Comparison of the free radical-scavenging activity in infected *Oidium* and sound Dolcetto grape cultivar grown in a terroir of Central Italy

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ABSTRACT

The importance of polyphenols, which are present in many vegetables and grapes too, is well-know and documented. Specific research works about the red grape and its derivative juices and wines show that the antioxidant and/or antiradical activities are directly correlated with the complex polyphenol matrix. The content of polyphenols in grapes is clearly affected by agroecological factors: the cultivar, climatic condition, the effect of geographic origin of grapes, soil, chemistry, fertilization, and the degree of maturation. Aim of this work was value the radical-scavenging activity of polyphenols extract from skins and seeds of Dolcetto cultivar derived from infected *Oidium* grape and from the same sound grape. Infested Dolcetto grapes and sound Dolcetto grapes were grown in same terroir and with same training system (cordon spur).
The antiradical activity was determined by assay of free radical– 2,2 Diphenyl-1-picryl-hydrazyl (DPPH) method and total polyphenols content was determined by Folin - Ciocalteu method. Data show significant increase of total polyphenols both in skins and seeds from infected *Oidium* Dolcetto grape with respect to skin and seed total polyphenols from sound grape. To the contrary antioxidant activity calculated as value of ARP ($1/EC50_{P/D}$) in infected grape decreased significantly respecting sound grape. Therefore a possible relationship between the change composition of polyphenols in infected grapes and antioxidant property is suggested. While the antioxidant activity, calculated as micromoles of Trolox/g sample, increase in skins and seeds from infested *Oidium* grape with respect to skins e seeds from sound grape.

KEY-WORDS

Skins - seeds - *Oidium* - antioxidant activity - polyphenols

INTRODUCTION

Antioxidants are generally compounds that are capable, even in small quantities, to prevent or reduce the oxidative destruction of biologically important compounds such as lipids, proteins, and nucleic acid (Halliwell, 1990). In many cases, during the plant metabolism, the production of ROS (Reactive Oxygen Species) is genetically programmed, induced during the course of development and by environmental fluctuations (Foyer, Noctor, 2005 a). The ROS have complex downstream effects on both primary and secondary metabolism. Plant cell produce ROS , particularly superoxide and H_2O_2, as second messengers in many process associated with plant growth and development. Situation which provoke enhanced ROS production have in past been categorized under the heading of "oxidative stress", which in its self is a negative term implying a harmful process, when in fact is probably in many cases

quite the opposite, enhanced oxidation being an essential component of the repertoire of signals that the plants use to make appropriate adjustments of gene expression and cell structure in response to environmental and developmental cues (Foyer, Noctor, 2005 b).
Antioxidant enzymes and metabolites increase under various biotic and abiotic stresses, with their comparatively higher activity in stress tolerant-cultivars, suggesting that higher antioxidant activity imparts tolerance (Bhattacharjee, 2005). The concerted action of low molecular weight antioxidants like anthocyanis (Chalker-Scott, 1999) polyphenols (Sgherri et al., 2004) flavonoids (Hernandez et al. 2004), Carotenoids (Strzalja et al. 2003 and glutathione (Foyer, Noctor, 2005 a)) can effectively scavenge harmful radicals and stabilize lipid oxidation. Polyphenols vine grapes are part of complex mixtures of compounds that may react with radicals mechanism and often interact synergistically or inhibitorily (Saucier, Waterhouse, 1999). The basic mechanism of grape polyphenols is based on the ability to provide the hydrogen atom from their hydroxyl groups to the free radical with high oxidative activity. The importance of polyphenols, which are present in many vegetables and grapes too, is well-know and documented. Specific research works (Wang et al., 1996, Stratil et al., 2006, Garofolo et al., 2009) about the red grape and its derivative juices and wines show that its antioxidant and/or antiradical activities are directly correlated with the complex polyphenol matrix. The content of polyphenols in grapes is clearly affected by agroecological factors: the cultivar, climatic condition, the effect of geographic origin of grapes, soil, chemistry, fertilization, and the degree of maturation. Aim of this work was value the radical-scavenging activity of polyphenols extract from skins and seeds of Dolcetto cultivar derived from infected *Oidium* grape and from the same sound grape. Infested Dolcetto grapes and sound Dolcetto grapes were grown in same terroir and with same training system (cordon spur).

