Multi-experimental Characterization of Grape Skin Extracts

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Abstract: Grape skins contain a plenty of different flavonoids, most of them revealing significant antioxidant properties. In this contribution, a complex study is presented of grape skin ethanol extracts, prepared from grape skins of two vine grape varieties, Svatovavřinecké (St. Laurent) and Alibernet. Extracts were prepared from two different amounts of lyophilised grape skin powders using the pressurised fluid extraction (PFE). The antioxidant activity of the extracts was tested by EPR spectroscopy in Fenton system generating reactive radicals ($^{\bullet}OH$, $O_2^{-\bullet}$, $^{\bullet}R$) followed by spin trapping technique. In addition, radical scavenging activity of the extracts was assessed applying 2,2-diphenyl-1-picrylhydrazyl ($^{\bullet}DPPH$) free radical and 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) cation radical ($ABTS^{\bullet+}$) assays. Total phenolic content (TPC) of the individual extracts and their tristimulus colour values (CIE Lab) were evaluated, using an UV-VIS spectrophotometer. All the data obtained were subsequently correlated and discriminated, using the multivariate statistics, involving the canonical discriminant analysis, principal component analysis, and canonical correlation analysis. Results obtained indicated that PFE is a suitable extraction technique, only slightly influencing antioxidant ability as well as composition of the so-prepared extracts. The influence of extraction conditions on the entire monitored characteristics was insignificant.

Keywords: grape skin; ethanol extracts; PFE; EPR; DPPH; ABTS

A growing attention has been recently focused on the improvement of human health by the consumption of food or food supplements rich in antioxidants. Grape skins contain a plenty of different flavonoids, e.g., quercetin, catechins, flavonols, anthocyanidins, phenolic acid derivatives, and other compounds. Anthocyanins, as the most abundant of them, are water-soluble pigments possessing many human health beneficial effects such as the enhancement of visual acuity, reduction of the incidence of coronary heart disease, anticarcinogenic, antimutagenic, anti-inflammatory, and antioxidative properties (Cantos *et al.* 2002; Czyzowska & Pogorzelski 2002; Ju & Howard 2003; Garcia-Alonso *et al.* 2005; Longo & Vasapollo 2006).

A great deal of consideration should be given to the choice of a suitable extraction system for anthocyanins, as these are highly reactive compounds, exceptionally sensitive to pH changes (Kähkönen *et al.* 2003; Minussia *et al.* 2003). Traditionally, the extraction into organic solvent mixtures (Cantos *et al.* 2002) or acidified aqueous solutions of organic solvents (Kähkönen *et al.* 2003; Postescu *et al.* 2007) have been used. In recent years, the supercritical fluid extraction

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with carbon dioxide (Karasek *et al.* 2003) and pressurised liquid extraction with acidified water, sulphured water, or acidified organic solvents (Ju & Howard 2003, 2005; Luque-Rodriquez *et al.* 2007) have been successfully applied for the extraction of different phenolic compounds from grapes and wines. In this contribution, a complex study of grape skin ethanolic extracts prepared by Pressurised Fluid Extraction (PFE) (Richter *et al.* 2006) from two vine grape varieties, St. Laurent and Alibernet is presented.

MATERIAL AND METHODS

Two grape varieties, St. Laurent and Alibernet from Velké Pavlovice and Mikulov sub-regions (south Moravia region, Czech Republic) were used in experiments. Full-blown grape berries of excellent quality were collected during the 2007 vintage.

A static PFE of polyphenols from red grape skins was performed using a *One*PSE extractor (Applied Separations, Allentown, USA). A portion (0.5 g or 1 g) of lyophilised grape skin powders was placed into a 22 ml extraction cell containing glass beads (570–700 µm) at the bottom. The PFE parameters were set as follows: pressure, 15MPa; extraction time, 3 × 5 min; rinsing time, 20 s; and nitrogen purge time, 90 s after each cycle and 120 s after the extraction run. To determine the effect of temperature on the content of polyphenolic compounds, individual extracts were prepared at temperatures of 40, 60, 80, 100, and 120°C, respectively, using ethanol as the extraction solvent. After each PFE run, the extracts were cooled to 5°C and stored in a fridge until the analysis (Richter *et al.* 2006). Analysis was performed within a one month after the extracts preparation.

