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INFLUENCES OF GRAPE CULTIVATION TECHNOLOGY ON CERTAIN AROMA COMPOUNDS SIGNIFICANT FOR THE DIFFERENTIATION OF ‘CABERNET SAUVIGNON’ AND ‘FETEASCĂ NEAGRĂ’ WINES

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Abstract

Red wine grape varieties ‘Fetească neagră’ and ‘Cabernet Sauvignon’ were produced in Murfatlar wine region under organic and conventional viticultural systems. For both varieties and viticultural systems, 30% cluster thinning was performed in some variants during summer, at the beginning of veraison. The resulted grapes, harvested in the autumn of 2014, were vinified using the same winemaking protocol and the differences in aromatic profile of the wine samples were assessed by using a GC e-nose from Alpha MOS. The chromatographic peaks were recorded and analysed with Alpha Soft ver. 12.42 and AroChemBase. The discrimination of the samples was based on multivariate DFA statistical analysis. The results showed that 30% cluster thinning did not influence significantly the major wine aroma compounds, only small differences being observed in the DFA diagram for samples with and without cluster thinning. However, the grape variety and the application of organic cultivation technology led to a good discrimination, with a validation score of 92, of the samples on the DFA diagram. An even better discrimination, with a validation score of 96, was observed on wine samples from grapes produced in conventional and organic system when the cluster thinning was disregarded and samples with or without this intervention in the vineyard were included in the same group. These results show that, as far as the aromatic profile is concerned, the wines produced from grapes to which cluster thinning was applied are difficult to differentiate from the controls produced from vines with normal yield, even with a sensitive equipment such as an e-nose. On the other hand, the organic and conventional growing led to wine samples easily differentiated by the electronic nose, suggesting that these differences can also be perceived by consumers. Some of the aroma compounds significant for the discrimination of wines produced from organic or conventional grapes were identified and their expected sensory effect is discussed.

Key words: *‘Fetească neagră’, ‘Cabernet Sauvignon’, electronic nose, aroma profile, organic viticulture, cluster thinning.*

INTRODUCTION

The global quality of wine aroma is well related with grape-growing conditions and healthy grapes, along with the winemaking technology involved, being an important attribute for consumers buying decisions and a challenge for the winemakers. The primary aroma of wines is derived from grapes and implicitly from cultivation technology, along with climate and soil variables, while the secondary aroma is related to the winemaking processing techniques and enzymatic transformations of grape compounds by microorganisms, especially during alcoholic fermentation. Viticultural practices and ecosystem factors influence the expression of certain genes involved in production of aroma compounds and precursors in grapes. Limited soil fertility influences the ripening process favouring aroma maturation and lowering the vegetative growth, which in turn favours the formation of flavours (Jackson, 2008). On the other hand, an increased potassium content in soil may lead to an increase in C_{13} norisoprenoids in grapes and of certain acetate esters produced during fermentation in wines (Falcao et al., 2008). Type of soil and drainage show an influence on the aroma composition of grapes and in the resulted wines, such as metoxypyrazines decrease in grapes on gravelly soil (Ribéreau-Gayon et al., 2006), more floral, sweet and fruity aromas develop in wines derived from clay soils, solvent-like and green notes appear in wines derived from sandy soils (Gómez-Míguez et al., 2007) while more terpenoids, sesquiterpenoids, and C_{13} norisoprenoids are found in wines derived from calcareous soils (Coelho et al., 2009). However, the type of soil and drainage, directly influence the soil temperature which appear to influence aroma compounds in wines with the highest accumulation of *β*-damascenone and geraniol in warmer soils and conversely, a lower accumulation *β*-damascenone but higher accumulation in *β*-ionone in cold soils. Dry weather at the end of summer improves fruit quality through the advance of ripening and favours the flavour formation (Jackson, 2008), while the temperature influences the development of C_6 aldehydes in cool regions and higher concentrations of monoterpenes in warmer regions (Ji and Dami, 2008). Excessive sunlight exposure or partially shading of grape clusters modify the terpenol contents. In Muscat cultivars, the highest concentrations of terpenols were found in artificially 50% shade (Belancic et al., 1997), while a 90% shade decreased the terpenols and C_{13} norisoprenoids (Bureau et al., 2000a, 2000b). The concentration of TDN (1,1,6-trimethyl-1,2-dihydronaphthalene) increased with sunlight exposure in Riesling (Gerdes et al., 2002), while in the other study on Shyrah, an extreme shading lead to a decrease in concentration of TDN (Ristic et al., 2007). Viticultural practices on different varieties regarding cluster thinning and basal leaf removal showed an increase of free terpenols and glycosidically bond terpenols (Reynolds et al., 1996b; Roberts et al., 2007; Hernandez-Orte et al., 2014; González-Barreiro et al., 2015). However, different training systems studied by various authors showed variations in terpenols concentrations (Ji and Dami, 2008; Reynolds et al., 1996a; Zoecklein et al., 2008). Water stress may increase blackberry, jam, raisin or dried fruit aroma in 'Cabernet Sauvignon' wines (Chapman et al., 2005), increase of cysteinylated thiol precursors in grapes of 'Sauvignon blanc' (Ribéreau-Gayon et al., 2006), increase of glycoconjugates of the main aromatic compounds observed in Agiorgitiko vines (Koundouras et al., 2006), increase of vitispirane, guaiacol, 4-methylguaiacol, 4-ethylguaiacol and 4-vinylguaiacol observed in Merlot vines (Qian et al., 2009). Fertilization of vineyard with nitrogen lead to higher cysteinylated thiol precursors in 'Sauvignon blanc' grapes (Choné et al., 2006), while in Riesling wines a rise in 1-butanol, *trans*-3-hexen-1-ol, benzyl alcohol and the majority of esters was observed (Webster et al., 1993). Plant protection with fungicides may lead to residues on the grapes that affect the yeasts and fermentative performance which in turn influence aroma quality (Noguerol-Pato et al., 2011; Gonzales-Rodriguez et al., 2011; Gonzalez-Alvarez et al., 2012a and 2012b).

