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trans-Resveratrol, Quercetin, (+)-Catechin, and (−)-Epicatechin Content in South Italian Monovarietal Wines: Relationship with Maceration Time and Marc Pressing during Winemaking

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The concentrations of *trans*-resveratrol, (+)-catechin, (−)-epicatechin, and quercetin were evaluated by means of high-performance liquid chromatography−diode array detection in red wines obtained from Aglianico, Piedirosso, and Nerello Mascalese grapes. The *trans*-resveratrol and epicatechin concentrations did not differ significantly between experimental wines. The concentration of quercetin in Nerello Mascalese wines was more than twice that observed in Aglianico and Piedirosso wines. Nerello Mascalese wines also significantly differed from other wines in the (+)-catechin content, which was significantly higher than those found in the other two wines. During maceration, the maximum extraction of *trans*-resveratrol was reached after 12 days for Aglianico and Piedirosso, after which a decline was observed. On the contrary, in the case of Nerello Mascalese, the concentration of *trans*-resveratrol increased steadily throughout the whole maceration process. After 2 days of maceration, the maximum concentration of quercetin was observed in Aglianico must, whereas the maximum quercetin extraction was reached after 12 days for Piedirosso and 17 days for Nerello Mascalese. The maximum levels of (+)-catechin and (−)-epicatechin were generally observed after 12 days of maceration for all wines, although a decline of (−)-epicatechin occurred after maximum extraction in Aglianico and Piedirosso wines. Following marc pressing, a significant increase in the concentration of *trans*-resveratrol for Aglianico, (+)-catechin and (−)-epicatechin for Piedirosso, and (−)-epicatechin for Nerello Mascalese was observed.

KEYWORDS: ***trans*-Resveratrol; (+)-catechin; (−)-epicatechin; quercetin; Aglianico; Piedirosso; Nerello Mascalese**

INTRODUCTION

Grapes and wines are known to contain large amounts of phenolic compounds of biological interest. Among these, *trans*-resveratrol is one of the most investigated in red wine because of its potential beneficial effects on human health, which include anticancer (*1*−*3*) and antioxidant (*4*−*6*) activities, inhibition of platelet aggregation (*7*), and inhibition of tissue factor expression in vascular cells (*8*). Besides *trans*-resveratrol, the flavonol, quercetin, as well as the flavan-3-ols, (+)-catechin and (−)-epicatechin, are present in red wine in significant concentrations and exhibit antioxidant activities (*9*−*11*). Moreover, it has been reported that quercetin has an anticancer activity (*3*, *12*, *13*), while (+)-catechin and (−)-epicatechin may act as inhibitors of low-density lipoprotein oxidation (*14*, *15*).

The bioavailability of polyphenols is important due to the potential benefits of their consumption on human health. Adsorption in humans and their distribution as various conjugated forms in blood have been shown for resveratrol (*16*, *17*), quercetin (*17*, *18*), catechin (*17*, *19*, *20*), and (−)-epicatechin (*21*, *22*). The determinant role of conjugated metabolites in polyphenol bioavailability is reported in a recent study, which shows a predominant presence of *trans*-resveratrol, (+)-catechin, and quercetin in serum and urine as glucuronide and sulfate conjugates (*23*).

The occurrence of these biological compounds in grapes has stimulated numerous studies focused on understanding the mechanisms that influence their concentrations in wine. Soleas et al. (*24*) suggested that the high intrinsic ability of some grape varieties to synthesize this compound and the positive response to environmental stresses can result in wines with higher levels of *trans*-resveratrol. Similar factors have also been shown to influence the concentrations of catechin, epicatechin, and quercetin (*25*, *26*).

In addition, it is known that the extension of maceration affects the transfer from the solid parts of grape berries to the must, influencing therefore wine phenolic composition. Extended maceration has been reported to improve the extraction

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of catechins (*27*, *28*) and quercetin (*29*). The same trend has been reported for *trans*-resveratrol extraction during the maceration of several grape varieties (*30*, *31*). Nevertheless, in some cases, a decrease during the final stages of maceration was observed (*24*).

The choice of specific winemaking practices aimed at the improvement of wine quality is related to the knowledge of intrinsic physiochemical characteristics of the grape cultivar employed in vinification, as well as to the evaluation of the influence of different practices on the final composition of wine. Because of the increasing demand for high quality wines obtained from South Italian autochthonous grapes, extensive studies are required on the factors potentially influencing the chemical compositions and biological effects of these wines. *Vitis vinifera* cvs. Aglianico and Piedirosso are the most common indigenous red grapes grown in the Campania region, while cv. Nerello Mascalese is an autochthonous red grape grown in Sicily. Both Campania and Sicily are among the South Italian winemaking regions most interested in the production of high-quality red wines.