Total phenolic compounds content (TPC) of the individual extracts was determined using the Folin-Ciocalteu assay and their trichromatic colour values L^*, a^*, and b^* of the extracts were estimated using an UV-VIS spectrophotometer Specord (Carl Zeiss, Jena, Germany) (Suhaj *et al.* 2006; Horváthová *et al.* 2007).

The antioxidant activity of the extracts was tested by EPR spectroscopy using the Bruker portable EPR spectrometer e-scan with the accessories in Fenton system (H_2O_2/Fe^{2+}), generating reactive hydroxyl radicals ($^{\bullet}OH$) followed by spin trapping technique, with the use 5,5-dimethylpyrroline-*N*-oxide (DMPO) as the spin trap (Polyakov *et al.* 2001). In addition, the radical scavenging activity of the extracts was assessed applying 2,2-diphenyl-1-picrylhydrazyl ($^{\bullet}$DPPH) free radical and 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) cation radical ($ABTS^{\bullet+}$) assays. All the experiments were performed at 298 K, using the same quartz flat cell. EPR spectra were processed similarly as previously described e.g., in Polovka (2006).

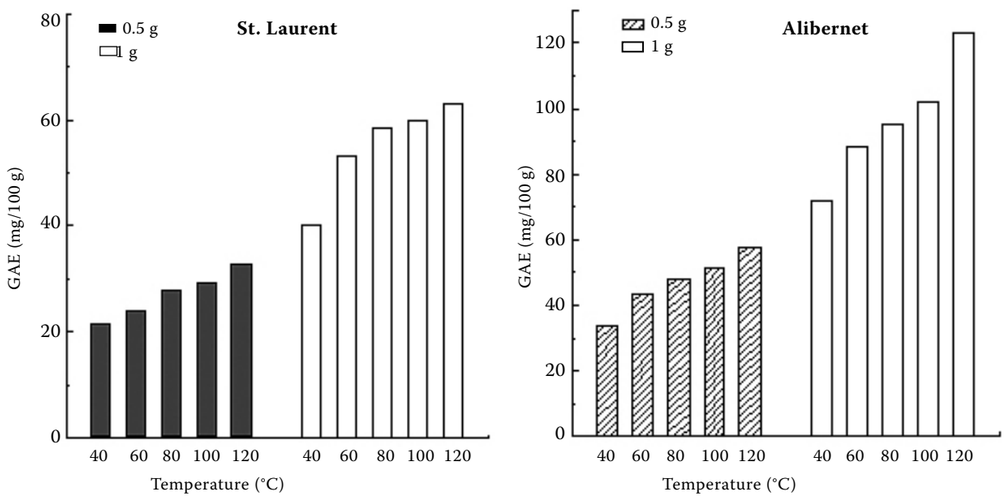

Figure 1. Polyphenols content in extracts prepared from different amount of grape skin powders (0.5 and 1 g) and at different temperature, measured in 25°C in comparison with gallic acid as standard (GAE)

Table 1. Correlation matrices between the TPC content and radical-scavenging abilities of grape skin extracts

	TPC	$TEAC_{ABTS^{\bullet+}}$	$TEAC_{^{\bullet}DPPH}$
St. Laurent			
TPC	1	0.9012	0.7349
$TEAC_{ABTS^{\bullet+}}$	0.9012	1	0.7208
$TEAC_{^{\bullet}DPPH}$	0.7349	0.7208	1
Alibernet			
TPC	1	0.9734	0.9750
$TEAC_{ABTS^{\bullet+}}$	0.9734	1	0.9654
$TEAC_{^{\bullet}DPPH}$	0.9750	0.9654	1

TPC – total phenolic compounds content; TEAC – Trolox-equivalent antioxidant capacity

In addition, pH values of all extracts were also measured using the combined glass electrode. The entire experiments were performed in triplicate.

All the data obtained were subsequently correlated and discriminated, using the multivariate statistics by means of Unistat® Statistical Package (Unistat Ltd, London, UK), involving the canonical discriminant analysis, principal component analysis, and canonical correlation analysis, respectively.

