How does aromatic composition of red wines, resulting from varieties adapted to climate change, modulate fruity aroma?

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Abstract

One of the major issues for the wine sector is the impact of climate change linked to the increasing temperatures, which affects physicochemical parameters of the grape varieties planted in Bordeaux vineyards and consequently, the quality of wine. In some grape varieties, the attenuation of their fresh fruity character is accompanied by the accentuation of dried-fruit notes (Pons *et al.* 2017). As a new adaptive strategy on climate change, some winegrowers have initiated changes in the Bordeaux blend of vine varieties (Van Leeuwen *et al.* 2019). This study intends to explore the fruitiness in wines produced from grape varieties adapted to the future climate of Bordeaux. 10 commercial single–varietal wines from 2018 vintage made from the main grape varieties in the Bordeaux region (Cabernet franc, Cabernet-Sauvignon and Merlot) as well as from indigenous grape varieties from the Mediterranean basin, such as Cyprus (Yiannoudin), France (Syrah), Greece (Agiorgitiko and Xinomavro), Portugal (Touriga Nacional) and Spain (Grenache and Tempranillo), were selected. Both sensory and instrumental analyses were coupled to investigate their fruity aroma expression. For sensory analysis, samples were prepared from wine, using a semi preparative HPLC method which preserves wine aroma and isolates fruity characteristics in 25 specific fractions (Pineau, 2007 ; Lytra *et al.* 2012b). Fractions of interest with intense fruity aromas were sensorially selected for each wine by a trained panel and mixed with ethanol and microfiltered water to obtain fruity aromatic reconstitutions (FAR) (Lytra *et al*. 2012). A free sorting task was applied to categorize FAR according to their similarities and dissimilarities and different clusters were highlighted. Instrumental analysis of the different wines demonstrated variations in their molecular composition. Results obtained from sensory and gas chromatography analysis enrich the knowledge of the fruity expression of red wines from "new" grape varieties opening up new perspectives in wine technology, including blending, thus providing new tools for producers.

Introduction

In the Bordeaux vineyards, the climatic conditions of recent vintages have led to a great heterogeneity in grape ripening. The global warming have resulted in higher quality wines made from late-ripening varieties, such as Cabernet-Sauvignon. From a sensory standpoint, oenologists have noted a strong influence of climate change on the aromatic quality and typicality of red wines. Among the multiple sensory characteristics involved in judging wine quality, olfactory notes are the most affected (Van Leeuwen *et al*. 2020). Thus, today, the fruity character of red wines, typical of a particular region, is considered synonymous with quality and highly sought-after by consumers. The work of Pineau (2007) highlighted the existence of a fruity aroma, specific to Bordeaux red wines, marked by notes of "black-berry" and "jammy" fruits. Climate change is tending towards warmer, drier conditions, increasing the risk that, in the medium term, grape varieties that currently produce satisfactory results in the various production regions, and more precisely Merlot, will no longer be adapted in the future. Therefore, it makes sense to research the possibility of modifying the blend and (re-)introducing grape varieties adapted to the future climate. In order to preserve the fruity typicality of Bordeaux region wines after the introduction of these "new" grape varieties in the vineyards, the sector wishes to evaluate their aromatic potential by comparing them to the main grape varieties currently grown in the Bordeaux region (Merlot, Cabernet-Sauvignon, and Cabernet franc).
The aim of this work is to compare single-varietal red wines made from indigenous grape varieties from the Mediterranean basin (France, Greece, Spain, Portugal and Cyprus) planted in areas with hot climates (simulating the future climate of Bordeaux), to single-varietal red wines made from the main grape varieties currently grown in the Bordeaux region. This work intends to explore the modulation of the fruity expression

of those single-varietal red wines using sensory analyses and instrumental analyses. A focus on the substituted ester compounds was chosen to explain their impact in the fruity aroma.

Materials and methods

Wine samples

7 commercial single-varietal red wines, made from mid-ripening and late-ripening grape varieties planted in a region with a high-temperature climate, were collected. The selected grape varieties planted around the Mediterranean basin were from different countries: Cyprus (Yiannoudin), France (Syrah), Greece (Agiorgitiko and Xinomavro), Portugal (Touriga Nacional) and Spain (Grenache and Tempranillo). 3 single-varietal red wines made from the main grape varieties in Bordeaux vineyard were also added to the study (Cabernet franc, Cabernet Sauvignon and Merlot). These red wines were from the 2018, as red wines express their typical fruity aroma, distinguishable from the fermentation aromas (Lytra *et al.* 2017).