Given the importance of compounds with antioxidant activity on overall wine quality and consumer acceptance, the aim of the current study was to determine the contents of *trans*-resveratrol, quercetin, (+)-catechin, and (−)-epicatechin in monovarietal wines produced, with the same vinification protocol, from Aglianico, Piedirosso, and Nerello Mascalese grapes and to establish the relationship between the content of these phenols found in the wine and the duration of maceration during winemaking. To evaluate the influence of marc pressing on these antioxidant phenols of red wine, the effect of the addition of pressing after dejuicing was also investigated.

MATERIALS AND METHODS

Wines. Grapes of *V. vinifera* cv. Aglianico, Piedirosso, and Nerello Mascalese were used in this study. Aglianico and Piedirosso grapes were obtained from vineyards located in the area surrounding the city of Benevento, in Campania. In the case of Nerello Mascalese, the grapes were harvested in vineyards located in the Etna region (Sicily). To ensure optimal maturity at harvest, sugar accumulation was constantly monitored during the ripening process, and the harvest was carried out at the last phase of maturity when the sugar concentration in the berries remained constant over 1 week. Fifteen different lots of Aglianico grapes from as many distinct vineyards were processed, while eight and six lots, from as many vineyards, were, respectively, employed for Piedirosso and Nerello Mascalese experiments. The average sugar contents of the grapes used for this experiment were 24.9 (Aglianico), 24.5 (Piedirosso), and 19.9 °Brix (Nerello Mascalese). For winemaking, 100 kg of grapes was destemmed and crushed. The must was treated with $K_2S_2O_5$ (60 mg/kg of grapes). Fermentation took place at 26 °C with indigenous yeast, and the cap was immersed twice a day. Maceration of the pomace lasted 21 days. Successively, the must was pressed (about 8 bar), and finished wine (free + 7% press run after dejuicing) was obtained. For each grape variety, three vinifications were randomly selected in order to study the extraction kinetics of the phenols during fermentation and maceration.

Chemicals. All chromatographic solvents were high-performance liquid chromatography (HPLC) ultra gradient grade and were purchased from Merck (Darmstadt, Germany). *trans*-Resveratrol and quercetin standards were purchased from Sigma Aldrich (Milan, Italy), while (+)-catechin hydrate (purity > 90%) and (−)-epicatechin (purity ≥ 90%) standards were purchased from Fluka (Milan, Italy).

Preparation of Standard Solutions. One hundred milliliters of mixed standard solution containing 2.0 g/L (+)-catechin, 1 g/L (−)-epicatechin, 0.5 g/L quercetin, and 0.084 g/L *trans*-resveratrol was prepared by diluting the standards in methanol. The solution containing the mixed standards was kept at 5 °C until use. The calibration standards were prepared by pipetting 0.5, 1, 5, and 20 mL of the solution containing the mixed standards into four 100 mL glass volumetric flasks, followed by dilution to 100 mL with methanol.

Table 1. Linearity of Assays for Four Phenolic Constituents of Wine Assessed by Regression Analysis

	equation of calibration line	r^2	linearity range (mg/L)
(+)-catechin	$y = 5E - 0.5x - 5.7995$	0.9982	1–200
(−)-epicatechin	$y = 4E - 0.5x + 2.3075$	0.9991	0.5–100
trans-resveratrol	$y = 3E - 0.6x - 0.0344$	0.9999	0.0425–8.5
quercetin	$y = 8E - 0.6x + 3.2744$	0.9992	0.25–50

Determination of Ethanol. The ethanol concentration was determined according to the Official European Regulation 2676/90.

