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Resveratrol-derivatives and antioxidative capacity in wines made from *botrytized* grapes

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Abstract

Eighteen quality wines from Tokaj (Hungary) and 15 quality wines with "Prädikat" made of *botrytized* grapes from Germany were analyzed for their resveratrol and total polyphenol contents, and their antioxidative capacities. Levels of total resveratrol in the quality wines from Tokaj were higher than in the quality wines with "Prädikat" from Germany, due to the different winemaking technology in making Tokaji Aszú. Compared to German white wines of normal quality (resveratrol 0.5–4.4 mg/l; mean: 2.1 mg/l), the wines made from *botrytized* grapes from Germany had lower concentrations of resveratrol and piceid (from <0.003 to 6.3 mg/l; mean: 0.9 mg/l). The Hungarian wines had slightly higher concentrations (from <0.003 to 7.8 mg/l; mean: 2.5 mg/l) due to their production technique.

The antioxidative capacities (TEAC values) for the German wines ranged from 0.6 to 2.8 mmol/l (mean: 1.4 mmol/l), while the wines from Tokaj showed much higher values: from 1.1 up to 10.8 mmol/l (mean: 4.2 mmol/l). These polyphenol-rich wines could therefore be an excellent source of polyphenols with antioxidative capacity.
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Keywords: Antioxidant capacity; *Botrytis cinerea*; Resveratrol; Piceid; Tokaj; HPLC

1. Introduction

Many epidemiological studies have indicated the decreasing risk of coronary heart disease through moderate wine consumption (Renaud & de Lorgeril, 1992; Renaud, Gueguen, Schenker, & d'Houtaud, 1998). Moderate wine consumption has also been related to prevention of cancer, Alzheimer's disease and dementia (Dorozynski, 1997; Orgogozo et al., 1997; Renaud et al., 1998). Resveratrol, a 3,4′,5-trihydroxy-*trans*-stilbene, has been linked to these effects. It has been cited as responsible for the inhibition of the oxidation of human low-density lipoprotein (LDL), the inhibition of the dioxygenase activity of lipoxygenase, and the inhibition of platelet aggregation, and it possesses preventive anti-inflammatory activities (Acquaviva et al., 2002; Ferrero et al., 1997; Frankel, Waterhouse, Teissedre, & Kinsella, 1993; Mérillon et al., 1996; Pinto, García-Barrado, & Macías, 1999). Resveratrol also exists in its glucosidic form, named piceid, Fig. 1. Their *cis*-isomers have been found in wine, grapevine stems and grape products, e.g., grape juices (Bavaresco, Fregoni, Trevisan, & Fortunati, 2000; Lamuela-Raventós, Romero-Pérez, Waterhouse, & de la Torre-Boronat, 1995; Pour Nikfardjam, Schmitt, Rühl, Patz, & Dietrich, 2000; Romero-Pérez, Lamuela-Raventós, Waterhouse, & de la Torre-Boronat, 1996; Vrhovsek, Wendelin, & Eder, 1997; Wang, Catana, Yang, Roderick, & van Breemen, 2002). *cis*-Resveratrol, *trans*- and *cis*-piceid are physiologically as

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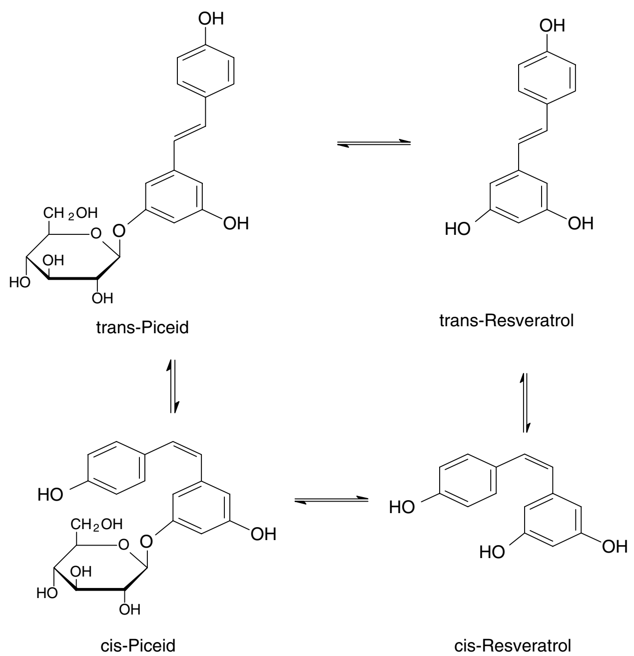