MATERIALS AND METHODS

The grapes used for this study were from the 'Cabernet Sauvignon' and 'Fetească neagră' cultivars, vintage 2014, cultivated in Murfatlar wine region. The vine parcels used in this study are located close to each other, in order to reduce the variability induced by soil and climate. The organic cultivation technology for both varieties are subject to actual regulations and controlled by Council Regulation (EEC) 834/2007. An additional viticultural practice applied consisted in 30% cluster thinning during summer, at the beginning of veraison, being evaluated against the control with no cluster thinning. The grapes were harvested on 16th September for the 'Fetească neagră' and on 24th September for 'Cabernet Sauvignon'. In this manner eight experimental variants were produced, according with viticultural practices briefly described in Table 1. More details regarding the grapes physico-chemical composition and other grape related parameters are described in other paper (Artem et al., 2015). The grape processing was done for batches of grapes of 50 kg, in 3 repetitions resulting 24 samples. The vinification process was the classical one, with maceration and fermentation on skins for 5-8 days for 'Fetească neagră' and for 8-10 days for 'Cabernet Sauvignon'. The fermentation was conducted at room temperature at about 22-24°C with native yeasts (no added yeast), without added nutrients or enzymes. At the end of fermentation the macerated grape marc of each variant was pressed using a laboratory press. After one month of clarification and cold stabilization, the resulted wines were racked off and treated with a dose of 50 mg/L of SO_2 to prevent oxidation and development of spoilage microorganisms. The resulted wine samples were analysed in other 3 repetitions on 7th May 2015, assessed with a GC e-nose from Alpha MOS in order to establish the differences among certain aroma compounds between experimental variants.

Alpha MOS Heracles e-nose analyser is a Fast GC using a Tenax trap for pre-concentration of volatile organic compounds, designed with two short capillary columns working together having a different polarity, one being non-polar DB5 (5% diphenyl, 95% dimethylpolysiloxane) and the other with a low/mid polarity DB1701 (14% cyanopropylphenyl, 86% dimethylpolysiloxane).

Table 1. Experimental variants and the related codes attributed

Variety	Grape cultivation technology	Additional practice	Variant code
'Cabernet Sauvignon'	Conventional	No cluster thinning	CS_Con_N
		30% Cluster thinning	CS_Con_CT
	Organic	No cluster thinning	CS_Org_N
		30% Cluster thinning	CS_Org_CT
'Fetească neagră'	Conventional	No cluster thinning	FN_Con_N
		30% Cluster thinning	FN_Con_CT
	Organic	No cluster thinning	FN_Org_N
		30% Cluster thinning	FN_Org_CT

The separated volatile organic compounds are detected by means of simultaneous dual flame ionization detectors (FID), located at the end of each chromatographic column. The injection mode was made with 2.5 ml HS syringe, extraction of volatiles from the head-space of vials, using a 250°C injector temperature with an initial column temperature 40°C with an increase rate of a 5°C/s up to 200°C and hydrogen in constant pressure (16 psi) as a carrier gas. The oven agitator temperature for sample preparation was 10 minutes at 60°C and 500 rpm. The flame ionization detectors temperature have been set at 220°C and a fuel pressure of 35 psi. Other details regarding the methodology and Alpha MOS Heracles can be found in other papers (Antoce et al., 2015; Antoce and Namolosanu, 2011). The identification of peaks was based on retention index (Kovats), calibrated by using a known standard n-alkane mixture (n-C_6 to n-C_{16}) with the same determination conditions as for the analysed wine samples. The discriminant factor analysis (DFA) was selected in Alpha Soft v 12.42 software as the most relevant statistical method to separate the experimental variants and groups.

