COMMUNICATION

Volatile Compounds in Red Wines Processed on an Industrial Scale by Short Pre-fermentative Cold Maceration

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Abstract Nero d'Avola and Shiraz grapes were subjected to pre-fermentative cold maceration at 4°C for about 24 h in industrial winemaking, in order to evaluate the effects of the pre-treatment on aroma profile. The volatile compounds were identified by gas chromatography-mass spectrometry and grouped into 14 chemical families 4 and 9 months after bottling. Principal components analysis (PCA) plots of the volatile compounds showed clear separation among wines from the Shiraz and Nero d'Avola varieties. For instance, Shiraz wines were characterized along the PC1 axe by esters of organic acids, norisoprenoids and C_6 alcohols, while Nero d'Avola wines were characterized by acetates and monoterpenic oxides and diols. Moreover, pre-treated samples showed significant $(p<0.05)$ changes in volatile compounds belonging mainly to ethyl esters of straight chain fatty acids, acetates and norisoprenoids. The sensorial analysis showed that cold maceration contributed to development of a different aroma profile during bottle maturation if applied on Nero d'Avola or Shiraz variety.

Keywords Coldmaceration . Nero d'Avola . Shiraz . Volatile compounds. Wine

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Introduction

Pre-fermentative cold maceration (CM) is a technological practice aimed to increasing the extraction of the aromatic compounds present in grape berry pericarp. The increase in aroma compounds, observed after CM, is due to the combined action of increased precursor conjugates concentration and subsequent enzymatic activity of the yeast on these precursors. In not aromatic varieties free forms of many aroma compounds occur in the grapes at concentrations lower than their perception thresholds. Only few papers focused on the effect of CM on red wine's aroma profile (Álvarez et al. [2006;](#page-5-0) Marais [2003](#page-6-0); Reynolds et al. [2001\)](#page-6-0), and its evolution during wine bottle maturation. In this phase, a number of changes affecting sensorial characteristics occur: these changes will depend not only on wine chemical composition, but also on storage duration and conditions (Pérez-Prieto et al. [2003\)](#page-6-0). In this study, Nero d'Avola and Shiraz grapes, considered poor in free aromatic compounds (Vidal & Aagaard [2008](#page-6-0); Esti & Tamborra [2006](#page-5-0)), were processed by short pre-fermentative cold maceration (SCM), on industrial scale. Thus, the aim of this research was to investigate the effect of SCM on aromatic compounds and sensory evaluation during bottling maturation of both wines.

Materials and Methods

Grapes and Winemaking Procedure

Grapes from Vitis vinifera L. cvs. Nero D'Avola and Shiraz (vintage year 2007), were harvested at commercial maturity: (Nero D'Avola, 22.5°Brix, pH 3.4, total acidity 7.2 g tartaric acid/l, Shiraz, Brix 23.8°, pH 3.4, total acidity 7.1 g tartaric

acid/l), in a vineyard located in Mazara del Vallo (TP), Sicily, Italy. The trials were conducted in an industrial plant by two batches for each variety and winemaking technique: pre-fermentative SCM and control. The grapes were destemmed/crushed and collected in four rotary cylindrical fermentation tanks (10,000 l). The batches were then added of SO₂ (10 g/100 l), pectinase enzymes (2 g/100 l) (Lafase HE Grand Cru, Laffort), and tannin (10°g/100 °l) (Tanin VR Supra, Laffort). Two winemaking procedures were applied:

- SCM: 1 day of cold-maceration at $4.0\pm0.5^{\circ}$ C, 12 h to reach 22°C, 6 days of alcoholic fermentation at 22°C plus a 2-day post-fermentation extended maceration
- Control: 6 days of alcoholic fermentation at 22°C, plus a 2-day post-fermentation extended maceration

At the end of malo-lactic fermentation the wines were collected in stainless steel tanks. Samples were bottled after post-fermentation stabilization process (January 2008), then 50°l for each wine sample were bottled and volatile compounds were analysed 4 months (April 2008) and 9°months (September 2008) after bottling. The bottles were stored in aging cellar at 15–19°C, before sampling.

