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Effect of Enological Practices on the Resveratrol Isomer Content of Wine

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Resveratrol (*trans*-3,5,4′-trihydroxystilbene) is a phytoalexin produced by grapevines in response to fungal infection, particularly to *Botrytis cinerea*, the causal organism for gray mold. This compound is known to occur in grapes as well as in wine and claimed to protect against heart diseases. Two factors that can modify resveratrol levels in wine were studied here: (1) the influence of classical white or red winemaking practices and (2) the effect of grape *Botrytis* levels on the resveratrol content of wines. Analysis of resveratrol was carried out by HPLC and GC-MS. Maceration on the grape skins increased the extraction of resveratrol by *ca.* 10-fold compared to nonmacerated wines. Paradoxically, lower concentrations of resveratrol were observed in wines made from highly *Botrytis* infected grapes than in those vinted from healthy and moderately infected grapes. Finally, this study has clearly established the presence of high quantities of the *cis* isomer of resveratrol in wine, a form only slightly detectable in grapes.

Keywords: *Resveratrol; gas chromatography–mass spectrometry; high-performance liquid chromatography; wine; Pinot noir; Chardonnay blanc*

INTRODUCTION

Resveratrol, as well as other wine phenolics (Waterhouse and Frankel, 1993), has been linked to the reduced cardiac disease rates observed in humans as a result of drinking wine (Seigneur *et al.*, 1990). Though this theory is open to discussion in view of the low concentration of resveratrol in wines (*i.e.* from 0.5 to 10 mg/L in red wines), *in vitro* studies have however shown that this compound may reduce human low-density lipoprotein oxidation (Frankel *et al.*, 1993) and platelet aggregation (Chung *et al.*, 1992). Since the pioneering work of Siemann and Creasy (1992), much interest has been focused upon the analysis of resveratrol in wine, which is a phytoalexin produced by grapevines in response to fungal infection or abiotic stresses (Langcake and Pryce, 1976; Pool *et al.*, 1981; Barlass *et al.*, 1987; Dercks and Creasy, 1989; Jeandet *et al.*, 1991, 1992, 1994a).

Previous investigations have been conducted to determine the resveratrol content in wine by using various analytical methods: HPLC (Siemann and Creasy, 1992; Lamuela-Raventos and Waterhouse, 1993; Mattivi, 1993; Pezet *et al.*, 1994; Roggero and Archier, 1994), GC and GC-MS (Jeandet *et al.*, 1993; Goldberg *et al.*, 1993; Soleas *et al.*, 1993). These studies established that white wines contain lower amounts of resveratrol than red wines (Siemann and Creasy, 1992; Jeandet *et al.*, 1993; Lamuela-Raventos and Waterhouse, 1993; Goldberg *et al.*, 1993). Bearing in mind that this compound is synthesized by grape skin cells (Creasy and Coffee, 1988; Jeandet *et al.*, 1991, 1994a), it has been suggested that resveratrol requires relatively long maceration time on the skins to be extracted (Siemann and Creasy, 1992). As resveratrol is a phytoalexin produced by grape berries in response to pathogen infection (*e.g.* *Botrytis cinerea*, the causal organism for gray mold), the fungal disease pressure in the vineyard, and therefore the resveratrol level in the grapes used for winemaking, was also thought to be a factor in determining the level of resveratrol in wine (Siemann and Creasy, 1992). Finally, we have reported the presence of the *cis* isomer of resveratrol in wine (Jeandet *et al.*, 1993).

From these observations, it appears that winemaking has marked effects on resveratrol levels in wine though it is not clear what is its specific role. The aim of this work was thus to study the influence of various enological practices on a wine's resveratrol content. In this way, we have sought to compare the effect of classical white or red winemaking practices and of *Botrytis* infection of the grapes on the resveratrol content of wines. In addition, we have also investigated the origin of the *cis* isomer of resveratrol in wine.

MATERIALS AND METHODS

Wine Samples. Wines were made at the enological station of the Bureau Interprofessionnel des vins de Bourgogne (B.I.V.B.) in Beaune (France) from grapes of Pinot noir and Chardonnay blanc, hand-harvested in 1993. After destemming the grapes, musts were subjected to different procedures as described below. In all cases, the musts were treated with sulfur dioxide (50 mg/L) and inoculated with 150 mg/L dried RC 212 yeast (*Saccharomyces cerevisiae*). After fermentation, the wines were then filtered, SO_2-adjusted prior to bottling (50 mg SO_2/L, *i.e.* 15 mg/L free SO_2), and stored at 14 °C in the dark. A minimum of three separate experiments were carried out for each procedure.