Determination of *trans*-Resveratrol, (+)-Catechin, (−)-Epicatechin, and Quercetin. Separation and quantification were carried out by HPLC, as described by Goldberg et al. (*32*) with slight modifications. The HPLC used was a Shimadzu apparatus (Shimadzu Italy, Milan) LC10 ADVP, consisting of a SCL-10AVP system controller, two LC-10ADVP pumps, a SPD-M 10 AVP detector, and an injection system full Rheodyne model 7725 (Rheodyne, Cotati, CA) equipped with a 20 μL loop. The column used for this separation was an ODS Hypersil column (250 mm × 4 mm, 5 μm particles diameter) equipped with an ODS Hypersil guard column (20 mm × 4 mm) (Thermo Quest, Hypersil Division). Samples of 20 μL of wine or calibration standards were directly injected onto the column. Methanol/acetic acid (97.5/2.5) and water/acetic acid (97.5/2.5) were used as the mobile phase (respectively, solvents A and B). The elution program was as follows: 0 min, 17.5% A, 82.5% B at a flow rate of 0.4 mL/min; 5 min, 22.5% A, 77.5% B at a flow rate of 0.5 mL/min; 30 min, 47.5% A, 52.5% B at 0.5 mL/min until the end of analysis at 50 min. This was followed by a 10 min equilibrium period with the zero-time solvent mixture prior to injection of the next sample. Detection was performed by monitoring the absorbance signals at 306 (*trans*-resveratrol), 369 (quercetin), and 280 nm ((−)-epicatechin, and (+)-catechin). The retention times of the four phenolic compounds, identified by comparison with the pure reference standards, were as follows: (+)-catechin, RT = 11.5 min; (−)-epicatechin, RT = 17.8 min; *trans*-resveratrol, RT = 34.9 min; and quercetin, RT= 46.4 min. For each sample of wine, extractions and analyses were carried out in triplicate.

Calibration Curves, Detection Limit, and Reproducibility of the HPLC Method. The calibration curves obtained by injecting mixed standard solutions containing (+)-catechin, (−)-epicatechin, *trans*-resveratrol, and quercetin were characterized by a correlation coefficient (r^2) > 0.998. The linearity range of the calibration curves was reported in **Table 1**.

The reproducibility of the method used was tested by 10 replicate analyses on two samples of red wine and on two samples of grape skin extract. The coefficient of variation (CV < 4.8%) demonstrated the good reproducibility of the HPLC analysis.

Statistical Analysis. Analysis of variance and Tukey's test were used to interpret differences in means, if any, at the 99% confidence level. Elaborations were carried out by means of JMP 4.0 (Cary, NC).

RESULTS AND DISCUSSION

Mean levels of *trans*-resveratrol, quercetin, (+)-catechin, and (−)-epicatechin of Aglianico, Piedirosso, and Nerello Mascalese wines are reported in **Table 2**.

trans-Resveratrol values ranged between 2.1 and 2.5 mg/L and did not differ significantly between wines obtained from different grape cultivars. All wines analyzed showed a concentration of this potent antioxidant within the range reported in other studies carried out on French, Canadian, Italian, Chilean, Spanish, and Australian wines (*32*−*34*). Nerello Mascalese had a significantly higher mean concentration of quercetin than the two other experimental wines. No significant difference was observed between the mean level of quercetin in Aglianico and Piedirosso wines. The higher concentration of quercetin in

Table 2. Mean Values[a] (mg per L of Wine) and Standard Deviations of *trans*-Resveratrol, Quercetin, (+)-Catechin, and (–)-Epicatechin in Aglianico, Piedirosso, and Nerello Mascalese Wines[b]

	trans-resveratrol	quercetin	(+)-catechin	(–)-epicatechin
Aglianico ($n = 15$)	2.1 ± 0.7 a	8.3 ± 2.6 a	65.0 ± 29.8 a	45.0 ± 17.4 a
Piedirosso ($n = 8$)	2.4 ± 0.4 a	6.6 ± 0.8 a	75.2 ± 6.1 a	42.4 ± 3.5 a
Nerello Mascalese ($n = 6$)	2.5 ± 0.5 a	17.7 ± 1.3 b	195.4 ± 23.5 b	46.6 ± 5.2 a

[a] Values followed by different letters on the column are significantly ($p < 0.01$) different. [b] Number of analyzed wines in parentheses.