Fig. 1. Resveratrol and its derivatives.

important as *trans*-resveratrol (Jayatilake et al., 1993; Varache-Lembège et al., 1996).

Some authors have suggested that vinification techniques have a great influence on resveratrol and piceid levels in wine (Bavaresco et al., 2000; Jeandet, Bessis, Sbaghi, Meunier, & Trollat, 1995; Mattivi, Reniero, & Korhammer, 1995; Roldán, Palacios, Caro, & Pérez, 2003; Yasui, Yunoki, Naito, Kawaguchi, & Ohnishi, 2002). Clarifying and filtering lead to a decrease in resveratrol and piceid levels (Lamuela-Raventós et al., 1995; Threlfall, Morris, & Mauromoustakos, 1999; Vrhovsek et al., 1997). Jeandet et al. (1995) and Roldán et al. (2003) show that high fungus pressure (*Botrytis cinerea*) in the vineyard led to a decrease of resveratrol in grape berries. Adrian, Rajaei, Jeandet, Veneau, and Bessis (1998) concluded that *B. cinerea* generates a laccase-like enzyme (a stilbene-oxidase), which oxidizes resveratrol and other stilbenic compounds. One major metabolite formed during this degradation process is a resveratrol-dehydrodimer, Fig. 2 (Breuil et al., 1998), which might be responsible for the self-intoxication of the fungus (Schouten, Wagemakers, Stefanato, Kaaij, & Kan, 2002).

From this point of view it was interesting to have a closer look at wines made from *botrytized* grapes. In the investigation, we wanted to see whether high *Botrytis* pressure in the vineyard leads to a decrease of resveratrol levels in wine. Also interesting, was whether the oxidative aging of Tokaji aszú in wooden barrels would lead to a strong decrease in polyphenol content and antioxidative capacity. As there are only three commonly known wine-types, in the world, which have to be made from *Botrytis* grapes: Tokaji Aszú (Hungary), Sauternes

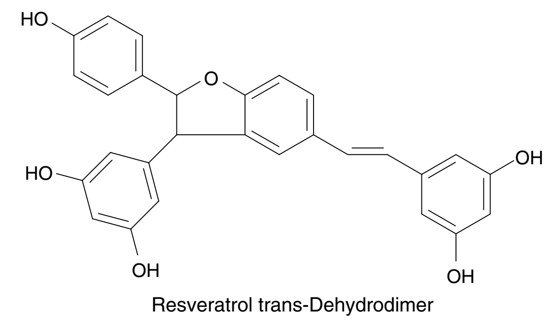

Fig. 2. Resveratrol *trans*-dehydrodimer.

(France) and German quality wines with "Prädikat Auslese, Beerenauslese, Eiswein, and Trockenbeerenauslese", we analyzed several aszú wines from Tokaj (Hungary) and wines from Germany of comparable quality for their resveratrol and piceid concentrations, total polyphenol content, and antioxidative capacity.

2. Materials and methods

2.1. Standards

trans-Resveratrol was from Sigma (Munich, Germany), *trans*-piceid from BioSPECS (Delft, Netherlands). *cis*-Resveratrol and -piceid were identified through UV-irradiation of a *trans*-resveratrol and -piceid standard, leading to the corresponding *cis*-isomers. Acetonitrile and acetic acid were from Merck (Darmstadt, Germany). All water used was de-ionized, nanopure. Quantification was performed using the molar absorptivities published by Trela and Waterhouse (1996).

