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Influence of UV and Ozonised Water Treatment on *Trans*-resveratrol Content in Berry Skins and Juices of Franc and Green Veltliner Grapes

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Abstract

Landfeld A., Tříska J., Balík J., Strohalm J., Novotná P., Vrchotová N., Totušek J., Lefnerová D., Híc P., Tománková E., Halama R., Houška M. (2015): **Influence of UV and ozonised water treatment on *trans*-resveratrol content in berry skins and juices of Franc and Green Veltliner grapes**. Czech J. Food Sci., 33: 267–276.

Grapes from two varieties – Franc (red) and Green Veltliner (white) were processed using UV radiation at selected powers and times. Irradiated grapes were stored for 24, 48, and 72 h at room temperature. A second set of grapes was dipped into ozonised water. We tested the influence of ozone concentration, dipping time, and storage time. All experiments were performed using grapes harvested in 2009, 2010, and 2011. The two treatments were compared relative to *trans*-resveratrol content in grape skins and juices (prepared from treated grapes).

Keywords: grape juices; stilbens content; UV irradiation; ozonisation

The cardiovascular benefits of resveratrol, a substance found in the grapes and wine of *Vitis vinifera*, are well known with multiple citations in the literature, e.g. Cui *et al.* (2002). Evidence for the chemopreventive effects of resveratrol was first published in 2003 (Dong 2003). Resveratrol was studied by Perečko *et al.* (2008) for the antioxidative activity and compared with other stilbene derivatives. Resveratrol has also been suggested as a substance that could influence longevity (Maroon 2009) and it also plays an important role in supporting immunity and reducing stress (Šmidrkal 2010; Harmatha 2011).

These studies form the basis for the medical recommendation of moderate and controlled consumption of red wine for the prevention of cardiovascular diseases. However, this form of prevention is unavoidably connected with the consumption of small amounts of alcohol, which leads to some scepticism from many nutritional experts. Therefore, our work is intended to alleviate this concern by preparing non-fermented grape juices with increased *trans*-resveratrol concentrations.

It has been shown that resveratrol has substantial anti-moulding activity (Adrian *et al.* 1997) and its increased production in grapes and also in the grapes of interspecific grapevine varieties (Bábíková *et al.* 2008) is related to several types of biological stress, but mainly stress caused by the *Botrytis cinerea* mould. Upon infestation of grapevine plants by *Plasmopara viticola* 2,4,6-trihydroxyphenanthrene-2-*O*-glucoside was identified as the main fluorescent natural product arising from *trans*-resveratrol glucoside in the grapevine leaves (Tříska *et al.* 2012). Some researchers have studied the influence of UV radiation, with spe-

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cific wavelengths, as a stressor on plants and grapes (Langcake & Pryce 1977) and several authors have published on this topic, e.g. Cantos *et al.* (2001), Versari *et al.* (2001), Berli *et al.* (2008), Resign and Kunter (2008) also with regard to the *trans*- to *cis*-resveratrol isomerisation (López-Hernández *et al.* 2007; Montsko *et al.* 2008). The *cis*-form of resveratrol is normally present to a lesser extent in grape berries and leaves. According to the literature (Otreba *et al.* 2006), the presence of *cis*-resveratrol in the leaves, berries, and wines is a consequence of so-called bioproduction during the treatment of *Vitis vinifera* plants in the vineyards. Isomerisation of *trans*-resveratrol solution in diffused daylight and its dependence on temperature and *trans*-resveratrol isomerisation in wine samples were investigated using liquid chromatography with electrochemical and UV detection (Kolouchová-Hanzlíková *et al.* 2004).

It has been shown that ozone can also be used as a stressor and leads to an increase in resveratrol production (González-Barrio *et al*. 2006); however, its effectiveness appears to be much lower than that of UV radiation.

A detailed review of physical methods that can be used to increase the concentration of resveratrol as well as other biologically beneficial substances in grapes and grape products was recently published by Tříska and Houška (2012).

Our goal was to try to experimentally increase the content of biologically active molecules, *trans*-resveratrol in particular, in grapes and juices prepared from grapes. However, we were concerned that current grape juice and wine preparation methods might not be able to preserve the increased content of these biologically active molecules. One of the main research goals was to try to increase *trans*-resveratrol production in grapes using physical methods (abiotic stress: i.e. application of UV radiation and dipping in ozonised water).

MATERIAL AND METHODS

Material. The experiments were carried out with selected *Vitis vinifera* L. (cvs Franc and Green Veltliner) grapes containing direct antioxidants and phenolic substances, and grown under standard conditions of wine agriculture at the Mendel Universtiy in Brno, Faculty of Horticulturae, work place in Žabčice, Czech Republic from three harvest years (2009, 2010 and 2011).

Selected sorts of Vitis vinifera over several years

2009 – Franc grapes were harvested on October 14, 2009, 22.0°Bx, total acids 5.4 g/l (acids were expressed as tartaric acid).

