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# Towards complex utilisation of winemaking residues: Characterisation of grape seeds by total phenols, tocols and essential elements content as a by-product of winemaking

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ABSTRACT

Seeds of twelve white and red vine varieties cultivated on six vine-growing areas were assessed as a by-product after winemaking on the contents of total polyphenols (TP) and phosphorus (P) spectrophotometrically, total tocols (TC) by HPLC-FLD, and metals (Ca, Mg, Na, K, Fe, Zn, Cu and Mn) by FAAS. Remaining TP and TC levels were mainly affected by the variety, while levels of microelements (Cu, Mn and Zn) and P or K by the vine-growing area. The highest TC levels were found in the seeds of the Müller Thurgau, Pinot Noir and Zweigeltrebe varieties. Different white and red winemaking methods have significant impact on the TP content with higher remaining levels in white varieties. Conversely, red varieties contained higher levels of macroelements except P, however no significant differences between varieties have been found. Red varieties contained higher Fe, Cu, Zn and comparable Mn levels. Results herein revealed the considerable potential of grape seeds, a by-product of the vinification process, as a valuable inexpensive source of high added value of nutritionally beneficial compounds – polyphenol and tocol antioxidants and macro- and microelements for use as feed additives in animal nutrition.

## 1. Introduction

*Vitis vinifera* L. production is widespread throughout the world, exceeding 68 million tonnes (FAOSTAT, 2010). As grape seeds comprise about 5% of the fruit weight (Choi and Lee, 2009), more than 3 million tonnes of grape seeds are discarded annually worldwide (Fernandes et al., 2012). Grape seeds are an important part of the pomace, corresponding to 38–52% of dry matter (Maier et al., 2009). Regarding this fact, grape seeds are often referred as significant agricultural and industrial waste (Freitas et al., 2008; Kim et al., 2008; Luque-Rodríguez et al., 2005; Lutterodt et al., 2011; Tounsi et al., 2009). By-products obtained after wine production, the seeds and the pomace, constitute a cheap source of antioxidant compounds, providing important economic advantages. Recently, efforts and attempts at comprehensive utilisation of winemaking residues have been reported (Mendes et al., 2013; Mendoza et al., 2013; Rondeau et al., 2013). Skins and seeds of grapes are produced in large quantities by the winemaking industry. These by-products have become valuable raw materials due to their high content of polyphenols, tocols and other macro- and micronutrients (Yilmaz and Toledo, 2006). Grape seed and skin constituents have been shown to have health-functional activities as LDL cholesterol-lowering functional foods (Chen et al., 2011). The composition of grape seeds is basically (w/w) 40% fibre, 16% essential oil, 11% protein, 7% complex phenolic compounds like tannins, and also sugars and minerals (Campos et al., 2008). Standardised grape seed extracts contain 74–78% oligomeric proanthocyanidins and less than approximately 6% of free flavanol monomers on a dry weight basis (Burdock, 2005). These can combine with gallic acid to form gallate esters and ultimately glycosides (Negro et al., 2003; Weber et al., 2007). The red colour and astringency taste can be attributed to polyphenol rich compounds, especially to proanthocyanidins which may affect the colour and sensory characteristics of the product when used at higher concentrations (Monteleone et al., 2004; Weber et al., 2007).

Tocopherols (Tcph) are found homogeneously dispersed throughout all tissues of the seed in concentrations ranging from

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*Abbreviations:* α-Tcph, α-tocopherol; β-Tcph, β-tocopherol; γ-Tcph, γ-tocopherol; δ-Tcph, δ-tocopherol; α-Tct, α-tocotrienol; β-Tct, β-tocotrienol; γ-Tct, γ-tocotrienol; δ-Tct, δ-tocotrienol; Tc, total tocols; DM, dry matter; HPLC-FLD, high performance liquid chromatography with fluorescence detector; TP, total polyphenols.

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**Table 1**
Characterisation of analysed vine seed samples.

| Variety | Type | Registration[a] | Origin | Vine-growing area |
|---|---|---|---|---|
| Hibernal | White | 2004 | DEU Geisenheim | Prague-Grébovka (B) |
| Pinot Gris | White | 1941 | FRA Burgundy | Karlštejn (B)<br>Prague-Grébovka (B) |
| Müller Thurgau | White | 1941 | CHE Thurgau | Karlštejn (B)<br>Mělník (B)<br>Prague-Grébovka (B)Velké Bílovice (M) |
| Chardonnay | White | 1987 | FRA Burgundy | Hustopeče (M) |
| Traminer Rot (Savagnin Rosé, Traminer Red) | White | 1941 | ITA Tramino, Trentino, Alto Adige, FRA, DEU | Karlštejn (B) |
| Welschriesling | White | 1941 | FRA | Hustopeče (M)<br>Mělník (B) |
| Pinot Noir | Red | 1941 | FRA Burgundy | Karlštejn (B)<br>Prague-Grébovka (B)<br>Velké Bílovice (M) |
| Cabernet Sauvignon<br>Cabernet Moravia | Red | 1980/2001 | FRA Bordeaux<br>Cabernet Moravia Zweigeltrebe × Cabernet Franc<br>CZE | Velké Bílovice (M) |
| Zweigeltrebe | Red | 1980 | AUT Klosterneuburg | Hustopeče (M)<br>Karlštejn (B)<br>Prague-Grébovka (B)<br>Velké Bílovice (M) |
| Laurot | Red | 2004 | CZE Velké Bílovice | Lednice (M) |
| Saint Laurent | Red | 1941 | FRA Alsace | Karlštejn (B)<br>Velké Bílovice (M) |
| Neronet | Red | 1984 | CZE Lednice | Prague-Grébovka (B) |

