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WINE METABOLOMICS AND SENSORY PROFILE IN RELATION TO TERROIR: A CASE STUDY FOCUSING ON DIFFERENT WINE-GROWING AREAS OF PIACENZA PROVINCE (ITALY)

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Abstract

Aim: In this work, we have optimized a robust methodology for investigating possible correlations between the phytochemical profile of wine and the terroir (including the climate), considering the specific wine-growing area. In particular, the untargeted metabolomic and sensorial profiles of Gutturnio DOC commercial wines (both still and “frizzante” types) from different production areas in the Piacenza province were determined. The geographical areas taken into consideration for this study consisted in Val Tidone, Val Nure and Val d’Arda.

Methods and Results: A metabolomic approach based on ultra-high-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UHPLC-ESI-QTOF) was used to investigate the untargeted phenolic profiles of “Gutturnio” DOC wines from different growing areas, namely Val Tidone, Val Nure, and Val d'Arda, located in Piacenza province (Emilia Romagna region, Italy, 45 °Lat N). In this regard, eight "Gutturnio" wines (both still and “frizzante”) from the same vintage (2016) were compared in order to highlight the impact of terroir on their chemical composition and sensory profile. Besides, correlations between wine chemical composition and climatic data of each of the three valleys have been investigated. The highest content of phenolic acids was recorded in still Gutturnio wines from Val Tidone and Val d'Arda (i.e., 389.9 and 388.2 mg/L, respectively). Both unsupervised and supervised multivariate statistical analyses (hierarchical clustering, principal component analysis, and partial least squares discriminant analysis) of metabolomics-based data allowed the different samples to be clearly discriminated according to the corresponding growing-areas. Interestingly, the most discriminant compounds allowing sample grouping belonged to phenolic acids (such as isomeric forms of diferuloylquinic acid) and alkylphenols (such as 5-heptadecylresorcinol). Besides, the Venn diagram analysis revealed seven common markers belonging to both conditions under investigation (i.e., terroir and winemaking practices). Besides, strong correlations were outlined between flavonoids, lignans, and phenolic acids with climatic data. Finally, sensory analysis allowed clear discrimination between still *vs”* frizzante” Gutturnio wines.

Conclusions: The untargeted phenolic profiling was able to discriminate Gutturnio wine samples according to both terroir and vinification methods. Also, strong correlation coefficients were outlined when considering polyphenol profiles and climatic data, although further ad-hoc studies are needed to confirm this occurrence.

Significance and Impact of the Study: Preliminary and potential correlations have been identified between the phytochemical profile and sensorial quality of Gutturnio wines as related to both growing areas and vinification type.

Keywords: Wine metabolomics, foodomics, terroir, polyphenols, sensory quality

Introduction

In the last decades, studies showing a strong correlation between the phytochemical/sensory profiles of wine and its growing areas (i.e., defined under the term "terroir") are rapidly increasing (Fregoni *et al.,* 1994; Bavaresco, 2003; Lucini *et al.,* 2020). Recently, the application of high-throughput techniques has allowed researchers to focus on the metabolome of grape, must and wine, thus representing a promising methodology for quality and traceability purposes (Flamini *et al.,* 2013; Rocchetti *et al.,* 2018). In this regard, untargeted metabolomics-based approaches (such as UHPLC-QTOF or UHPLC-Orbitrap mass spectrometry strategies) are able to reveal several thousand signals of candidate biomarkers in a single analysis, thus providing high sensitivity and good resolution. Overall, red wine is reported to contain a variety of bioactive secondary metabolites belonging to several phenolic classes, namely flavonoids (i.e. anthocyanins, dihydroflavonols, flavan-3-ols, flavanones, and flavonols), phenolic acids (i.e. hydroxycinnamics, hydroxybenzoics, and hydroxyphenylacetics), stilbenes, and lower-molecular-weight compounds (such as tyrosol derivatives). Starting from the previous considerations, in this work, an untargeted metabolomics approach was used to comprehensively screen the polyphenolic profiles of Gutturnio DOC wines (blend of cvs. Barbera and Croatina) from different growing areas in Piacenza province (i.e., Val Tidone, Val Nure, Val d'Arda), thus providing correlations with the sensory profiles as well. The novelty of the work lies in the proposal of a fast and effective approach (i.e., phenolic profiling with a combination of unsupervised and supervised multivariate statistics) to investigate the phenolic composition of the Gutturnio wines considered, thus highlighting in a preliminary fashion, the potential impact of growing areas on its chemical and sensory quality.

