CHEMICAL CHARACTERISTICS OF WINE MADE BY DISEASE TOLERANT VARIETIES

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INTRODUCTION

Vitis vinifera L. is the most widely cultivated *Vitis* species around the world which includes a great number of cultivars. Owing to the superior quality of their grapes, these cultivars were long considered the only suitable for the production of high quality wines. However, the lack of resistance genes to fungal diseases like powdery and downy mildew (*Uncinula necator* and *Plasmopara viticola*) which were introduced from North America to Europe at the end of the 19th century, makes it necessary the application of huge amounts of plant protection products in vineyard. Recently, the continuous use of these chemical products and the growing awareness of their negative consequences on environment and human health have led to search for alternative and low impact strategies to control the major grapevine pathogens. In this contest, new disease tolerant varieties of *V. vinifera* represent one of the most promising tools for a more sustainable viticulture. Indeed, these varieties combine wine quality and resistance to pathogens since they have been obtained by crossing European grapevines characterised by high wine quality and American genotypes with a high resistance to pathogens. This explains why disease tolerant varieties are nowadays receiving great attention which is related to their great potential to significantly reduce the application of chemical products as well as to have a direct impact on the production costs allowing significant cost savings for winegrowers. Nevertheless, these varieties still suffer from negative prejudice related to the poor wine quality of the first varieties developed at the beginning of the 20th century.

The disappointing organoleptic characteristics of the first hybrids didn't meet the expectations of winegrowers and consumers contributing to the assumption that also the new disease tolerant varieties produce low-quality wines. In particular, undesirable flavour compounds represent the main concern as regards disease disease tolerant varieties (Fig. 1). Studies have reported that some of these compounds are mainly attributable to *Vitis labrusca* and show fewer occurrences in other American *Vitis* species (Sun, Gates, Lavin, Acree, & Sacks, 2011). As a result, there are no evidences that all wild American species and the resulting hybrids contain foxy aroma compounds. It is worth noting that some undesirable compounds can be detected also in wines from traditional *V. vinifera* varieties in the case of troubles in fermentation. The second issue with disease tolerant varieties regards the anthocyanin profile of red grapevine varieties. Unlike *V. vinifera* varieties, disease tolerant varieties are generally

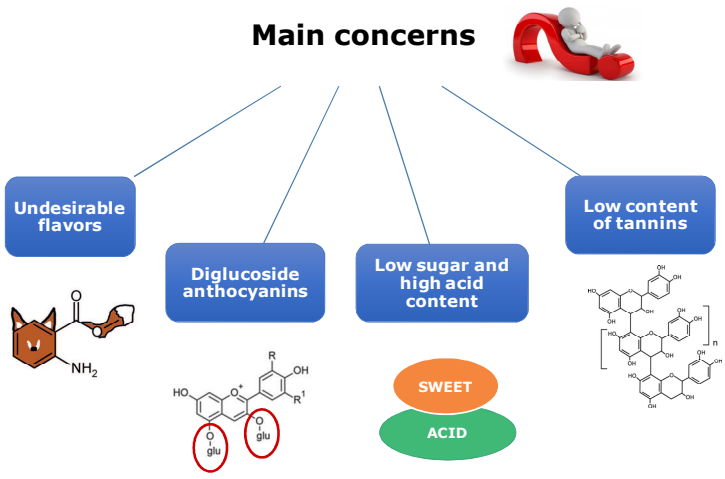

Fig. 1. Schematic representation of the main concerns in disease tolerant varieties.

characterised by the presence of diglucoside anthocyanins which are characteristic of wild *Vitis* species. In fact, these compounds are used as markers for the classification of grapes and wines and in general, grapes and wines from disease tolerant varieties contain high amount of diglucosides. What is more, although no negative evidence of the influence of these compounds on wine quality exists, the maximum acceptable limit of malvidin 3,5-*O*-diglucoside content is 15 mg/L according to the OIV recommendations. Another problem concerns the sugar and acid contents of the grape berries from disease tolerant varieties. It is supposed that these varieties give wines relatively poor in alcohol and not well balanced for acid. Finally, it has also been reported that wines from disease tolerant varieties generally contain low amounts of tannins which are responsible for astringency and bitterness of wine (Manns, Coquard Lenerz, & Mansfield, 2013). Therefore, to promote their use and diffusion for wine production, it is necessary to gain more information about their qualitative traits.

