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# Constitutive stilbene contents of grapevine cluster stems as potential source of resveratrol in wine

by

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S u m m a r y : Ripe clusters of 8 *Vitis vinifera* L. varieties (Cabernet franc, Gewürztraminer, Marzemino, Merlot, Moscato, Pinot gris, Sauvignon, Tocai friulano) were analysed to determine the constitutional levels of *trans-* and *cis*-resveratrol, ε-viniferin and pterostilbene. Grape stems were extracted by an hydroalcoholic solution, in order to simulate the resveratrol extraction from stems that might occur during alcoholic fermentation, due to the presence of little pieces of stems in the fermenting must. The average stem concentrations of constitutive *trans-*, *cis*-resveratrol and ε-viniferin were 142, 0.30, and 70 $\mu g \cdot g^{-1}$ FW, respectively; pterostilbene was not found. Varieties differed significantly in their stilbene content. The percentage of *trans*-resveratrol extracted from the stems by a hydroalcoholic solution varied from 2.5 % (Gewürztraminer) to 32.9 % (Marzemino), the contribution of the stems to the *trans*-resveratrol concentration of the hydroalcoholic solution being, on the average, 34 $\mu g \cdot l^{-1}$.

K e y w o r d s : grapevine, stems, *trans*-resveratrol, *cis*-resveratrol, ε-viniferin.

## Introduction

After the landmark work of SIEMANN and CREASY (1992) regarding resveratrol as a wine component, many studies have been done on resveratrol concentration in wine all over the world (JEANDET *et al.* 1993, 1995 a; LAMUELA-RAVENTOS and WATERHOUSE 1993; MATTIVI 1993 a, b; FREGONI *et al.* 1994; MC. MURTREY *et al.* 1994; ROGGERO and ARCHIER 1994; GONZALO *et al.* 1995; GOLDBERG *et al.* 1995 a and b, 1996; MATTIVI *et al.* 1995; SOLEAS *et al.* 1995; VACCA *et al.* 1995; CELOTTI *et al.* 1996; CRAVERO *et al.* 1996; ECTOR *et al.* 1996; MOZZON *et al.* 1996; OKUDA and YOKOTSUKA 1996; REVEL *et al.* 1996; ROMERO-PEREZ *et al.* 1996 a, b). Stilbene is deserving a lot of interest because it is supposed to be the active principle of red wines that have been shown to reduce heart diseases (SEIGNEUR *et al.* 1990; RENAUD and DE LORGERIL 1992). In fact resveratrol is involved in antiplatelet activity (BERTELLI *et al.* 1995, 1996), in inhibition of eicosanoid synthesis (KIMURA *et al.* 1985, quoted by ROMERO-PEREZ *et al.* 1996), and recently *trans*-resveratrol has been shown to have cancer chemopreventive activity in mice (JANG *et al.* 1997). It is likely that the most important source for resveratrol in wine is the berry skin, and that the resveratrol solubility is enhanced in an alcoholic solution such as wine (PEZET and CUENAT 1996). It has been assumed that in ripe berries skins can be elicited to synthesize resveratrol (JEANDET *et al.* 1995 b, c) as a free compound or as a glucoside (piceid); these compounds are supposed to be extracted during alcoholic fermentation and degradated (the latter) during malolactic fermentation, by enzymatic activity of malolactic bacteria (PEZET and CUENAT 1996). Berry skins may also be elicited to synthesize ε-viniferin (BAVARESCO *et al.* 1997), which could be extracted and degradated during fermentation, liberating resveratrol.

It is likely that during destemming and crushing little pieces of stems drop into the fermenting must; they are possibly a potential resveratrol source, since grape stems possibly contain constitutive stilbenes.

The aim of this research was to determine the levels of constitutive stilbenes of stems and their possible role as resveratrol source in wine.

## Materials and methods

P l a n t m a t e r i a l : The following *Vitis vinifera* L. varieties, cultivated mostly in northern Italy were used: Cabernet franc, Gewürztraminer, Marzemino, Merlot, Moscato, Pinot gris, Sauvignon, Tocai friulano. Ripe clusters were sampled in the grapevine germplasm repository of the Istituto agrario S. Michele all'Adige (Trento), and berries were destemmed.

E x t r a c t i o n o f c o n s t i t u t i v e s t e m s t i l b e n e s : Grape stems (= grape stalks, consisting of rachis, branches and pedicels) were cut in little pieces by stainless steel scissors 1 g of which was added to 100 ml methanol and left for 48 h in the dark at room temperature. After filtration with GF/A Whatman filters the filtrate was evaporated *in vacuo* at 35 °C. Stilbenes were recovered by 2 x 1 ml methanol (50 %), and analysed by HPLC.