Fruity Aromatic Reconstitutions (FAR)

Sample preparation. Samples were prepared from 10 single-varietal red wines previously mentioned, using a methodology optimized by (Lytra *et al*. 2012b). A 500 mL wine sample was extracted successively using 80, 80 and 40 mL of dichloromethane for 15 min. Organic phases were collected, dried over sodium sulphate, and concentrated using a Rotavapor (Laborota 4010 digital Rotary Evaporator, Heidolph, Germany) to obtain a final amount of wine extract of 1.25mL. Aroma extracts of wines were eluted with a Reversed-Phase (RP) High-Performance-Liquid-Chromatography (HPLC) methodology, developed by Ferreira *et al*. (1999). The HPLC fractionation was realised by using a Nova-Pak C18 column (300 × 3.9 mm i.d., 4 µm, 60 Å, Waters, Saint-Quentin, France), without a guard cartridge (Pineau, 2007). The HPLC system consisted of an L-6200A pump (Merck-Hitachi, Germany). Chromatographic conditions were as optimized by Pineau (2007): 20 flow rate, 0.5 mL/min; injection volume, 250 µL wine extract; program gradient, phase A, water, phase B, ethanol; 0−2 min, 100% A, linearly programmed until 100% B at minute 50. The effluent was collected in 1 mL fractions. This methodology was used to isolate fractions with intense fruity notes (fractions 17−21), thus creating a fruity pool. For FAR, fractions of the fruity pool (fractions 17-21) were blended together to reproduce the initial concentrations in the original wines, adding ethanol and microfiltered water to obtain an ethanol level of 12% (v/v) (Lytra *et al*. 2012b).

Quantitation of substituted ester compounds in FAR made from single-varietal red wines

Sample Preparation. Samples were prepared as described by Lytra *et al.* (2017). A 50 mL FAR sample was spiked with 300 µg/L ethyl 2-hydroxy-2-methylpropanoate as an internal standard. The sample was then extracted successively using 4, 4 and 2 mL of dichloromethane, with magnetic stirring (700rpm), for 10 min each and separated in a separating funnel for 5 minutes. Organic phases were collected, dried over sodium sulphate, and concentrated under nitrogen flow (100 mL/min) to obtain 250 µL of wine extract.

Gas Chromatographic – Mass Spectrum analyses (GC-MS). GC-MS analyses were carried out as described Lytra *et al*. (2017) on an HP 5890 GC system coupled to an HP 5972 quadrupole mass spectrometer (Hewlett-Packard), equipped with a Gerstel MPS2 autosampler. Injections were in split mode (split ratio, 30:1), using a 2 mm i.d. non-deactivated direct linear transfer (injector temperature, 200 °C; interface temperature, 280 °C). A BP21 capillary column (50 m × 0.32 mm i.d.; film thickness, 0.25 µm; Astec, Whippany, NJ, USA) was used for quantitation of substituted esters. The oven temperature was programmed at 40 °C for 1 min, then increased at a rate of 1 °C/min to 100 °C, and finally raised by 3 °C/min to a final isotherm at 180 °C, maintained for 3 min. The carrier gas was helium 5.5 (Linde, France) with a constant flow of 1 mL/min. The mass spectrometer was operated in electron impact mode at 70 eV with selected-ion monitoring (SIM). Assays were performed in triplicate.

Statistical Analyses. Statistical data were analysed using the Kruskall–Wallis statistical non-parametric test (XLSTAT software, Addinsoft, Paris, France), with a statistically significant level of 5% ($p < 0.05$).

Sensory Analyses of Fruity Aromatic Reconstitutions (FAR)

10 FARs were all presented randomly to a panel, composed by 19 judges from the research laboratory staff at ISVV (University of Bordeaux), to compare the fruitiness of samples. A free sorting task was applied to explore perceptive similarities among FARs (Maître *et al*. 2010). Assessors were instructed to classify each FAR according to their similarities or dissimilarities. No constraints were required for this test and they had the

possibility to do the number of groups they wanted (with a minimum of 2 groups and at least 2 samples per group). Statistical data were analyzed using Hierarchical Cluster Analyses (HCA, XLSTAT software, Addinsoft, Paris, France) and analysis of variance (ANOVA) was applied to the data obtained by quantitation coupled to HCA data.

Results and discussion

Sensory Analyses of FAR

25 fractions with various flavours were obtained using the HPLC fractionation method and were evaluated by the panel using a free sorting task test. The fruity character of each selected wine (marked by black-berry, red-berry, fresh and jammy fruits) was preserved into the fruity pool (fractions 17-21). Data collected from the free sorting task test were organized into a dissimilarity matrix (Faye *et al.* 2011). Then, a Hierarchical Cluster Analysis (HCA) was performed on the dissimilarity matrix to obtain a dendrogram (Figure 1). Samples were gathered according to their similarities or dissimilarities comparing their fruity expression. Statistical analysis highlighted the existence of 3 distinct groups constituted by different FAR made from different single-varietal red wines (Figure 1). The 1st group in green colour was composed by 5 FAR made from single-varietal red wines: Yiannoudin (Cyprus), Syrah (France), Touriga Nacional (Portugal) and Tempranillo (Spain). Regarding the 2nd group in brown colour, 3 FAR of single-varietal red wines made from different countries were gathered: Cabernet franc (France), Grenache (Spain) and Xinomavo (Greece). Finally, the FAR of Merlot red wine from France was classified into the 3rd group (in pink colour) with the FAR of Agiorgitiko red wine from Greece.

