VARIABILITY OF THE CONTENT OF TOTAL POLYPHENOLS AND RESVERATROL IN TRAMINER BOTTLES OF THE SAME BATCH*

K. Faitová, A. Hejtmánková, J. Lachman, J. Dudjak, V. Pivec, M. Šulc

Czech University of Agriculture, Faculty of Agronomy, Department of Chemistry, Prague, Czech Republic

In twelve bottles of Traminer (vintage 2000, late harvest, vine growing area Žernoseky), total content of polyphenols (TPC) and resveratrol (which are ascribed to have a positive impact on human body) were measured to determine the variability of the content of measured substances in various bottles of the same batch and from the same manufacturer. TPC was measured spectrophotometrically using Folin-Ciocalteau's reagent with Heλios γ spectrophotometer at wavelength $\lambda = 765$ nm. The content of resveratrol was measured using HPLC method with isocratic elution on the chromatograph Waters^{TM} at wavelength $\lambda = 313$ nm. TPC ranged from 257.7 to 282.5 $mg.l^{-1}$ (average content 272.6 $mg.l^{-1}$) and the content of resveratrol ranged from 0.05–0.08 $mg.l^{-1}$ (the average content 0.06 $mg.l^{-1}$). From the results obtained it is possible to conclude that even in the samples with the same maturation conditions, bottling and storage, a certain role has also the choice of the bottle.

polyphenols; resveratrol; Traminer; variability

INTRODUCTION

Polyphenols are a group of chemical substances widely spread in nature. They can be found in vegetables, tea and wine (mostly in the red wine) and they are ascribed to have positive effects on human organism (Soleas et al., 2002). Every glass of wine contains approximately 200 various polyphenols, which are known as antioxidants. Wine is a significant source of antioxidants in human nutrition (Fauconneau, 1997; Burns et al., 2000). Its positive influence has been brought up in various studies as the antioxidant, antithrombotic (Wang et al., 2002) and anti-proliferatic effects; may also stimulate the detoxification enzymatic system and decrease the risk of coronary heart disease (Wu et al., 2001). The antioxidant impact of various polyphenols can vary from the results given by the synergic influence of various polyphenolic compounds (esp. in red wine) (Soleas et al., 1997). It has been proved, that reasonable wine consumption (it is recommended 200–400 ml of red wine a day) (Matějová, Gut, 2000) reduces the risk of peripheral arterial and coronary cardiac disease. Alcohol comprised in wine also helps the absorption of polyphenols in the intestine. Antioxidant effects of wine are due the influence of alcohol on the absorption of polyphenols better than the effects of other sources (pure grape juice) (Burns et al., 2001; Racek et al., 2001).

Resveratrol is a polyphenolic compound from the group of stilben character flavonoids. Resveratrol shows significant anti-coagulation and anti-oxidant characteristics, inhibits low-density lipoproteins (LDL) and sharply enhances the proportion of high-density lipoproteins (HDL), and thereby reduces the risk of cardiovascular diseases and the risk of cancer in several sites (Bianchini, Vainio, 2003) and also absorbs free radicals (Fauconneau et al., 1997; Burns et al., 2000). Resveratrol is probably one of the main substances of the vegetal extracts used by oriental medicine against the cardiovascular diseases and for tumour medication. Relatively rich and widespread source of resveratrol are the grapevine grapes. Average concentration of resveratrol in red wines is approximately 2–6 $mg.l^{-1}$, in white wines is the concentration lower approx. 0.2–0.8 $mg.l^{-1}$. The structure of the resveratrol is 3, 4', 5-trihydroxystilben (Fig. 1) and it also exists in the form of

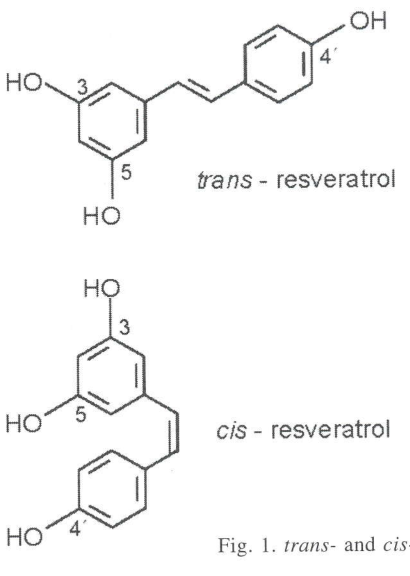

Fig. 1. *trans-* and *cis-* isomers of resveratrol

* This work was supported by the grant of Ministry of Education and Youth of CR MSM 41200002 and grant of the Faculty of Agronomy of Czech University of Agriculture in Prague No. 21120/1312/213134.