This project aimed to give a more comprehensive and detailed overview about disease tolerant varieties. For this reason, bearing in mind the role of wild *Vitis* genotypes as a source of genetic resistance to pests and diseases used to obtain resistant varieties, the grape metabolomic profile of seven non-*V. vinifera* genotypes in different vintages was explored. Then, our attention has been specially focused to characterise the composition of grape and wine obtained by a wide selection of some promising disease tolerant varieties.

STUDY OF NON-V.VINIFERA GENOTYPES

Wild American species represent an important source of genetic diversity within the *Vitis* genus. Due to the long coevolution with grapevine pathogens, they gained varying degrees of resistance or tolerance to pests and diseases. This remarkable characteristic caused close attention by grape breeders in the last decades of the 19th century: wild genotypes were first used as rootstocks with the aim to provide protection against phylloxera and they were successively crossed with *V. vinifera* varieties in order to obtain new varieties having the positive traits of both species. Despite non-*V. vinifera* genotypes have been long used in grapevine breeding programs, their composition has not been extensively studied. Therefore, the aim of this work was to study the metabolomic profile of seven red non-*V. vinifera* genotypes in different vintages. In particular, five wild American genotypes (*V. californica*, *V. cinerea*, *V. arizonica Texas*, *V. champinii* and *V. andersonii*), two hybrid varieties (41B and Kober 5BB) and two high quality *V. vinifera* varieties, Pinot noir and Cabernet Sauvignon, were considered in this study. The grape berries were collected at the experimental vineyards of Fondazione Edmund Mach in San Michele all'Adige in six different vintages (from 2007 to 2014). A targeted strategy, based on a combination of LC-MS and LC-DAD methods, was adopted to study the metabolomic profile of the grape berries in terms of simple phenols, anthocyanins, proanthocyanidins and lipids (Fig. 2). This scientific work has also been published on Food Research International (Ruocco et al., 2017).

The results obtained highlighted differences in the composition of anthocyanins and simple phenolics among the genotypes studied. Especially, the analysis of anthocyanins in grape berry skins showed that total anthocyanins varied significantly with the genotype but that the anthocyanin profiles for each genotype were relatively stable among years. Not all wild genotypes contained both mono- and di-glucoside derivatives, which were found in variable amounts in non-*V.vinifera* gentoypes. In particular, diglucoside anathocyanins are known to be characteristic of wild genotypes and their occurrence is a quality indicator used in distinguishing *vinifera* from non-*vinifera* grapes and their products. Furthermore, it was observed that the accumulation of proanthocyanidins was not significantly influenced by environmental factors rather than by a strong genotypic influence and that proanthocyanidins of non-*V. vinifera* genotypes were mainly rich in oligomers and short-chain

polymers. With regards to lipids, they have been described for the first time in wild *Vitis* genotypes. It was observed that non-*V. vinifera* genotypes, with the exception of *V. andersonii*, had a higher total lipid content as compared to *V. vinifera* cultivars. In addition to this, the existence of a certain diversity in the lipid composition in the wild *Vitis* genotypes according to the genotype and also with regards to the vintage was observed. In particular, high variability observed in different vintages suggests the strong influence of environmental factors on lipid content.

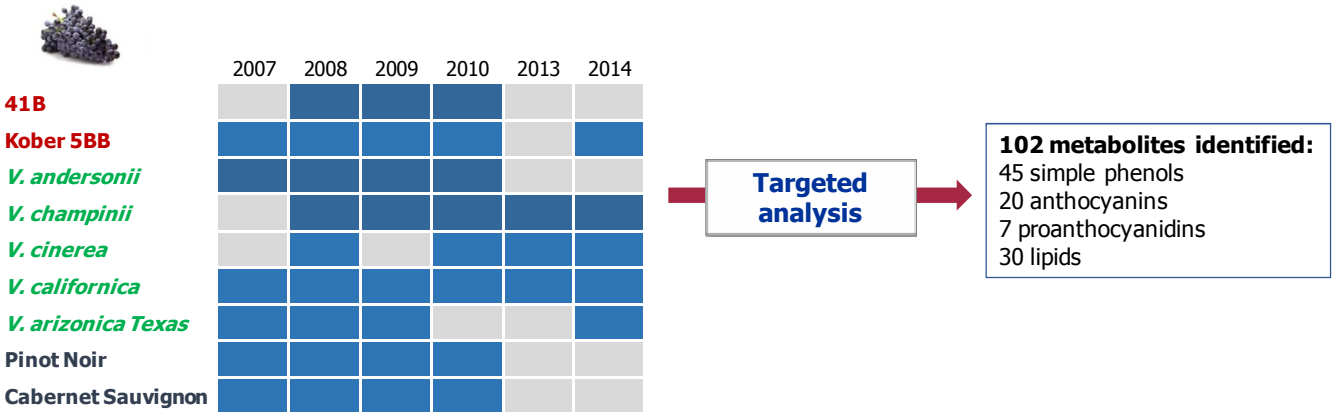

Fig. 2. Schematic overview of the experimental design of the study.