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Effect of Maceration on the Resveratrol Content of Wine. For this study, four different lots (where bunches of grapes were less than 10% affected by *Botrytis*) were randomly prepared and vinified separately. The first lot of Pinot noir was fermented on the skins and maintained at 25 °C for 7 days. The second lot of Pinot noir was just pressed lightly and spent no time on the skins. The first lot of Chardonnay blanc wines was prepared in the same way as the first lot of Pinot noir (*i.e.* fermentation on the skins at 25 °C for 7 days prior to pressing). The second lot of Chardonnay blanc was prepared in the same way as the second lot of Pinot noir grapes.

Influence of Grape *Botrytis* Levels on the Resveratrol Content of Wine. The effect produced on resveratrol levels when using grapes infected by *Botrytis* was studied. In this way, four different lots were prepared from healthy grapes of Pinot noir and from grapes affected 10, 40, and 80% by *Botrytis*. They were vinted in the same way as described above.

***Cis–Trans* Isomerization of Resveratrol in Wines.** To study whether light-induced isomerization of *trans*-resveratrol may occur during the winemaking process, grapes of the variety Pinot noir were vinted in the dark by classical red winemaking practices (see above). Fruits were also analyzed to determine whether *trans*-resveratrol may be isomerized partially by sunlight subsequent to its formation in grape berries.

Extraction of Resveratrol from Wine and Grape Berries. Resveratrol in wine or grape berries was extracted as previously described in Jeandet *et al.* (1993) and Jeandet *et al.* (1991, 1994a). All extractions were carried out in subdued light to avoid photochemical isomerization of resveratrol.

Analysis of Resveratrol. *HPLC Analysis.* Analysis of resveratrol was performed by HPLC on a Lichrocart Merck C18 reverse phase column (5 μm; 250 × 4.6 mm) with a mobile phase of 55% water/45% acetonitrile at a flow rate of 0.6 mL/min. Since wine samples contained the two isomers of resveratrol, the eluate was monitored at 307 and 280 nm (corresponding to the *trans*- and *cis*-resveratrol absorbance maxima, respectively) (Siemann and Creasy, 1992). Limits of detection were 10 μg/L wine and 0.1 μg/g fresh weight.

GC-MS Analysis. Identification of resveratrol isomers in wine was confirmed by GC-MS analysis of their trimethylsilyl derivatives (Jeandet *et al.*, 1993), by using a quadrupole mass spectrometer (Nermag R10-10C). The separation was performed on a SE-30 capillary column (25 m × 0.32 mm) operating from 200 to 240 °C with a gradient temperature of 2 °C/min. The pressure of the carrier gas (helium) was maintained at 50 kPa at a flow rate of 1 mL/min. Selective ion monitoring was performed at m/z = 444 (M^+) and m/z = 429 (M − 15).

Chemical Standards and Quantification of Resveratrol Isomers. Pure *trans*-resveratrol was synthesized as described in Jeandet *et al.* (1991). The *cis*-resveratrol standard was obtained by UV irradiation (*i.e.* at 366 nm for 45 min) of a *trans*-resveratrol solution in the form of its trimethylsilyl derivative (10 μg/mL in BSTFA; Jeandet *et al.*, 1993). Under these conditions, the *trans* form of resveratrol was converted quantitatively (*i.e.* in 95% yield) to the *cis* isomer. The response as area counts obtained on GC chromatograms was always equivalent to that of *trans*-resveratrol at the same molar concentration. The *cis* isomer was characterized by its retention time in GC and its mass spectrum.

The quantification of resveratrol isomers in wine or grape skins was carried out by GC as follows: the resveratrol concentration was estimated by simultaneous injection of hexacosane as an internal standard (limits of detection were *ca.* 10 μg/L wine and 0.1 μg/g fresh weight) [see Jeandet *et al.* (1993)]. Linear correlation was excellent from 10 μg to 4 mg/L wine (correlation coefficient r = 0.998). Chromatographic conditions were identical to those described for GC-MS.