2010 – Green Veltliner grapes were harvested on October 4, 2010, 20.5°Bx, total acids 13.2 g/l and Franc grapes on October 13, 2010, 19°Bx, total acids 13.8 g/l acids.

2011– Green Veltliner grapes were harvested on October 11, 2011, 20°Bx, total acids 9 g/l and Franc grapes on October 4, 2011, 20.5°Bx, total acids 9.25 g/l (expressed as wine acid).

Methods

The year 2010 was selected to test the influence of process parameters in broad ranges on *trans*-resveratrol content. In case that these changes in process parameters had no or bad effect on *trans*-resveratrol content, these ranges were not applied in 2011.

Equipment for grape treatment with UV irradiation. A wooden box (inner dimensions 1050 mm × 1500 mm, height 1300 mm) was constructed for the grape treatment using germicide UV-C irradiation (wavelength 254 nm). All inner surfaces were covered with reflective aluminium foil. There were two UV panels (five UV bulbs per panel, one panel on each side) with a total power of 525 W. For health and safety reasons, the tubes had external switches that allowed the tubes to be turned off before opening the treatment box (Figure 1).

Each treatment was applied to 3 grape clusters. Radiation time (30 and 60 s), UV power (250 and 525 W), and storage times (24 and 48 h) were the

Figure 1. Arrangement of UV irradiation equipment

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experimental variables. A distance of grapes from the UV radiation panels was 40 cm. Treated samples were stored in plastic sieves. After storage, 60 grapes from each grape cluster were tested. Individual grapes were stored, in groups of 30, in plastic bags and frozen.

We applied two UV treatment methods for the year 2010 – Method 1 irradiated whole grapes while Method 2 irradiated isolated grape berries.

Equipment for grape treatment with ozonised water. The main piece of equipment for this part of the experiment was a washing machine of our own design, having three laminar boxes with antimicrobial filters; the machine was made by the Labox Co. (Jirny, Czech Republic). This machine was equipped with a stainless steel bath in which a box containing grapes was placed. The box served as a storage bath for the ozonised water and was fitted with a water inlet and a drain. The Ozontech Company (Zlín, Czech Republic) provided the generator used to produce ozonised water. The generator produced 2 g of ozone per hour. The ozone was mixed with water in a cooled static mixer (Frigera Metal, Kolín, Czech Republic). The ozonised water was kept in a stainless steel heat-insulated vessel, with an inner volume of 300 litres.

A circulating pump kept the water moving through the static mixer allowing high ozone concentrations in the final product (mean ozone concentration was 0.85 ppm at 6°C). Ozonised water was pumped into a dip chamber in the laminar box. Water with an average ozone concentration of 0.42 ppm was produced by mixing ozonised water with chilled tap water. A schematic of the equipment is presented in Figure 2. This schematic is valid for experiments done during 2009. Experiments done in 2010 and 2011 used an improved dip chamber and a closed system for moving ozonised water to and from the storage vessel. Ozone concentration was measured at the dip chamber outlet, so it was possible to know the exact ozone concentration during processing.

Production of grape juice by thermal treatment D20 after UV treatment or dipping in ozonised water. Grape juices were prepared shortly after UV or ozonised water treatment. One juice sample was prepared using each of the three different types of treated grapes.

300 g of grapes were separated from treated grape clusters. 30 mg of ascorbic acid was added to that grape mass. The grapes were squeesed and mixed, and thermal maceration followed. The mixture was heated in a double jacketed vessel to a temperature of 80°C (heating time 6–7 min). After reaching 80°C the mixture was put into a 500 ml beaker and placed in a thermostatic bath (holding time was 20 min at 80°C). The mixture was poured into a plastic sieve and the juice was left to freely flow out (10–30 min). The process was concluded with 100 g samples of juice being placed into plastic bags made from layered PE/PA, the bags were then heat sealed and frozen.

Trans-resveratrol content in juices and grape skins. A 15 ml volume of juice samples was separated, then centrifuged and the supernatant was collected. The sediment was extracted 3 times with 5 ml of 100% methanol at room temperature. The first extraction was held for 30 min, while the second and third extractions were held for 10 minutes. All supernatants were mixed together and centrifuged to produce a pure clear extract. This extract (stored before measurement at –18°C) was then analysed using HPLC.

The determination of *trans*-resveratrol content in grape skins was based on a similar procedure used for juices. Extracts from lyophilised grape skins (about 0.7 g) were prepared using 100 ml of 75% methanol.

