

EFFECT OF *BOTRYTIS CINEREA* AND ESCA ON PHENOLIC COMPOSITION OF BERRIES AND WINES¹

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Key words: *Botrytis cinerea*, esca, phenolic compounds

1. INTRODUCTION

Aquitaine region's vineyard represents 17.3 % of the total French vineyard area. This region is subjected to several diseases in particular those related to pathogenic fungi such as *Botrytis cinerea* rot (an epidemic disease with an infectious process) which causes important losses in yield or esca, a grapevine trunk disease (a parasitic model disease with an infectious process). A decision undertaken by the "Grenelle de l'environnement" on October 2007 suggests the increase of surfaces dedicated to organic farming and the reduction of chemical additives. The viticultural world begins to worry about the implementation of this measure. The aim of this study was to determine the quantity of either botrytized grapes or grapes from vine stocks affected by esca which can be used without modifying wine phenolic composition and sensory properties.

2. MATERIALS AND METHODS

Three different batches of microvinification (B, B.ov and E) were carried out in duplicate. For the first batch (B), due to the unfavorable condition for a natural development of *Botrytis cinerea* (Bc), 'Merlot' grapes at maturity were inoculated with a selected strain 213A of *Botrytis cinerea* (Bc) and 4 % and 13 % of these inoculated grapes were added to healthy grapes to attend 9 kg per tank. For the second batch (B.ov), in order to have a natural development of *Botrytis cinerea*, overripe grapes were harvested. 5 % and 20 % of grapes were added with healthy grapes to attend 9 kg per tank. For the third batch (E), 5 %, 25 % 50 % 75 % and 100 % of 'Cabernet sauvignon' grapes from plants affected by esca were added to healthy grapes to attend 9 kg per tank. For each batch, control tank with 100 % of healthy grapes were also carried out.

Total polyphenols, tannins and anthocyanins contents were determined from skin and seed extracts, in must and in wine by the Folin-Ciocalteu, the Bate-Smith and the sodium bisulfite decoloration procedures, respectively. Moreover the proanthocyanidin monomers [(+)-catechin, (-)-epicatechin, (-)-epicatechin-*O*-gallate] and oligomers were identified and quantified by HPLC-UV-Fluo (Chira *et al.*, 2009). The mean degree of polymerization

¹ QUAD. VITIC. ENOL. UNIV. TORINO, 2009-2010

(mDP), the percentage of galloylation (%G) as well as the percentage of prodelfinidins (%P) of grape tannins oligomers were estimated by HPLC-UV-MS (Drinkine *et al.*, 2007) by using the phloroglucinolysis reaction. The anthocyanic composition (glucosides, acetyl glucosides and coumaroyl glucosides) in grape skins extracts were determined by HPLC-MS. Furthermore, sensory analyses were conducted by triangular tests and profile tests with previously trained judges and correlations between sensory analysis data and phenolic compounds analysis were established.

3. RESULTS AND DISCUSSION

3.1 *Botrytis cinerea*

Analysis of musts showed that between the control and the 13 % (B) and 20 % (B.ov) tanks, total anthocyanins significantly decrease for both B and B.ov while total phenol content remained stable. This fact suggested that *Botrytis cinerea* firstly damages the most sensitive molecules which are the anthocyanins and also activate the host defense mechanisms.

Total anthocyanins level is lower in wines made with the higher percentage of botrytised grapes in the two conditions (B and B.ov). Total phenolic content and total tannins are stable. Moreover, HPLC-UV analysis confirmed the results obtained about total anthocyanins, as malvidin-3-*O*-glucoside, the major anthocyanin, decreases strongly as well as for the acetyl glucoside and coumaroyl glucoside forms. Furthermore, we observed an increase of the monomers (+)-catechin and (-)-epicatechin as well as of the dimers B2, B3 and B4. This observation supports the hypothesis that *Botrytis cinerea* may synthesize monomers and/or break interflavanoid linkages by its enzymatic activities.

Phloroglucinolysis reaction showed that for the condition B, the mDP decreased up to 5% and the %G increased up to 30%. For the condition B.ov, these values remained stable. Concerning the %P, it decreased up to 25% for the two conditions. This result showed that *Botrytis cinerea*'s enzymatic action has an impact on skin proanthocyanidins.

Sensory analysis was also undertaken. Triangular tests revealed that only 5% of botrytised grapes can affect negatively the organoleptic properties of a wine. During profile tests, judges were also asked to evaluate wines on the criteria of fruit (nose and mouth), astringency and bitterness using a 0 to 7 point scale. Any significant difference between wine made from healthy grapes and wines containing botrytised grapes were determined ($p \geq 0.05$) for any of the criteria. Botrytised wines were perceived as reduced, earthy, wild mushroom and herbal like.

3.2 Esca

Berries harvested from trunk affected by esca showed a lower rate of total phenolic content, total tannins and total anthocyanins as for skins and for seeds. These results were confirmed by HPLC analysis which a downward trend of proanthocyanidins monomers, a decrease of anthocyanin monoglucosides as well as a lower rate of the mDP and %G in berries from a healthy than in and affected trunk.

Analysis of musts obtained from healthy and affected trunk revealed a lower content of reducing sugar (4%), a higher rate of total acidity (12%), total nitrogen (27%) and malic

acid (8%). Moreover, the total phenolic content increases and total anthocyanins decrease. These observations, which are in accordance with those obtained by Calzarano *et al.* (2004), point to a maturation delay. This fact is possibly due to a loss of functionality of the tiger striped leaves of trunks affected by esca.

Regarding wines, HPLC-UV-fluo analysis demonstrated that between the control and the wine made with 100% of berries from affected plants, the proanthocyanidin monomers tended to increase. Besides, the mDP, the %P and the anthocyanic composition decreased. These results are in agreement with those obtained from skin extract.

Further investigation concerning sensory properties of esca wine firstly showed, by a triangular test, that judges cannot distinguish a wine made from healthy grapes or from affected plants. The overall taste of wine is not altered. In order to characterize each wine, judges were asked to evaluate wines on the criteria of fruit (nose and mouth), astringency and bitterness using a 0 to 7 point scale. ANOVA statistic analysis revealed significant difference between control and 100 % esca wine which is perceived as less bitter and less astringent. Esca wines are also perceived as reduced, phenolic and herbal like.

Abstract

This study showed that *Botrytis cinerea* could degrade the phenolic compounds by its enzymatic activity. It led to a diminution of skin's anthocyanins from 20 % to 50 % and an increase level up to 40 % of individual proanthocyanins, 30 % of the %G and 25% of the %P. Indeed, only 5 % of botrytised grapes are enough to modify negatively the phenolic composition and the organoleptic properties of a wine.

Furthermore, this work revealed that esca caused a delay in ripening and led to modifications on grapes: decrease of the level of reducing sugar (4 %), anthocyanins (40 %), mDP (5 %) and %P (17 %) and increase of individual proanthocyanins. The tasting allowed to bring to light significant differences on astringency and bitterness which decreased with the percentage of affected grapes.

Literature cited

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