[a] Registration in the Czech State Register of Grape Varieties: B, Bohemia; M, Moravia.

20 to 100 mg tocopherol per kg dry weight (Horvath et al., 2006a). It has been shown that during seed development there are significant differences in localisation and accumulation kinetics of tocopherols (Tcph) and tocotrienols (Tct). Tocopherol levels decrease gradually during seed development. In contrast, tocotrienols were found only in the endosperm of the seeds, accumulating in a sigmoid fashion during the maturation period of seed development of *V. vinifera* L. Evaluation of ten traditional Portuguese grape varieties showed that the grape seed oils were a good source of γ-Tct, α-Tcph, and α-Tct (Fernandes et al., 2012).

The mineral content of grape seeds before the vinification and winemaking process has been shown to be an important source of nutrients and essential elements. In some grape seeds collected from different locations in Turkey the mineral contents of macro- and microelements (Al, B, Ca, Co, Mo, Cr, Fe, K, Mg, Mn, Na, P, S, Se and Zn) were determined (Ozcan, 2010). Ca, K, Mg, Na and P were established as the major minerals contained in grape seeds. While Al, B, Fe, Mn and Zn mineral contents of seeds were found to be partly similar in all the seeds, the Co, Mo, Cr and Se contents were found to be very low. Recently eighteen trace elements and 15 rare earth elements were investigated in the skin, pulp, and seeds of the red varieties Cabernet Sauvignon and Marselan and the white variety Welschriesling (Yang et al., 2010). The two red varieties showed significantly higher concentrations of Cu, Cr, Ba, Mo, Cd, Ga, Ge and Tl and lower concentrations of B, Mn, Sr, and U than the white variety Welschriesling. Similar tissue-specific distribution in their concentrations in the order seeds > skin > pulp has been found, but concentrations varied between the three varieties. Concentrations of most trace elements, such as Mn, Fe, Cu, Zn, Sr, Ba, Mo, Pd, Cd, Ga, and Ge, in the seeds were very significantly higher than in the skin and pulp. The distribution pattern of rare earth elements (Y, La, Ce, Nd, Pr, Sm, Eu, Gd, Tb, Dy, Mo, Er, Tm, Yb and Lu) in the various tissues of the grape berries was different than that of trace elements and largely depended on the grape variety.

Grape seeds are increasingly on demand as a by-product of wine industry. They can be used at the step of retanning process for lowering free formaldehyde (Bayramoğlu, 2013), their extract modulates proliferation and apoptosis of pancreartic beta-cells (Cedó et al., 2013). Grape seed flour has been shown to be a viable ingredient to reduce lipid oxidation of frankfurters (Özvural and Vural, 2011). Grape seed extract positively effects the oxidative and microbial stability of mutton slice (Reddy et al., 2013), may be a photochemoprotective agent against UVB-induced skin cancer (Perde-Schrepler et al., 2013), exerts antihypertensive effect (Quiñones et al., 2013) and could be considered as an expensive source of natural antioxidants (Tounsi et al., 2009).

Wine by-products and wastes produced by agro and food industries are not yet fully economically utilised (Tangolar et al., 2009). The use of these wastes in feed or food supplements can contribute to lower production costs and to creating new feed mixtures and sources to improve the nutritive value of the animal or human nutrition. In this context, the aim of this study was to assess remaining selected essential macro- and microelements, individual and total tocols (tocotrienols and tocopherols) and total phenolic content of grape seeds after white and red winemaking, with a view to exploiting their potential as a source of bioactive compounds and possible use as additives in feed-stuffs. While the content of these compounds in grape seeds was recently relatively well studied, the remaining levels of micro- and macro-metals, tocotrienols and tocopherols and total polyphenols in grape seeds after the red and white winemaking processes were not yet refereed. The effect of winemaking, varieties and vine-growing areas was considered for possible use as an additive in animal feed.