Materials and Methods

In this work, eight commercial DOC Gutturnio wines, both still and "frizzante", vintage 2016 (four from Val Tidone, two from Val Nure, two from Val d'Arda) were analyzed using untargeted methodology based on liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-ESI-QTOF-MS). Sample preparation, sample analysis and data processing were done according to the method described in a previous work from our research group (Rocchetti *et al.,* 2018). Regarding the sensory analyses, a panel of expert judges was requested for the visual, olfactory, gustatory, and retro-olfactory analysis, through the Trialtest Plus, optimized by the Centro Studi Assaggiatori-Brescia. This approach allowed a complete sensory description of the wines under investigation to be obtained with a high degree of reliability. The data obtained were subsequently processed using the Big Sensory Test management software, able to link the different sensory descriptors to the frequency of detection. Climatic data and bioclimatic indices of the year taken into consideration (2016), like Winkler index, Huglin index and Selianinov index, for the three viticultural areas were considered and provided by the management software of the Piacenza Phytosanitary Consortium. Finally, these data have been correlated (by linear regressions) with the main phenolic classes found in the chemical analyses, by using Pearson's correlation coefficients ($p < 0.05$).

Results and Discussion

The untargeted and comprehensive metabolomic profiling allowed us to detect the presence of a great number of phenolic compounds, mainly belonging to flavonoids (72 compounds), tyrosol derivatives (34 compounds), phenolic acids (25 compounds), lignans (21 compounds), and stilbenes (4 compounds). Table 1 shows the total phenolic content (as sum of the different phenolic subclasses) in the different Gutturnio wines. Overall, the total phenolic content ranged from 198.4 mg/L ("frizzante" Gutturnio wine from Val Tidone) up to 389.9 mg/L (still Gutturnio wine from Val Tidone). Interestingly, looking to specific phenolic subclasses, anthocyanins showed the higher cumulative content (expressed as cyanidin equivalents) in two still wines, namely Gutturnio from Val Tidone (123.1 mg/L) and Gutturnio from Val d'Arda (119.7 mg/L). Among the most abundant anthocyanins, we found glycosidic forms of pelargonidin, delphinidin, malvidin, and peonidin. Also, evident when inspecting the metabolomic dataset was an abundance of flavonols (62.7-168.3 mg/L) and phenolic acids (42.4-85.5 mg/L). Overall, the composition revealed by using the untargeted phenolic profiling approach was in strict agreement with data reported in literature for red wine (Granato *et al.*, 2011; Sun *et al.,* 2009; van Leeuwen *et al.*, 2014). However, it is important to underline that, in our analytical conditions, we have not evaluated the distribution of tannins. In this regard, it is known that the presence of a proper ratio between native anthocyanins and tannins could affect the color stabilization of wines because of oxidation reactions during storage and aging (Picariello *et al.,* 2017).

As can be observed from Figure 1, the heat map produced from unsupervised cluster analysis (built considering the fold-change variation of each metabolite across sample replicates), resulted in three main sub-groups: the

first cluster consisted of Gutturnio wines from Val Tidone area, whilst the remaining samples (i.e., from Val Nure and Val d'Arda) showed a similar phytochemical profile. Also, the growing area was highlighted as a hierarchically major discrimination factor when compared to the vinification type, thus allowing to postulate a higher impact of the terroir on the secondary metabolites profile of wines under investigation, although further *ad-hoc* works based on a higher sample dataset are strongly required to include more variability and to confirm our preliminary findings. Interestingly, the same analytical approach (i.e. untargeted phenolic profiling following by multivariate statistical analyses) has been previously applied on white wines from different geographical areas (Rocchetti *et al.,* 2018), thus confirming the potential of metabolomics as related to the confirmation of chemical quality and traceability of wine. Besides, in a previous work, Izquierdo-Llopart and Saurina (2019) demonstrated that polyphenolic profiling combined with a chemometric data analysis (i.e. principal component analysis) resulted as an excellent approach for potential authentication purposes of wines.

Table 1: Total phenolic content per subclass of compounds considering the eight Gutturnio wines under investigation. Results are provided as mean value (n = 3 replicate bottles) and expressed as mg Equivalent (Eq.)/L. *= Val Tidone; **= Val Nure; ***= Val d'Arda; GSp = Gutturnio "frizzante" type; GS = Gutturnio still type; TPC = total phenolic content.