MATERIALS AND METHODS

The study was carried out on a experimental vineyard 14 years old of the Institute of Enology, located in Velletri (Rome) in the Lazio region (Italy) (41° 40.5' N latitude, 12° 50.7' E longitude) at 355 m up the sea level. The trials were made using V*itis vinifera* L. red Dolcetto cultivar. Grapes were harvested at technological maturation. The cultivars had training system to Cordon Spur. From 10 Dolcetto sound grape cluster (vintage 2009), 150 berries were randomly divided in three groups from 50 berries for total polyphenol extraction and DPPH analysis. The extraction solution (buffer to pH 3,2) composition per litre was: tartaric acid 5g; NaOH 1N 22 mL, $Na_2SO_2O_5$ 2 g; 120 mL ethanol 96% and distilled water up to 1000 mL. The skins, and the seeds were manually separated, and incubated with 125 ml of 3.2 pH Tartaric buffer , and left 48 hours for skins , and 6 days for seeds at 30°C. Then the samples were homogenized and centrifuged at 4000 rpm, for 15 min, and the supernatant was collected for analysis. The same procedure was used for Dolcetto *Oidium* infected grape.
Determination of total polyphenols: The SO_2 present in the Skin and seed extracts, was removed by separation using C18 column according to the methods proposed by Di Stefano et al. (1991). Total polyphenol content was determined according to the Folin Ciocalteau method using (+) cathechin as a standard. The results were expressed as mg (+) cathechin / Kg of grape ratio. Free radical scavenging activity was determined by the DPPH spectrophotometric method**.** The radical scavenging activity of the samples against $DPPH^{\cdot}$ was measure according to the method of Sanchez Moreno, et al., 1999 and slightly modified in our laboratory Garofolo et al.2006, as follow: 0.1 mL of sample, opportunely diluted, were

added to 3.9 mL of DPPH solution (0.0473 g/L) and to 3.9 mL of methanol (spectrophotometric blank) .
The kinetic of absorbance of DPPH for different sample concentrations were monitored at constant temperature (23°C), wavelength 515 nm, every 10 minutes, until reaching the plateau (60 min.). For each antioxidant concentration tested, the percentage of remaining DPPH (%DPPH $_{rem}$) at plateau was calculated. The EC50, which represents the amount of antioxidant (i.e. polyphenols) or of sample (skins or seeds) needed to 50% reduction of initial DPPH concentration, was calculated both in moles of total polyphenols per moles of DPPH (EC50 $_{P/D}$) and in grams of sample per litre of extraction solution (EC50 $_{g/L}$) The reciprocal value, 1/EC50, represents a measure of efficiency or antiradical power or activity (ARP). The antiradical activity was also related to a standard solution of Trolox (EC50 = 0.3220 g/L) and calculated as μmoles of Trolox / g of sample (skins or seeds).
Statistical analysis: the statistical analysis was carried out with analysis of variance (ANOVA) and the means were compared with Least Significant Difference (LSD) test. For data analysis, the "Statistical package" (version 7.1, StatSoft., Italy) was used.

RESULTS AND DISCUSSION

Data show that during *Oidium* attack the weight of the berries, skins and seeds significantly decreased (tab.1), probably due at the precocious senescence induced from pathogen infection. In agreement to Bhattacharjee, 2005 and Foyer, Noctor, 2005 b, during the pathogenic attack the grape produce a large quantities of reactive oxygen species (ROS) as response of the biotic stress. ROS play a critical role during the natural course of senescence and during oxidative stress. Lipid peroxidation is a inherent feature of a senescing cell and a source of ROS, especially alkoxy, peroxyl radicals and a singlet oxygen ,which are highly toxic.

Tab.1. Weight in g ± ds (standard deviation) of berry skins and seeds of Dolcetto cultivar

Dolcetto grape samples	Mean Weight (g) (50 berries)	Weight Skins (g)	Weight Seeds (g)
Sound	2.22 ± 0.44	14.50 ± 0.30	5.96 ± 0.27
Oidium infested	1.07 ± 0.28	6.68 ± 0.15	4.50 ± 0.15

The values refer to triplicate replication

The fig. 1 shows the total polyphenols in Sound and *Oidium* infected skins and seeds of Dolcetto Grape cultivar. The statistical analysis point out that significant differences ($P \leq 0.05$) between sound skins and *Oidium* infected ones, and between sound seeds and *Oidium* infected ones. The higher value of polyphenols was found in infested skins and seeds in relation to their high biochemical activity during the pathogen infection.

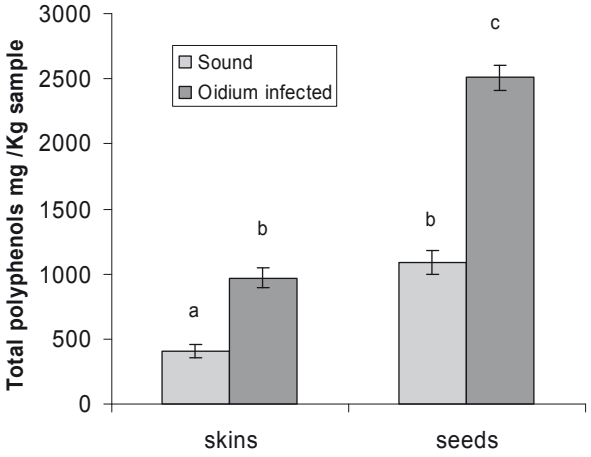

Fig.1 Total polyphenols in sound and *Oidium* infected skin and seeds. Bars indicate standard deviation .The values represent the mean of triplicate replication. Different letters mean significant difference at P≤ 0.05 (LSD test)