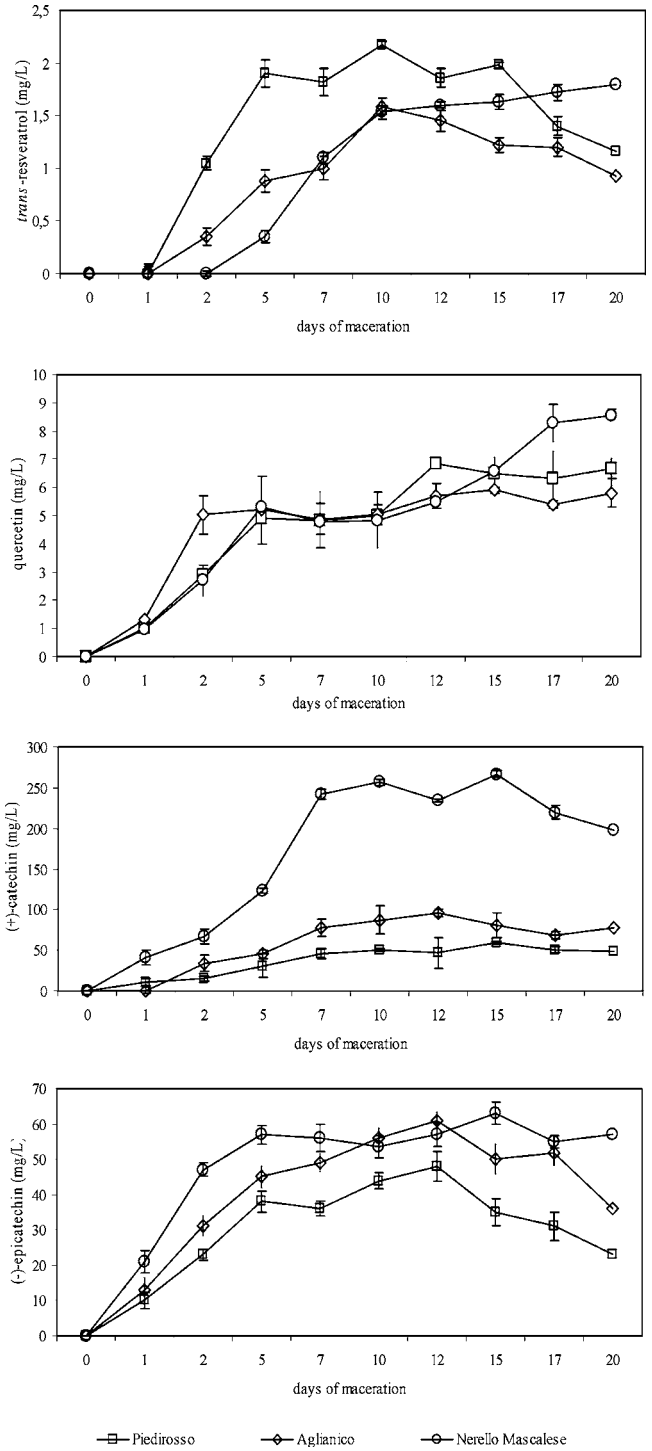

Figure 1. Concentrations of *trans*-resveratrol, quercetin, (+)-catechin, and (–)-epicatechin during skin maceration of Piedirosso, Aglianico, and Nerello Mascalese grapes.

Nerello Mascalese wines could be due to a higher ability of this grape variety to synthesize this flavonol.

Table 3. Ethanol Content (% v/v) during Maceration of Piedirosso, Aglianico, and Nerello Mascalese Grapes

maceration time	Piedirosso	Aglianico	Nerello Mascalese
day 1	1.3 ± 0.8	1 ± 0.5	1.6 ± 0.7
day 3	5.4 ± 1.3	3.9 ± 1.1	4.2 ± 1.2
day 5	7.2 ± 1.1	8.1 ± 1.4	10 ± 2.1
day 7	9.6 ± 1.2	9.9 ± 1.2	11.2 ± 1.0
day 10	14 ± 1.0	12.5 ± 1.1	11.3 ± 0.8
day 12	14.1 ± 1.1	14.6 ± 0.9	11.3 ± 0.9
day 15	14.2 ± 0.9	14.5 ± 0.7	10.9 ± 1.1
day 17	14.4 ± 0.7	14.7 ± 0.9	11 ± 0.6
day 20	14.3 ± 0.5	14.8 ± 0.8	11.2 ± 0.9

Independently of the grape cultivar, (+)-catechin occurred at higher concentrations than the other antioxidant compounds, and its content in Nerello Mascalese wines was more than twice as much as in Aglianico and Piedirosso wines. It was interesting to observe that the level of (+)-catechin detected in Nerello Mascalese wines was similar to that previously observed in Pinot noir wines, described by Goldberg et al. (*26*), as characterized by an extremely high content of this flavanol. Therefore, the high concentration of (+)-catechin in Nerello Mascalese wines may also be due to an intrinsic genetic ability of this grape variety to accumulate this flavanol in berries. Finally, the concentration of (−)-epicatechin ranged between 46.6 mg/L for Nerello Mascalese wines and 42.4 mg/L for Piedirosso wines and did not differ significantly between the three groups of varietal wines studied.

