibit yeast growth.

Maceration day	Merlot skins macerated in distilled water		Merlot skins macerated in distilled water with 100 mg/l pimaricin	
	Aroma description[1]	Intensity[2]	Aroma description[1]	Intensity[2]
1	-	-	-	-
2	grape juice herbaceous, grassy	very light light	grape juice herbaceous, grassy	very light light
3	herbaceous, grassy grape juice	medium medium to strong	grape juice dusty, earthy herbaceous, hay	light light medium
4	herbaceous, grassy grape juice raspberry	light medium medium to strong	grape juice herbaceous, grassy, hay	light medium to strong
5	grape juice raspberry	medium intense	grape juice herbaceous, cucumber, hay	light strong
6	red-berry fruit, raspberry	intense	grape juice herbaceous, cucumber, hay	light intense
7	cider, winy red-berry fruit, strawberry jam	strong intense	grape juice herbaceous, cucumber, hay	light intense
8	banana winy red-berry fruit, strawberry jam, raspberry	strong strong intense	grape juice soap herbaceous, cucumber, hay	light light intense

[1] Sensory assessments were performed by a panel of three experienced assessors who were asked to describe the dominant aromas they perceived in the musts. Only descriptors common to all three assessors were retained.
[2] Perceived intensities were evaluated using a 6-point scale (very light, light, medium, strong, intense, and very intense).

Finally, no lactic acid bacteria were found in samples with or without inhibitor (data not shown), which was quite surprising, as the literature reports average concentrations around 10^3 to 10^4 CFU/mL in must, before and during the early part of alcoholic fermentation. Lactic acid bacteria are nevertheless known to be very sensitive to medium conditions, particularly pH (Davis *et al*., 1986; Lafon-Fourcade *et al*., 1983). Their total absence may be due to the restrictive conditions in this maceration medium.

As presented in table 5, macerating skins with specific inhibitors of yeast and bacterial growth (test 3A) did not produce any fruity aromas. The absence of microorganisms at the end of the maceration was confirmed by cell counts. Consequently, the aromas of red- and black-berry fruit perceived cannot be explained by the presence of aroma compounds extracted in the maceration medium from the grapes, as is the case with Muscat (Cordonnier and Bayonove, 1974; Günata *et al*., 1985).

Tests 3B and 3D confirmed the results presented in table 4. Lactic acid bacteria did not grow during maceration, and acetic acid bacteria were inhibited in the presence of yeast. Furthermore, bacterial populations were only associated with herbaceous aromas in maceration media. In view of these findings, the fruity aromas that developed in the winery samples cannot be of bacterial origin.

Overall, yeasts were the only microorganisms systematically associated with fruity aromas in maceration media under both laboratory and winery conditions. In laboratory experiments, a considerable increase in the intensity of the fruity aromas was also systematically correlated with gas release in the maceration media. This may provide indirect evidence of yeast metabolic activity.

3. Alcoholic fermentation and the development of fruity aromas

As shown in figure 2, rosé and red wines fermented in the winery from both directly pressed and pre-fermentation-macerated must exhibited significant differences in the perceived intensity of the overall aroma of red- and black-berry. Rosé wine made from non-macerated must had the least intense aroma, followed by the rosé wine made with pre-fermentation-macerated must. Both red wines had a much more intense red- and black-berry aroma, irrespective of any pre-fermentation maceration.

There was a remarkable difference in the intensity of fruity aromas between the rosé and red wines. The only difference between the winemaking methods used for the rosé and red wines was that the grape skins remained in the must throughout alcoholic fermentation for the reds, maintaining continuous maceration. In these four wines, the perceived intensity of the fruity aroma apparently reflected the length of maceration before and during fermentation.

As already discussed by Delfini *et al*. (2001), the major difficulty was to maintain a reproducible skin-to-juice ratio under both winery and laboratory conditions. Several skin-to-juice ratios were tested in the laboratory (table 2), with 1:1 corresponding to the ratio in the winery. However, it is only possible to compare models with identical skin-to-juice ratios. Nevertheless, the extraction process during alcoholic fermentation of red grapes in the winery is much more intense than in microvinification models. While vats are commonly pumped over 1 to 3 times per day, without deteriorating the aromas of the resulting wine, applying a similar procedure to 300 ml microvinifications introduces

Table 5 - Aroma evolution of Merlot skin maceration in distilled water spiked with 4 g/L tartaric acid, pH adjusted to 3.5 and supplemented with specific microbial growth inhibitors.