2.2. Samples

A total of 18 wines from Hungary and 15 wines from Germany was analyzed. The wines were from different sources, representing the whole spectrum of Tokaj and German wines with "Prädikat Auslese, Beerenauslese, Eiswein, and Trockenbeerenauslese".

2.3. HPLC analysis

All samples were filtered through Schleicher & Schuell Brown Rim L filters (Spartan 30/0.45 RC, 0.45 μm) and directly injected onto a Merck-Hitachi L-6200A HPLC equipped with a Merck-Hitachi AS-2000 autosampler, and a Merck LaChrom L-7210 diodearray detector (Merck, Darmstadt, Germany). Chromatograms were recorded at 310 nm for the *trans*-isomers and at 286 nm for the *cis*-isomers. The system was equipped with a Fluofix® 120 E (NEOS Company Ltd., Kobe/Japan) RP-column (250 × 4.6 mm), 5 μm particle size, with a pre-column of the same material maintained at 20 °C with a Bio-Rad Column Heater (Bio-Rad Laboratories, Munich, Germany) column oven. The injection volume was 25 μl.

The HPLC elution profile was as follows: 0 min, 82% A, 18% B; 10 min, 82% A, 18% B; 17 min, 77% A, 23% B; 21 min, 75% A, 25% B; 27 min, 68% A, 32% B; 30 min, 0% A, 100% B; 40 min, 0% A, 100% B; 40.1 min, 82% A, 18% B, 55 min, 82% A, 18% B, where solvent A was glacial acetic acid in water (56.2:900) (v/v) and solvent B was 20% phase A and 80% acetonitrile (v/v).

The HPLC method was validated according to Huber (1993). The limit of detection was 0.003 mg/l and the limit of quantification 0.01 mg/l.

2.4. Total polyphenols

Total polyphenols were estimated using the Folin–Ciocalteu method (Singleton & Rossi, 1965). The results were calculated as (+)-catechin.

2.5. Antioxidative capacity (TEAC-test)

The antioxidative capacity was estimated using a modified method of Re et al. (1999), as published by Pour Nikfardjam (2002).

3. Results and discussion

3.1. Resveratrol and derivatives

Table 1 shows the results for the quality wines from Tokaj (Hungary). The concentrations for the glucosides (piceids) ranged from <0.003 (not detectable) to 1.8 mg/l (mean: 0.6 mg/l) for *trans*-piceid and from <0.003 (not detectable) to 6.6 mg/l (mean: 1.4 mg/l) for *cis*-piceid. The aglycones could not be detected in most of the wines, yet values for *trans*- and *cis*-resveratrol reached concentrations from <0.003 (not detectable) to 0.4 mg/l with a mean of 0.03 mg/l and from <0.003 (not detectable) to 2.8 mg/l with a mean of 0.5 mg/l, respectively. Mean total resveratrol content was 2.5 mg/l ranging from <0.003 (not detectable) to 7.8 mg/l.

Table 2 shows the results for the German wines. The concentrations for the glucosides (piceids) ranged from <0.003 (not detectable) to 3.4 mg/l (mean: 0.4 mg/l) for *trans*-piceid and from <0.003 (not detectable) to 2.9 mg/l (mean: 0.4 mg/l) for *cis*-piceid. For the aglycones the values range from <0.003 (not detectable) to 0.5 mg/l (mean: 0.1 mg/l) for *trans*-resveratrol and from <0.003 (not detectable) to 0.6 mg/l (mean: 0.04 mg/l) for *cis*-resveratrol. Mean total resveratrol content was 0.9 mg/l.

