The Contents of Total Polyphenolic Compounds and *Trans*-Resveratrol in White Riesling Originated in the Czech Republic

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Abstract

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Wine is a significant source of antioxidants in human nutrition. Every glass of wine contains approximately 200 different phenolic compounds, several of which have been noted as antioxidants because they have been shown to slow down the potentially damaging cell oxidation process. In white Riesling from different wine-growing sub-regions, kinds of wine, years of harvest and vintners, the content of total polyphenols (TP) was determined using spectrophotometric method, and that of *trans*-resveratrol (R) by HPLC method. The TP content was presented as gallic acid equivalent per litre of wine, and the content of R as *trans*-resveratrol per litre of wine. TP values in the wine-growing region of Bohemia ranged from 223.0 to 532.7 mg/l (average content 330.3 mg/l), in the wine-growing region of Moravia from 175.0 to 465.0 mg/l (average content 271.7 mg/l), while R values in the wine-growing region of Bohemia ranged from < 0.033 to 0.421 mg/l (average content 0.117 mg/l), in the wine-growing region of Moravia from < 0.033 to 0.875 mg/l (average content 0.123 mg/l). The highest average TP content (370.1 mg/l) and R content (0.262 mg/l) were found in the sub-region Roudnická (the wine-growing region of Bohemia). The harvest year of 1994 was evaluated as that providing the highest average levels of TP (386.5 mg/l) and R (0.201 mg/l). The kind of wine with the highest average TP was the kind of "selected grapes" (327.2 mg/l), while the highest average R content was found in the late harvest wine (0.141 mg/l). The R and TP contents were not significantly affected by vintage, wine-growing sub-region or the kind of wine. The statistically significant correlation between TP and R content was not demonstrated (5.73%).

Keywords: wine; white Riesling; total polyphenol content; *trans*-resveratrol

Polyphenols are very important for the taste and nature of wine. Inside the grapes, they also take part in the formation of taste, nature and colour of grapes (Farkaš 1980). The adduced content of total polyphenolics (presented as a gallic acid equivalent) ranges in red wines from 1800 to 4059 mg/l and in white wines from 165 to 331 mg/l, respectively. Their structures and contents are influenced by various factors such as variety, vintage, and wine-growing sub-regions (Frankel *et al*. 1995). They have positive effects on the human body. Every glass of wine contains approximately 200 various polyphenols. Some of them are known as antioxidants; wine is a significant source of them in human nutrition. Its positive influence has been demonstrated in various studies (Lavy *et al*. 1994; Fauconneau *et al*.

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1997; Burns *et al*. 2000). Epidemiological surveys have shown positive effects of wine, especially red wine, due to its antioxidant, antithrombotic, and antiproliferatic effects (especially related to *trans*-resveratrol and quercetin). Polyphenolics are anti-inflammatory, they reduce the risk of cardiovascular diseases and cancer. Antioxidant effects of individual polyphenols can differ from the results given by the synergic influence of various polyphenolic compounds in wine (Soleas *et al*. 1997a, b). Protective effects of wine, especially red wine, are ascribed to the antioxidant effects of the polyphenolic compounds (Burns *et al*. 2000; Matějková & Gut 2000). Extensive *in vitro* studies have confirmed the results of epidemiological surveys and revealed a whole range of mechanisms within the polyphenols, which likely affect their protective properties.

Antioxidants have scavenging effect on free radicals owing to their ability to act as substrates in reactions where free radicals take part. Taking into account that antioxidants react with free radicals, they influence the final result of many reactions and reduce the damaging cell oxidation process caused by free radicals. The concentration of substances with the antioxidant effect in wine fluctuates, depending on cultivars, wine-growing sub-regions, and the length of sunshine. Lowland plants (higher oxygen pressure) and plants from areas with a higher intensity of UV rays (more free radicals arise) are considered to have a higher antioxidation capacity, because they protect themselves against the oxidation damage (Racek *et al*. 2001).