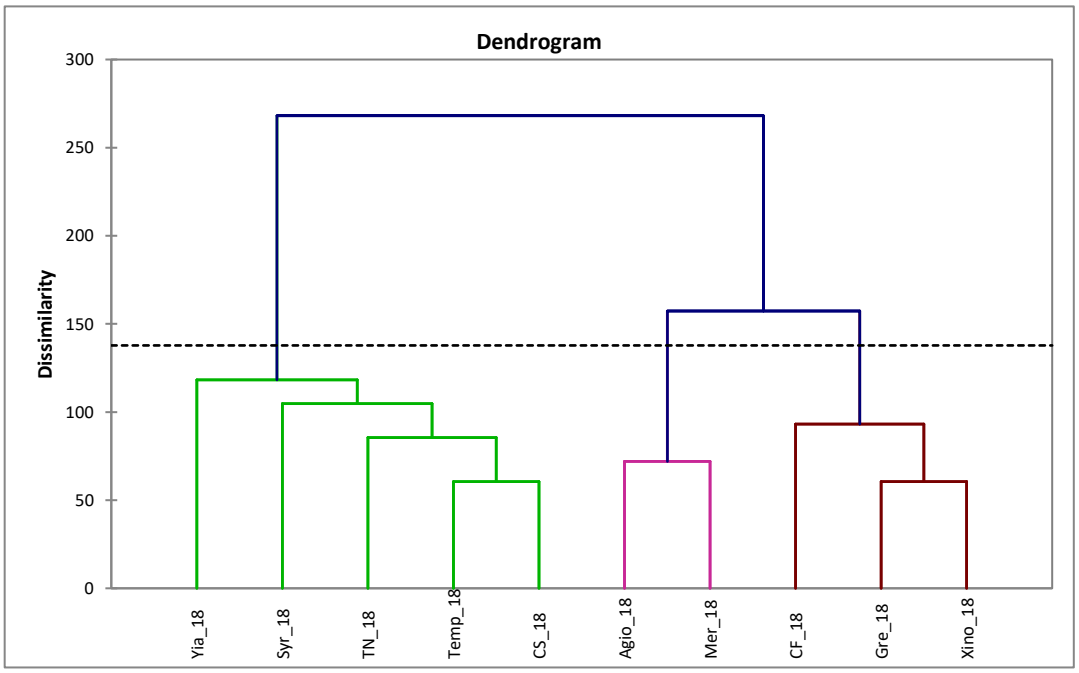

Figure 1. Dendrogram obtained by HCA using a dissimilarity matrix applied on free sorting data (Agio_18: Agiorgitiko; CF_18: Cabernet franc; CS_18: Cabernet Sauvignon; Gre_18: Grenache; Mer_18: Merlot; Syr_18: Syrah; Temp_18: Tempranillo; TN_18: Touriga Nacional; Xino_18: Xinomavro; Yia_18: Yiannoudin).

Substituted ester composition in FAR

6 substituted ester compounds were quantified in the 10 different FAR using GC-MS analyses (Table 1). Statistical analysis, performed by the Kruskall-Wallis non-parametric test, shown significant differences regarding the levels of the different substituted ester in samples, excepted for the ethyl 6-hydroxyhexanoate which no significant variations were observed.

Table 1. Levels of substituted ester compounds in red wines.

