FROM GRAPES TO SPARKLING WINES: AROMAS EVALUATION IN A VINE-SPACING EXPERIMENT

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Abstract

Aim: Wine aromatic profile is a combination of viticulture and oenological practices and it is related to character, quality, and consumer acceptance. Based on the competition between soil capacity and canopy development, and on the potential to produce sparkling wines at Caldas, in the south region of Minas Gerais (Brazil) (21°55´S and 46°23´W, altitude 1.100m), the aim of this work was the evaluation of the development of aromas (secondary metabolites) from grapes to sparkling wines in a vine-spacing experiment and whether the distance between the vines can influence the aromatic profile of the sparkling wines (final product).

Methods and Results: The study was conducted with grapes from a 7-year-old vine-spacing experimental vineyard located at Caldas city and their respective must, base wine, and sparkling wine from vintage 2016 of the cultivar Chardonnay (*Vitis vinifera* L.) grafted onto 1103 Paulsen rootstock, in a clayey soil, and trained on a vertical shoot positioned trellis. Grapes were harvested in the maturity stage for sparkling wines, which were obtained by the traditional method. The volatile compounds in the specimens described were analysed by HS-SPME/GC-MS. Considering the five vine-spacing systems studied (0.5 m, 0.75 m, 1 m, 1.5 m, and 2 m), around 60-80 volatile compounds (secondary metabolites) were identified in the free form for all the specimens studied and PCA analysis showed discrimination among them. Thus, some compounds were slightly higher in must and wines than in berries (e.g., terpenoids, carotenoids). While in the grapes and must the high number of compounds was related to aldehydes and alcohols, in the base and sparkling wines it was related to esters, benzenoids and fatty acids compounds. These compounds resemble pleasant, powerful, floral, fruity odours of apricot and pineapple-banana note, and have an influence on foam.

Conclusions: All the processes (grapes metabolism, first fermentation and *sur-lie*) influenced the development of the aromas. Although the aromatic profile of the five vine-spacing systems sparkling wines was slightly different, a sensorial analysis would be an additional tool to this work to assess if these variations would be noticed by final consumers.

Significance and Impact of the Study: This study impacts on the knowledge of the products obtained in this terroir, in which conditions one can have a product that pleases the final consumer more and also has a good production. In other words, the consideration for the balance among grape-growing and winemaking practices, productivity/economy, and quality of the final product.

Keywords: *Vitis vinifera*, food analysis, aromatic profile, PCA analysis, HS-SPME, GC-MS, Chardonnay grapes, vine-spacing systems

Introduction

The aromatic profile of any beverage is a key point concerning quality, character, and consumer acceptance, and this topic plays an important role in the wine industry due to the complex mixture of volatile organic compounds contributing to the *bouquet*. According to Carlin *et al*. (2016), Welke *et al*. (2014) and Swiegers *et al*. (2005), a main feature that differentiates wine quality is the aroma, influenced by grape-growing and oenological practices.

Among viticultural practices, the study of vine-spacing (vine density) comprises the colonisation of the soil by the root system and the colonisation of the air by the aerial part of the plant. Mendonça (2015) and Almeida (2017) explained that when there is a high vine density, the roots of plants compete for water, and other nutrients, thus contributing to reduce vegetative growth, as the plants divert nutrients to the fruit to reach adequate maturity. On the other hand, in a low density there will be competition between the inflorescence and the vegetative part, altering the hormonal balance of the plant and consequently the ripeness and quality of the berry. Jones and collaborators (2014) in a revision concerning viticulture for sparkling wines, pointed out that when soil conditions and fertility are not a limitation, the impact of vine-spacing on production and fruit composition is minimal.

Based on the fact that Brazil is a tropical country, which favors the consumption of fresh and light wines such as sparkling wines, EPAMIG (Agronomic Research Company of Minas Gerais) develops researches that are later used to improve and expand the productions in wineries in the southeast region of Brazil. One of the experiments conducted in those researches is the grapevine spacing system study (planting density), thus this work aims to valuates how spacing can influence sparkling wine composition.

Materials and Methods

Plant Material and Fermentation (Experimental Conditions)

This study was carried out in the experimental vineyard (7-year-old) and winery located at EPAMIG in Caldas city, a mountain region (1,100 m) in the south of Minas Gerais/Brazil (21°55´S, 46°23´W, minimum average temperatures of 12°C and maximum of 25°C, and 1500 mm of precipitation levels during fruit ripening). This trial was performed in the vintage 2016 with Chardonnay grape berries (clone 76) grafted onto 1103 Paulsen in a clayey soil, trained on vertical shoot positioned trellis, and in 5 vine-spacing systems (0.5 m, 0.75 m, 1 m, 1.75 m, and 2 m, respectively, reported as VS-1, VS-2, VS-3, VS-4, and VS-5). Four lines at the same spacing.