Six minor stilbene-character flavonoids have been found in wine. The most significant is resveratrol – 3,4',5-trihydroxystilbene. From its structure it is obvious that two geometric isomers can exist – *cis* and *trans*. *Trans*-resveratrol is predominant in vegetable material (Šmidrkal *et al*. 2001). It is classified as phytoestrogen. Resveratrol is a stress metabolite (phytoalexin) formed in grapevine in response to abiotic (UV rays, mechanical damage) and biotic stresses (*Botrytis cinerea*) (Melzoch *et al*. 2000). UV rays transform *trans* isomers into *cis* form. UV irradiation of the vegetable tissue has an interesting impact on the metabolism of phenol substances. UV-B rays seem to be connected with the increase of the enzyme content responsible for the biosynthesis of the flavonoids which protect the vegetal cells from UV rays (Cantos *et al*. 2000).

Resveratrol shows significant anticoagulation and antioxidant characteristics, inhibits low-density lipoproteins (LDL) and sharply enhances the proportion of high-density lipoproteins (HDL), thereby reducing the risk of cardiovascular diseases and cancer (in some cases), and it also absorbs free radicals (Fauconneau *et al*. 1997). A relatively rich and widespread source of resveratrol is the grapevine grapes. Average concentration of resveratrol in red wines is approximately 2–6 mg/l, in white wines the concentration is lower, approximately 0.2–0.8 mg/l (Šmidrkal *et al*. 2001). Pazourek and Havel (2001) adduce that the content of *trans*-resveratrol in Riesling from Wolxhaim (France) ranges from 0.1 to 0.34 mg/l. Other white wines have the content of *trans*-resveratrol in the range of 0.05–0.32 mg/l.

The objectives of this study are to chart the content of the total polyphenols TP and *trans*-resveratrol R in white Riesling originating from various wine-growing sub-regions of the Czech Republic, and to determine the influence of the vintage, area and kind of wine on the contents of TP and R.

MATERIAL AND METHODS

Material. 76 samples of white Riesling wine were obtained from different wine-growing regions of the Czech Republic. Wines were produced in the wine-growing region of Bohemia (in the wine-growing sub-regions: Mělnická, Mostecká, Pražská, Roudnická, Žernosecká), and in the wine-growing region of Moravia (in the wine-growing sub-regions: Brněnská, Bzenecká, Kyjovská, Mikulovská, Mutěnická, Podluží, Strážnická, Uherskohradišťská, Velkopavlovická, Znojemská). The wines differed by the vintage, vintner and kind of wine – "quality wine" and wines with attributes. The kind of wine "quality wine" is produced from grapes with the sugar content of at least 15 degrees of the standardised must meter. The analysed kinds of wines with attributes were "kabinet wine" (it can be produced from grapes with the sugar content of at least 19 degrees of the standardised must meter), "late harvest" (its can be produced from grapes with the sugar content of at least 21 degrees of the standardised must meter), and "selected grapes" (it can be produced from grapes with the sugar content of at least 24 degrees of the standardised must meter). The wines were obtained from retail shops.

Measurements of total polyphenol content TP. TP was measured spectrophotometrically using Folin-Ciocalteau's reagent after Lachman *et al*.

(1998). Amount of 1 ml of wine aliquots was pipetted into 50 ml volumetric flask and diluted with 5 ml of distilled water. All analyses were performed in three parallel determinations (R^2 = 0.9959).

Determination of trans-resveratrol content R. The samples were filtered using Spartan 0.45 µm filter. The content of *trans*-resveratrol was determined using HPLC method with isocratic elution on the chromatographic equipment Waters™ for the identification in UV and visible light (pump Waters™ 616, autosampler Waters™ 717 plus, detector Waters™ PDA 996). Mobile phase – acetonitrile (Merck AG)/water (25:75, v/v), pH was adjusted to 1.5 using TFA (trifluoracetic acid). Wavelength λ = 313 nm; flow rate 1 ml/min; standard – *trans*-resveratrol (3,4´,5-trihydroxy-*trans*-stilbene, 99% purity, Sigma Aldrich®). The limit of detection was evaluated from the calibration curve constructed in the range of 0.05–1.00 mg/l *trans*-resveratrol/1 ml calibration solution by standard statistical procedure in Excel program (Meloun & Militký 1994). The detection limit for wine was 0.033 mg/l. The content of *trans*-resveratrol was expressed in mg/l of wine. All analyses were performed in two parallel determinations (R^2 = 0.9994).