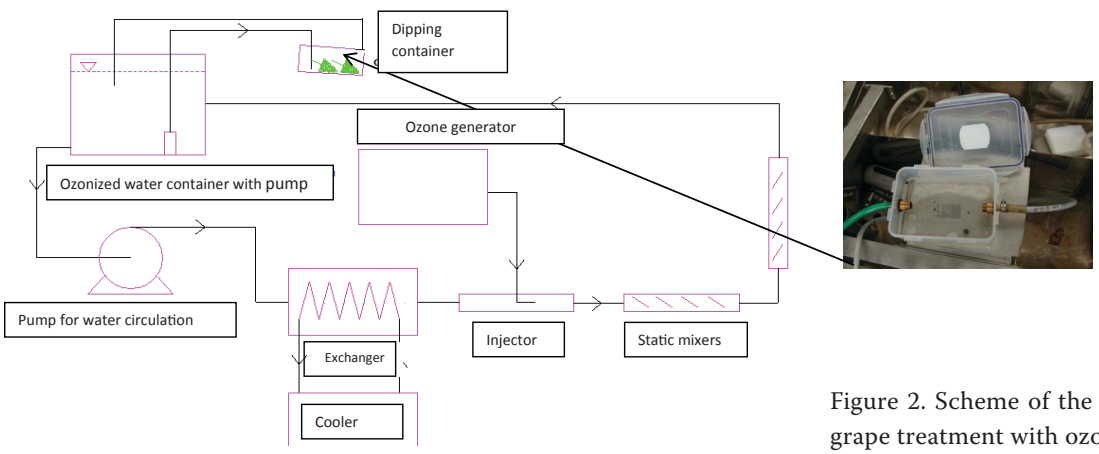

Figure 2. Scheme of the apparatus for grape treatment with ozonised water

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Results were recalculated based on 1 g of dry matter of grape skins and also on 1 g of fresh grapes.

Determination of trans-resveratrol by HPLC

Determination of *trans*-resveratrol was performed according to Tříska *et al.* (2012). The samples were analysed using an HP 1050 HPLC instrument (Hewlett Packard, Palo-Alto, USA) with HP G1315B diode array detector on 3 µm, 150 mm × 2 mm, Luna C18(2) column with water-acetonitrile-*o*-phosphoric acid mobile phase. Mobile phase A used 5% of acetonitrile + 0.1% of *o*-phosphoric acid; mobile phase B used 80% of acetonitrile + 0.1% of *o*-phosphoric acid. The gradient was increased from 20% to 80% of B during 20 min and from 80% to 100% of B during 5 minutes. Flow rate was 0.250 ml/min and column temperature 25°C. Detection of *trans*-resveratrol was done at 315 nm. The relative standard deviation (RSD) of *trans*-resveratrol determination was 2.5%.

Antimutagenic activity of grape skin extracts. Samples prepared from cvs Green Veltliner and Franc, harvested during the year 2010, were tested for microbial contamination before testing for antimutagenic activity. The results confirmed that the samples were not contaminated. The methanol extracts of grape skins were treated before testing. Methanol was evaporated on a vacuum evaporator at a temperature of 40°C and the residue was lyophilised at a temperature of –55°C and pressure 7 Pa. Lyophilised material was diluted in dimethyl sulphoxide (DMSO) considering that 100 µl of the solution applied to a plate corresponded to the dose of 30 mg of lyophilised skins.

Antimutagenic activity was tested by the Ames test procedure on *Salmonella typhimurium* TA 98 bacterial strain on Petri dishes. The 2-amino-3-methyl-3H-imidazo-(4,5-f)-quinoline (IQ) was applied as a model mutagen in a dose of 10 ng per dish. This particular mutagen requires metabolic activation with activator S9, which was added to the system.

Tests were replicated three times. Samples were tested also with the absence of IQ mutagen for potential occurrence of mutagenicity of the pure sample. Results of this test were negative. Inhibition of mutagenicity (inhibition rate I) was calculated using the following equation:

$$I\ (\%) = 100 - 100\ [R\ (v + m)/R\ (m)]$$

where: R (v + m) – number of colonies of revertants in test (sample + mutagen); R (m) – number of colonies of revertants in test (mutagen)

Evaluation scale of inhibition: 0–20 – negative; 20–40 – weakly positive; 40–60 – positive; > 60 – strongly positive.

Statistical evaluation. The *trans*-resveratrol content for each sample was calculated three times (using three independent samples). The mean values and standard deviations of results were also calculated. We used the Datafit 6.1 software (Engineering America Inc, Oakdale, USA) and searched for an optimum correlation function. The highest correlation coefficient was predicted for a non-linear correlation function:

$$Y = \exp(ax_1 + bx_2 + cx_3 + dx_4 + e) \quad (1)$$

where: x_1–x_4 – process parameters typical of a specific treatment process; Y – *trans*-resveratrol concentration in berry skins or wine juices

Table 1. The influence of UV radiation on *trans*-resveratrol content in Franc grapes (2009)