To our knowledge, this study is the first detailed and extended survey of non-*V. vinifera* grape metabolites. The information gained could potentially be useful providing important information for future grapevine breeding programs.
In conclusion, this work provides a comprehensive and systematic survey of the range of variation for all major classes of metabolites in non-*V. vinifera* genotypes. By evaluating these wild genotypes, it was possible to assess the value of this grape germplasm and the information acquired can be very helpful for further breeding programs. To the best of our knowledge this work is the most extended metabolomic profiling study on non-*V. vinifera* genotypes.

ANALYSIS OF DISEASE TOLERANT VARIETIES

Nowadays, a huge number of disease tolerant varieties are available representing one of the most promising tools for low impact, low cost and time-saving viticulture. To date, different studies have investigated the composition of disease tolerant varieties however they have mainly focused on a small group of varieties analysing one subset of chemical compounds. Therefore, in an effort to increase knowledge about these varieties, the aim of this project was to produce a comprehensive study characterising the metabolite profiling of grape and wine of a wide group of disease tolerant varieties which were grown in two experimental fields in two different countries (San Michele all'Adige in Italy and Geisenheim in Germany) in different vintages. The experimental design of this work is illustrated in Figure 3.

ANALYSIS OF GRAPE SAMPLES

Since grape quality is a crucial prerequisite for wine quality, the chemical composition of grapes from a wide selection of some promising disease tolerant varieties grown in the two experimental fields in Italy and Germany in 2013 vintage was investigated. In particular, the red disease tolerant varieties considered were: Regent, Rondo, Prior, Bolero, Nero, Accent, Cabernet Carbon and Cabernet Cortis

the white grape varieties under study included Bianca, Bronner, Muscaris, Phoenix, Helios, Johanniter, Solaris, Souvignier Gris and Jasmine. Grape reference varieties, chosen among *V. vinifera* cultivars of recognized high quality, were considered as well. The red varieties selected were Pinot Noir, Cabernet Sauvignon and Teroldego, while the white ones included Chardonnay, Riesling and Moscato Giallo.