The samples were coded as follows:

- Nero d'Avola: control after 4 months (NDC4), cold-macerated after 4 months (NDM4)
- Control after 9 months (NDC9), cold-macerated after 9 months (NDM9)
- Shiraz: control after 4 months (SC4), cold-macerated after 4 months (SM4); control after 9 months (SC9), cold-macerated after 9 months (SM9)

Analytical Assays

Enological parameters such as: Brix, pH value, total acidity (g tartaric acid/l) were determined according to official AOAC methods (AOAC 1990).

Preparation of Volatile Extracts and GC-MS Analysis

The extraction of the aroma compounds and the gas chromatographic conditions for their analysis were made according to Cocito et al. [1995.](#page-5-0) The identification and determination of the aroma compounds present in the samples (250 ml) were made using GC coupled to a mass spectrometer analysis (Trace MS plus, Thermo Finnigan, USA), and by GC-FID (HP 6890, Agilent), both equipped with a capillary column (Supelcowax 10; 60 m \times 0.25 mm \times 0.25 µm; Supelco, USA). The identification was based on comparing the GC retention times

and mass spectra with authentic standards from Sigma-Aldrich (St. Louis, MO, USA), or on comparing with the spectral data of the NIST and Wiley libraries and the chromatographic data from literature. The quantitative analyses were done assuming the response factors equal to the 2-octanol, used as internal standard.

Sensory Analysis

Ten wine tasting panelists with several years' experience in wine sensory analysis participated in sensory evaluation of wine samples. Each of the two winemaking methods, for each of the two maturation periods and for each variety, was assessed in duplicate presentation replicates in an incomplete block randomised design. The perceived intensity of each aroma attribute was rated using a structured scale ranging from 0 to 5 points.

Statistical Analysis

Each sample was extracted in triplicate, and the concentration of volatile compounds was determined as the mean value of three repetitions. The data were subjected to a monofactorial variance analysis, by a significance of differences of $p < 0.05$ (IBM SPSS Statistics 19). Principal Component Analysis (Unscrambler version 9.8, CAMO 2008) was used to study volatile compounds– sample relationships.

Results and Discussion

A total of 53 volatile compounds were identified in wines, among them, only volatiles with significant differences $(p<0.05)$, due to the SCM or aging, and those with an odour activity value: $(OAV) > 1$ $(OAV) > 1$, were shown (Table 1). OAV is the concentration/threshold ratio that allows an estimate of the contribution of a specific compound essential to wine aroma. Anyway, total volatiles were grouped into 14 chemical families and quantified considering the total volatiles identified by GC-MS (data not reported).

In Shiraz wine SC4 total volatile compounds were equal to 845.45 mg/l, while in Nero d'Avola NDC4, they were equal to 714.36 mg/l. As expected, the dominant volatile compounds in both varieties were higher alcohols, which contribute for about 70% of total volatiles in SC4, and 57% in NDC4, followed by esters and acids. C_6 alcohols, characterized by a "vegetal" and "herbaceous" aroma, such as 1 hexanol and *cis*-3-hexen-1-ol, were present in higher concentrations in Shiraz (Piňeiro et al. [2006\)](#page-6-0), than in Nero d'Avola wines. Acetates of higher alcohols play a modulating role in wine aroma quality (Lebaka et al. [2011;](#page-5-0) Ferreira et al. [1995\)](#page-5-0). By considering that ethyl acetate can give an

Different letters (a,b) indicate significant differences (p<0.05) between the samples belonging to the same variety, with different vinification methods and the same time of maturation in bottle.

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Different letters (x,y) indicate significant differences $(p<0.05)$ between the samples belonging to the same variety, with the same vinification and different time of maturation in bottle