glucoside. From its structure it is obvious that two geometric isomers could exist – *cis* and *trans* when *trans* resveratrol is predominant in vegetal material (Šmidrkal et al., 2001). Resveratrol is a plant stress secondary metabolite (phytoalexin), it is low molecular compound with antimicrobial properties (Kuč, 1995; Bavaresco et al., 1997), formed in grapevine as the response to non-biotic (UV rays, mechanical damage, ozone) and biotic stress (*Botrytis cinerea*) (Goldberg et al., 1999).

The aim of this study was to determine the possible variability of TP and resveratrol contents in various samples (bottles) of Traminer wine of the same batch.

MATERIAL AND METHODS

Samples of wine: 12 bottles of Traminer, vintage 2000, late harvest, wine growing region Žernoseky, were obtained from the manufacturer.

Determination of total polyphenols content (TPC): TPC was measured spectrophotometrically using Folin-Ciocalteau's reagent (Penta, Czech Republic). 1 ml of wine was pipetted into 50 ml measuring flask and diluted by 5 ml of distilled water. In the second step 2.5 ml of Folin-Ciocalteu reagent and 7.5 ml of 20% Na_2CO_3 solution were added. Then the volume was adapted up to the mark by distilled water, stirred, and then left for two hours to stand at laboratory temperature. After this the samples were filtered out using Spartan 0.45 µm filter (Macherey-Nagel, Germany). The same technique was used for the blank to construct the calibration curve using gallic acid (Merck Ltd., Germany) as standard. Absorbance was measured using Heλios γ spectrophotometer (Spectronic Unicam, United Kingdom) at wavelength $\lambda = 765$ nm. The TPC values were expressed in milligrams of gallic acid (Fig. 2) per litre of wine. All analyses were performed in three parallel determinations (max. measuring error 3.3%). Statistical evaluation was calculated in the Statistica 6.0 programme, at the level of significance $\alpha = 0.01$ by variance analysis of simple grouping.

Determination of resveratrol content: The wine samples were filtered using Spartan 0.45 µm filter (Macherey-Nagel, Germany). The content of resveratrol was measured by HPLC (high performance liquid chromatography) method with isocratic elution on the chromatograph Waters™ (USA) (pump Waters™ 616, autosampler Waters™ 717 plus, UV-VIS detector Waters™ PDA 996). As mobile phase mixture acetonitrile (Merck Ltd., Germany) – water (25 : 75, V/V) was used. pH value of mobile phase was lowered to 1.5 using trifluoroacetic acid (25% water dilution, Merck Ltd., Germany). Chromatographic conditions: column ODS – Hypersil – 250 x 4.6 mm, 5 µm; wave length – 313 nm; flow – 1 $ml.min^{-1}$, elution time of resveratrol – approx. 25 min; time of sample analysis – 60 min (Burns et al., 2000). Results were obtained from two parallel determinations (measuring error 2.5%). As standard *trans* – resveratrol (99% purity, Sigma Aldrich®, Germany) was used.

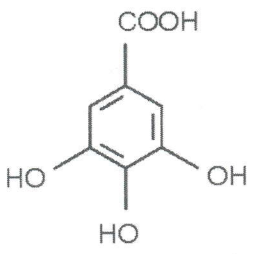

Fig. 2. Gallic acid

RESULTS AND DISCUSSION

The TPC (Table 1) in twelve bottles of Traminer ranged from 257.7 to 282.5 $mg.l^{-1}$ (average content 272.6 $mg.l^{-1}$), which shows variability from –5.47% to +3.63% from the average TPC in the samples. Within the absolute figures the variability is lower than 24.8 mg TPC per litre of wine.

Statistical evaluation of the variance analysis of simple grouping (Tables 2, 3) confirmed with probability higher than 99% statistically significant differences among some of the bottles. More detailed evaluation of the analysis of variance using the Tukey method (Table 4) allows us to declare that bottles (of the same batch) significantly statistically differ from 18.2%. Major statistically significant differences in the content of polyphenols were found in the bottle number 12, which varied from 2/3 of analysed bottle samples.

