

Concentration of the Phytoalexin Resveratrol in Wine

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Resveratrol is a phytoalexin found in grapes. Recent research had shown that orally administered resveratrol lowered lipid levels in the liver of rats. Wine had been shown to lower serum lipid levels in humans. The concentration of resveratrol was measured in selected wines using HPLC and spectrophotometry. Geographical origin, variety, growing methods, and winemaking procedures all seemed to affect resveratrol concentration. Analysis of wines indicated that resveratrol may be the active ingredient in wines causing reduction of serum lipids.

KEY WORDS: phenolics, stilbenes, resveratrol, Polyclar-AT

Total phenolic content and the concentration of many individual components of wine have been reported (1,20,21). In this experiment, wines were analyzed for their content of one specific phenolic compound, the phytoalexin *trans*-resveratrol.

Research on *trans*-resveratrol in grapes has been due to interest in its anti-fungal properties (3,4,5,6,7,12,13,14,15,16,18,22). Resveratrol is considered to be a phytoalexin, and its formation in grape leaves has been correlated with disease resistance (12,13). Phytoalexins are a group of plant chemicals of low molecular weight which are inhibitory to microorganisms and whose accumulation in plants is initiated by interaction of the plant with microorganisms. Resveratrol is not unique to *Vitis*, occurring in numerous other genera, e.g., *Veratrum*, *Arachis*, and *Trifolium* (7,8,23). Resveratrol was identified as being an active ingredient of the Japanese and Chinese folk medicine "kojo-kon" (2), which is used for the treatment of suppurative dermatitis, gonorrhoea, favus, athlete's foot, hyperlipemia, arteriosclerosis, and allergic and inflammatory diseases (2,9,10,11). This traditional medicine is composed of the dried, powdered root of Japanese Knotweed (*Polygonum cuspidatum* Sieb. et Zucc.).

Resveratrol was reported in the skin of grapes (4) and, therefore, might be expected to occur in grape products. Resveratrol in wine could be related to therapeutic effects similar to those for isolated resveratrol. Recently, there has been a renewal of interest in wine as a medical treatment for various disorders. It is commonly accepted that moderate consumption of alcoholic beverages results in some protective action against heart disease. Seigneur *et al.* (19) determined that consumption of red wine (Bordeaux), but not white wine or ethanol, induced platelet hypoaggregation and an increase in HDL-cholesterol which have cardioprotective activities in humans. Interestingly, in 1985, Kimura *et*

al. (11) determined that orally administered resveratrol isolated from the roots of *Polygonum cuspidatum* also induced platelet hypoaggregation in an *in vitro* system using rat cells; in 1982, Arichi *et al.* (2) found that resveratrol had lipid-lowering action in rats with hyperlipemia. This study suggests a link between the study reporting decreased lipid deposition in rats' liver from resveratrol and clinical studies showing reduced serum lipid levels in humans from drinking wine. The measurements were carried out using HPLC and spectrophotometry on ethyl acetate extracts of commercially available wines of the United States and France.

Materials and Methods

Trans-resveratrol was obtained and purified from grape canes (6) and confirmed by spectroscopy. Wines used in this study were purchased from retail liquor stores.

Wines were extracted at room temperature three times with ethyl acetate which was combined and washed twice with distilled water. Extractions of distilled water, 15% ethanol, and wine with known amounts of added resveratrol indicated that resveratrol could be extracted completely from any of these systems. Water content of the ethyl acetate extract was reduced by freezing out water overnight and filtering through Miracloth (Behringer Diagnostics, La Jolla, CA) at 0°C to remove ice crystals. The filtered samples were dried by rotary evaporation and taken up in methanol. Half of the sample was injected into an HPLC silica Lichrosorb (Merck, Germany) column and eluted with a 3% methanol/ 97% methylene chloride mobile phase. A fraction was collected at the proper time determined by injection of known *trans*-resveratrol. The collected fraction was exposed for 45 minutes to 730 μ W/cm² ultraviolet radiation with a peak wavelength of 312 nm. The *trans*-resveratrol was converted quantitatively to *cis*-resveratrol to achieve better peak resolution in the second system described below and to be more easily detected by the 280-nm UV detector. The spectra of *trans*-resveratrol and an equimolar solution of *cis*-resveratrol are shown in Figure 1. The UV spectrum for *trans*-resveratrol is [λ_{max} (EtOH) nm (ϵ), 218 (19 100), 306 (26 800), 320 (25 900), and 336 (16 700)] (15); the UV