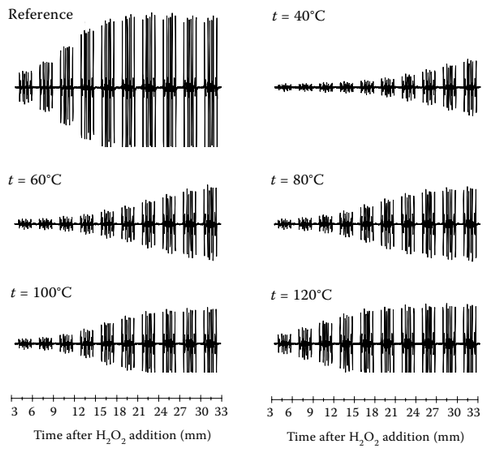

Figure 2. The time evolution of EPR spectra recorded in system containing ethanol (reference) and ethanolic extracts of Alibernet variety prepared from 1 g of crude grape skins at 40–120°C, respectively; Fenton's reagents and DMPO spin trap. Spectra were recorded at 298 K using magnetic field sweep, SW = 8 mT

RESULTS AND DISCUSSION

UV-VIS experiments

The TPC content was evaluated and expressed in all extracts as gallic acid equivalent (GAE) (Suhaj *et al.* 2006). The obtained results indicate that the content of polyphenols in the Alibernet variety extracts was on average twofold than that of the variety St. Laurent (Figure 1). This may explain the generally higher antioxidant and radical-scavenging activities of Alibernet extracts. A significant correlation was found between TPC and TEAC (Trolox-equivalent antioxidant capacity) with both types of extracts, in the dependence on the mass of grape skins used for the extract preparation. As follows from the data presented in Table 1, the correlation is better for Alibernet variety, probably due to the different composition of polyphenols.

As a result of the growing extraction temperature from 40°C up to 120°C, approximately 70% and 90% increase of the TPC content was noticed in the extracts of St. Laurent and Alibernet cultivars, respectively.

The influence of the extraction temperature on CIE L^*, a^*, b^* colour characteristics or on pH values was only negligible.

EPR experiments

The antioxidant properties of the grape skin extracts were tested in experimental systems in which free radicals were generated via Fenton reaction. Figure 2 shows a typical time evolution of EPR spectra recorded in the system containing the respective grape skin extract of St. Laurent vine variety and Fenton's reagents in the presence of DMPO spin trap. As follows from the simulation analysis, a dominant formation of $^{\bullet}$DMPO–CH2–CH2–OH, $^{\bullet}$DMPO–OH, and $^{\bullet}$DMPO–CX spin adducts was observed, respectively, in accord with our previous experiments and the data already published (Polyakov *et al.* 2001; Valdez *et al.* 2002; Kovács *et al.* 2004; Polovka 2006). The addition of the grape skin extracts into the system resulted in a decrease of the spin adduct concentration as a result of competitive reactions between the antioxidants, generated free radicals, and spin trap (Polovka *et al.* 2003; Polovka 2006; Staško *et al.* 2007).

Staško *et al.* (2006) suggested previously the expression of the antioxidant activity of Tokay