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unpleasant odour at concentration higher than 100 mg/ l (Dubois [1994](#page-5-0)), SCM greatly lowered the amount of this compounds below such value in Nero d'Avola wines. Instead, Shiraz wines showed an ethyl acetate content always below 50 mg/l, and a greater amount in β-phenylethyl acetate (AOV >1), related to rose petal aroma. Ethyl esters of straight chain fatty acids with even number of carbon atoms contribute to young wine aroma and exhibit floral and fruity odours. This class of volatiles increased significantly in NDCM4 compared with NDC4. Ethyl octanoate always with AOV > 1, increased in both short cold macerated varieties after 4 months, while ethyl decanoate increased only in SM4, compared to SC4 $(p<0.05)$. For medium chain fatty acids ethyl esters, precursor availability, namely amino acid must composition (Hernandez-Orte et al. [2002\)](#page-5-0), rather than expression of biosynthetic enzymes, has been suggested to control their formation (Saerens et al. [2008](#page-6-0)). β-Damascenone increased in SM4 compared with SC4, a significant increase was observed also during the aging in SC9 and SM9, as well in NDM9, compared with NDC9 (p<0.05). β-Damascenone can be formed by acid-catalyzed conversion of compounds derived from enzymatic transformation of the lutein (Pineau et al. [2007\)](#page-6-0). Like all glycoconjugated precursors, β-Damascenone precursors may be acid hydrolyzed during wine aging and storage, thus increasing the free β-Damascenone concentration. Overall results, considering the total levels of chemical families of volatile compounds, were analyzed by principal component analysis (PCA). The results of PCA analysis are shown as bi-plot score loading (Fig. 1). The two first principal components described are closed to 77% of the total variance of samples. PC1 explains the 58% of the variance, whereas the PC2 the 19%. PCA showed the potential to discriminate between Nero d'Avola and Shiraz varieties, and at a lower extent, for separate winemaking technique (i.e., SCM), and wine maturation stage (Ivanova et al. [2011\)](#page-5-0). It was apparent from PCA bi-plot a positive correlation among acetates,

Fig. 2 Aroma profiles of Nero d'Avola wine samples after 4 months (a) and 9 months (b) of maturation in bottle. NDC4 control after 4 months in bottle, NDM4 crio-macerated after months in bottle, NDC9 control after 9 months in bottle, NDM9 crio-macerated after 9 months in bottle. Different letters (a, b) indicate significant differences $(p<0.05)$

Fig. 1 PCA bi-plot for Nero d'Avola and Shiraz samples at 4 and 9 months of maturation and amount of volatile compound groups. (1 C_6 compounds, 2 alcohols, 3 fatty acid ethyl esters lipid metabolism, 4 ester of organic acids, 5 acetates, 6 acids, 7 aldehydes, 8 lactones, 9 ketones, 10 monoterpenic alcohols, 11 monoterpenic oxides and diols, 12 phenols, 13 norisoprenoids, 14 amides)

monoterpenic oxides and diols and Nero d'Avola wines. Instead, Shiraz samples were characterized by higher levels of esters of organic acids and phenols.

Sensory Analysis

In Nero d'Avola wines, among the aromatic descriptors individuated by the panel, significant differences (p < 0.05), were found only in sour black cherry descriptor: higher in NDM4 than in NDC4 samples (Fig. [2a](#page-4-0)). During maturation all descriptors decreased greatly in NDC9, whereas in NDM9 aroma descriptors were maintained and violet descriptor increased (Fig. [2b\)](#page-4-0). More differences in aromatic profile were detected between SC4 and SM4 (Fig. 3a). SC4 samples were characterized by

Fig. 3 Aroma profiles of Shiraz wine samples after 4 months (a) and 9 months (b) of maturation in bottle. SC4 control after 4 months in bottle, SM4 crio-macerated after months in bottle, SC9 control after 9 months in bottle, SM9 crio-macerated after 9 months in bottle. Different letters (a, b) indicate significant differences $(p<0.05)$

a higher values for carob, geranium leaves and lees descriptors, than in SCM4 wines $(p<0.05)$. Shiraz samples showed during maturation an increase for almost all descriptors in both samples, in particular, for rose, cooked fruit and carob attributes (Fig. 3b). These descriptors may be associated to some volatile compounds; among them, for instance, β-Damascenone and 2-phenylethanol (both with AOVs >1), were related to cooked fruit and rose odour, respectively.

Conclusions

The results of PCA analysis showed the potential to discriminate between Nero d'Avola and Shiraz varieties, and at a lower extent, for separate winemaking technique, and wine maturation stage

Moreover, SCM on Shiraz and Nero d'Avola varieties had significantly varied their volatile compounds belonging to ethyl esters of straight chain fatty acids, acetates and norisoprenoids classes. The sensorial analysis showed that SCM contributed to development of a different aroma profile during bottle maturation if applied on Nero d'Avola or Shiraz variety.

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