RESULTS AND DISCUSSION

Effect of Maceration on the Resveratrol Content of Wine. As expected, maceration increased the extraction of resveratrol (see Table 1). When maceration occurred, the resveratrol content of the white wines (1.11 ± 0.18 mg/L) increased *ca.* 10-fold the values found in wines which were just pressed lightly and spent no time on the grape skins (0.12 ± 0.04 mg/L). In contrast, the resveratrol content from nonmacerated red wines (0.23 ± 0.07 mg/L) was much lower (*ca.* 13-fold) than that obtained in the corresponding macerated wines (3.04 ± 0.53 mg/L). Since resveratrol is mainly located at the skin level and is absent from, or low, in the fruit flesh (Creasy and Coffee, 1988; Jeandet *et al.*, 1991, 1994a), there is a better extraction of resveratrol in wines which spend a long fermentation time in contact with grape skins than for wines which are not fermented on the skins prior to pressing.

Table 1. Effect of Maceration on the Resveratrol Content of Wine

	resveratrol[a] (mg/L) ± SD			
	macerated wines[b]		nonmacerated wines	
	red wine	white wine	red wine	white wine
trans isomer	1.84 ± 0.35	0.81 ± 0.13	0.17 ± 0.05	0.09 ± 0.03
cis isomer	1.20 ± 0.18	0.30 ± 0.05	0.06 ± 0.02	0.03 ± 0.01
total resveratrol	3.04 ± 0.53[c]	1.11 ± 0.18	0.23 ± 0.07	0.12 ± 0.04

[a] Quantification of resveratrol was carried out by GC [see Materials and Methods and also Jeandet *et al.* (1993)]. SD = standard deviation of replicate experiments. [b] Maceration experiments were done in triplicate (2 extracts/bottle; standard errors of these two measurements were < 2%). [c] Mean of three replicates.

It is interesting to note that the resveratrol content of wines made with red grapes (var. Pinot noir) is *ca.* 3 fold higher than in those made with white grapes (var. Chardonnay blanc), when vinted under the same conditions. This may reflect varietal differences in the ability to synthesize resveratrol in response to fungal infection (Jeandet *et al.*, 1994a).

Influence of Grape *Botrytis* Levels on the Resveratrol Content of Wines. As resveratrol is a phytoalexin produced by grapevines in response to pathogen infection and namely to *B. cinerea*, which is the cause of the worst plagues in Burgundy vineyards at harvest time, one would expect to find more resveratrol in wines made with grapes that have suffered a high *Botrytis* infestation than in those vinted with healthy or moderately *Botrytis*-infected grapes. However Table 2 shows that, contrary to what we expected, wines obtained from grapes affected 40 or 80% by *Botrytis* have the lowest resveratrol levels (respectively, 2.02 ± 0.50 and 1.01 ± 0.15 mg/L) while wines prepared from healthy grapes or grapes affected 10% by *Botrytis* have higher resveratrol contents (respectively, 2.71 ± 0.24 and 3.88 ± 0.10 mg/L). This result could be explained by assuming that resveratrol formed in highly *Botrytis*-infested grapes might have undergone degradation by exocellular enzymes of this fungus, *e.g.* a laccase like stilbene oxidase (Pezet *et al.*, 1991). This enzyme which is also active in the must could thus lower the resveratrol content of the wines obtained. These results confirm the findings of Lamuela-Raventos and Waterhouse (1993), who demonstrated a low resveratrol content in Sauternes wines (*i.e.* wines made with grapes fully parasitized by *Botrytis*).

Surprisingly, there was a high resveratrol level in wines obtained from healthy grape clusters. This is in good agreement with the fact that, even on apparently noninfected grape clusters, we have observed a pronounced stimulation of phytoalexin synthesis (Jeandet *et al.*, 1994a). Such a response is due to the presence, at harvest time, of *Botrytis* on fruits even though

Table 2. Influence of Grape *Botrytis* Levels on the Resveratrol Content of Wine

	resveratrol[a] (mg/L) ± SD			
	healthy grapes	grapes affected 10% by *Botrytis*	grapes affected 40% by *Botrytis*	grapes affected 80% by *Botrytis*
trans isomer	1.86 ± 0.20	2.62 ± 0.08	1.45 ± 0.35	0.74 ± 0.10
cis isomer	0.85 ± 0.04	1.26 ± 0.02	0.57 ± 0.15	0.27 ± 0.05
total resveratrol[b]	2.71 ± 0.24 a[c]	3.88 ± 0.10 b	2.02 ± 0.50 a	1.01 ± 0.15 c
	$n = 8$[d]	$n = 4$	$n = 6$	$n = 4$

