

Effect Of Viticultural And Enological Methods On The Resveratrol Content Of Wines

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Abstract *Resveratrol is a key component of the vine's ability to protect against infection. Wines produced from grapevines synthesizing resveratrol may be expected to contain resveratrol. The resveratrol content in wine is not only dependent on variety of grapes but also viticultural and enological methods applied during the winemaking process. The standard additions method was used for analysis of resveratrol in wine. Reproducibility of the method was verified with three replications and three injections per replication, which were not significantly different. Screening studies showed the effect of viticultural treatments on Cynthiana wine. Resveratrol concentrations were higher in wines produced from a bilateral cordon, non-positioned canopy, 30+10 pruning severity, and six nodes per bearing unit. Cynthiana wine contained higher amounts of resveratrol than Noble wine. Wines produced from grapes exposed to ultraviolet light prior to processing had significantly higher levels of resveratrol. Skin contact time affected the resveratrol level but not significantly. Carbon fining and filtering (0.45p) did not significantly affect resveratrol level. Fining agents and fining agent levels can negatively affect resveratrol levels.*

Introduction

Resveratrol (3,5,4'-trihydroxystilbene) is produced by plants in response to stress conditions, including infection by microorganisms, application of chemicals, injury from light exposure, and change in temperature (Agrios, 1988; Bailey and Mansfield, 1982). Researchers have illustrated the positive effect of alcohol consumption on lipid metabolism and lowering the risk of coronary disease (Bisson et al., 1995; Blue, 1993; Ellison, 1994; Holmgren, 1993; Renaud and Lorgeril, 1992; St. Leger et al., 1979). Seigneur et al. (1990) concluded that increased high density lipoprotein cholesterol was associated with consumption of red wine and not white wine or ethanol. The active ingredient of Japanese and Chinese folk medicine "kojo-kon" was identified as resveratrol (Arichi et al., 1982). Two Cornell University plant scientists, E.H. Siemann and L.L. Creasy (1992), con-elated these findings with research done on consumption of red wine in relation to health benefits.

Analysis of wine indicated that resveratrol concentration is high in red wines, while those produced from white grapes contained smaller amounts of resveratrol (Siemann and Creasy, 1992). Jeandet et al. (1993) confirmed these findings and extended the study to include Burgundy wines. The resveratrol concentration in Japanese red wines was approximately six-fold that in white wines (Okuda and Yokotosuka, 1996). In a global survey, Goldberg et al. (1995) analyzed over 100 white wines and found very few with concentrations greater than 0.1 mg/L.

Further studies were done to determine influence of classical white or red winemaking styles. The researchers concluded that grape skin maceration increased resveratrol levels in wine approximately 10-fold compared to wine made from nonmacerated grape skin (Jeandet et al., 1995). Okuda and Yokotosuka (1996) found that resveratrol concentrations increased during fermentation on the skins but the amount extracted was dependent on the variety and enological conditions.

In several studies, researchers have determined that wines made from Pinot Noir contained the largest amounts of resveratrol (Goldberg et al, 1995; Lamuela and Waterhouse, 1993; Shore, 1995). However, Soleas et al. (1995) studied resveratrol concentrations in wines from Ontario and concluded that resveratrol content was higher in Cabernet Sauvignon than Pinor Noir. Mattivi (1993) analyzed 32 monovarietal wines produced in Italy and found that resveratrol content was ten times higher than measurements in wines from the United States. The highest contents of resveratrol were observed in wines made with Mourvedre, Pinot, and Cabernet Sauvignon varieties, and the lowest in those made with Gamay, Cabernet Franc, or Grenache (Roggero and Archier, 1994).

Objectives The objectives of the experiment were to determine and demonstrate that resveratrol content in wine is affected by varietal differences, viticultural practices (training systems, nodes per bearing unit, pruning severity, and canopy management), ultraviolet light exposure, and etiological methods (skin contact time, fining agents, and filtering).

Materials and methods

Study 1. A reproducibility study was done to evaluate the use of standard additions method for High Pressure Liquid Chromatography (HPLC) analysis of wine. Standard additions method was replicated three times with three injections per replication.

Study 2. A statistical screening study was done using a Quality Control Statistical Analysis System (SAS) to provide initial data to breakdown and optimize factors that most effect the resveratrol content in wine. Wines already made from Cynthiana (*Vitis aestivalis*) from a Fayetteville research plot were analyzed to determine resveratrol content. The viticultural treatment included training system (double curtain vs. bilateral cordon), shoot position (positioned vs. nonpositioned), nodes per bearing unit (3 nodes vs. 6 nodes), pruning severity (30+10 vs. 60+10).