RESULTS AND DISCUSSIONS

In order to differentiate wine samples, discriminant factor analysis was applied to certain chromatographic peaks area (sensors), selected by using an extensive iterative process and their discriminant power provided by Alpha Soft v 12.42. Using this process, the main volatile organic compounds identified to differentiate wine samples in this study are briefly described in Table 2. The results show small differences between the experimental variants with 30% cluster thinning as compared to no cluster thinning variants, this being especially evident for conventional 'Fetească neagră' (Figure 1). This clearly led to the idea that cluster thinning practice did not significantly influence the major volatile organic compounds in wine samples and only small changes being observed, which are hardly recognized even by a powerful tool such as GC-e-nose. However a good validation score of 92 in DFA diagram (Figure 1) is explained by the higher variability induced by both grape cultivation technology (organic or conventional) and variety. According to Figure 1, the DFA bi-plot explained 96.554% of the total variance in the data through the first two dimensions, with 85.069% and 11.485% explained by DF1 (mostly grape variety related) and DF2, respectively. In general in Figure 1 and 4, no cluster thinning, appear to be associated with more negative volatile organic compounds as 2,4-hexadienal, 2,3-octanedione and valeric acid, while 30% cluster thinning, appear to be associated with more positive volatile organic compounds as 3-methyl-1-pentanol, dehydro-*p*-cymene, *β*-phenylethanol, nerol oxide and (*E*)-2-hexenal. The results regarding cluster thinning practice did not show a clear difference between those samples as can be seen in Figure 4, where DFA bi-plot have a validation score of 69.

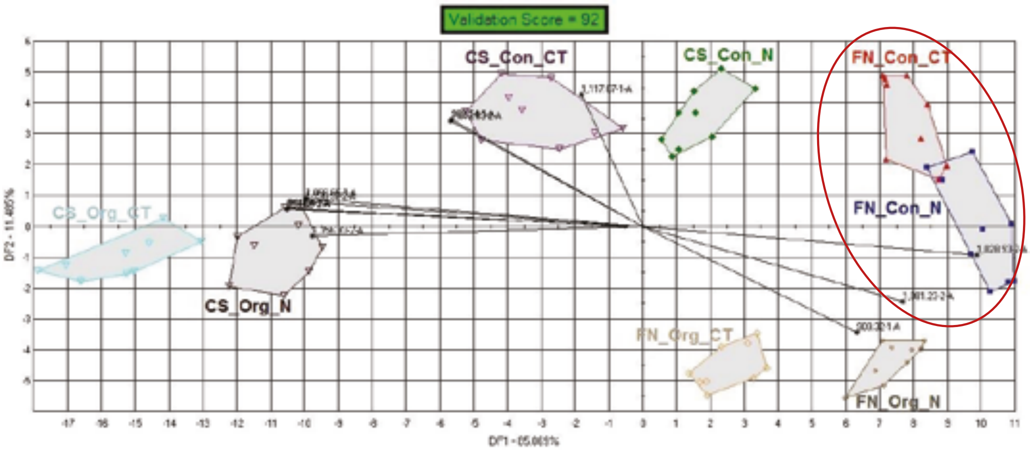

Figure 1. DFA bi-plot of aroma compounds peaks used for differentiation of wine sample groups in accordance to grape variety, grape cultivation technology (organic/conventional) and cluster thinning practice

Table 2. Relevant aroma compounds identified as being discriminative in wine samples of ‘Cabernet Sauvignon’ and ‘Fetească neagră’