[a] Quantification of resveratrol was carried out by GC [see Materials and Methods and also Jeandet *et al.* (1993)]. SD = standard deviation of replicate experiments ($n = 4-8$). [b] Mean of n replicates ($n = 4-8$). [c] Total data were analyzed using Kruskal–Wallis test (H = 17.138; significant difference at $p = 0.007$) and were compared by using the Wilcoxon–Mann–Withney test; wines followed by the same letter are not significantly different at the 0.05 level. [d] Number of replicate experiments (one extract/bottle).

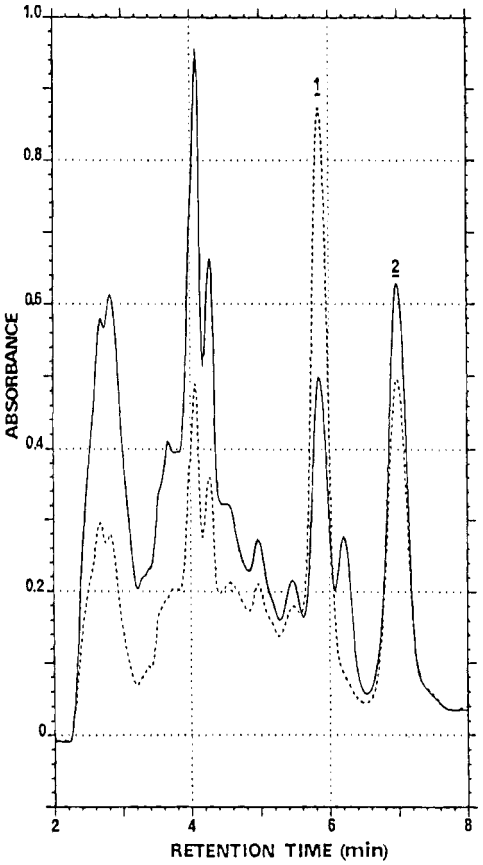


Figure 1. HPLC analysis of resveratrol. Profile of a Pinot noir wine obtained by separation on a 5 μm Lichrocart Merck C18 column using a mobile phase of 55% water/45% acetonitrile at a flow rate of 0.6 mL/min, monitoring the eluant at 307 nm (···) and 280 nm (—): **1**, *trans*-resveratrol; **2**, *cis*-resveratrol. (Retention times: *trans*-resveratrol, 5.85 min; *cis*-resveratrol, 6.99 min).

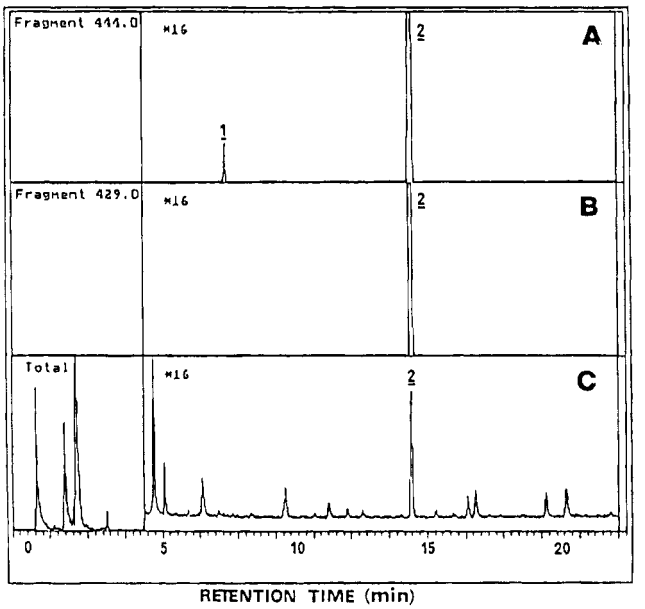
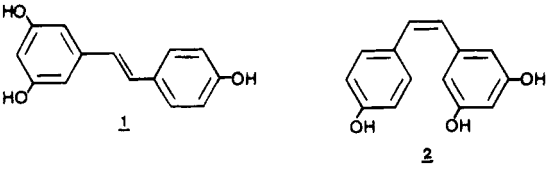