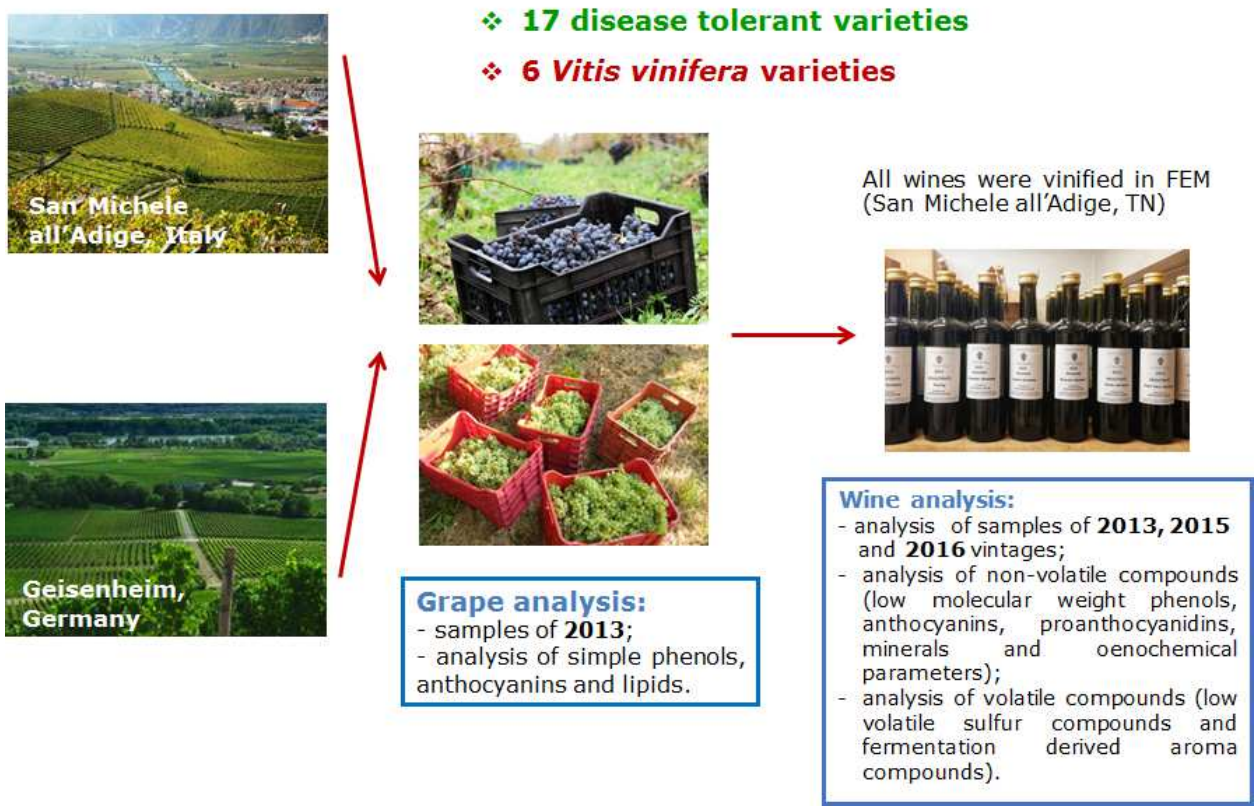

Fig.3 Scheme illustrating the experimental design of the study.

Grape berries were harvested at technological maturity. A targeted strategy by means of LC-MS was used to investigate the phenolic and lipid composition of both red and white grapes of disease tolerant varieties. The phenolic composition of the berries for some disease tolerant varieties under study has not been previously investigated in the literature. Furthermore, to the best of our knowledge, this is the first time that the lipid profiles of these varieties have been described.

The analysis of the composition of grape from disease tolerant varieties revealed a clear difference among the varieties in the total content of anthocyanins and phenolic compounds. As regards the anthocyanin profile, the range of total anthocyanins in red disease tolerant varieties was higher as compared to red references (Fig. 4a). Diglucosides were found in variable amounts in disease tolerant varieties which are known to produce both monoglucoside and diglucoside anthocyanins. Cabernet Carbon was found to be the variety with the highest percentage of diglucosides (more than 50%), followed by Prior, Cabernet Cortis, Rondo, Accent and Regent which contained 20% of diglucosides. However, diglucosides were not detected in the disease tolerant variety Nero. Then, as regards the phenolic compounds it was observed the total amount varied among the varieties studied. Within the red grape samples, Pinot noir and Cabernet Carbon had the highest total amounts of phenolic compounds while Bianca and Jasmine were the white varieties with the highest total content. Furthermore, it was also observed that the grape samples from Germany were generally richer in polyphenols as compared to those from Italy. A positive diversity of the lipid profile among the varieties under study as well as the good stability of the lipid content of a given variety was observed.

In particular, it was observed that the range of variation of these compounds was wider in disease tolerant varieties in comparison to references (Fig. 4b). Nevertheless, the range of variation of total lipids was more similar to that found in *V. vinifera* references in comparison to the American wild genotypes studied which were found to contain higher levels of total lipids. Then, it was possible to note that the amount of total lipids detected was very similar and stable for the varieties cultivated in both countries.