The resveratrol content (Table 1) ranged from 0.05 to 0.08 $mg.l^{-1}$ (average content 0.06 $mg.l^{-1}$), which shows variability from –16.7% to +33.3% of the average content of resveratrol in the samples. Within the absolute figures the variability is lower than 0.02 mg of *trans*-resveratrol per litre of wine.

Although the manufacturer declared that the bottles are of the same batch with entirely similar conditions for bottling and bottle storage, the content of measured substances (TPC and resveratrol) slightly oscillated. Interesting is the fact that every single bottle (although of the same manufacturing succession) slightly differed each other. Finally the consumer with „unlucky hand" could choose wine with lower TPC and resveratrol content and thus he could lower intake of health beneficial substances. The differences in the content of measured substances could be likely explained by storage and transport conditions (bottles placed in the upper part of storage area are more exposed to light and temperature changes than bottles stored in lower layers (Goldberg et al., 1996). The packaging material can also influence the content of the measured substances. The outer conditions (light and heat exposure) can cause also the degradation of one group of polyphenols – anthocyanins (Bakowska et al., 2003; Garcia-Beneytez et al., 2002; Morais et al., 2002). It is possible to conclude that the light exposure causes significant changes in the amount of anthocyanins, which can cause the differences in the content of total polyphenols.

Table 1. TPC and resveratrol content in Traminer wine

Sample number	1	2	3	4	5	6	7	8	9	10	11	12	Average
Content of total polyphenols (mg.l^{-1})	276.0	**282.5**	271.1	281.2	273.5	282.3	278.4	264.9	273.6	262.8	267.5	**257.7**	272.6
Deviation from average content (%)	1.25	3.63	–0.55	3.16	0.33	3.56	2.13	–2.83	0.37	–3.60	–1.87	–5.47	–
Content of resveratrol (mg.l^{-1})	0.07	**0.05**	0.07	0.06	0.07	0.07	0.07	0.05	0.05	0.07	**0.08**	0.05	0.06
Deviation from average content (%)	16.7	–16.7	16.7	0.0	16.7	16.7	16.7	–16.7	–16.7	16.7	33.3	–16.7	–

Table 2. Variance analysis of single grouping for TPC content in Traminer wine

Source of variability	Degree of freedom	Sum of squares	Variance	*F*-test	α
Number of sample	11	2153	196	6.9	0.0000
Residual	24	680	28		
Total	35	2833			

Table 3. Basic statistical characteristics for TPC in Traminer wine

Effect	Level of factor	Frequency	Average	Standard deviation	Mean error of average of class	– 95%	+ 95%
Number of sample	1	3	276.0	9.212	5.318	253.133	298.900
	2	3	282.5	8.422	4.862	261.556	303.398
	3	3	271.1	0.770	0.445	269.190	273.016
	4	3	281.2	9.523	5.498	257.547	304.859
	5	3	273.5	3.511	2.027	264.742	282.185
	6	3	282.3	0.968	0.559	279.936	284.744
	7	3	278.4	3.368	1.945	269.996	286.731
	8	3	264.9	3.963	2.288	255.022	274.712
	9	3	273.6	4.388	2.534	262.685	284.488
	10	3	262.8	3.708	2.141	253.585	272.009
	11	3	267.5	4.330	2.500	256.779	278.290
	12	3	257.7	1.004	0.580	255.250	260.237

Table 4. Detailed evaluation of analysis of variance using Tukey method for TPC in Traminer wine