All grapes were harvested at the technological maturation stage for sparkling wines (January 2016). The procedures in the winery for the 5 vine-spacing systems were carried out separately and in experimental conditions. After *débourbage* of the 5 musts, the first and second fermentations were performed with *Saccharomyces cerevisiae bayanus* (Maurivin PDM) and coadjuvants. It was not necessary to proceed to the malolactic fermentation and the second fermentation following the *Champenoise* method. After 18 months in *sur-lie*, the sparkling wine proceeded to *remuage*, *dégorgement* and corking.

Sample Preparation for Volatile Compounds Analysis

Once the berries were picked for the volatile compounds analysis, they were frozen and stored at -80°C. The skins and seeds were removed and the pulp was crushed under liquid nitrogen and stored in an ultrafreezer until sample preparation. Samples for volatiles compounds were prepared as follows: 3 g of the powder were weighed, then completed to 10 g with NaCl saturated solution (ACS grade, Sigma Aldrich). An amount of the must after *débourbage* and the base wine were frozen and stored at -80 °C prior to volatile compounds analysis. The sparkling wine after *dégorgement* was stored in the underground cellar and before analysis it was refrigerated, aliquots were taken prior to the extraction procedure and sonicated during 2 minutes. These samples were prepared as follows: 5 mL of the specimen and added 5 mL of NaCl saturated solution. The experiments for the specimens and vine-spacing systems were undertaken in triplicate.

Extraction of the volatile compounds by headspace-solid phase microextraction (HS-SPME) was established as described: the samples were equilibrated for 10 min (grape berries and must) and 5 min (base and sparkling wines) in a water bath adjusted to 40°C and then the SPME fibre, 50/30µm 2-cm DVB/CAR/PDMS Stableflex (divinylbenzene/carboxen/polydimethylsiloxane) from Supelco was inserted and equilibrated into the headspace during 50 min. Afterward, the fibre was removed and exposed in the GC injection port at 250°C during 5 min for desorption.

GC-MS was performed using a HP6890 (Series GC System G1530A) coupled to a HP model 5973 mass selective detector (Agilent Technologies, Palo Alto, CA) fitted with a Carbowax column (30 m x 0.25 mm x 0.25 µm). The method used for grape berries and must was adapted from the procedure described by Sun *et al.* (2011), and for the base and sparkling wines the method was adjusted from Carlin *et al.* (2016). Mass spectrometer parameters were set at 70eV, 230°C (EI source temperature) and 150°C (quadrupole temperature). The data was analysed and confirmed by Agilent MassHunter Qualitative Analysis software (version: B.07.00), NIST library, and Kovats retention index.

Statistical Analysis

The data were expressed by the areas of volatile compounds and then employed Principal Component Analysis (PCA), using normalized data, log transformation and pareto scaling, in addition to other multivariate analyses ing MetaboAnalyst 4.0 software.

Results and Discussion

Volatile compounds in free form were evaluated by HS-SPME/GC-MS for all specimens according to the parameters described above. Volatile compounds analysis presented 67, 64, 78 and 68 compounds, respectively, in grape berries, must, base wine and sparkling wine in the 5 vine-spacing systems evaluated. In order to compare the specimens and perform multivariate analysis, the volatile compounds were clustered and distributed in classes based on the chemical structure, biosynthesis, and yeast metabolism, as follows: volatile sulfur, sulfides, volatile fatty acids, norisoprenoids, monoterpenoids, higher alcohols, glycerol, furanoid, ethyl ester, ethanol, carbonyl (aldehyde, lactone, ketone), branched-chain ester, benzenoid, and acetate ester (Swiegers *et al*., 2005; Schwab *et al*., 2008; Burdockg, 2010; Ugliano and Henschke, 2011; Ilc *et al.*, 2016).

In Figure 1, a relatively low amount of free aromatic compounds in Chardonnay grape berries and must can be noticed, whereas in the base wine and sparkling wine, the peak areas for the odoriferous compounds are higher. Two points can be mentioned for this difference: in grape berries and must the compounds are in the glycosylated, conjugated forms (Ilc *et al*., 2016), and other point discussed by Ugliano and Henschke (2011) is that yeasts play an important role in the biosynthesis and transformation of nutrients, flavour precursors and non-precursors.