Wine	Anthocyanins (Cyanidin Eq.)	Flavones (Luteolin Eq.)	Flavonols (Quercetin Eq.)	Alkylphenols (Cardol Eq.)	Tyrosols (Tyrosol Eq.)	Phenolic acids (Ferulic acid Eq.)	Lignans (Sesamin Eq.)	Stilbenes (Resveratrol Eq.)	TPC
GSp*	50.3	12.0	106.3	2.5	24.7	42.4	2.9	5.3	246.5
GSp*	48.3	10.1	62.7	2.9	15.1	53.1	2.2	4.0	198.4
GSp**	77.6	14.4	125.9	2.8	17.4	82.6	2.9	5.7	329.4
GSp***	78.3	14.7	124.3	4.9	18.8	75.3	3.1	4.7	324.2
GS*	123.1	12.1	142.6	2.1	15.9	84.2	3.3	6.8	389.9
GS*	69.5	6.7	68.2	2.9	10.4	46.1	2.1	6.9	212.9
GS**	66.5	17.1	168.3	5.3	19.6	83.2	3.2	8.8	372.1
GS***	119.8	20.3	132.9	3.0	22.9	85.2	3.0	0.5	388.2

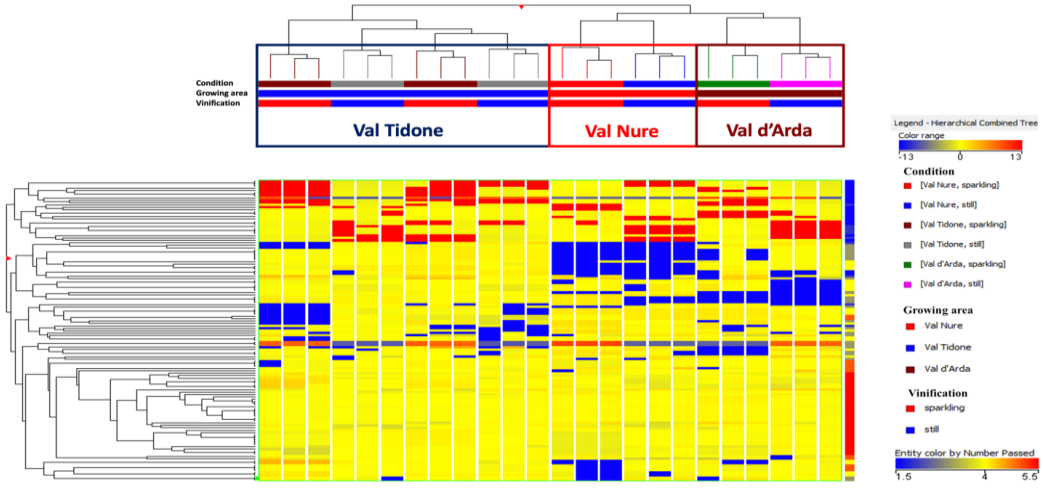

Figure 1: Non-averaged unsupervised hierarchical cluster analysis (HCA) of the phenolic profile of Gutturnio wines (similarity: Euclidean; linkage rule: Ward).

Thereafter, two PLS-DA supervised prediction models were built to better account for chemical markers of the differences in the phenolic profile, when considering both the growing area and the vinification type (data not shown). Overall, we found 43 compounds possessing the highest discrimination potential ($p < 0.05$) as a function of the growing area, whilst 30 compounds were highlighted as most discriminant variables ($p < 0.05$) as a function of the vinification method. Also, the most discriminant compounds regarding the different growing areas were

phenolic acids (mainly hydroxycinnamates) and alkylphenols (such as 5-heptadecylresorcinol). Interestingly, these compounds were majorly abundant in Gutturnio wines from Val Tidone and Val d'Arda when compared to those from Val Nure. Interestingly, few data are actually available in literature regarding the distribution of alkylphenols in red wine, therefore further works based on targeted metabolomics-based approach are mandatory to structurally confirm these potential marker compounds.