Treatment	Power (W)	Holding time of UV treatment (s)	Storage time (h)	*Trans*-resveratrol (µg/g ± SD)
000-0-0	0	0	0	0.63 ± 0.03
250-30-48		30	48	0.66 ± 0.03
250-60-24	250	60	24	1.28 ± 0.06
250-60-48		60	48	0.87 ± 0.04
500-30-24		30	24	0.88 ± 0.04
500-30-48		30	48	0.69 ± 0.04
500-60-24	525	60	24	0.62 ± 0.03
500-60-48		60	48	0.22 ± 0.01
525-120-24		120	24	1.36 ± 0.07*

SD – standard deviation; the given value was determined in the whole berry, not in the berry skin; *grapes were placed on an Al film and UV treated from the top of the unit

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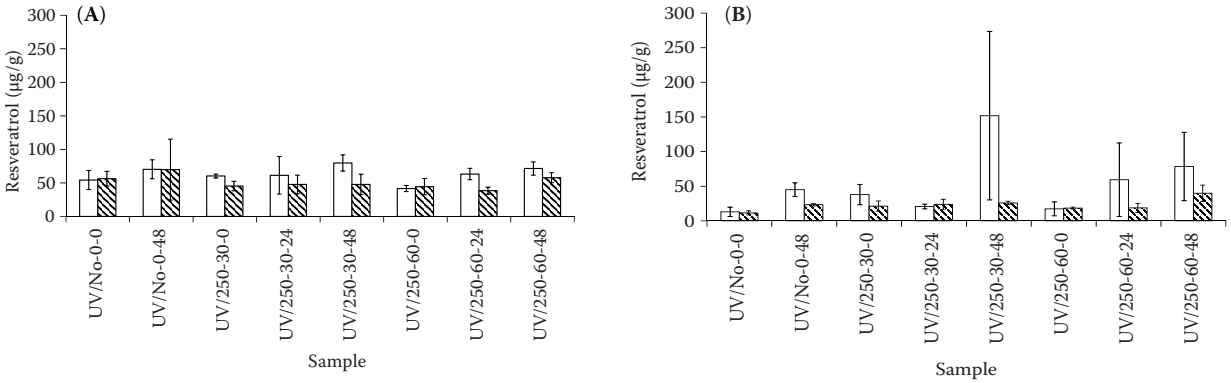

Figure 3. *Trans*-resveratrol content in lyophilised grape skins of cvs (A) Franc and (B) Green Veltliner – □ grapes (method 1) and ▨ berries (method 2) treated with UV radiation (2010)

RESULTS AND DISCUSSION

Influence of UV irradiation on trans-resveratrol content and antimutagenicity

Trans-resveratrol content in grape skins of Franc was influenced by the power, irradiation time and storage time. Franc grapes were used to test the effect of UV radiation. Results of the analysis are shown in Table 1. The highest *trans*-resveratrol content in grape skins was measured during the last experiment using 525 W for 120 s and grape storage for 24 hours. This data set was correlated with Eq. (1) (without parameter x_4, where x_1 – power of UV lamps in W, x_2 – holding time of UV treatment in s, x_3 – storage time of grapes after treatment in h). The final correlation equation for the Franc grape skins was: $Y = \exp(-0.00095x_1 + 0.00968x_2 - 0.00649x_3 - 0.19072)$, where: Y – *trans*-resveratrol content in mg/g of mass of the whole grapes.

The correlation coefficient of the above equation was $R = 0.726$ ($R_{crit} = 0.874$, number of measurements = 9) and was lower than the critical value. Tested parameters x_1–x_3 were not statistically significant ($P > 0.05$ for all parameters).

Results of trans-resveratrol content for Franc (2010) and Veltliner green (2010) are given in Figure 3.

Results from the UV irradiation of grape clusters and individual grapes are presented separately. Franc grapes had higher *trans*-resveratrol contents compared to Green Veltliner grapes (Figure 4A). Higher *trans*-resveratrol content was found in grape skins after the irradiation of whole grapes. Figure 4B contains analytical results of *trans*-resveratrol in grape skins for UV treated grapes, Franc and Green Veltliner, 2011.

The data set valid for cv. Franc grape skins (2010 and 2011) was correlated with Eq. (1), where: x_1 – power of UV lamps in W, x_2 – holding time of UV treatment in s, x_3 – storage time of grapes after treatment in h, x_4 – year of harvest. The final correlation equation for Franc grape skins was as follows: $Y = \exp(0.00051x_1 - 0.00102x_2 + 0.00527x_3 - 0.8667x_4 + 1746.1)$, where: Y – *trans*-resveratrol content in mg/g of mass of lyophilised skins; the correlation coefficient of the above equation was $R = 0.846$ ($R_{crit} = 0.420$, number of measurements = 54) and it was much higher than the critical value; parameters x_1 and x_2 were not statistically significant ($P > 0.05$) but parameters x_3 and x_4 significantly influenced the *trans*-resveratrol content in grape skins.