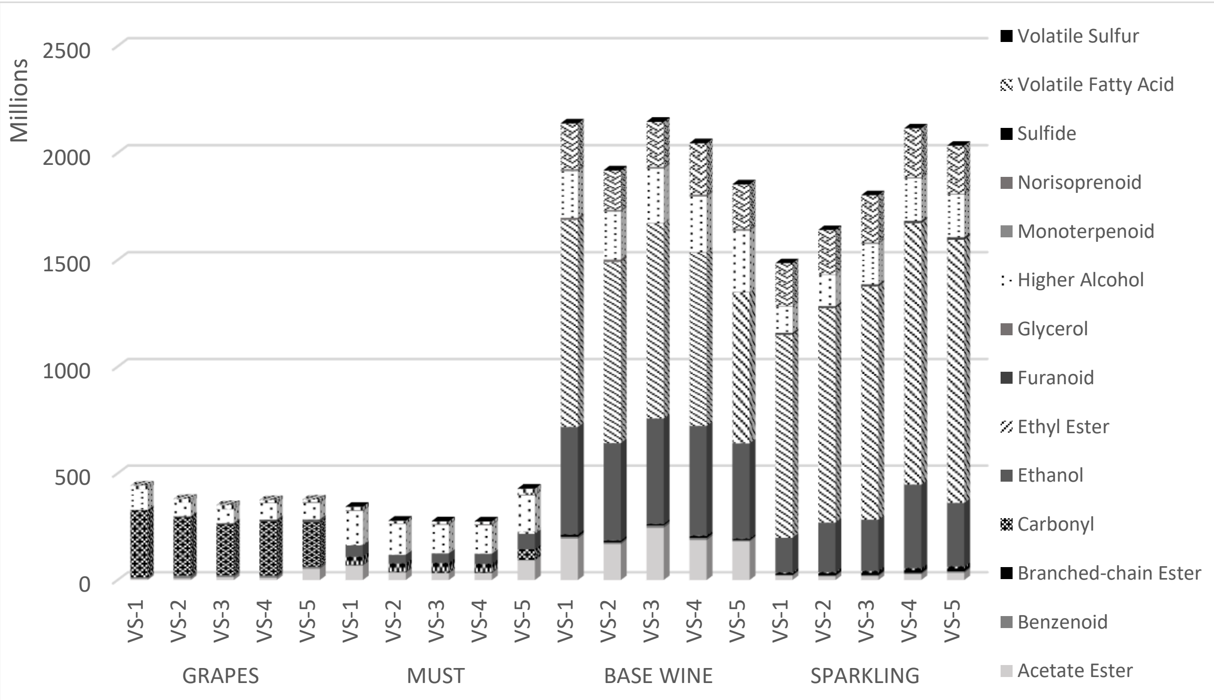

Figure 1: Total peak area according to GC-MS of the volatile compounds separated in classes and evaluated in grape berries, must, base wine and sparkling wine of Chardonnay grape berries in the harvest 2016.

The predominant aroma compounds in grape berries is concerning C6-aldehydes and then C6-alcohols, with this profile being previously reported by Yuan and Qian (2016) in Pinot Noir berries. These products of the lipoxygenase pathway are related to plant defence (stress biotic and abiotic, as diseases and injuries) (Lin *et al.*, 2019). However, Pott *et al.* (2019) described that in plants, lipoxygenase is involved in other process besides defence, such as seed germination and fruit ripening. Although these compounds have herbaceous, green, leafy aroma and are not pleasant (Waterhouse *et al.*, 2016; Lin *et al*., 2019), they participate in the development of other compounds during fermentation. As seen in Figure 1, the amount of carbonyl compounds decreased in the base wine and sparkling wine. According to Waterhouse *et al.* (2016), the main route for the decomposition of carbonyl compounds such as C6-aldehydes during fermentation is the reduction to their respective alcohols and the oxidation to fatty acids may partially occur.

Besides the points raised above, observing the classes of compounds from base wine to sparkling wine (Figure 1), there is an increase of ethyl esters and a decrease of ethanol and acetate esters in the sparkling wines. It can be taken under consideration the formation of ethyl esters occurs mainly enzymatically, with the reaction between ethanol and fatty acid, it probably explains the decrease of ethanol in the sparkling wines. Regarding the decrease of acetate esters, this can be explained by some factors: the employment of strains with high esterase activity, fermentation conditions, temperature, volatilization during storage (Waterhouse *et al*., 2016). Torrens and collaborators (2010) evaluated volatile compounds in the base wine to cava and they described the reduction of acetate esters until they are no more detected. This corroborates the data described in the literature that these compounds can be used as aging markers and, consequently, the loss between base wine and cava of fresh and fruity aromatic descriptors. The fact that acetate esters tend to reduce during storage was also mentioned by Waterhouse *et al.* (2016).

Multivariate analysis was applied to evaluate the sparkling wines of the 5 vine-spacing systems (Figure 2) due to the number of variables analysed, 14 classes of volatile compounds. Principal Component Analysis (PCA) revealed that 60.2% of the data is explained by two components. PC1 (35.5%) seems to discriminate the sparkling wines according to the vine-spacing systems, distinguishing VS-1 and VS-2 (0.5 m and 0.75 m) from the others, which could be confirmed by the heatmap. They show high concentration of norisoprenoid, volatile sulfur, ethyl ester, volatile fatty acid, furanoid, sulfide, on the other hand these compounds are lower in VS-3, VS-4 and VS-5. Another observation in the hierarchical clustering was the distinction of sparkling wine VS-5 (2 m) from VS-3 and VS-4 (1 m and 1.75 m, respectively) based on the prominent presence of acetate ester and low amount of ethanol among the wines of these systems under discussion. These remarks allowed for differentiating the sparkling wines by the spacing between the vines.