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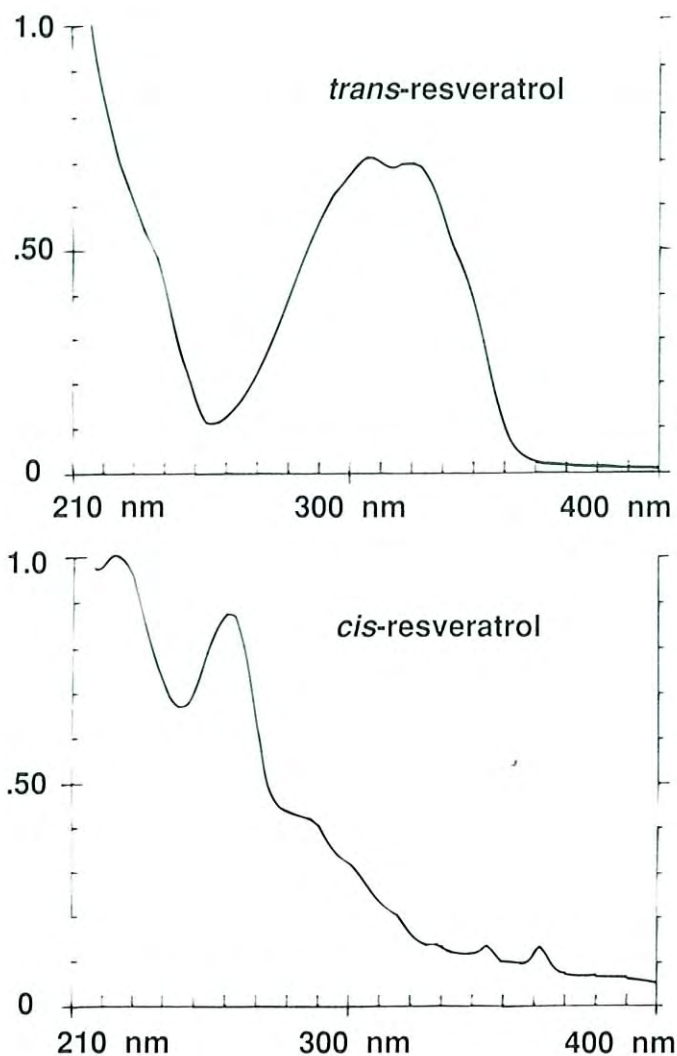


Fig. 1. The absorption spectra of *trans*- and *cis*-resveratrol in ethanol.

spectrum for *cis*-resveratrol is [λ_{\max} (EtOH) nm (ϵ), 225 (26 100), 261 (22 800), 345 (4400), and 362 (4300)]. After UV irradiation, fractions were dried under a stream of nitrogen, dissolved in methanol, and injected into an ODS-II (Whatman, England) reverse phase column and a mobile phase of 70% water/ 29.9% acetonitrile/ 0.1% acetic acid. The resulting peak was measured and the molar content determined by comparison with known samples of pure resveratrol. Identity of the peak was confirmed using spectrophotometry.

Polyclar-AT (GAF Corp., NY) treated wines were passed through a slurry-packed column to represent clarifying wine with this resin. The eluted wines were subsequently extracted and analyzed by the standard procedure.

Polygonum cuspidatum root was extracted using the same procedure described above and also using the method used in the medical studies (17). Pure *trans*-resveratrol was obtained using both methods.

Results

There was a broad range in the concentration of

resveratrol in the different wines analyzed (Table 1). Within red wines, there was a range in concentration from 2.861 to less than 0.003 $\mu\text{mol/L}$. Within the group of white wines there was a range in concentration from 0.438 to less than 0.001 $\mu\text{mol/L}$. One micromole/L corresponds to 171 $\mu\text{g}/750\text{-mL}$ bottle. Within a variety, vintage, or geographical region, there were also large differences.

There were significant differences in resveratrol concentration between New York Chardonnays, all of the same appellation, whereas very little difference between California Chardonnays was detected. All California samples contained low concentrations of resveratrol, and only one of the wines, CA-5, was significantly different from the others. However, one New York Chardonnay, NY-6, had a low resveratrol concentration similar to the California Chardonnays.

Included in the wines analyzed were a red and a white Zinfandel vinted by the same California producer. The red Zinfandel was much higher in resveratrol content than the white Zinfandel.

Three wines, Chardonnays NY-1 and NY-3 and a non-vintage blended California red, were treated with Polyclar-AT, a resin that could be used to remove tannins or pigments from wine. There was significantly less resveratrol in the treated wines as compared to the non-treated wines.

The wines used in the study by Seigneur *et al.* (19) were red and white Bordeaux with no more specific appellation. Four such wines, a red and a white Bordeaux from each of two producers, were analyzed for resveratrol content. It was found that both reds had very high resveratrol contents while both whites had very low contents.