Substantial differences were observed in the ratio (+)-catechin/(−)-epicatechin. This ratio was 4.3 in Nerello Mascalese wines, 1.5 in Aglianico wines, and 1.8 in Piedirosso wines. In previous studies, the biosynthesis of (+)-catechin and (−)-epicatechin was supposed to be strongly dependent on the genetic factors comprising each grape cultivar, and the ratio between these flavanols was used to differentiate the cultivars employed during winemaking (*26*, *35*). As the wines collected for the present study were produced during the same vintage, with the same winemaking protocol, the differences observed in the (+)-catechin/(−)-epicatechin ratio should be due to the genetic characteristics of the grapevine.

Effect of Maceration on the Extraction of *trans*-Resveratrol, (+)-Catechin, (−)-Epicatechin, and Quercetin. To investigate the evolution of the four antioxidants during vinification, samples were taken on a three day basis from three randomly selected vinification lots of each one of the grape varieties studied. The extraction kinetics of phenolic compounds during maceration of Piedirosso, Aglianico, and Nerello Mascalese grapes are reported in **Figure 1**. Because the phenolic release from the solid part of the cluster to must is strongly influenced by the alcoholic content of the medium, the ethanol content of experimental musts is also reported (**Table 3**).

After 10 days of fermentation on grape skins, *trans*-resveratrol reached a maximum concentration in Piedirosso and Aglianico musts while for Nerello Mascalese grapes the highest level of *trans*-resveratrol was observed after 20 days from the beginning

Table 4. Mean Values[a] (mg per L of Wine) and Standard Deviations of *trans*-Resveratrol, Quercetin, (+)-Catechin, and (–)-Epicatechin before Press in Free-Run Wine (FR) and in Mixture (Free + 7% Press Run after Dejuicing)

	Aglianico		Piedirosso		Nerello Mascalese	
	FR	mixture	FR	mixture	FR	mixture
trans-resveratrol	0.9 ± 0.1 a	1.5 ± 0.1 b	1.2 ± 0.3 a	1.7 ± 0.2 a	1.8 ± 0.2 a	2.0 ± 0.1 a
quercetin	5.8 ± 0.1 a	6.4 ± 0.8 a	6.7 ± 0.7 a	6.5 ± 0.9 a	8.6 ± 0.5 a	8.3 ± 0.5 a
(+)-catechin	78.0 ± 4.5 a	95.4 ± 18.3 a	48.8 ± 5.6 a	67.1 ± 6.9 b	198.5 ± 15.6 a	202.3 ± 35.1 a
(–)-epicatechin	36.2 ± 17.6 a	57.4 ± 14.7 a	23.1 ± 3.2 a	42.0 ± 4.7 b	57.1 ± 7.6 a	79.3 ± 6.1 b

[a] Values followed by different letters on the column are significantly ($p < 0.05$) different.

of fermentation, although the maximum extraction had probably still not been achieved. Piedirosso grape skins released more of this compound into the must; the maximum level of *trans*-resveratrol detected was almost 0.5 mg/L higher than the maximum concentration of *trans*-resveratrol in Aglianico and Nerello Mascalese. However, even though there may be differences in grapevine characteristics, in the skin content of *cis*- and *trans*-resveratrol and their glycosides, and in skin cell permeability, the ethanol concentration can also result in different rates of *trans*-resveratrol extraction (*24*, *36*−*38*). Because Piedirosso and Aglianico musts had, even at 10 days of maceration, a higher alcohol content with respect to Nerello Mascalese must, it seems that the main factor determining the time required for the achievement of maximum extraction was the ethanol content of the medium.

When maceration was prolonged for more than 10 days, a decrease in the *trans*-resveratrol content was detected in Piedirosso and Aglianico musts (**Figure 1**), consistent with the behavior observed by Soleas et al. (*24*) during vinification of Merlot grapes. Precipitation, adsorption on yeast lees or marc, and isomerization to *cis*-resveratrol may cause a reduction of the concentration of this compound during winemaking.