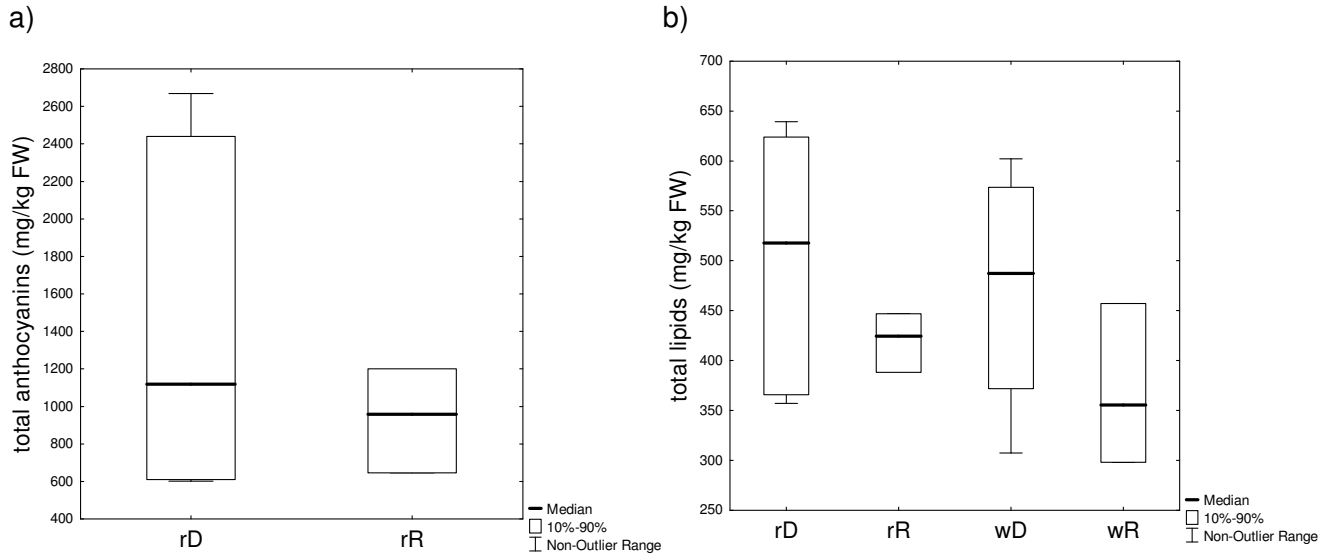

Fig. 4. Boxplots ot the total anthocyanin (a) and lipid (b) content. Abbreviations: red disease tolerant varieties (rD), red reference varieties (rR), white disease tolerant varieties (wD) and white reference varieties (wR).

In conclusion, this study contributes to the chemical characterisation of the grapes from new disease tolerant varieties providing important information for future grapevine breeding programs. What is more, the analysis of polyphenols and lipids provides useful information due to their important role in oenology: polyphenols are among the main contributors to the quality of wine and lipids are key factors in oenology capable to affect the properties of the resulting wines.

ANALYSIS OF WINE SAMPLES

Experimental wines were obtained from seventeen disease tolerant varieties and six reference *V. vinifera* varieties which were cultivated in the two sites (Italy and/or Germany) for the 2013, 2015 and 2016 vintages (Fig. 5). Grapes were harvested at technological maturity (20˚Brix) and were all vinified at pilot scale by applying standard winemaking protocols in the experimental winery of Fondazione Edmund Mach (San Michele all'Adige, Trento, Italy). A total of 92 wines from disease tolerant varieties and *V. vinifera* ones were produced.

A multi-targeted approach using different analytical techniques (GC-MS, HS-GC-PFPD, UPLC-MS/MS, NMR and FT-MIR analysis) according to optimised methods was adopted to investigate the main classes of volatile and non-volatile compounds playing a key role in the organoleptic and sensory properties of wine. Among the non-volatile compounds, the composition of wines in terms of low molecular weight phenols, proanthocyanidins, anthocyanins, minerals and other oenochenical parameters). While, the analysis of the volatile profile included low volatile sulphur compounds and fermentation derived aroma compounds. A total of 134 parameters were identified and quantified.