Statistical evaluation. The results were evaluated by Statistica program 6.0 at the level of significance α = 0.05 and Scheffé-test.

RESULTS AND DISCUSSION

The highest TP content was found in the sample coming from the vintage of 1994, the kind of wine "quality wine", the wine-growing region of Bohemia sub-region Roudnická (532.7 mg/l). The highest R content was found in the sample coming from the vintage of 2000, the kind of wine "late harvest", the wine-growing region of Moravia, sub-region Mikulovská, wine village Valtice, vineyard estate Hintertály. The average TP content was higher

Table 1. Content of TP and R (in (mg/l) in white Riesling related to the wine-growing sub-regions (first part of table is wine-growing region of Moravia and second part of table is wine-growing region of Bohemia)

Wine-growing sub-regions	Frequency	Average TP content	Range of TP content	Average R content	Range of R content
Brněnská	1	–	265.0	–	0.045
Bzenecká	3	264.6	261.1–269.5	0.086	< 0.033–0.169
Kyjovská	1	–	243.4	–	0.004
Mikulovská	18	258.5	175.0–371.8	0.144	< 0.033–0.875
Mutěnická	9	238.7	171.7–288.3	0.132	< 0.033–0.447
Podluží	3	266.0	226.3–328.1	0.063	0.044–0.095
Strážnická	3	277.1	241.7–315.5	0.113	0.077–0.161
Uherskohradišťská	1	–	280.6	–	0.072
Velkopavlovická	6	298.9	212.1–465.0	0.091	0.038–0.158
Znojemská	12	263.1	171.7–358.0	0.149	< 0.033–0.466
Mělnická	7	305.1	223.0–443.8	0.092	< 0.033–0.145
Mostecká	1	–	353.5	–	–
Pražská	1	–	378.4	–	0.185
Roudnická	4	370.1	282.6–532.7	0.262	0.050–0.421
Žernosecká	6	321.3	245.0–392.0	0.051	< 0.033–0.070
Wine-growing region of Bohemia	19	330.3	223.0–532.7	0.115	< 0.033 –0.421
Wine-growing region of Moravia	57	271.7	175.0–465.0	0.121	< 0.033–0.875

< 0.033 – below range of detection

in the samples from the wine-growing region of Bohemia (average 330.3 mg/l), where it ranged from 223.0 to 532.7 mg/l. There were no significant differences in the average R contents.

The average results obtained are given in Table 1. The highest average TP contents were found in the wines from the sub-region Roudnická (370.1 mg/l) in the wine-growing region of Bohemia, and in those from the sub-region Velkopavlovická (298.9 mg/l) in the wine-growing region of Moravia. On the contrary, the lowest average TP contents were found in the samples from the sub-region Mělnická (305.1 mg/l) in the wine-growing region of Bohemia, and in those from the sub-region Mutěnická (238.7 mg/l) in the wine-growing region of Moravia. The highest average R content was found in the samples from the sub-region Roudnická (0.262 mg/l) in the wine-growing region of Bohemia, and in those from the sub-region znojemská (0.149 mg/l) in the wine-growing region of Moravia. The lowest average R content was found in the samples from the sub-region Žernosecká (0.051 mg/l) in the wine-growing region of Bohemia, and in those from the sub-region Podluží (0.063 mg/l) in the wine-growing region of Moravia. The highest TP and R contents found in the sub-region Roudnická can be due to the northern location of the vineyards and the exposition to a more significant effect of the stress factors. The highest average TP content was found in the kind of wine "selected grapes" (327.2 mg/l), the lowest content in the kind of wine "late harvest" (264.7 mg/l) (Table 2). The highest average R content was found in the kind of wine "late harvest" (0.128 mg/l), and the lowest one in "selected grapes" (0.070 mg/l). Regarding the kind of wines, the highest TP content was found in wines which had been harvested from the vineyard at a later time (after they had reached the demanded level of saccharinity that predicts the quality of wine). This can be explained by the activity of the stress factors. On the contrary, the lowest R content in the kind of wine "selected grapes" can be explained by the objective selection of nonaffected grapes, made by the producer during the wine harvest for this kind of wine. The highest average TP content (386.5 mg/l) and average R content (0.201 mg/l) were found in the samples from the vintage of 1994 (Table 3). The lowest average TP content (260.3 mg/l) was found in the samples from the vintage of 1999, and the lowest average R content (0.076 mg/l) in those from the vintage of 2002.