During maceration, the content of quercetin increased, consistent with Merida et al. (*29*) who found a positive correlation between maceration time and quercetin extraction (**Figure 1**). The level in the Aglianico must was 5.03 ± 0.69 mg/L after 2 days of maceration, and this value increased slowly throughout the maceration period. In the case of Piedirosso, the quercetin extraction was slower than Aglianico and the maximum extraction (6.5 ± 0.8 mg/L) occurred at 12 days of maceration. Finally, the level of quercetin in Nerello Mascalese must increased during the first 17 days of maceration and the maximum value detected was 8.3 ± 1.1 mg/L.

The content of (+)-catechin in must during maceration of Nerello Mascalese was twice as much as in Aglianico and Piedirosso musts (**Figure 1**). Nerello Mascalese must achieved the maximum level of (+)-catechin extraction after 15 days of maceration. In the case of Aglianico and Piedirosso cultivars, the maximum content of (+)-catechin was observed after 12 days of maceration. As for (−)-epicatechin, the maximum concentration occurred within 15 days from the beginning of maceration for Nerello Mascalese and 12 days for the other two cultivars (**Figure 1**). At the end of the maceration period, the content of (−)-epicatechin in Nerello Mascalese wine was higher than Aglianico and Piedirosso. As observed by Fuleki and Ricardo Da Silva (*39*), the concentration of these flavanols in the grape juice was strongly influenced by cultivar.

Effect of Adding Marc Pressing. Inconsistent with the findings of Mattivi et al. (*30*, *37*) on the variety Lambrusco a foglia frastagliata and of Vrhovsek et al. (*38*) on Pinot noir, a significant increase in the concentration of *trans*-resveratrol was observed following the addition of marc pressing for Aglianico wines (**Table 4**). Pressing also caused a significant increase in the concentration of (−)-epicatechin in Piedirosso and Nerello Mascalese and a minor increase in the concentration of (+)-catechin in Piedirosso, similar to the behavior observed by Mayén et al. (*40*) on Cabernet Sauvignon wines.

In summary, the results obtained in this study showed that within the three cultivars studied, grape variety can influence the concentrations of (+)-catechin and quercetin, while the levels of *trans*-resveratrol and (−)-epicatechin were generally not influenced by the type of grape employed for vinification. Concerning the effect of maceration time on wine phenolics, while the contents of (+)-catechin and quercetin remained generally constant after maximum extraction, a significant decline in the concentrations of *trans*-resveratrol and (−)-epicatechin may occur when the maceration time is excessively prolonged. Finally, an addition of 7% of press run after dejuicing can result in a significant increase in the concentrations of antioxidant phenolics of wine. Further studies on the phenolic contents of these grapes and on their behavior during winemaking will help to clarify the role of different winemaking practices on the antioxidant compounds of wine.

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LITERATURE CITED

(1) Jang, M. S.; Cai, E.; Udeani, G. O.; Slowing, K. V.; Thomas, C. F.; Beecher, C. W. W.; Fong, H. H. S.; Farnsworth, N. R.; Kinghorn, A. D.; Mehta, R. G.; Moon, R. C.; Pezzuto, J. M. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* **1997**, *275*, 218.

(2) Clement, M. V.; Hirpara, J. L.; Chawdhury, S. H.; Pervaiz, S. Chemopreventive agent resveratrol, a natural product derived from grapes, triggers CD95 signaling-dependent apoptosis in human tumor cells. *Blood* **1998**, *92*, 996−1002.

(3) El Attar, T. M.; Virji, A. S. Modulating effect of resveratrol and quercetin on oral cancer cell growth and proliferation. *Anticancer Drugs* **1999**, *10* (2), 187−193.

(4) Frankel, E. N.; Waterhouse, A. L.; Kinsella, J. E. Inhibition of human LDL oxidation by resveratrol. *Lancet* **1993**, *341*, 1103−1104.

(5) Alonso, A. M.; Guillén, D. A.; Barroso, C. G. Mise au point d'une nouvelle méthode de calcul du pouvoir antioxidant des composés phénoliques présent dans le vin. *Bull. O.I.V.* **2000**, *73* (837−838), 794−808.

(6) Acquaviva, R.; Russo, A.; Campisi, A.; Sorrenti, V.; Di Giacomo, C.; Barcellona, M. L.; Avitabile, M. A. Vanella. Antioxidant activity and protective effect on DNA cleavage of resveratrol. *J. Food Sci*. **2002**, *67* (1), 137−141.