Figure 2. GC-MS analysis of a TMS-derivatized grape skin extract (var. Pinot noir) (**1**, *cis*-resveratrol; **2**, *trans*-resveratrol): (A) reconstructed gas chromatogram (RGC) obtained when selecting the molecular ion at $m/z = 444$ (relative abundance = 100%); (B) RGC obtained when selecting the ion fragment at $m/z = 429$ (M − 15) (relative abundance = 4%); and (C) total ion current (TIC) chromatogram. (Retention times: *cis*-resveratrol, 7.50 min; *trans*-resveratrol, 14.10 min.) For chromatographic conditions see text.

Chart 1. Structures of the *Trans* Isomer (1) and the *Cis* Isomer (2) of Resveratrol



clusters do not exhibit disease symptoms (Bessis, 1972). Finally, the highest resveratrol levels were observed in wines vinted from grapes 10% affected by *Botrytis*, thus confirming that mild attacks of gray mold, which have no consequences on wine quality, are needed to have a high resveratrol wine (Jeandet *et al.*, 1994b).

***Cis–Trans* Isomerization of Resveratrol in Wine.** All the wine samples analyzed by HPLC contained two compounds whose retention times were consistent with the *cis* and *trans* isomers of resveratrol (Figure 1). These two peaks were also identified by GC-MS analysis as the trimethylsilyl ethers of both *cis*- and *trans*-resveratrol. The presence of either the *cis* or the *trans* isomer of resveratrol (Chart 1) in wine is very surprising since only the *trans* isomer was detectable on total ion current chromatograms of the TMS-derivatized grape berry extracts (Figure 2C), thus confirming the previous report of Langcake and Pryce (1976). However, the *cis* isomer was detected in low amounts on the reconstructed gas chromatograms (Figure 2A) when selecting the ion mass at 444, which represents the molecular ion of the TMS derivative of resveratrol (relative abundance = 100%) (Jeandet *et al.*, 1991, 1993). Thus, it can be assumed that the presence of the *cis* isomer in wine is not a consequence of *trans*-resveratrol being isomerized by sunlight exposure subsequent to its formation in grape berries. In contrast, the *cis* isomer was present in high amounts (41% of total resveratrol) in wines vinted in the dark (see Figure 3A), while it represents 46% in wines which were not protected from light (data not shown). Since we have shown that wines contained both the *cis* and the *trans* isomers of resveratrol, then further nutritional studies

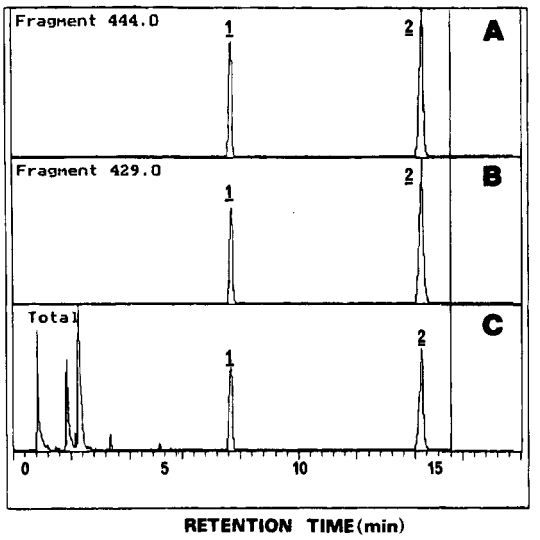


Figure 3. GC-MS analysis of a TMS-derivatized Pinot noir wine extract vinted in the dark (**1**, *cis*-resveratrol; **2**, *trans*-resveratrol): (A) RGC obtained when selecting the molecular ion at m/z = 444 (relative abundance = 100%); (B) RGC obtained when selecting the ion fragment at m/z = 429 (M − 15) (relative abundance = 4%); and (C) TIC chromatogram. (Retention times: *cis*-resveratrol, 7.50 min; *trans*-resveratrol, 14.10 min.) For chromatographic conditions see text.

should include the possible effect of these two isomers on human health.

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Supplementary Material Available: Mass spectra of *cis*- and *trans*-resveratrol as their trimethylsilyl derivatives, UV spectra of *cis*- and *trans*-resveratrol, and purity check of resveratrol peaks (5 pages). Ordering information is given on any current masthead page.

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