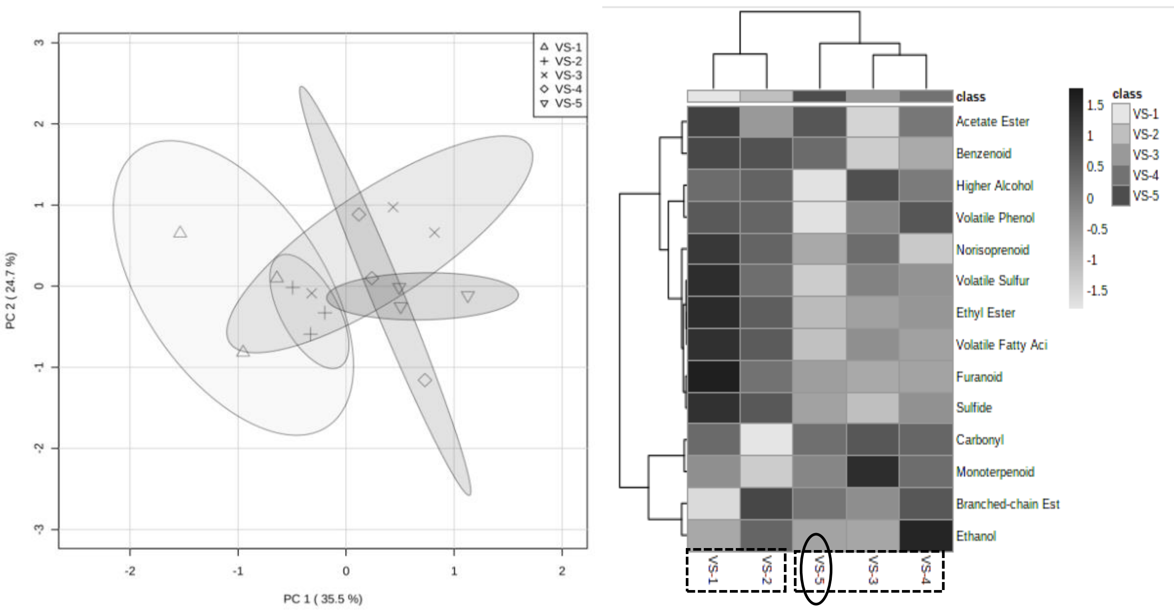

Figure 2: Principal component analysis (left) and hierarchical clustering and heatmap (right) of volatile compounds in the sparkling wine of five vine-spacing systems of *Vitis vinifera* cv. Chardonnay in the south of Minas Gerais/Brazil in 2016.

Although the sparkling wines were differentiated mainly by the vine-spacing, the fermentation conditions, must composition, yeast strain, nutrients, and stage in *sur-lie* could also affect the equilibrium reactions, aroma, colour, and flavour (Ugliano and Henschke, 2011).

In the sparkling wines, the product that will be marketed, the classes of compounds with the largest chromatographic area (Figure 1) are ethyl ester, higher alcohols volatile fatty ester, and ethanol. The compounds formed from ethyl esters and higher alcohols resemble a pleasant, powerful, floral, fruity odours of apricot and pineapple-banana note (Burdock, 2010). Regarding the foam properties, some fatty acid esters have a positive influence, while long chain fatty acids have a negative impact on foam quality as well as alcohol concentration (Martínez-Rodríguez and Pueyo, 2009).

Conclusions

This is the first study concerning volatile composition from Chardonnay cultivar grapes to sparkling wines in Caldas/Minas Gerais (south-eastern Brazil) focusing on the spacing between the grapevines. There are variables that affect berry metabolism (soil nutrition, climatic conditions etc), must composition, fermentation conditions, which could be evaluated in additional harvests and large scale production. In this study, there were differences assessed in the aromatic profile (classes of compounds and compounds area) of the matrices *vs* the vine-spacings studied. Thus, a sensorial analysis would be an additional tool to appraise whether these variances impact the final consumer or whether other aromas in lower concentration has a greater impact in aromatic perception.

Acknowledgments

This investigation was supported by University of São Paulo and FAPESP (Grant number: 2013/07914-8) for laboratory practices (infrastructure, equipments, consumables) and by FAPEMIG (Grant number: CAG-PPM-00301-16) and Vitacea Bra for the vineyard implementation in the agronomical center. Thanks to all EPAMIG staff who helped in the vineyards and in the winery.

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