(7) Pace-Asciak, C. R.; Hahn, S.; Diamandis, E. P.; Soleas, G.; Goldberg, D. M. The red wine phenolis trans-resveratrol and quercetin block human platelet aggregation and eicosanoid synthesis: Implications for protection against coronary heart disease. *Clin. Chim. Acta* **1995**, *235*, 207−219.
(8) Pendurthi, U. R.; Williams, J. T.; Rao, L. V. M. Resveratrol, a polyphenolic compound found in wine, inhibits tissue factor expression in vascular cells. A possible mechanism for the cardiovascular benefits associated with moderate consumption of wine. *Arterioscler., Thromb., Vasc. Biol.* **1999**, *19*, 419−426.
(9) Frankel, E. N.; Waterhouse, A. L.; Teissedre, P. L. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *J. Agric. Food Chem.* **1995**, *43*, 890−894.
(10) Soleas, G. J.; Tomlinson, G.; Diamandis, E. P.; Goldberg, D. M. Relative contributions of polyphenolic constituents to the antioxidant status of wines: development of a predictive model. *J. Agric. Food Chem.* **1997**, *45*, 3995−4003.
(11) Ghiselli, A.; Nardini, M.; Baldi, A.; Scaccini, C. Antioxidant activity of different phenolic fraction separated from an italian red wine. *J. Agric. Food Chem.* **1998**, *46* (2), 361−367.
(12) Stavric, B. Quercetin in our diet: from potent mutagen to probable anticarcinogen. *Clin. Biochem*. **1994**, *27*, 245−248.
(13) Richter, M.; Ebermann, R.; Marian, B. Quercetin-induced apoptosis in colorectal tumor cells: Possible role of EGF receptor signaling. *Nutr. Cancer* **1999**, *34* (1), 88−99.
(14) Kanner, J.; Frankel, E.; Granit, R.; German, B.; Kinsella, J. E. Natural antioxidants in grapes and wines. *J. Agric. Food Chem.* **1994**, *42*, 64−69.
(15) Hayek, T.; Fuhrman, B.; Vaya, J.; Rosenblat, M.; Belinky, P.; Coleman, R.; Elis, A.; Aviram, M. Reduced progression of atherosclerosis in the apolipoprotein E deficient mice following consumption of red wine, or ist polyphenols quercetin and catechin, is associated with reduced susceptibility of LDL oxidation and aggregation. *Arterioscler., Thromb., Vasc. Biol.* **1997**, *17*, 2744−2752.
(16) Kaldas, M. I.; Walle, U. K.; Walle, T. Resveratrol transport and metabolism by human intestinal Caco-2 cells. *J. Pharm. Pharmacol.* **2002**, *55*, 307−312.
(17) Soleas, G. J.; Yan, J.; Goldberg, D. M. Measurement of trans-resveratrol, (+)-catechin, and quercetin in rat and human blood and urine by gas chromatography with mass selective detection. *Methods Enzymol.* **2001**, *335*, 130−145.
(18) Graef, E. U.; Witting, J.; Mueller, S.; Riethling, A. K.; Uehleke, B.; Drewelow, B.; Pforte, H.; Jacobasch, G.; Derendorf, H.; Veit, M. Pharmacokinteics and bioavailability of quercetin glycosides in humans. *J. Clin. Pharmacol.* **2001**, *41*, 492−499.
(19) Donovan, J. L.; Bell, J. R.; Kasim-Karakas, S.; German, J. B.; Walzem, R. L.; Hansen, R. J.; Waterhouse, A. L. Catechin is present as metabolites in human plasma after consumption of red wine. *J. Nutr.* **1999**, *129*, 1662−1668.
(20) Bell, J. R.; Donovan, J. L.; Wong, R.; Waterhouse, A. L.; German, J. B.; Walzem, R. L.; Kasim-Karakas, S. E. (+)-Catechina in human plasma after ingestion of a single serving of reconstituted red wine. *Am. J. Clin. Nutr.* **2000**, *71*, 108−108.
(21) Natsume, M.; Osakabe, N.; Oyama, M.; Sasaki, M.; Baba, S.; Nakamura, Y.; Osawa, T.; Terao, J. Structures of (−)-epicatechin glucuronide identified from plasma and urine after oral ingestion of (−)-epicatechin: Differences between human and rat. *Free Radical Biol. Med.* **2003**, *34* (7), 840−849.