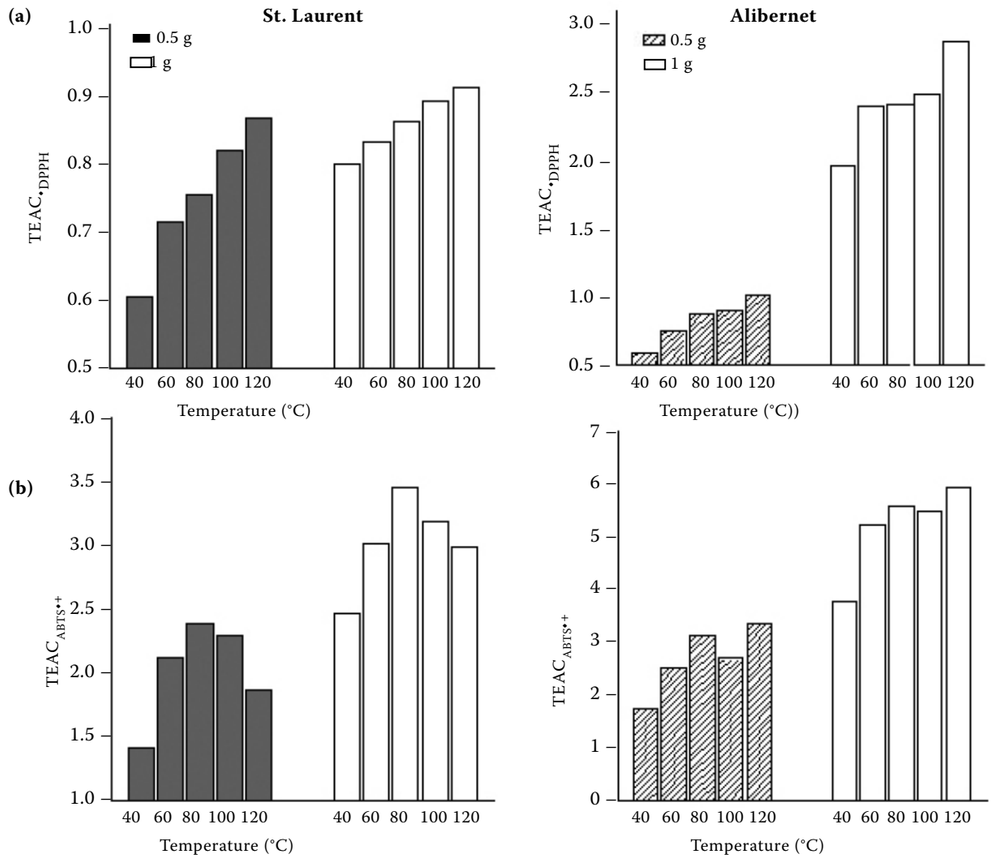

Figure 3. Trolox equivalent antioxidant capacity (TEAC) of St. Laurent and Alibernet extracts, prepared at different temperature from different mass of crude grape skins in the reaction with (a) •DPPH, (b) ABTS•+

wines as the relative amounts of the radicals scavenged. Using this approach, we can conclude that the antioxidant properties of the St. Laurent variety extract are significantly weaker than those of the Alibernet variety, reaching maximum 50% of that of Alibernet ones. Moreover, as we proved, the antioxidant activities of the extracts were in both cases strongly dependent on the extraction temperature.

•DPPH and ABTS•+ radicals assays are traditionally used for food samples radical-scavenging ability (RSA) evaluation. They were previously successfully used, e.g., for tea or wine samples characterisation (Polovka *et al.* 2003; Polovka 2006; Staško *et al.* 2006, 2007).

As we confirmed, the extracts of both varieties prepared at any of the temperatures demonstrated a significant ability to terminate •DPPH and ABTS•+.

Pellegrini *et al.* (2003) suggested an effective comparison between RSA of different food products, based on Trolox-equivalent antioxidant capacity calculation. Following this approach, TEAC value of each extract was calculated for the reactions with both •DPPH and ABTS•+. The obtained results indicated, that the increasing extraction temperature lead to significant (up to 100%) increase of $TEAC_{ABTS•+}$ values of the Alibernet variety (Figure 3a), while those of St. Laurent increased only slightly. Regarding the $TEAC_{DPPH•}$ (Figure 3b) values, in the case of St. Laurent extracts prepared from 1.0 g of lyophilised grape skins, they ranged from 0.80 to 0.90, whereas in the case of Alibernet from 2.0 to 2.9.

Statistical evaluation

All the above mentioned characteristics were used for the classification and mutual recognition of both grapevine varieties using the principal component analysis and canonical discrimination analysis (Koreňovská & Suhaj 2005).

Both statistical approaches provided practically 100% correct classification of both varieties. As also follows from the discriminant analysis, all the monitored characteristics are dependent on the mass of grape skins used for the respective extract preparation.

CONCLUSIONS

Multi-experimental analysis performed with ethanolic extracts prepared from grape skins of two grapevine cultivars (St. Laurent and Alibernet) by PFE revealed their significant antioxidant and radical-scavenging abilities, in significantly positive correlation with the TPC contents. The influence of the extraction temperature on radical-scavenging abilities, trichromatic values or TPC content was non-insignificant.

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