Only a few German wines contained considerable amounts of resveratrol and piceid. Thus, levels of total resveratrol in the wines from Tokaj were higher than in the wines from Germany. While Aszú is made by adding specific amounts (so called "puttony": 20–25 kg) of *botrytized* grapes to a known amount (so called "Gönci barrel": 135 l) of base wine, the winemaking technique in Germany is quite different. In Germany, healthy and *botrytized* grapes are processed together, except in the case of "Trockenbeerenauslese", which is made from *botrytized* grapes only. In Hungary, the relatively long contact of the alcoholic base wine with the Aszú berries leads to a better extraction of the resveratrol isomers than during the "standard procedure" during the German winemaking. Besides, the aroma and the sugar free

Table 1
Resveratrol, piceid, total phenols, and TEAC Levels in wines from Tokaj (Hungary)

Wine	Vintage	*trans*-Piceid (mg/l)	*cis*-Piceid (mg/l)	*trans*-Resveratrol (mg/l)	*cis*-Resveratrol (mg/l)	Total amount (mg/l)	Total phenols (mg/l)	TEAC (mmol/l)
aszu 5 puttonyos	1993	0.0	n.q.	n.q.	n.q.	n.q.	717	3.0
aszu 5 puttonyos	1993	1.0	1.3	n.q.	n.q.	2.3	690	2.0
aszu 5 puttonyos	1993	n.q.	0.9	n.q.	n.q.	0.9	647	2.2
aszu 5 puttonyos	1994	1.3	n.q.	n.q.	n.q.	1.3	621	2.4
aszu 5 puttonyos	1995	1.8	0.9	n.q.	n.q.	2.8	744	3.1
aszu 5 puttonyos	1996	n.q.	n.q.	n.q.	n.q.	n.q.	710	2.5
aszu 5 puttonyos	1998	n.q.	4.5	n.q.	2.5	7.0	1403	7.4
aszu 5 puttonyos	1998	1.4	4.5	n.q.	0.2	6.2	1168	6.1
aszu 5 puttonyos	1999	1.6	3.2	0.4	2.0	7.2	1085	5.9
aszu 5 puttonyos	1999	1.2	6.6	n.q.	n.q.	7.8	911	3.9
aszu 6 puttonyos	1981	n.q.	n.q.	n.q.	n.q.	n.q.	609	1.1
Esszencia	1999	n.q.	n.q.	n.q.	n.q.	n.q.	1154	2.8
Forditás	1996	0.9	1.2	0.1	0.6	2.8	681	3.4
Forditás	1999	n.q.	2.2	n.q.	0.3	2.5	1033	5.5
Forditás	1999	0.8	n.q.	n.q.	n.q.	0.8	1725	10.8
Szamorodni	1995	n.q.	n.q.	n.q.	n.q.	n.q.	537	3.2
Szamorodni	1998	n.q.	n.q.	n.q.	n.q.	n.q.	730	4.9
Szamorodni	1998	n.q.	n.q.	n.q.	2.8	2.8	787	4.7
Mean		0.6	1.4	0.03	0.5	2.5	886	4.2

n.q. = not quantified.

Table 2
Resveratrol, piceid, total phenols, and TEAC levels in German quality wines with "Prädikat"

Variety	"Prädikat"	Vintage year	*trans*-Piceid (mg/l)	*cis*-Piceid (mg/l)	*trans*-Resveratrol (mg/l)	*cis*-Resveratrol (mg/l)	Resveratrol amount (mg/l)	Phenols (Folin) (mg/l)	Capacity (TEAC) (mmol/l)
Ehrenfelser	Beerenauslese	1990	n.q.	n.q.	0.1	n.q.	0.1	411	0.8
Kerner	Auslese	1997	n.d.	n.d.	n.d.	n.d.	n.d.	259	1.8
Riesling	Auslese	1996	n.q.	n.q.	0.4	n.q.	0.4	369	1.4
Riesling	Auslese	1998	n.d.	n.d.	n.d.	n.d.	n.d.	248	0.7
Riesling	Auslese	1999	3.4	2.9	n.d.	n.d.	6.3	259	1.4
Riesling	Beerenauslese	1997	1.5	0.7	n.d.	n.d.	2.2	498	1.6
Riesling	Eiswein	1998	n.d.	n.d.	n.d.	n.d.	n.d.	605	2.8
Riesling	Eiswein	1990	n.q.	n.q.	n.n.	n.q.	n.d.	465	0.6
Riesling	Eiswein	1999	n.d.	n.d.	n.d.	n.d.	n.d.	286	0.7
Riesling	Trockenbeerenauslese	1999	0.5	1.2	n.d.	n.d.	1.7	600	1.8
Ruländer	Auslese	1994	n.q.	n.q.	0.3	n.q.	0.3	615	1.4
Scheurebe	Trockenbeerenauslese	1999	n.d.	n.d.	n.d.	n.d.	n.d.	747	2.4
Silvaner	Beerenauslese	1999	0.5	0.5	0.5	0.6	2.1	400	1.5
Pinot blanc	Beerenauslese	1992	n.q.	n.q.	0.2	n.q.	0.2	377	0.6
Pinot blanc	Trockenbeerenauslese	1999	n.d.	n.d.	n.d.	n.d.	n.d.	479	1.4
Mean			0.4	0.4	0.1	0.04	0.9	441	1.4