Researchs (Wang et al., 1996, Stratil et al., 2006, Garofolo et al., 2009) about the red grape and its derivative juices and wines show that the antioxidant and/or antiradical activities are directly correlated with the complex polyphenol matrix. The fig. 2 shows the antioxidant activity in sound and infected skins and seeds. A significant differences ($P \leq 0.05$) was found between the value of ARP ($1/EC50_{P/D}$) of skins and seeds from infested grape, and skins and seeds from sound grape. The lower antioxidant activity in skins and seeds from infested grape is probably due to a qualitative change of polyphenols compounds during pathogen infection. It is hypothesized that the qualitative change of polyphenols, is also responsible of the specific antiradical activity (ARP ($1/EC50_{P/D}$)) versus the free radical (DPPH).

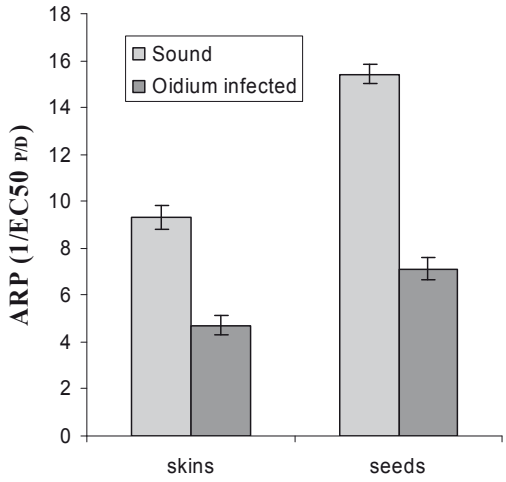

Fig. 2. ARP ($1/EC50_{P/D}$) in sound and *Oidium* infected skins and seeds. Bars indicate standard deviation .The values represent the mean of triplicate replication.

The fig. 3 shows the antiradical activity expressed as µmoles of Trolox/g sample (skins or seed). A significant differences ($P \leq 0.05$) was found between the antioxidant activity, calculated as micromoles of Trolox/g sample, of skins and seeds from infested grape and skins and seeds from sound grape.

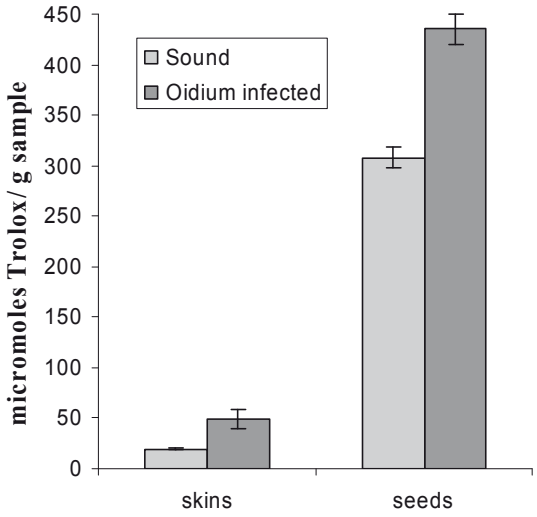

Fig. 3. µmoles of Trolox/g sample (skin or seed) in sound and *Oidium* infected skin and seeds. Bars indicate standard deviation .The values represent the mean of triplicate replication.

In agreement with Balik et al. 2009 that found a positive correlation in infested (grey mould) grape berries between antioxidant activity and polyphenols content, the higher antioxidant activity in skins and seeds from infested grape was found, when expressed as µmoles of Trolox/g sample (skins or seeds). The higher antioxidant activity was due to the increase of the polyphenols content. The results demonstrate the importance of the methodological approaches to express the antioxidant activity as ARP ($1/EC50_{P/D}$), or as µmoles of Trolox/g sample. The first is, well correlated with the molecular nature of polyphenols, the second is more correlated with their unitary concentration of the sample (skins or seeds).

CONCLUSION

The contents of the total polyphenols and the antioxidant activity were investigated in the skins and seeds sound Dolcetto grapes and in skins and seeds of the same grape infested with *Oidium.* The Dolcetto grape cultivar was grown in terroir of Central Italy .The analysis point out that the total polyphenols increase in infected skins and seeds compared to skins and seeds of the same sound grape. The antiradical activity expressed as ARP ($1/EC50_{P/D}$) decreased in infected skins and seeds. Therefore a possible relationship between the change composition of polyphenols in infected grapes and antioxidant property is suggested. While antiradical activity expressed as µmoles Trolox/g sample increased in infested skins and seeds with respect to sound skins and seeds.

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