Number of sample	1	2	3	4	5	6	7	8	9	10	11	12
1		0.9306	0.9898	0.9845	1.0000	0.9392	1.0000	0.3502	1.0000	0.1566	0.7186	**0.0131**
2	0.9306		0.3240	1.0000	0.6441	1.0000	0.9976	**0.0186**	0.6617	0.0062	0.0715	0.0005
3	0.9898	0.3240		0.4880	1.0000	0.3399	0.8647	0.9443	1.0000	0.7422	0.9993	0.1475
4	0.9845	1.0000	0.4880		0.8130	1.0000	0.9999	**0.0359**	0.8271	**0.0122**	0.1288	**0.0008**
5	1.0000	0.6441	1.0000	0.8130		0.6636	0.9900	0.7029	1.0000	0.4109	0.9600	0.0489
6	0.9392	1.0000	0.3399	1.0000	0.6636		0.9983	**0.0200**	0.6811	0.0067	0.0763	0.0005
7	1.0000	0.9976	0.8647	0.9999	0.9900	0.9983		0.1389	0.9918	0.0528	0.3899	0.0037
8	0.3502	**0.0186**	0.9443	0.0359	0.7029	0.0200	0.1389		0.6858	1.0000	1.0000	0.8778
9	1.0000	0.6617	1.0000	0.8271	1.0000	0.6811	0.9918	0.6858		0.3949	0.9541	**0.0460**
10	0.1566	**0.0062**	0.7422	0.0122	0.4109	0.0067	0.0528	1.0000	0.3949		0.9923	0.9873
11	0.7186	0.0715	0.9993	0.1288	0.9600	0.0763	0.3899	1.0000	0.9541	0.9923		0.5318
12	**0.0131**	**0.0005**	0.1475	**0.0008**	**0.0489**	**0.0005**	**0.0037**	0.8778	**0.0460**	0.9873	0.5318	

CONCLUSION

In the set of sample of twelve Traminer bottles of the same batch, the content of measured substances slightly oscillated. We can infer that the content of total polyphenols and resveratrol in the distribution chain is, to a certain degree, influenced by the outer conditions. The TPC and resveratrol are affected not only by the cultivars, vintage, wine growing area and the manufacturer, but also by the choice of an individual bottle.

REFERENCES

BAKOWSKA, A. – KUCHARSKA, A. Z. – OSZMIANSKI, J.: The effects of heating, UV-irradiation, and storage on stability of the anthocyanin – polyphenol copigment complex. Food Chem., *81*, 2003: 349–355.

BAVARESCO, L. – PETEGOLLI, D. – CANTÚ, E. – FREGONI, M. – CHIUSA, G. – TREVISAN, M.: Elicitation and accumulation of stilbene phytoalexins in grapevine berries infected by *Botrytis cinerea*. Vitis, *36*, 1997: 77–83.

BIANCHINI, F. – VAINIO, H.: Wine and resveratrol: mechanism of cancer prevention. Eur. J. Can. Prev., *12*, 2003: 417–425.

BURNS, J. – GARDNER, P. T. – O'NEIL, J. – CRAWFORD, S. – MORECROF, I. – McPHAIL, D. B. – LISTER, C. – MATTHEWS, D. – MacLEAN, M. R. – LEAN, M. E. J. – DUTHIE, G. G. – CROZIER, A.: Relationship among antioxidant activity, vasodilatation capacity and phenolic content of red wines. J. Agric. Food Chem., *48*, 2000: 220–230.

BURNS, J. – GARDNER, P. T. – MATTHEWS, D. – DUTHIE, G. G. – LEAN, M. E. J. – CROZIER, A.: Extraction of phenolics and changes in antioxidant activity of red wines during vinification. J. Agric. Food Chem., *49*, 2001: 5797–5808.

FAUCONNEAU, B. – WAFFO-TEGUO, P. – HUGET, F. – BARRIER, L. – DECENDIT, A. – MERILLON, J. M.: Comparative study of radical scavenger and antioxidant properties of phenolic compounds from *Vitis vinifera* cell cultures using in vitro test. Life Sci., *16*, 1997: 2103–2110.

GARCIA-BENEYTEZ, E. – REVILLA, E. – CABELLO F.: Anthocyanin pattern of several red grape cultivars and wines made from them. Eur. Res. Technol., *251*, 2002: 32–37.

GOLDBERG, D. M. – KARUMANCHIRI, A. – SOLEAS, G. J. – TSANG, E.: Concentration of selected polyphenols in white commercial wines. Am. J. Enol. Vitic., *50*, 1999: 185–193.

GOLDBERG, D. M. – TSANG, E. – KURAMANCHIRI, A. – DIAMANDIS, E. P. – SOLEAS, G. – NG, E.: Method to assay the concentrations of phenolic constituents of biological interest in wines. Analyt. Chem., *68*, 1996: 1688–1694.

KUČ, J.: Phytoalexins, stress metabolism, and disease resistance in plants. Ann. Rev. Phytopathol., *33*, 1995: 275–297.