*Retention time	Column	*Sample Kovats	Database Kovats	Sensor number	Identified volatile organic compounds	Sensory descriptor
8.05	DB5	844,04	843	844,04-1-A	3-methyl-1-pentanol	chocolate, wine-like, green
10.36	DB5	909,32	908	909,32-1-A	2,4-hexadienal	green, citrus, floral
12.54	DB5	962,54	962	962,54-1-A	*γ*-valerolactone	anise, herbal, sweet, warm, tabacco, cocoa, woody, hay-like, coumarinic odor,
17.72	DB5	1090,66	1094	1090,66-1-A	dehydro-p-cymene	citrus, pine
18.70	DB5	1117,07	1117	1117,07-1-A	*β*-phenylethanol	sweet, floral, fresh, bready, rose, honey
11.25	DB1701	953,38	956	953,38-2-A	(*E*)-2-Hexenal	sweet, vegetable, almond, apple, green, plum
14.34	DB1701	1028,93	1031	1028,93-2-A	valeric acid	cheese, sweat
16.10	DB1701	1072,03	1073	1072,03-2-A	*γ*-valerolactone	anise, herbal, sweet, warm, tabacco, cocoa, woody, hay-like, coumarinic odor,
16.49	DB1701	1081,23	1082	1081,23-2-A	2,3-octanedione	dill, cooke, broccoli, buttery
19.58	DB1701	1156,97	1157	1156,97-2-A	dehydro-p-cymene	citrus, pine
27.26	DB1701	1356,93	1353	1356,93-2-A	nerol oxide	flower, oil

*average values resulted from all chromatograms;

Nevertheless, the cultivation technology and variety are the most important variables in this study, for which reason we have redefined the groups according to only cultivation technology and variety, removing the cluster thinning variable (Figure 2). Using this approach, a better validation score (96) is obtained and a very good separation on the DFA bi-plot (Figure 2). In Figure 2, the DFA bi-plot explained 96.852% of the total variance in the data through the first two discriminant functions, with DF1=76.516% and DF2=20.336%. From the loadings of the discriminant peaks also included in Figure 2 it can be seen that conventional ‘Fetească neagră’ is associated with more valeric acid than the organically cultivated ‘Fetească neagră’, which is associated with 2,4-hexadienal, while the conventional ‘Cabernet Sauvignon’ is associated with *β*-phenylethanol and organic ‘Cabernet Sauvignon’ with nerol oxide, dehydro-*p*-cymene and (*E*)-2-hexenal.

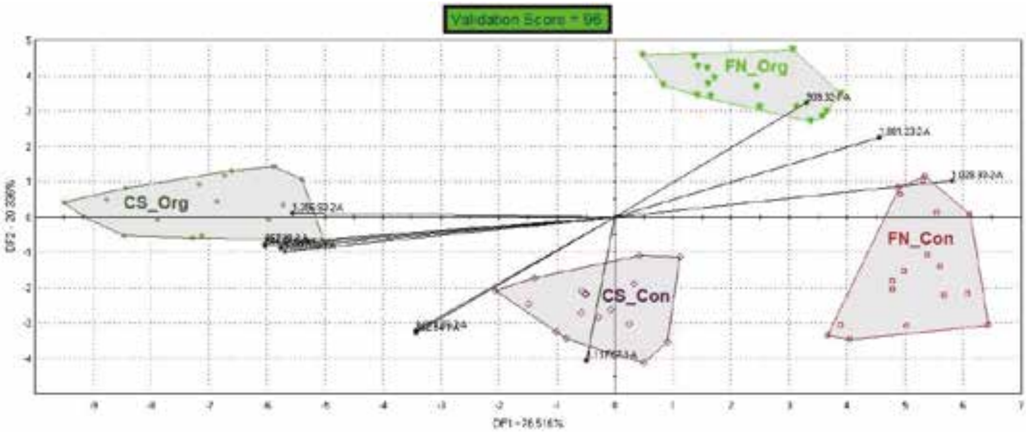

Figure 2. DFA bi-plot of aroma compounds peaks used for differentiation of wine samples depending on grape variety and grape cultivation technology (organic/conventional)

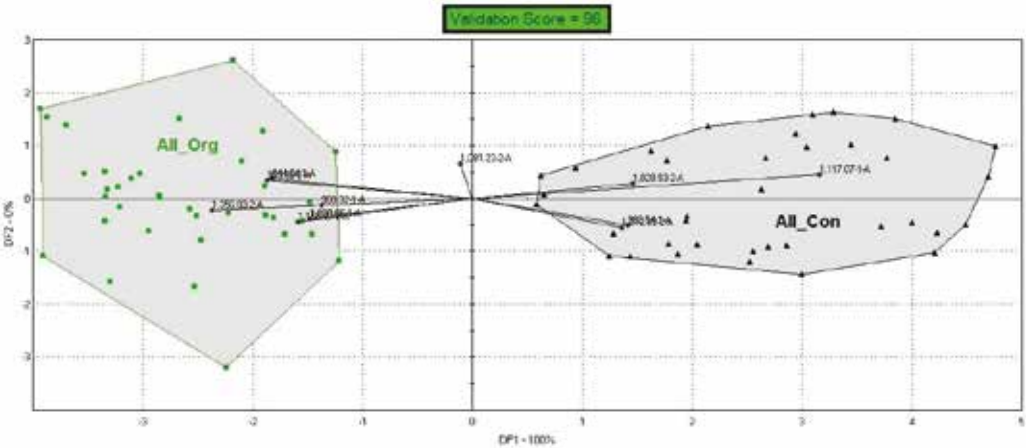

Figure 3. DFA bi-plot of aroma compounds peaks used for differentiation of wine samples depending on grape cultivation technology (organic/conventional)