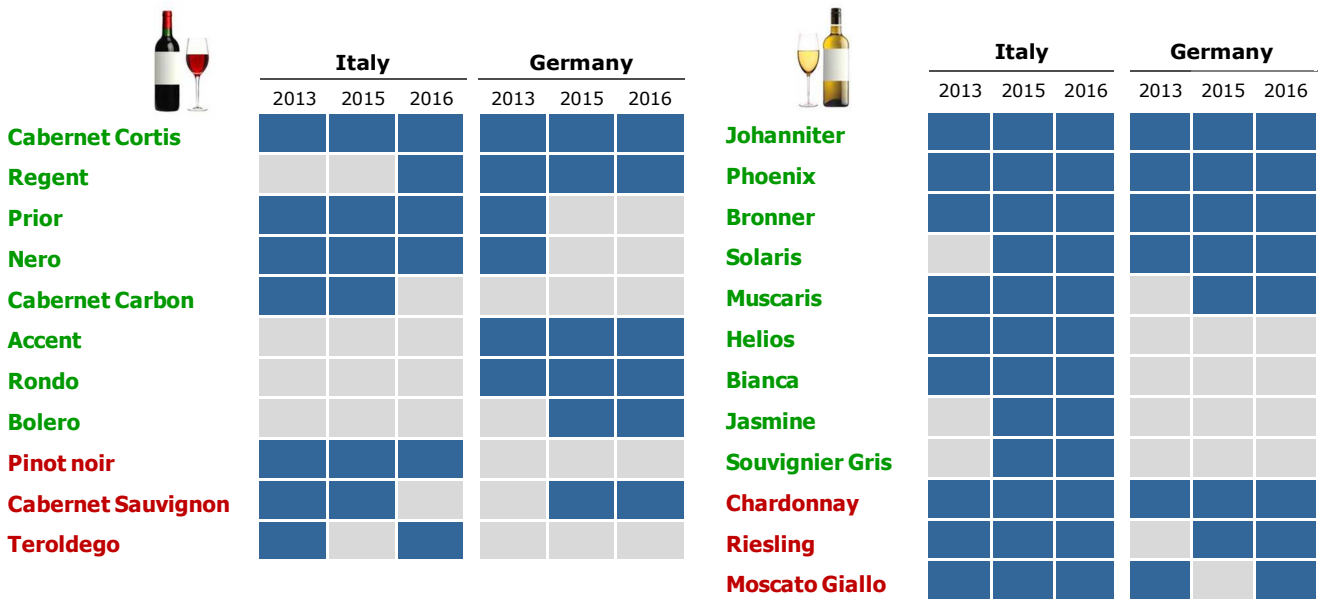

Fig. 5 Schematic representation of the wines investigated in this study. Wines from disease tolerant varieties and reference varieties are indicated in green and red, respectively. Blue colour indicates the samples taken into account while grey colour the samples not analysed.

PCA analysis of the overall chemical composition for both red and white wines showed, as expected, grouping of the samples based on the wine colour. Considering the vintage of grape harvest, PCA analysis also permitted to clearly separate the wine samples into three groups corresponding to the vintages considered. Nevertheless, such a distinct differentiation of the wines also according to where the grape varieties were grown was not found. Rather it was possible to note that wines of the same variety, for the same vintage of both countries were located close. Therefore, vintage had a strong influence on the composition of wines produced from a given grape variety and it was found to be a major factor for wine sample discrimination as previously reported (Pereira et al., 2006; Roullier-Gall et al., 2014). Furthermore, it was observed that the environmental factors at the site of cultivation were not so discriminant to mask the influence of genotype. In addition to this, comparing wines made from disease tolerant and *V. vinifera* varieties, PCA analysis showed a not clear separation between them based on their overall composition, with the exception of anthocyanins. In general, and not considering the anthocyanins in red wines, this study highlighted substantial overlapping of the chemical space of traditional and disease tolerant cultivars.

In particular, the analysis of the non-volatile compounds showed differences in the phenolic composition since i) some of the disease tolerant varieties had a higher amount of total simple phenols in comparison to references; ii) the presence of diglucoside anthocyanins characterised the majority of red wines from disease tolerant varieties wines and, iii) variations in the concentration and composition of tannins were more evident for some disease tolerant varieties than others. In particular, as concerns the main problems associated to disease tolerant varieties (such as low sugar content resulting in wines relatively poor in alcohols and the high acid content) it was observed that the titratable acidity of red wines made from disease tolerant varieties fell into the range of that found for reference varieties, while it was comparable between white wines from disease tolerant varieties and references. As regards the total alcohol, it was found to be similar between both red and white wines made from disease tolerant varieties and references.

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In relation to the other issues of disease tolerant varieties such as the low levels of tannins and degree of polymerization, the results obtained showed that not all disease tolerant varieties contained a lower content of tannins as compared to references. Furthermore, the range for mDP (mean degree of polymerization) and %G (percentage of galloylation) were comparable between references and disease tolerant varieties for both red and white wines. The investigation of the volatile profile of wines made from disease tolerant varieties revealed that the presence of low volatile sulphur compounds at lower concentrations than their aroma detection threshold could contribute to the wine aroma complexity rather than to create undesirable “off-flavours” in the wines analysed.

To conclude this study contributes to increase knowledge and provide a detailed survey of the chemical profile of the wine obtained from a wide selection of some disease tolerant varieties. In particular, it appears that with the exception of anthocyanins, the wine produced from the modern disease tolerant varieties have a general composition closely resembling that of the well know *V. vinifera* wines. On the basis of the results obtained, these new varieties face as equally valuable varieties that are promised to produce high quality wines. Therefore, the information gained may be useful to change the bad reputation of disease tolerant varieties in the wine industry and for future grapevine breeding programs as well as to adapt oenological practices to the new varieties. Up to our knowledge this is the first time that the overall chemical composition of wine produced by a wide selection of disease tolerant varieties grown in climatically different regions in different vintages have been described.

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