The fact, which also appears to be very interesting, is that since 1998 the average content of *trans*-resveratrol in the samples of wine has gradually declined. It may be possible to conclude that the global warming of the climate improves the conditions for the vine growing even in the Czech Republic, where warmer and drier weather conditions can possibly lower the development of grey mould, which may reduce the content of *trans*-resveratrol produced in the grape vine as a reply to the effects of this grey mould – *Botrytis cinerea*. The R content depends on many different factors such as location, climatic conditions, agricultural techniques used, the techniques of the wine production (Goldberg *et al*. 1996). This fact could explain the oscillation of the R content in the samples of the same vintage and wine-growing sub-regions differing by the producer.

The R and TP contents are affected by various conditions such as the weather (Melzoch *et al*. 2000), soil quality, conditions of grapes, the sort of cultivars (Frankel *et al*. 1995), geographical location of the vineyard, the technology of the wine production (Goldberg *et al*. 1996) and the storage of the bottles of wine (Faitová *et al*. 2004). The wine-growing regions in the Czech Republic are

Table 2. TP content and R content in white Riesling related to the kind of wine (in mg/l)

Kind of wine	Frequency	Average TP content	Range of TP content	Average R content	Range of R content
Quality wine	34	282.5	197.7–532.7	0.127	< 0.033–0.466
Late harvest	29	264.7	175.0–362.1	0.128	< 0.033–0.875
Kabinet wine	11	300.3	193.6–443.8	0.102	< 0.033–0.242
Selected grapes	2	327.2	282.6–371.8	0.070	0.050–0.090
Average	76	279.5	175.0–532.7	0.122	< 0.033–0.875

< 0.033 – below range of detection

Table 3. TP content and R content in white Riesling related to vintage (in mg/l)

Vintage	Frequency	Average TP content	Range of TP content	Average R content	Range of R content
2002	7	261.4	171.7–394.5	0.076	< 0.033–0.144
2001	16	267.2	175.0–465.0	0.096	< 0.033–0.242
2000	24	293.7	171.7–443.8	0.135	< 0.033–0.875
1999	15	260.3	215.3–334.8	0.125	< 0.033–0.447
1998	6	281.3	188.8–370.2	0.156	0.057–0.218
1997	1	–	255.1	–	0.123
1996	1	–	201.2	–-	0.075
1994	3	386.5	235.2–532.7	0.201	0.070–0.421
1990	1	–	243.4	–	0.162
1989	1	–	279.0	–	0.136
1985	1	–	349.0	–	0.057
Average	76	279.5	175.0–532.7	0.122	< 0.033–0.875

< 0.033 – below range of detection

also located on the northern border of the wine-growing sub-regions. The higher TP content in the white Riesling mostly grown in the Czech Republic may depend on that wines from the northern locations are exposed to more significant effects of the stress factors (biotic and abiotic) which can result in a higher TP content. *Trans*-resveratrol content ranged in the wine-growing region of Bohemia from < 0.033 to 0.421 mg/l (average 0.117 mg/l), and in that of Moravia from < 0.033 to 0.875 mg/l (average 0.123 mg/l), these amounts are corresponding to those found in white wines by Šmidrkal *et al.* (2001) which ranged from 0.2 to 0.8 mg/l. These amounts of *trans*-resveratrol are higher than its content in Wolxhaim Riesling (France) mentioned by Pazourek and Havel (2001) which varied from 0.10 to 0.34 mg/l. Romero-Peréz *et al.* (1996) present the R content in Riesling from Spain as ranging from 0.041 to 0.061 mg/l (average 0.054 mg/l). The levels of R in Czech white Riesling were very similar, i.e. between 0.033 and 0.875 mg/l.