The final correlation equation for Green Veltliner grape skins (2010 and 2011) was like this: $Y = \exp(0.00591x_1 - 0.01268x_2 + 0.03843x_3 - 0.8945x_4 + 1799.9)$.

The correlation coefficient of the above equation was $R = 0.717$ ($R_{crit} = 0.420$, number of measurements = 54) and it was much higher than the critical value. All process parameters were statistically significant ($P < 0.05$).

Cantos *et al.* (2001) presented results for the *trans*-resveratrol content in skins of red Napoleon grapes treated with UV radiation pulses. They tested the influence of power (maximum 510 W), irradiation times (0–60 s), and storage times (0–7 days). Maximum concentrations were reached using 510 W, 60 s exposure times and storage time of 3 days. The maximum *trans*-resveratrol concentration was approximately 100 µg/g of fresh skin weight. This value agrees well with our results presented in Figure 4.

The *trans*-resveratrol content in juices made of Franc grapes, harvested in 2011, was statistically analysed and the optimum correlation was found out [Eg. (1)] (parameter x_4 is missing, only one year data set was applied): $Y = \exp(0.00094x_1 - 0.00184x_2 + 0.0133x_3 + 0.6107)$.

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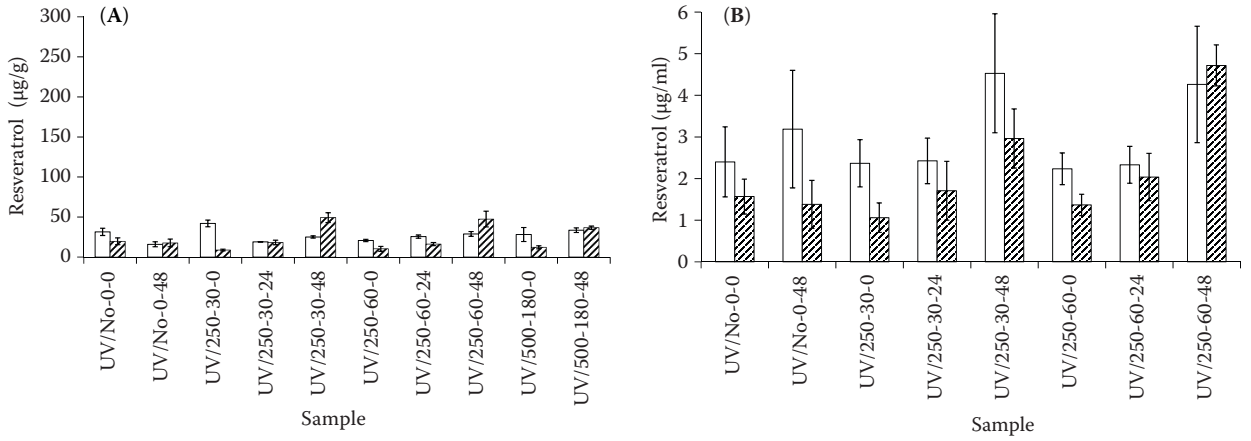

Figure 4. *Trans*-resveratrol content (A) in grape skins of UV treated Franc (□) and (B) in juices made from UV treated Green Veltliner (▧) grapes (2011)

The correlation coefficient of the above equation was $R = 0.651$ ($R_{crit} = 0.563$, number of measurements = 24) and it was higher than the critical value; parameters x_1 and x_2 were not statistically significant ($P > 0.05$) but parameter x_3 (storage time) significantly influenced the *trans*-resveratrol content in juice.

The same analysis was done for data of *trans*-resveratrol content in juices prepared from Green Veltliner grapes. The optimum correlation equation was: $Y = \exp(0.000704x_1 + 0.0130x_2 + 0.2374x_3 - 0.6063)$.

The correlation coefficient of the above equation was $R = 0.863$ ($R_{crit} = 0.563$, number of measurements = 24) and it was higher than the critical value. Parameter x_1 was statistically insignificant ($P > 0.05$) but parameters x_2 and x_3 had a statistically significant influence on the *trans*-resveratrol content in juice.

Inhibition rate of antimutagenic activity of grape skin extract after UV treatment. Antimutagenic activities of grape skins from Franc grapes harvested in 2010 are shown in Figure 5A. It is apparent that the UV treatment did not influence antimutagenic activity. Figure 5B shows that the UV irradiation of grapes had no statistically significant influence on antimutagenic activity. Skins from both varieties exhibited strong antimutagenic activity.