Test	Inhibitor used[1]	Aroma description[2]	Intensity[3]	Gas release[4]
3A	pimaricin, penicillin, chloramphenicol	herbaceous: Granny Smith apple, fresh grass	medium	-
3B	pimaricin	herbaceous: cucumber, green pepper	strong	-
		off-flavour: fermented fish	strong	
3C	penicillin, chloramphenicol	herbaceous: humus, cardboard	strong	+
		fruity: strawberry, raspberry	light	
3D		fermentation: cider, banana	strong	++
		fruity: raspberry	strong	
		soap aroma	light	

[1] Pimaricin: specific inhibitor of yeast growth; penicillin: specific inhibitor of acetic acid bacteria growth; chloramphenicol: specific inhibitor of bacterial growth.
[2] Sensory assessments were performed by a panel of three experienced assessors who were asked to describe the dominant aromas they perceived in the musts. Only descriptors common to all three assessors were retained.
[3] Perceived intensities were evaluated using a 6-point scale labelled very light, light, medium, strong, intense, and very intense.
[4] -: absence +: little gas released ++: large amounts of gas released

too much oxygen, considerably modifying the aromatic profile of the wine. Microvinification models were partially closed after inoculation in order to release the gas produced by fermenting yeasts while preventing oxidation. To compensate for the less intense extraction, skins were arbitrarily added to the must at a 2:1 skin-to-juice ratio.

The aroma nuances revealed by alcoholic fermentation in a must alone are presented in table 6. Wines made from both model must and non-macerated Merlot must (tests 4A and 4B) exhibited no characteristics of red- or black-berry. According to the assessors, they generally had « fermentation » or « vinous » aromas, with no specific white, rosé-, or red-wine character.

In contrast, wines made by microvinification with Merlot skins had clear, intense red- and black-berry aromas, reminiscent of « strawberries », « raspberries », and « cherries » (table 6, tests 4C and 4D). The overall aroma was described by the assessors as similar to that of a « nouveau » red wine. Consequently, fruity aromas only developed when red grape skins were present during alcoholic fermentation, irrespective of the maceration medium.

Furthermore, as reported above, macerating Merlot skins in dilute alcohol solution only produced herbaceous aromas, described as « hay » and « crushed leaves». The increase in ethanol, characteristic of alcoholic fermentation, cannot therefore be responsible for revealing these specific fruity aromas.

Both physical and chemical effects of skins contact were further investigated. The results in table 6 show that wines produced by adding either Italia or Merlot skins previously macerated in ethanol to must did not develop any red- or black-berry aromas (tests 4E, 4F and 4G). This indicated that the fruity aroma did not simply develop when yeast fermented in the physical presence of red grape skin solids.

Merlot skin extract in ethanol had only a slight grape juice-like aroma, with no specific character of red- or black-berry. Nevertheless, as shown in table 6, wines made from this extract by microvinification revealed fresh red-berry fruit (test 4H). These wines were generally reminiscent of « Clairet » or « nouveau » red wines. On the contrary, wine made from Italia grape skin extract in ethanol exhibited no aroma of red- or black-berry (table 6, test 4I). Finally, microvinification with both Merlot skin extract and grape skins, irrespective of whether they were Merlot or Italia skins, revealed more intense fruity character (tests 4J and 4K). These wines were perceived by the panel as very similar to the winery-fermented red wines.

The latter results clearly showed, for the first time, that fruity aromas can develop when an aromatically-neutral ethanol extract, made from specific red grape skins, is fermented. This indirectly demonstrates that inodorous skin constituents are responsible for the development of a specific aroma of red- and black-berry after alcoholic fermentation by yeast. The most intense fruity aromas developed under conditions similar to those in the winery, i. e. the presence of both red grape skin extract and grape skins.