FAR made from single-varietal red wines	Ethyl 2-methylpropanoate	Ethyl 2-methylbutanoate	Ethyl 3-methylbutanoate	2-methylbutyl acetate	Ethyl 2-hydroxy-3-methylbutanoate	Ethyl 6-hydroxyhexanoate
Agiorgitiko	34,82 ± 0.14 **abc**	12,07 ± 0.17 **abc**	21,10 ± 0.26 **ab**	30,93 ± 0,26 **c**	2,30 ± 0,07 **ab**	1,80 ± 0,20 **a**
Xinomavro	n.d.	17,47 ± 0,30 **abc**	31,99 ± 0,45 **ab**	11,80 ± 0,15 **ab**	2,32 ± 0,08 **ab**	1,85 ± 0,15 **a**
Yiannoudin	8,86 ± 0,68 **abc**	18,39 ± 0,24 **abc**	30,07 ± 0,10 **ab**	18,16 ± 0,10 **abc**	2,21 ±0,08 **a**	2, 28 ± 0,31 **a**
Grenache	1.62 ± 0,43 **ab**	38,22 ± 0,25 **bc**	23,81 ±0,06 **ab**	25,63 ± 0,12 **abc**	2,57 ± 0,20 **ab**	1,88 ± 0,11 **a**
Tempranillo	38,71 ± 1,31 **bc**	25,58 ±0,17 **abc**	38,14 ± 0,31 **ab**	11,34 ±0.07 **a**	2,31 ± 0,10 **ab**	3,42 ± 0,36 **a**
Touriga Nacional	9,13 ± 1,80 **abc**	11,24 ±0,05 **abc**	21,11 ± 5,80 **ab**	25,85 ± 0,16 **ab**	2,37 ± 0,13 **bc**	2,59 ± 0,50 **a**
Cabernet franc	22,80 ± 0,57 **abc**	20,32 ± 0,16 **abc**	31,29 ± 0,27 **ab**	20,11 ± 0,05 **abc**	2,43 ± 0,03 **ab**	1,90 ± 0,08 **a**
Cabernet Sauvignon	34,14 ± 1,01 **abc**	30,56 ± 0,26 **abc**	43,53 ± 0,28 **b**	19,52 ± 0,11 **abc**	2,45 ± 0,19 **ab**	2,05 ± 0,23 **a**
Merlot	49,27 ± 0,22 **c**	46,60 ± 0,20 **c**	68,20 ± 0,22 **c**	21,61 ± 0,09 **abc**	2,42 ± 0,02 **ab**	1,96 ± 0,08 **a**
Syrah	10,37 ± 1,07 **abc**	7,49 ± 0,13 **a**	10,40 ± 0,12 **a**	24,11 ± 0,21 **abc**	2,80 ± 0,15 **b**	2,33 ± 0,37 **a**

± Standard deviation over the average concentration, n.d.: not-detected, values with different letters within each column are significantly different (Kruskall-Wallis Test (Dunn / Billateral test), $p < 0,05$).
An analysis of variance (ANOVA) was performed to quantitation data in order to highlight significant variations of the concentrations of substituted ester in the 3 different groups. A substituted ester seams to discriminate statistically the clusters obtained from the free sorting task data: ethyl 2-methylpropanoate (Figure 2).

Conclusion

3 different groups of similarity were formed. FAR made from 5 single-varietal red wines made from different countries (Yiannoudin, Syrah, Touriga Nacional, Tempranillo and Cabernet Sauvignon) were gathered together. The FAR of Merlot single-varietal red wine was classified with the FAR of Agiorgitiko single-varietal red wine, highlighting their similar fruitiness and the possible replacement of Merlot by this variety in the Bordeaux traditional blend. Significant differences regarding the levels of substituted ester compounds in the FAR were observed. Among the quantified esters, it seems that ethyl 2-methylpropanoate may discriminate statistically the clusters obtained from the free sorting task data. These results show that the family of substituted ester may be partly responsible for the fruity expression of wine, thus opening up new future perspective of further sensory analyses as well as instrumental analyses of other families of wine compounds.

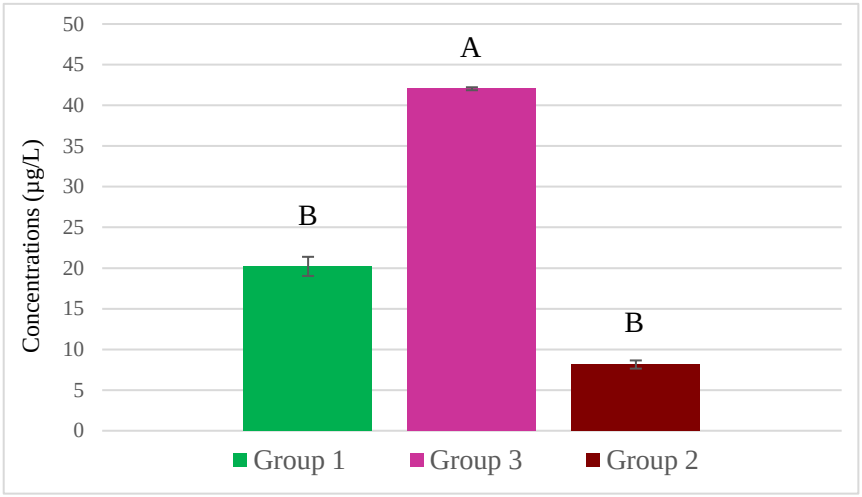

Figure 2. Mean concentrations of ethyl 2-methylpropanaote in the 3 groups. Group 1: Yiannoudin, Syrah, Touriga Nacional, Tempranillo and Cabernet Sauvignon; Group 2: Agiorgitiko and Merlot; Group 3: Cabernet franc, Grenache and Xinomavro. (ANOVA, $p < 0,05$). Error bars indicate standard deviation.

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