Influence of dipping into ozonised water on *trans*-resveratrol content and antimutagenic activity

Trans-resveratrol content in Franc grape skins, relative to ozone concentration, dipping time, and storage time. Table 2 contains experimental variables associated with dipping grapes (Franc grapes, 2009) into ozonised water. Additionally, there are results

Table 2. The influence of dipping grapes into ozonised water relative to *trans*-resveratrol content in Franc grapes (2009)

Sample	Average concentration of ozone in water (ppm)	Time of dipping into ozonised water (min)	Storage time (h)	*Trans*-resveratrol (µg/g ± SD)
0A-0-0	0	0	0	0.31 ± 0.02
0A-0-24		30	24	0.18 ± 0.01
0A-0-48			48	0.60 ± 0.03
A-10-24	0.42	10	24	0.46 ± 0.02
A-10-48			48	0.77 ± 0.04
A-60-24		60	24	0.61 ± 0.03
A-60-48			48	0.38 ± 0.02
2A-10-24	0.85	10	24	0.30 ± 0.02
2A-10-48			48	0.83 ± 0.04
2A-60-24		60	24	0.97 ± 0.05
2A-60-48			48	3.71 ± 0.19

SD – standard deviation

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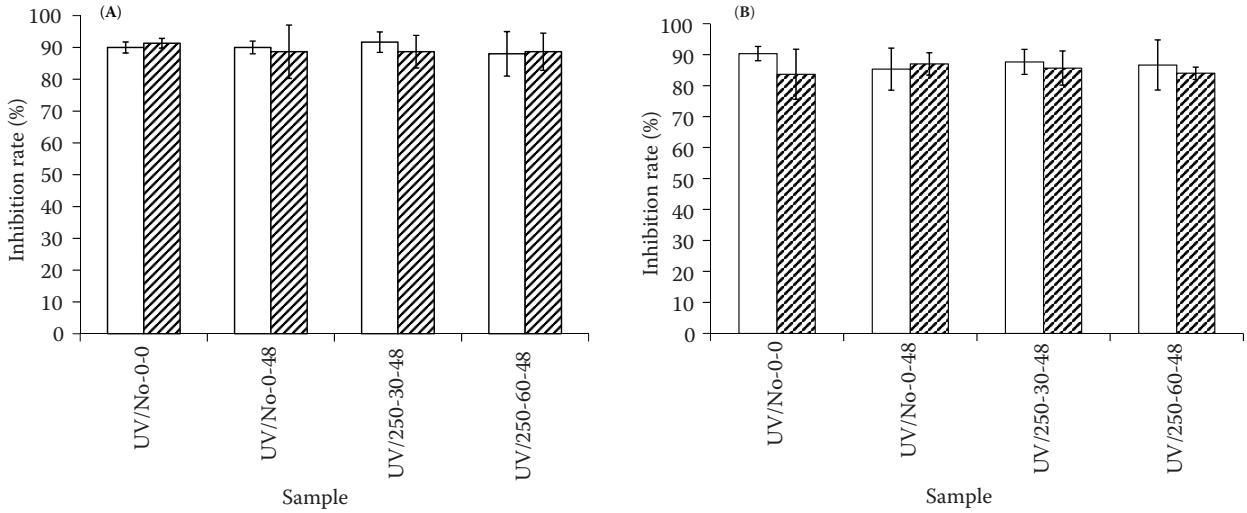

Figure 5. Inhibition rate of antimutagenicity I, mutagen IQ, extract of grape skins of UV treated (A) Franc grapes and (B) Green Veltliner grapes, 2010 (□ grapes – method 1, ▧ berries – method 2)

from the *trans*-resveratrol analysis of grape skins recalculated to the mass of whole grapes. Presented values confirmed that increased *trans*-resveratrol content can be achieved by dipping grapes into ozonised water (up to 0.85 ppm) for 10–60 min followed by storage for 48 hours. Substantially lower *trans*-resveratrol values were found in untreated samples.

This data set was correlated with Eq. (1) (without parameter x_4, x_1 – ozone concentration in water in ppm, x_2 – holding time of dipping into ozonised water in min, x_3 – storage time of grapes after treatment in h). The final correlation equation for Franc grape skins was: $Y = \exp(3.6771x_1 + 0.0258x_2 + 0.0505x_3 - 5.811)$.

The correlation coefficient of the above equation was R = 0.934 (R_{crit} = 0.807, number of measurements = 11) and it was higher than the critical value; all tested process parameters x_1–x_3 had a statistically significant influence ($P < 0.05$ for all parameters).

Figure 6A contains the results of *trans*-resveratrol content in skins of Green Veltliner and Franc grapes harvested in 2010 and dipped into ozonised water. Results are valid for lyophilised grape skins. It is apparent from Figure 6A that the *trans*-resveratrol content is not higher after treatment (with ozonised water + storage) compared with untreated samples.

Figure 6B shows a significant increase in *trans*-resveratrol content in grape skins of Green Veltliner and Franc, but only in those subjected to a post-treatment storage time of 24 hours. Figure 6B also shows that Franc grapes did not respond to ozonised water treatment and storage.