CONCLUSION

The alcoholic fermentation of red grapes was shown to produce characteristic fruity aromas, reminiscent of red- and black-berries, paralleling the growth of the yeast population. These fruity aromas were, for the very first time, shown to be directly linked to inodorous red grape skin constituents, which were successfully isolated from an ethanol extract. The yeast activity characteristic of alcoholic fermentation was then shown to transform the inodorous precursors into aromatic compounds, revealing clearly perceived red- and black-berry aromas in the wine. Moreover, the intensity of these fruity aromas was enhanced by the physical presence of grape skins during alcoholic fermentation.

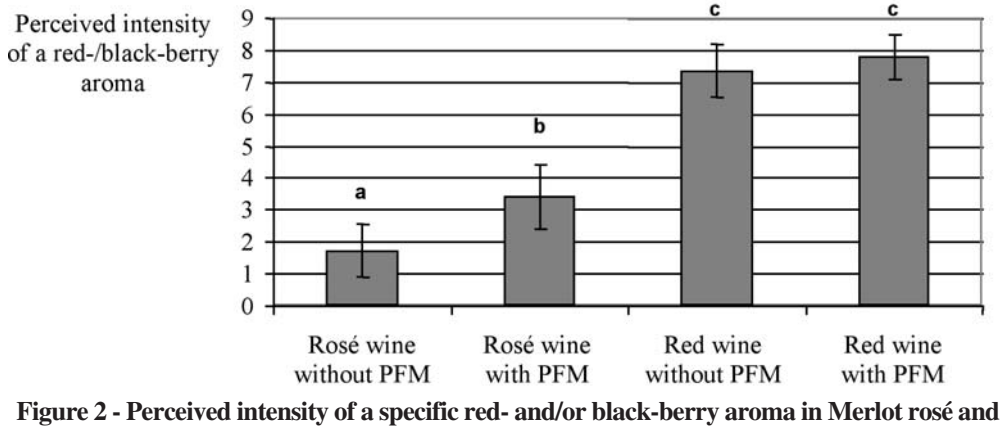

Figure 2 - Perceived intensity of a specific red- and/or black-berry aroma in Merlot rosé and red wines at the end of alcoholic fermentation.

For the first time, these findings shed light on the extremely important question of the origins of fruity aromas in red wines. However, the identity of the inodorous constituents involved remains unknown and further experiments are required to determine their chemical nature. Microvinification provides an excellent tool for indirect observation of the development of red- and black-berry aromas, coupled with analysis of grape composition.

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Table 6 - Aroma description of wine obtained by laboratory microvinification of model or Merlot musts, with or without grape skins and/or grape skin extract added before inoculation.

Test	Must	Addition before vinification	Aroma description[1]	Intensity[2]
4A	model	-	fermentation aroma*	strong
4B	Merlot			
4C	model	Merlot skins	fresh red-berry fruit aroma: strawberry, raspberry → "nouveau" red wine	intense
4D	Merlot		fresh red- and black-berry fruit aroma: strawberry, cherry	intense
4E	model	Italia skins	fermentation aroma*	medium
4F	Merlot		fermentation aroma*	medium
			+ herbaceous aroma → white wine	light
4G	model	Merlot skins previously extracted and washed	fermentation aroma*	medium
4H	Merlot	Merlot skin extract	fresh red-berry fruit aroma: strawberry → "nouveau" red wine	intense
4I	Merlot	Italia skin extract	fermentation aroma*	strong
			herbaceous aroma → white wine	light
4J	Merlot	Italia skins + Merlot skin extract	red- and black-berry fruit aroma: strawberry, strawberry jam, cherry	intense
4K	model	Merlot skins previously extracted and washed + Merlot skin extract	black-berry fruit aroma: blackberry, cherry → red wine-like	intense

[1] Sensory assessments were performed by a panel of three experienced assessors who were asked to describe the dominant aromas they perceived in the musts. Only descriptors common to all three assessors were retained.
[2] Perceived intensities were evaluated using a 6-point scale (very light, light, medium, strong, intense, and very intense).
* Aroma described as banana, cider, cucumber, fatty acids, and higher alcohols.

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