The R and CP contents were not significantly affected by the vintage, the wine-growing sub-region or the kind of wine. No statistically significant correlation between TP and R content was demonstrated (5.73%). Melzoch *et al.* (2000) obtained similar results. They did not find any relation to the age of wine. Contrary to this finding, Jeandet *et al.* (1995) found a significant influence of vintage on the R content. The authors realised that the content of the investigated compounds was influenced by many factors (winemaking, storage). They also found that, according to the bill 115/1995 and 216/2000 which tolerates the use of 15% of different cultivars, the producers can affect TP and R contents – this fact could have a definite impact on the TP and R contents. The statistical evaluation is only tentative and shows the possible impact of the vintage, the wine-growing sub-regions or the kind of wine on the contents of the substances measured.

CONCLUSION

The average TP content was higher in the samples from the wine-growing region of Bohemia where it ranged from 223.0 to 532.7 mg/l (average content 330.3 mg/l). No significant differences existed in the average R content. The R and TP contents were not significantly affected by the vintage, the wine-growing sub-region or the kind of wine.

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Souhrn

Faitová K., Hejtmánková A., Lachman J., Pivec V., Dudjak J. (2004): **Obsah celkových polyfenolických látek a *trans*-resveratrolu v Ryzlinku rýnském vyrobeném v České republice.** Czech J. Food Sci., **22**: 215–221.

Víno je významným zdrojem antioxidantů v lidské výživě. Každá sklenice vína obsahuje přibližně 200 různých fenolických sloučenin, ze kterých jsou některé označovány jako antioxidanty, protože zpomalují potenciální poškození buněk oxidačními pochody. Byl stanoven obsah celkových polyfenolů (TP) spektrofotometrickou metodou a obsah *trans*-resveratrolu (R) metodou HPLC v bílém Ryzlinku rýnském z různých vinařských oblastí a roků sklizně. Obsah TP byl vyjádřen jako ekvivalent galové kyseliny a obsah R jako obsah *trans*-resveratrolu v litru vína. Hodnoty TP v českém regionu se pohybovaly v rozmezí 223,0–532,7 mg/l (průměrný obsah 330,3 mg/l), v moravském regionu v rozmezí 175,0–465,0 mg/l (průměrný obsah 271,7 mg/l) a R hodnoty v českém regionu v rozmezí < 0,033– 0,421 mg/l (průměrný obsah 0,117 mg/l), v moravském regionu od 0,033 do 0,875 mg/l (průměrný obsah 0,123 mg/l). Nejvyšší průměrný obsah TP – 370,1 mg/l a R – 0,262 mg/l byl nalezen v oblasti Roudnická (český region). Rok 1994 byl vyhodnocen jako rok sklizně s nejvyšším průměrným obsahem TP – 386,5 mg/l a R – 0,201 mg/l. Druh vína s nejvyšším obsahem TP byla třída „pozdní sběr" (327,2 mg/l) a nejvyšší obsah R byl nalezen u archivního vína (0,141 mg/l), které také mělo druhý nejvyšší obsah TP – 321,6 mg/l. Obsahy TP a R nebyly významně ovlivněny ročníkem, oblastí pěstování a druhem vína. Nebyla nalezena statisticky významná korelace mezi obsahem TP a R (5,73 %).

Klíčová slova: víno; bílý Ryzlink rýnský; celkový obsah polyfenolů; *trans*-resveratrol

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