The data set valid for Green Veltliner grape skins for the years 2010 and 2011 was statistically evalu-

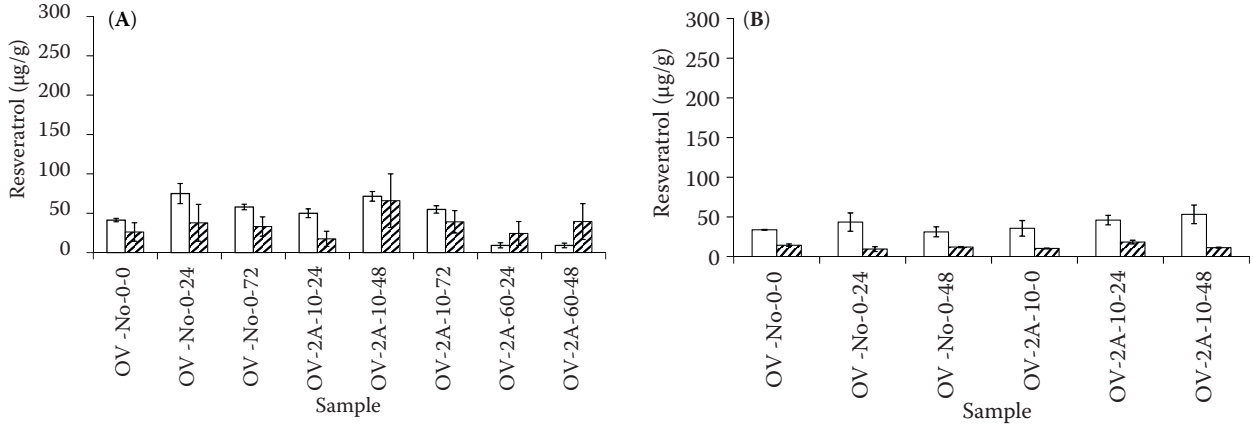

Figure 6. *Trans*-resveratrol content in lyophilised grape skins of Green Veltliner (▧) and Franc (□) grapes (A) 2010 and (B) 2011 (OV – grapes dipped into ozonised water)

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Table 3. Franc grapes and reen Veltliner grapes (2011) dipped into ozonised water (OV), the influence of ozone concentration, dipping time, and grape storage time on juice (3 grape clusters)

Treatment	Symbol (treatment-storage-repeating)	Dipping time (min)	Storage time (h)	O_3 concentration (ppm)
Franc grapes				
	M-No-0-0-1,2,3	0	0	0
Control	M-No-0-24-1,2,3	0	24	0
	M-No-0-48-1,2,3	0	48	0
	M-2A-10-0-1,2,3	10	0	2.9–2.4
OV	M-2A-10-24-1,2,3	10	24	2.4–1.8
	M-2A-10-48-1,2,3	10	48	2.4–1.4
Green Veltliner grapes				
	M-No-0-0-1,2,3	0	0	0
Control	M-No-0-24-1,2,3	0	24	0
	M-No-0-48-1,2,3	0	48	0
	M-2A-10-0-1,2,3	10	0	3.2–2.4
OV	M-2A-10-24-1,2,3	10	24	2.6–1.8
	M-2A-10-48-1,2,3	10	48	2.2–1.5

Mean storage temperature 21.1°C; mean temperature at treatment 9.3°C

ated and the optimum non-linear correlation Eq. (1) (where: x_1 – ozone concentration in water in ppm, x_2 – dipping (holding) time in ozonised water in minutes, x_3 – storage time of grapes after treatment in h, x_4 – year of harvest) was constructed: $Y = \exp(0.1143x_1 - 0.00165x_2 + 0.00549x_3 - 1.151x_4 + 2317.4)$, where: Y – *trans*-resveratrol content in mg/g of mass of lyophilised grape skins. The correlation coefficient of the above equation was $R = 0.621$ ($R_{crit} = 0.471$, number of measurements = 42) and it was higher than the critical value. Only the year of harvest (x_4) had a statistically significant influence ($P < 0.05$).

The same analysis was done for data of *trans*-resveratrol content in grape skins of Franc grapes (also for the years 2010 and 2011). The optimum correlation equation was: $Y = \exp(0.1868x_1 - 0.0280x_2 + 0.00303x_3 - 0.544x_4 + 1096.9)$.

The correlation coefficient of the above equation was $R = 0.842$ ($R_{crit} = 0.471$, number of measurements = 42) and it was higher than the critical value; only the storage time after treatment (x_3) was statistically insignificant ($P > 0.05$); all other parameters had a statistically significant influence on *trans*-resveratrol content.

Table 3 contain the experiment protocol for dipping Franc and Green Veltliner grapes (2011). After treatment the grapes were stored for specified times and then used for the preparation of juices and lyophilised grape skins.