E x t r a c t i o n o f s t e m s t i l b e n e s b y h y d r o a l c o h o l i c s o l u t i o n : In order to simulate stilbene extraction from the stems during alcoholic fer-

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mentation, 0.3 g of stems were added to 100 ml hydroalcoholic solution: 11 % (v/v) ethanol and 250 ppm (v/v) methanol. The ratio was calculated as follows: during destemming and crushing at least 2 % of stalks can drop into the fermenting must. If we consider a 100 g cluster (consisting of, on the average, 90 g berries and 10 g rachis), 0.2 g rachis can drop into ca. 70 g juice (without skins and seeds) , that means 0.28 g rachis in 100 g must (ca. 92 ml at 19 °Brix) corresponding to ca. 90 ml wine with 10.9 % alcohol. The samples were kept in the dark at 25 °C for 4 d. Afterwards the stalks were separated from the liquid by filtration and stilbenes were extracted by the method already described for wine (FREGONI *et al.*1994).

Stilbene identification: Stilbenes were analysed by HPLC according to the method of PEZET *et al.* (1994) utilizing the following standard compounds: *trans*-resveratrol (Sigma); *cis*-resveratrol by *trans*-resveratrol exposure to UV rays ($\lambda$ = 366 nm) for 40 min (90 % of conversion); ε-viniferin (obtained from G. Hoos, formerly BFA für Rebenzüchtung Geilweilerhof, Germany); pterostilbene (obtained from R. PEZET, Fed. Agric. Res. Station of Changins, Nyon, Switzerland). A liquid chromatograph (Hewlett Packard 1090 L, Waldbronn, Germany) equipped with an autosampler (10 µl of injection volume) and DAD (diode array detector) set at $\lambda$ = 310 nm, was utilized. As column we used a Lichrospher 100 RP (Merck) 125 x 4 mm, 5 µm particle size, eluting with a gradient of methanol (A) and formic acid (0.24 %) (B). The gradient was from 20 to 80 % of A in 27 min at a flow rate of 1.0 ml·min$^{-1}$.

Statistical analysis: A one-way-ANOVA was used, and means were compared by Tukey test (5 % level). Constitutive stilbene values are expressed in µg·g$^{-1}$ fresh weight of stem and, in the case of extraction by hydroalcoholic solution, in µg stilbenes extracted from 1 g stem, and µg stilbenes contained in 1 l hydroalcoholic solution.

## Results

Constitutive *trans*-resveratrol ranged from 31 (Marzemino) to 393 µg g$^{-1}$ FW (Gewürztraminer), while *cis*-resveratrol varied from 0.10 (Tocai friulano) to 0.59 µg g$^{-1}$ FW in Gewürztraminer and Marzemino (Tab. 1). The highest ε-viniferin level was found in Sauvignon (171 µg·g$^{-1}$ FW), while in Marzemino this compound was not detected. Pterostilbene was not found in any sample.

The hydroalcoholic solution extracted only *trans*-resveratrol out of the stems (Tab. 2); the values ranged from 6.0 (Sauvignon) to 17.8 µg g$^{-1}$ FW (Cabernet franc), with an average of 11.4 µg g$^{-1}$ FW, while the percentage of extraction varied from 2.5 % (Gewürztraminer) to 32.9 % (Marzemino). Sauvignon showed the lowest *trans*-resveratrol concentration in the hydroalcoholic solution (18 µg l$^{-1}$), and Cabernet franc the highest (53 µg l$^{-1}$); the average value was 34 µg l$^{-1}$.

A negative, highly significant correlation (r = -0.92) between constitutive *trans*-resveratrol and the percentage of extraction due to hydroalcoholic solution was calculated (Figure).

Table 1

*Trans*- and *cis*- resveratrol and ε-viniferin in cluster stems of eight grapevine varieties

| | Resveratrol (µg·g$^{-1}$ FW) *trans*- | *cis*- | ε-Viniferin (µg·g$^{-1}$ FW) |
|---|---|---|---|
| Cabernet franc | 238 b | 0.22 a | 138 bc |
| Gewürztraminer | 393 c | 0.59 a | 69 abc |
| Marzemino | 31 a | 0.59 a | n.d. |
| Merlot | 38 a | 0.12 a | 54 ab |
| Moscato | 100 ab | 0.17 a | 56 ab |
| Pinot gris | 159 ab | 0.33 a | 34 ab |
| Sauvignon | 95 ab | 0.25 a | 171 c |
| Tocai friulano | 82 a | 0.10 a | 35 ab |
| Mean | 142 | 0.30 | 70 |

Values followed by the same letter are not significantly different at the 0.05 level as determined by the Tukey test.
n.d.: not detected.

Table 2

Extraction of *trans*-resveratrol from cluster stems of eight varieties in a hydroalcoholic solution

| | *trans*-resveratrol µg·g$^{-1}$ stem | percentage of extraction % | µg·l$^{-1}$ hydroalc. solution |
|---|---|---|---|
| Cabernet franc | 17.8 b | 7.5 | 53 b |
| Gewürztraminer | 9.8 a | 2.5 | 29 a |
| Marzemino | 10.2 a | 32.9 | 31 a |
| Merlot | 10.2 a | 26.8 | 31 a |
| Moscato | 12.0 ab | 12.0 | 36 ab |
| Pinot gris | 12.9 ab | 8.1 | 39 ab |
| Sauvignon | 6.0 a | 6.3 | 18 a |
| Tocai friulano | 12.5 a | 15.2 | 37 ab |
| Mean | 11.4 | | 34 |

Values followed by the same letter are not significantly different at the 0.05 level as determined by Tukey test.