(22) Baba, S.; Osakabe, N.; Yasuda, A.; Nasume, M.; Takizawa, T.; Nakamura, T.; Terao, J. Bioavailability of (−)-epicatechin upon intake of chocolate and cocoa in human volunteers. *Free Radical Res.* **2000**, *33*, 635−641.
(23) Goldberg, D. M.; Yan, J.; Soleas, G. J. Adsorption of three wine-related polyphenols in three different matrixes by healthy subjects. *Clin. Biochem.* **2003**, *36*, 79−87.
(24) Soleas, G. J.; Goldberg, D. M.; Karumanchiri, A.; Diamandis, E. P.; Ng, E. Influence of viticultural and oenological factors on changes in *cis*- and *trans*-resveratrol in commercial wines. *J. Wine Res.* **1995**, *6* (2), 107−121.
(25) Price, S. F.; Breen, P. J.; Valladao, M.; Watson, B. T. Cluster sun exposure and quercetin in Pinot noir grapes and wine. *Am. J. Enol. Vitic.* **1995**, *46* (2), 187−194.
(26) Goldberg, D. M.; Karumanchiri, A.; Tsang, E.; Soleas, G. J. Catechin and epicatechin concentrations of red wines: Regional and cultivar-related differences. *Am. J. Enol. Vitic.* **1998**, *49* (1), 23−34.
(27) Ribèreau-Gayon, P.; Glories, Y.; Maujean, A.; Dubourdieu, D. In Trattato di enologia. Ed. Ed agricole, 2003.
(28) Sun, B. S.; Pinto, T.; Leandro, M. C.; Ricardo-Da-Silva, J. M.; Spranger, M. I. Transfer of catechins and proanthocyanidins from solid parts of the grape cluster into wine. *Am. J. Enol. Vitic.* **1999**, *50* (2), 179−184.
(29) Merida, J.; Moyano, L.; Millan, C.; Medina, M. Extraction of phenolic compounds in controlled macerations of Pedro Ximenez grapes. *Vitis* **1991**, *30*, 117−127.
(30) Mattivi, F.; Nicolini, G. Influenza della tecnica di vinificazione sul contenuto di resveratrolo dei vini. *L'Enotecnico* **1993**, *7/8*, 81−88.
(31) Pezet, R.; Cuenat, P. Resveratrol in wine: Extraction from skin during fermentation and post-fermentation standing of must from Gamay grapes. *Am. J. Enol. Vitic.* **1996**, *47* (3), 287−290.
(32) Goldberg, D. M.; Karumankiri, A.; Diamandis, E. P.; Soleas, G.; Ng, E. Method to assay the concentrations of phenolic constituents of biological interest in wines. *Anal. Chem.* **1996**, *68*, 1688−1694.
(33) Goldberg, D. M.; Yan, J.; Ng, E.; Diamandis, E.; Karumanchiri, A.; Soleas, G.; Waterhouse, A. L. A global survey of trans-resveratrol concentrations in commercial wines. *Am. J. Enol. Vitic.* **1995**, *46* (2), 159−165.
(34) Mattivi, F. Il contenuto di resveratrolo nei vini rossi e rosati trentini in commercio. *Riv. Vitic. Enol.* **1993**, *1*, 37−45.
(35) Sakkiadi, A. V.; Stavrakakis, M. N.; Haroutounian, S. A. Direct HPLC assay of five biologically interesting phenolic antioxidants in varietal Greek red wines. *Lebensm. Wiss. Technol*. **2001**, *34*, 410−413.
(36) Okuda, T.; Yokotsuka, K. *trans*-Resveratrol concentrations in berry skins and wines from grapes grown in Japan. *Am. J. Enol. Vitic* **1996**, *47* (1), 93−99.
(37) Mattivi, F.; Reniero, F.; Korhammer, S. Isolation, characterization and evolution in red wine vinification of resveratrol monomers. *J. Agric. Food Chem.* **1995**, *43*, 1820−1823.
(38) Vrhovsek, U.; Wendelin, S.; Eder, R. Effects of various vinification techniques on the cobncentration of cis- and trans-resveratrol and resveratrol glucoside isomers in wine. *Am. J. Enol. Vitic.* **1997**, *48* (2), 1997.
(39) Fuleki, T.; Ricardo Da Silva, J. M. Effects of cultivar and processing method on the contents of catechina and procyanidins in grape juice. *J. Agric. Food Chem.* **2003**, *51*, 640−646.
(40) Mayén, M., Mèrida, J.; Medina, M. Flavonoid and nonflavonoid compounds during fermentation and post-fermentation standing of musts from Cabernet Sauvignon and Tempranillo grapes. *Am. J. Enol. Vitic.* **1995**, *46* (2), 255−261.

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