MATĚJOVÁ, Š. – GUT, I.: Polyfenoly v potravě jako protektivní látky v aterosklerotickém procesu (Polyphenols in food as protective substances in aterosclerotic process). Remedia, *10*, 2000: 272–281.

MORAIS, H. – RAMOS, C. – FORGACS, E. – CSERHATI, T. – MATOS, N. – ALMEIDA, V. – OLIVEIRA, J.: Stability of anthocyanins extracted from grape skins. Chromatographia, *56* (Suppl.), 2002: 173–175.

RACEK, J. – HOLEČEK, V. – TREFIL, L.: Víno jako antioxidant (Wine as an antioxidant). Česká a slovenská gastroenterologie a hepatologie, *55*, 2001: 110–113.

SOLEAS, J. G. – TOMLINSON, G. – DIAMANDIS, P. E. – GOLDBERG, M. D.: Relative contributions of polyphenolic constituents to the antioxidant status of wines: Development of a predictive model. J. Agric. Food Chem. *45*, 1997: 3995–4003.

SOLEAS, G. J. – GRASS, L. – JOSEPHY, P. D. – GOLDBERG, D. M. – DIAMANDIS, E. P.: A comparison of the anticarcinogenic properties of four red wine polyphenols. Clin. Biochem., *32*, 2002: 119–124.

ŠMIDRKAL, J. – FILIP, V. – MELZOCH, K. – HANZLÍKOVÁ, I. – BUCKIOVÁ, D. – KŘÍSA, B.: Resveratrol. Chem. Listy, *95*, 2001: 602–609.

WANG, Z. R. – ZOU, J. G. – HUANG, Y. Z. – CAO, K. J. – XU, Y. N. – WU, J. M.: Effect of resveratrol on platelet aggregation in vivo and vitro. Chin. Med. J., *115*, 2002: 378–380.

WU, J. M. – WANG, Z. R. – HSIEH, T. C. – BRUDER, J. L. – ZOU, J. G. – HUANG, Y. Z.: Mechanism of cardioprotection by resveratrol, a phenolic antioxidant present in wine. Int. J. Mol. Med., *8*, 2001: 3–17.

Received for publication on February 24, 2004
Accepted for publication on March 16, 2004

FAITOVÁ, K. – HEJTMÁNKOVÁ, A. – LACHMAN, J. – PIVEC, V. – ŠULC, M. (Česká zemědělská univerzita, Agronomická fakulta, katedra chemie, Praha, Česká republika):

Kolísání obsahu celkových polyfenolických látek a resveratrolu v lahvích Tramínu stejné šarže.

Scientia Agric. Bohem., *35*, 2004: 64–68.

Ve dvanácti lahvích Tramínu (ročník 2000, pozdní sběr, žernosecká vinařská oblast) byl měřen obsah celkových polyfenolických látek a resveratrolu (je jim připisován příznivý vliv na lidský organismus) s cílem určit kolísání obsahu sledovaných látek mezi jednotlivými lahvemi stejné šarže od stejného výrobce. Obsah celkových polyfenolických látek byl měřen spektrofotometricky s užitím Folin-Ciocalteuova činidla na spektrofotometru Heλios γ při vlnové délce $\lambda = 765$ nm. Obsah resveratrolu byl stanoven metodou HPLC na chromatografu Waters™ isokratickou elucí při vlnové délce $\lambda = 313$ nm. Obsah celkových polyfenolických látek se pohyboval v rozmezí hodnot 257,7–282,5 mg.l^{-1} (průměrný obsah 272,6 mg.l^{-1}) a obsah resveratrolu v rozmezí hodnot 0,05–0,08 mg.l^{-1} (průměrný obsah 0,06 mg.l^{-1}). Ze získaných výsledků je možné vyvodit závěr, že i u vzorků se stejnými podmínkami při dozrávání, lahvování a skladování vína hraje určitou roli i výběr jednotlivé lahve.

polyfenolické látky; resveratrol; Tramín; variabilita

Contact Address:

Ing. Alena H e j t m á n k o v á , CSc., Česká zemědělská univerzita v Praze, Agronomická fakulta, katedra chemie, Kamýcká 129, 165 21 Praha 6-Suchdol, Česká republika, tel.: +420 224 382 715, fax: +420 234 381 840, e-mail: hejtmankov@af.czu.cz