n.q. = not quantified; n.d. = not detected.

extract are also increased. As resveratrol is mostly located in the berry skin (Jeandet, Bessis, & Gautheron, 1991), the longer the contact of the skins with the mash or the base wine, the better can resveratrol and piceid be extracted from the skins. The generally high values of *cis*-piceid in the Hungarian wines can not be explained; normally, the *trans*-isomer is the more common (Jeandet et al., 1991). Maybe the high UV-irradiation in the warm and sunny climate in Hungary leads to isomerization of the *trans*-compound, as reported by Jeandet et al. (1991).

There is only a slight difference between the Aszú and the Forditás wines in their resveratrol-contents. Forditás is obtained from the hask of the Aszú after the pressing and extraction of the fermented Aszú berries. The hask is combined with a base wine, which is a selected, high quality wine with high sugar-free extract, specific to the grape variety and high alcohol content (14–15 vol%). Due to this technology, it has more phenolics, but less aroma and value compared to the Aszú (Katona, 1987; Pour Nikfardjam, 2002). If resveratrol or piceid remained in the grapes during Aszú production, it could be extracted during Forditás making, because the hask and the base wine are left together for 12–48 h before pressing. But resveratrol and piceid concentrations are not increased compared to the corresponding Aszú wines. Presumably, most of the resveratrol is already extracted during Aszú production, and only small amounts are left in the remaining pulp; but this is not true for the total phenols. During production of Forditás, the remaining polyphenols can be extracted from the Aszú berries' skins; high pressure during pressing also elevates the polyphenol content. This leads to these high amounts of polyphenols and antioxidative capacity (up to 1725 mg/l and 10.8 mmol/l, respectively) in the Forditás wines.

Szamorodni wines are normally produced in dry or cold years with only slight yields of Aszú berries, where picking of the Aszú berries does not pay for it. They are produced by crushing, destemming, and fermenting *botrytized* and healthy grapes together. In contrast to the Forditás wines, the Szamorodni wines contained very small amounts of resveratrol. Only one sample contained considerable amounts (2.8 mg/l of *trans*- and *cis*-resveratrol).

Compared to German white wines of normal quality, the wines made from *botrytized* grapes from Germany have lower concentrations of resveratrol and piceid. We found total resveratrol amounts between 0.5 and 4.4 mg/l (mean: 2.1 mg/l) in German Riesling wines of standard quality (Pour Nikfardjam, 2002). The mean concentrations in the Tokaj and German wines from *botrytized* grapes were 2.5 and 0.9 mg/l, respectively. This might be due to the fact, that the *Botrytis*' stilbene-oxidase oxidizes most of the resveratrol to its corresponding metabolites. As reported by Jeandet et al. (1995) and Roldán et al. (2003), extensive fungus pressure leads to a reduction of resveratrol production in the grapes. The shrivelling of the grapes – caused by *Botrytis* – might concentrate the contents of the berries, but at the same time the stilbene-oxidase decreases resveratrol and piceid concentrations, in which oxidation is obviously the predominant process. Yet, during the production of Tokaji Aszú, the long skin contact might aid in the extraction of remaining and un-oxidized resveratrol derivatives in the grape skins and, thus, explain their higher mean values (2.5 mg/l) compared to the German wines from *botrytized* berries.