Figure 7 presents the *trans*-resveratrol content in juices prepared from Green Veltliner and Franc grapes (2011). It is obvious that there was no statistically significant increase of *trans*-resveratrol content, associated with grape storage for 24 and 48 h after treatment. Longer storage times did not significantly increase the *trans*-resveratrol content in juice. It is apparent from Figure 7 that dipping into ozonised water increases the *trans*-resveratrol content in juice

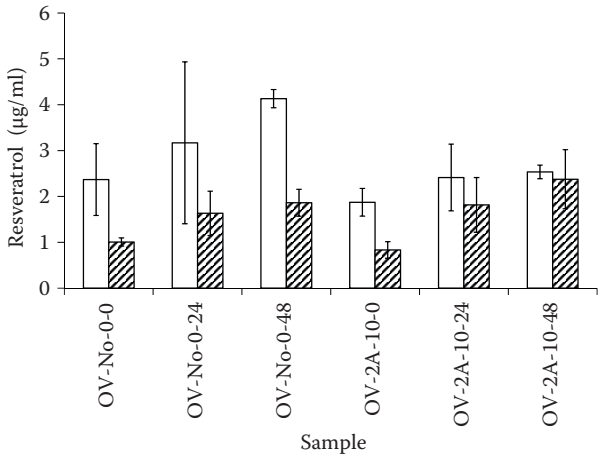

Figure 7. *Trans*-resveratrol content in juices made from Green Veltliner (▨) and Franc (□) grapes dipped into ozonised water, 2011 (OV – grapes dipped into ozonised water before the juice preparation)

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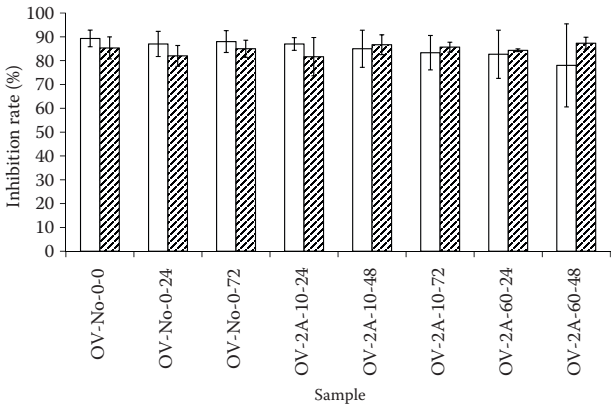

Figure 8. Inhibition rates of antimutagenicity I, mutagen IQ, extract of grape skins of Green Veltliner (▨) and Franc (□) grapes, dipped into ozonised water (influence of ozone concentration, dipping time and storage time of grapes), 2010

from Franc grapes, mainly associated with storage after treatment, however, the increase is not statistically significant compared to untreated samples.

The data set valid for Green Veltliner grape juices for the year 2011 was statistically evaluated and an optimum non-linear correlation Eq. (1) was developed. The following parameters were tested: x_1 – the ozone concentration in water in ppm, x_2 – the dipping (holding) time into ozonised water in min, x_3 – the storage time of grapes after treatment in hours. Parameter x_4 the year of harvest was not considered because only one harvest was evaluated. The optimum correlation equation was: $Y = \exp(-0.5116x_1 + 0.1228x_2 + 0.0105x_3 + 0.1390)$, where: Y – *trans*-resveratrol content in mg/ml of the juice; the correlation coefficient of the above equation was $R = 0.810$ ($R_{crit} = 0.646$, number of measurements = 18) and it was higher than the critical value; none of the tested parameters had a statistically significant influence on *trans*-resveratrol content in juice ($P > 0.05$).

The same analysis was done for the data of *trans*-resveratrol content in juices prepared from Franc grapes. The optimum correlation equation was: $Y = \exp(0.2513x_1 - 0.0904x_2 + 0.01095x_3 + 0.8868)$, where: Y – *trans*-resveratrol content in mg/ml of the juice; the correlation coefficient of the above equation $R = 0.710$ ($R_{crit} = 0.646$, number of measurements = 18) was higher than the critical value; only the storage time (x_3) was statistically significant ($P < 0.05$); none of the other parameters was statistically significant.

We can also compare literature results with results of juices prepared from ozone-treated grapes (Figure 7). Our results for Veltliner grapes range from 0.8 µg/g to 2.4 µg/g of fresh weight, and from 1.9 µg/g to 4.1 µg/g of fresh weight of Franc grapes. These ranges coincide with ranges published by Bábíková *et al.* (2008).

Inhibition rate of the antimutagenic activity of grape skin extract after ozonisation. It is evident from Figure 8 that inhibition rates of antimutagenic activity are usually strongly positive and almost independent of the treatment method and storage time after treatment. The mean value of the rate of antimutagenic activity was around 80%.

CONCLUSIONS

We can briefly conclude that both the tested technologies proved to be potentially applicable methods for increasing the *trans*-resveratrol content in skins and juices prepared from treated grapes. Skins with increased *trans*-resveratrol content can be used for extraction of the substance into the wine or alcoholic beverages of various types. For detailed conclusions see supplementary on-line materials.

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