Discussion

Geographical origin appears to be a factor in determining the level of resveratrol in wine. There was significantly more resveratrol in New York Chardonnays than in California Chardonnays. As resveratrol is a phytoalexin, more would be expected to be produced in an area where there is higher fungal disease pressure. Since New York has a more moist growing climate and a higher fungal disease pressure, we expected that New York wines would have more resveratrol than their California counterparts of the same variety. Another indication of a geographical variable is the very high content of red Bordeaux compared to the California Cabernet Sauvignon. However, since the California wine had been aged for five years longer (allowing possible breakdown of resveratrol) and the Bordeaux probably spent a long time on the skins, this may not be a purely geographical effect. Geographical differences could be due to differences in growing practices and winemaking styles rather than to environmental differences.

Winemaking techniques probably make an important contribution to the resveratrol content of wine. An extreme example was the California Zinfandels. The

Table 1. Concentration of resveratrol in selected wines.

Variety	Origin #	Vintage	N	$\mu\text{mole/L} \pm \text{SD}$	
Chardonnay	NY-1	1988	4	0.438 \pm 0.058	a ¹
	NY-1 ²	1988	3	0.059 \pm 0.024	fg
Chardonnay	NY-2	1988	4	0.255 \pm 0.041	b
Chardonnay	NY-3	1988	4	0.220 \pm 0.008	c
	NY-3 ²	1988	2	0.087 \pm 0.012	efg
Chardonnay	NY-4	1988	5	0.159 \pm 0.027	d
Chardonnay	NY-5	1988	4	0.129 \pm 0.004	de
Chardonnay	NY-6	1988	4	0.081 \pm 0.022	f
Chardonnay	CA-1	1988	4	0.064 \pm 0.014	fg
Chardonnay	CA-2	1987	4	0.058 \pm 0.013	fg
Chardonnay	CA-3	1989	8	0.054 \pm 0.011	fg
Chardonnay	CA-4	1989	4	0.049 \pm 0.009	fg
Chardonnay	CA-5	1989	8	0.028 \pm 0.010	g
Red blend	CA	non	1	0.239	
	CA ²	non	1	<0.005	
Red Zinfandel	CA	1988	6	0.361 \pm 0.118	
White Zinfandel	CA	1989	1	0.002	
Red Bordeaux	FR-R1	1987	2	1.367 \pm 0.129	
White Bordeaux	FR-W1	1988	2	0.011 \pm 0.006	
Red Bordeaux	FR-R2	1988	2	2.861 \pm 0.393	
White Bordeaux	FR-W2	1989	2	0.018 \pm 0.004	
Muscadet	FR	1988	2	0.034 \pm 0.005	
Red blend	NY	non	4	1.217 \pm 0.194	
Blush blend	CA	non	1	0.241	
Cabernet Sauv.	CA	1982	1	0.010	
Red blend	CA	non	3	<0.005	
Red blend	NY	non	1	<0.003	
Cayuga White	NY	1988	2	<0.002	
White blend	NY	non	1	<0.001	

¹Wines followed by the same letter are not significantly different at the 0.05 level as determined by the Tukey-Kramer test.

²Wine passed through Polyclar AT before analysis.

N = Number of replicate samples drawn from the same bottle.

red had a much higher resveratrol content than the white. Resveratrol is found in the skin but not in the flesh (4). Therefore, the white wine which is pressed lightly and spends no time on the skins would be expected to have less than the red which is fermented initially on the skins. In a less extreme case, a free run or lightly pressed wine would be expected to have less resveratrol than its full press counterpart. Likewise, a wine which spends more time fermenting on the skins would accumulate more resveratrol. Methods of clarification, such as use of Polyclar-AT resin which binds phenolics, have a clear effect on how much resveratrol remains in the final wine.

If the grapes contain low amounts of resveratrol when they reach the winemaker, the wine will invariably be low in resveratrol. Clearly, how the grapes are grown and what varieties are grown are also important. While no clear vintage effect was found, the weather in a growing season would be expected to play a part. Control of disease pressure could also play a critical role,

though it is not clear what that role is. Finally, the choice of variety seems to be of prime importance. Since resveratrol production potentials of berries varies greatly, with high and low potentials for both reds and whites (4), selection of varieties is needed to have a high resveratrol wine.

While it is not always true that white wines contain less resveratrol than red wines, in the Bordeaux wines we analyzed this appears to be true. Therefore, Seigneur's treatments were potentially valid for investigating the effects of resveratrol rather than unknown phenolics. If the wine used in the study was approximately the same as the red Bordeaux wines analyzed, the daily dose of resveratrol for the subjects consuming red wine would have been approximately 150 to 320 μg while those consuming white wine would have received less than 2 μg per day.

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