Study 3. A factorial experiment was done involving ultraviolet light exposure, skin contact time, carbon fining, and filtering on wines produced from Cynthiana and Noble grapes. Grapes grown in Altus and Fayetteville, AR were harvested and sent to the University of Arkansas Food Science Department on the same day. Grapes were spread in one cluster layers and exposed to ultraviolet (UV) light using a Black-Ray 254 Bin shortwave UV light for 5 min. The clusters were turned and exposed for 5 additional minutes. The UV light was completely contained within the area. The grapes were held at 21 °C for 20 hours. An equal amount of grapes not exposed to UV light and held at the same temperature were used as a control. The grapes were de-stemmed and crushed. The must was fermented on the skins until dryness and/or extended for one week after dryness and pressed upon completion. The fermentation was completed and the wine was carbon fined at a rate of 0.06/1000 ml of wine and/or filtered into 220 mL bottles for storage.

Study 4. The fining agents, polyvinylpyrrolidone (PVPP), Bentonite, Egg White and Gel+Kieselsol, were used at the following amounts in Cabernet Sauvignon.

Fining Agents	Control	Level	Level	Level
		1	2	3
PVPP(lbs/1000 gal)	0	1.5	3	6
Bentonite(lbs/100 gal)	0	1	2	4
Egg White(lbs/1000 gal)	0	0.625	1.25	2.5
Gel+Kieselsol(lbs/1000 gal)	0	0.2	0.4	0.8

Analytical Procedure

Analysis was conducted on wines produced at the University of Arkansas Food Science Department. Resveratrol concentrations were determined with High Pressure Liquid Chromatography (HPLC) instrumentation using a Hitachi L-4200 UV VIS Detector, L-6200 Intelligent Pump, and D-2000 Chromato-Integrator. The column was a Phenomenex 10p Phenyl 300 x 3.9 mm. A linear gradient of water adjusted to pH 2.5 with 0.6M perchloric acid and methanol was used for the analysis (Ector et al., 1996).

Time (min.)	Water(%)	Methanol(%)
0	90	10
2	90	10
12	0	100
15	0	100
17	90	10
30	90	10

A commercial standard of resveratrol was purchased from Sigma Chemical Co. (St. Louis, MO). The Standard Addition Method (Nielsen, 1994) was used for analysis of samples. A series of 10 mL flasks were used and a constant volume of the unknown (V_u) was added to the flask to determine analyte concentration (C_u). A known volume (V_s) of standard solution of concentration C_s was added to each flask, so that each flask received a unique volume of standard. The flasks had the same total volume (V_T). Each sample in the flask was analyzed. If Beer's Law was obeyed, then measured peak height of each flask was proportional to total analyte concentration as follows: resulting peak height as the dependent variable. Rearranging the equation above gives the following formula:

$$A = k(V_s C_s + V_u C_u / V_T)$$

where:

- V_s =volume of standard
- C_s =concentration of standard
- V_u =volume unknown
- C_u =concentration of unknown
- V_T total volume
- k =proportionality constant

The results were plotted from the assays with the volume of standard added to each flask (V_s) as the independent variable and the

$$C_u \text{ -(measured intercept/measured slope) } (C_s/V_u).$$

This formula was used to determine the concentration of resveratrol in the samples.

Results and discussion

Study 1. Reproducibility of the standard additions method was verified. Three replications and three injections per replication were found to be not significant.

Study 2. Screening studies showed the effect of viticultural treatments on Cynthiana wine. Resveratrol levels were higher in wines produced from a bilateral cordon, non-positioned canopy, 30+10 pruning severity, and six nodes per bearing unit.

Study 3. Cynthiana wine (mean of 0.37306 mg/L) contained higher amounts of resveratrol than Noble wine (mean of 0.09749mg/L). Cynthiana and Noble wines produced from grapes exposed to ultraviolet light prior to processing had significantly higher levels of resveratrol (Table 1). Skin contact time affected the resveratrol but not significantly. Carbon fining and filtering (0.45q) did not affect the resveratrol level in Cynthiana or Noble wine.

Study 4. Fining agents and fining agent levels can negatively affect resveratrol levels (Table 2). Polyvinylpolypyrrolidone significantly decreased resveratrol levels in wine.

Table 1. Effect of ultraviolet (UV) exposure on the Resveratrol concentration of Cynthiana and Noble Wine.

Variety	Treatment	Resveratrol (mg/L)
Cynthiana	UV	0.50361A
	Control	0.02425B
Noble	UV	0.12684A
	Control	0.06662B

Means with the same letter within variety are not significantly different.
LSD for Cynthiana= 0.1328

Table 2. Effect of fining agents on the Resveratrol content of Cabernet Sauvignon.

Fining Agent	Resveratrol (mg/L)
Egg White	0.29468A
Gel+Kieselso	0.27739AB
Bentonite	0.26165B
PVPP*	0.22701C

Means with the same letter are not significantly different.
LSD = 0.0247

*Polyvinylpolypyrrolidone

Conclusions

The standard additions method can be used for HPLC analysis of resveratrol in wine. Viticultural factors affect resveratrol content of Cynthiana wine. Cynthiana wine contains more resveratrol than Noble wine. Ultraviolet exposure of Cynthiana and Noble grapes results in higher resveratrol concentration in wine. Fining agents can lower resveratrol concentration in wine.

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