When we have redefined the groups according to only cultivation technology removing the variety and cluster thinning variables (Figure 3), all organic samples are well differentiated by conventional samples with a good validation score of 96 observed in the DFA bi-plot. In figure 3 we can observe the main differences between organic and conventional samples and the DFA bi-plot explained 100% of the total variance in the data through the first dimension. The results from the figure 3 reveal that conventional growing technology in this study is associated with more *β*-phenylethanol, *γ*-valerolactone and valeric acid, while the organic grape production is associated with more nerol oxide, dehydro-*p*-cymene, 3-methyl-1-pentanol, 2,4-hexadienal and (*E*)-2-hexenal and slightly with 2,3-octanedione. However, the organic and conventional growing led to wine samples easily differentiated by the electronic nose, suggesting that these differences can also be perceived by consumers. In one study of sensory evaluation of red wines deriving from organically and conventionally grown grapes, trained assessors and regular wine consumers attributed the differences mainly to sour and bitter taste, with astringency sensations, while aroma have a minor role (Pagliarini et al., 2013).

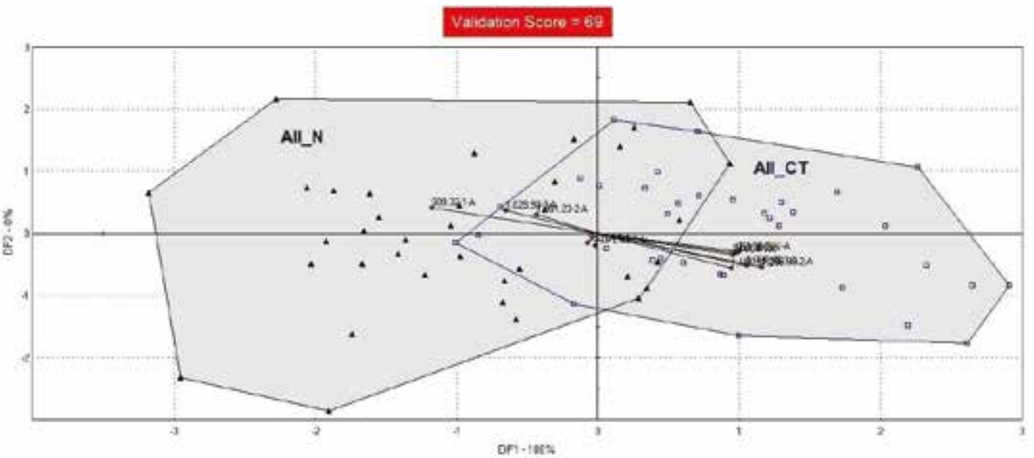

Figure 4. DFA bi-plot of aroma compounds peaks used for differentiation of wine samples depending on cluster thinning

CONCLUSIONS

Grape variety followed by the type of grape technology (organic/conventional) were the most discriminative factors for the wines produced and evaluated in this study, allowing for a good separation of wine samples in the DFA bi-plots, with high validation scores (96). This means that the grape growing technology has a significant impact on grape aroma compounds and implicitly in wines, meaning that these modifications are probably perceived even by regular wine consumers. The 30% cluster thinning did not influence significantly these aroma compounds compared with the samples with no cluster thinning and in this case, certainly, discrimination of wine samples by the electronic nose could not be achieved in this experiment, leading us to the idea that this viticultural practice did not have sufficient impact on the overall aroma quality. The organic grape technology affected more the wine aroma, resulting in associations with monoterpenes (nerol oxide and dehydro-*p*-cymene), higher alcohols (3-methyl-1-pentanol), aldehydes (2,4-hexadienal and 2-hexenal) derived from grape lipid enzymatic oxidation, and slightly with some ketones (2,3-octanedione). On the other hand, the conventional grape technology impact on wine aroma resulted in associations with other higher alcohols (β-phenylethanol), lactones (γ-valerolactone) and volatile acids (valeric acid). All these compounds may not be perceptible in wine as the pure substances are perceived and described in literature, as their mixture in the wine matrix changes very much this perception. However, these findings serve as hints to winemaker to find ways to improve the quality of grapes and wines.

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