## Discussion

The paper reports, for the first time, constitutive *trans*- and *cis*-resveratrol contents in cluster stems of different *V. vinifera* varieties at maturity. There is evidence in literature for constitutive resveratrol in lignified organs of grapevine, such as canes (LANGCAKE and PRYCE 1976; POOL *et al.* 1981; BOUKHARTA *et al.* 1996) and seeds (PEZET and CUENAT 1996). The recorded ε-viniferin content in cluster stems confirms previous findings of BOURHIS *et al.* (1996); this stilbene was also found in roots (MATTIVI and RENIERO 1992; OSHIMA *et al.* 1995), canes (BOUKHARTA *et al.* 1996) and stems (WEN-WU *et al.* 1996).

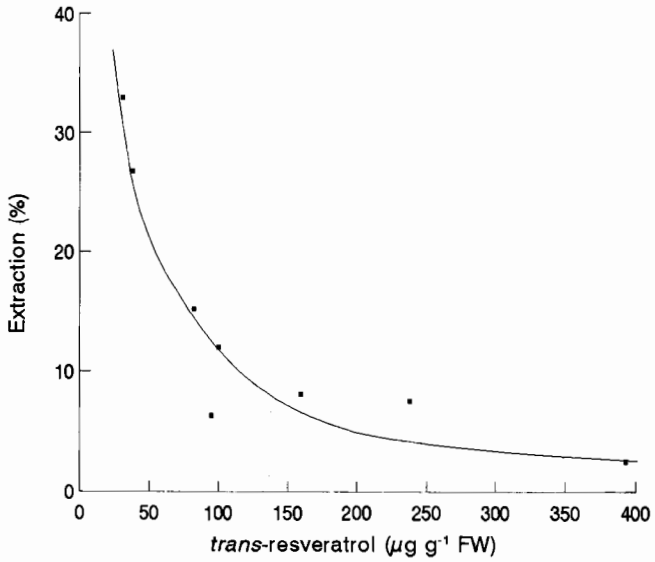


Figure: Correlation between constitutive *trans*-resveratrol stem concentrations and corresponding percentages of extraction in a hydroalcoholic solution. r = -0.92 **

Our *trans*-resveratrol values are in the same range as those from canes of some American *Vitis* species and *V. vinifera* cv. Sultanina as reported by POOL *et al.* (1981), lower if compared with data of LANGCAKE and PRYCE (1976), and much higher than concentrations in seeds (PEZET and CUENAT 1996). To our knowledge no data on ε-viniferin concentrations in lignified grapevine organs are available in literature.

The variation among varieties was wide; the highest *trans*-resveratrol values were found in Gewürztraminer and Cabernet franc which are considered to be less susceptible to grey mould (FREGONI 1983). Other authors reported variations in cluster stem compounds such as leucoanthocyanins (CANTARELLI and PERI 1965) and procyanidin (RICARDO-DA-SILVA *et al.* 1991) depending on grape variety. Pterostilbene was not found; other oligostilbenes, different from ε-viniferin, that might be present (WEBER *et al.* 1996), were not investigated.

The simulation of the alcoholic fermentation was not entirely realistic since ethanol was kept constant, i.e. did not increase with time; however, according to data from literature (MATTIVI and NICOLINI 1993; MATTIVI *et al.* 1995; SOLEAS *et al.* 1995; PEZET and CUENAT 1996), the maximum rate of resveratrol extraction from skin occurs within 4 d after the onset of the ethanol increase; therefore 4 d of stem contact with an 11 % alcohol wine seems to be a good model to simulate alcoholic fermentation. A time course of resveratrol extraction could be the next step for a better understanding of the phenomenon.

The negative correlation between constitutive *trans*-resveratrol levels and the corresponding percentages of extraction can possibly be explained by the saturation of the extracting solution at a given concentration. In fact the *trans*-resveratrol concentrations of the extracting solutions are similar.

The contribution of stems to the *trans*-resveratrol pool of wine is therefore low, but further studies are in progress, focussing on the effect of increasing ratio of stem to hydroalcoholic solution in modifying extraction performance.

According to PEZET and CUENAT (1996), who designed an elegant experiment on resveratrol extraction from skins of Gamay during fermentation, the stilbene concentration in must was slightly higher than that extracted from skins up to day 6 of alcoholic fermentation. The average amounts of *trans*-resveratrol extracted from stems in the present experiment (34 µg $l^{-1}$) could explain that difference, since it is likely that little pieces of stalk accidentally drop into the fermenting must during destemming and crushing.

The results presented in this paper were obtained in one year only. Data of varietal differences, for instance, might be influenced by ambient factors, thus experiments need to be investigated in another year.

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