The synthesis and accumulation of phenolic compounds are greatly influenced by the environment conditions, including light, air temperature, altitude, soil type, water availability, nutritional status, disease incidence and other developmental processes (Brighenti *et al.,* 2017). In this work, regarding potential correlations between the climatic data of 2016 and phytochemical composition highlighted by UHPLC-QTOF mass spectrometry, we found intriguing results. Overall, flavones, flavonols, lignans, and phenolic acids were found to significantly increase ($p < 0.05$) with the corresponding decrease of rainfalls and increase of temperature (mainly after May-June). The precipitation is an important climatic factor, able to affect the synthesis of phenolic compounds in the skin of the grapes. Typically, in places where there are high rainfall levels, there is also a stress for the plant (e.g. fungal diseases), thus promoting the production of polyphenols as defense mechanism (Bavaresco *et al.,* 2016; Meng *et al.*, 2017). However, in most of wine-producing regions in the worldwide, excessive rainfall during grape ripening dramatically affects grape berry quality (Meng *et al.,* 2017). On the other hand, the incidence of solar radiation in grape is also reported to contribute to the activation of secondary metabolism and the accumulation of phenolic compounds, especially flavonoids (Brighenti *et al.,* 2017). Finally, the results of the sensory analysis in the case of "frizzante" wines (Figure 2A) showed that samples from Val Tidone had the highest levels of color saturation and structure (score 7/9), while the wines from Val Nure have the highest roundness perception and were found to be the most aromatic characterized by both fruity notes of black cherry, spicy and vegetal wood notes. Little biochemical notes like aspartame and moldy wood are perceived in the Val d'Arda wines.

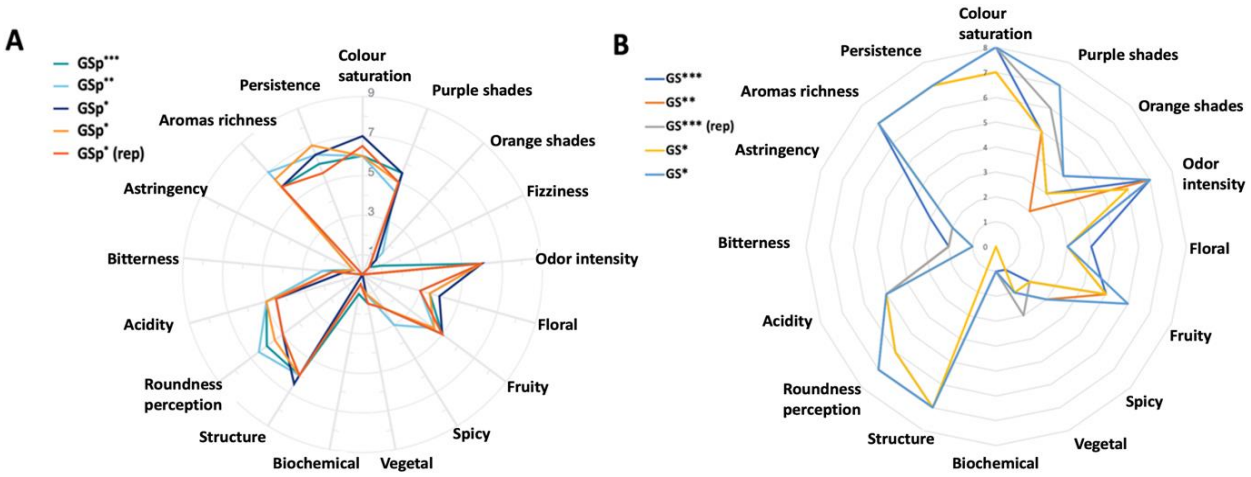

Figure 2: Sensory analysis results when considering four Gutturnio DOC "frizzante" wines (A) and Gutturnio DOC still wines (B) under investigations. *= Val Tidone; **= Val Nure; ***= Val d'Arda; GSp = Gutturnio "frizzante" type; GS = Gutturnio still type.

The results of the sensory analysis in the case of still wines (Figure 2B) showed that wines from Val d'Arda had the highest levels of color saturation like orange shades and roundness perception, while the samples from Val Tidone have the highest aromatic notes like fruity notes (black cherry, red fruits) and spicy notes like vanilla.

Conclusions

In this preliminary work, starting from the untargeted metabolomics, phenolic compounds with pH and acidity, coupled with sensory analysis and climatic data showed a promising and potential discrimination of Gutturnio DOC wine profiles belonging to different growing areas in the Piacenza province. Further analysis with a higher number of samples taken from the three Piacenza areas and a greater number of vintages will be conducted.

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