Even compared to wines from other wine-producing regions of the world, the resveratrol concentrations of the analyzed wines are relatively low. Ribiero de Lima et al. (1999) found 5.2 mg/l total resveratrol concentration in white wines from Portugal. Goldberg, Karumanchiri, Soleas, and Tsang (1999) found mean concentrations of 4.0 mg/l for *trans*-piceid and 3.0 mg/l for *trans*-resveratrol in white wines from Canada, France and California. *Botrytized* grapes obviously lead to wines with decreased resveratrol levels compared to wines made from "normal" grapes.

3.2. Polyphenol content (estimated by Folin-method)

In total polyphenol content, the wines from Tokaj show very high concentrations, due to the high amounts of *botrytized* berries and the longer skin contact with the Aszú berries and the Aszú berries' hask, respectively. Values ranged from 537 to 1725 mg/l with a mean of 886 mg/l. The wines from Germany had much lower concentrations: from 248 up to 747 mg/l (mean: 441 mg/l), because long berry skin contact with the mash is generally avoided in Germany to protect the wine from bitter taste, "negative" browning reactions and astringency. This leads to a loss in total polyphenol content and, inevitably, also in antioxidative capacity (Table 2).

Compared to German wines of normal quality, these values are still very high, which is particularly true for the Tokaj wines. We found a mean concentration of 264 mg/l (maximum: 610 mg/l) of total polyphenol content in our recent investigations (Pour Nikfardjam, Rechner, Patz, & Dietrich, 1999). The German quality wines with "Prädikat" therefore contain nearly twice and the Tokaj quality wines three times more total phenols than the German wines of "normal" quality.

3.3. Antioxidative capacity (TEAC-values)

The TEAC values for the German wines ranged from 0.6 to 2.8 mmol/l (mean: 1.4 mmol/l), while the wines from Tokaj show much higher values: from 1.1 up to 10.8 mmol/l (mean: 4.2 mmol/l), due to their higher polyphenol contents. Compared to values published in the literature, these values are very high, particularly those from Tokaj. In former investigations, we found TEAC values between 0.1 and 1.7 mmol/l with a mean of 0.7mmol/l in German white wines of standard quality (Pour Nikfardjam et al., 1999). Fogliano, Verde, Randazzo, and Ritieni (1999) found 1.4–1.9 mmol/l in Italian white wines.

As far as we know, TEAC values above 5 mmol/l have never been reported in the literature for white wine. Values above 10 mmol/l are only known for red wines (Fogliano et al., 1999; Pour Nikfardjam et al., 1999; Pour Nikfardjam, 2002). The same holds for the polyphenol content: values over 800 mg/l have not yet been reported for a white wine. Though the Aszú wines from Hungary were made by using only small amounts of SO_2 (30 mg/kg mash) and, thus, there was only a slight "chemical" protection against oxidation, except through the polyphenols themselves, they still show very high polyphenol contents and antioxidative capacities, which means only slight oxidative loss during production. During oxidation and aging, some processes (e.g., Maillard reactions) might convert monomeric phenols, proteins, sugars, and acids to high molecular compounds with higher molar antioxidative capacities. This could explain the high TEAC and total phenol values in the Tokaji wines.

4. Conclusions

While German wines from *botrytized* grapes contained less resveratrol than German wines made from "normal" grapes, the Hungarian wines showed slightly higher contents of these substances, which might be due to the long skin contact during Tokaji Aszú production. Total polyphenol content and antioxidative capacity in Hungarian Tokaj and *botrytized* German wines were higher than in the normal German wines, particularly so for the Tokaj. They provide an excellent source of polyphenols and antioxidative capacity. German quality wines with "Prädikat" show lower values, due to their short berry skin contact with the mash during production. High antioxidative capacities and polyphenol contents are therefore no longer a privilege for red wines, because of the comparable values of some Tokaj wines. Their full and rich taste also prove that polyphenol-rich white wines need have neither bitter or astringent tastes nor tendencies for "negative" browning reactions.

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