## 2. Materials and methods

### 2.1. Plant material

Seeds were obtained from grapes from winemaking of twelve vine varieties (six white and six red ones) cultivated in six vine-growing areas from the 2011 harvest (Table 1). Seeds of white varieties were immediately separated from grape marc (pomace) after stemming, crushing and pressing, while red varieties were processed after harvesting by "red vinification–red winemaking", i.e. they were stemmed, crushed, mashed, macerated and fermented classically for a period of 6–10 days at 18–20 °C. Seeds were obtained after subsequent pressing from pomace (grape marc) by manual separation. The seed samples were air dried and stored at room temperature before being analysed.

### 2.2. Determination of selected metals by flame atomic absorption spectroscopy (FAAS)

For determination of Ca, Mg, Na, K, Fe, Zn, Cu and Mn in wine seeds, aliquots of seeds (0.8–1 g) were mineralised by dry ashing (Mader et al., 1996, 1998).

All metals have been determined by flame atomic absorption spectrometry (FAAS) with the Varian SpectrAA 110 instrument (Varian, Inc., Mulgrave, Victoria, Australia, Agilent Technologies Inc., Palo Alto, CA, USA) in acetylene-air flame at the wavelength 422.7 nm (Ca), 285.2 nm (Mg), 589.0 nm (Na), 766.5 nm (K), 248.3 nm (Fe), 324.7 nm (Cu), 213.9 nm (Zn), and 279.5 nm (Mn), respectively. Width of spectral intervals was 1 nm (Zn, K and Na), 0.5 nm (Cu, Ca and Mg), and 0.2 (Fe and Mn), respectively.

During measuring of Zn, Fe, Mn, and Mg the background was corrected by deuterium lamp. In the Ca and Mg determination, 1% solution of lanthanum nitrate was added as a releasing agent. SIPS (Sample Introduction Pump System) was used for creation of calibration dependence.

### 2.3. Sample preparation and total phenols (TP) assay by Folin-Ciocalteau reagent

Finely powdered grape seeds in a HR 2185 Philips electric mill (Amsterdam, the Netherlands) (ca. 0.5 g) were extracted with 10 mL 80% methanol for 10 min in an ultrasound bath under stirring. Extract was centrifuged and transferred into 25 mL volumetric flask and the extraction was repeated. The extract was adjusted to 25 mL with 80% methanol and carefully stirred. For the TP determination 1 mL aliquots of sample solutions were pipetted. The sample extract (1 mL) was transferred into a 50 mL volumetric flask and diluted with approximately 5 mL distilled water. Then, 2.5 mL Folin-Ciocalteau reagent (Penta Chrudim, Czech Republic) and 7.5 mL of 20% (w/w) $Na_2CO_3$ (Lach-Ner, Ltd., Neratovice, Czech Republic) were added, adjusted with distilled water to 50 mL, agitated and left to stand for 2 h. Absorbance of the sample was measured on the UV–vis spectrophotometer Spectronic Heλios γ (Thermo Spectronic, Cambridge, Great Britain) at $\lambda = 765$ nm against a blank prepared with distilled water. Gallic acid (G.R. purity, Merck KGaA, Darmstadt, Germany) was used for calibration. The results were expressed as gallic acid equivalents (GAE) in mg/kg DM from three replicates.

### 2.4. Determination of tocols by HPLC-FLD

Approximately 0.5 g of homogenised grape seed sample was placed into a 10 mL test tube, 10 mL methanol was added, then sonicated for 10 min with occasional stirring, and consequently centrifuged at 5000 RCF × g for 5 min. The supernatant was transferred into a 100 mL evaporating flask. The remaining pellet was reextracted with 10 mL methanol and centrifuged. The supernatants were combined and evaporated until dry using Büchi rotovapor R-215 (Büchi Labortechnik GmbH, Essen, Germany) at 65 °C. For HPLC analysis the samples were redissolved in 10 mL methanol and transferred through nylon microfilter (0.45 μm) into a dark vial. Analysis was carried out using a High Performance Liquid Chromatograph Ultimate 3000 (Thermo Fisher Scientific, Dionex, Sunnyvale, CA, USA) with a quarternary pump, refrigerated autosampler, column heater and FLD Ultimate 3000 RS detector. Tocols in samples were determined by HPLC-FLD under the following conditions: analytical column Develosil 5μ RP AQUEOUS (250 mm × 4.6 mm) (Phenomenex, Torrance, CA, USA); precolumn Develosil 5μ C30-UG 100A (10 mm × 4 mm) (Phenomenex, Torrance, CA, USA); mobile phase methanol:water (97:3, v/v), HPLC super gradient methanol Lach-ner, Ltd. (Neratovice, Czech Republic) and water Milli-Q water, isocratic elution; flow rate 1 mL/min; injection 10 μL, column temperature 30 °C; detection FLD (excitation 292 nm, emission 330 nm). All results were expressed as mean value of three replicates.

### 2.5. Determination of phosphorus spectrophotometrically

#### 2.5.1. Mineralisation of sample

Approximately 2 g of finely powdered sample of minced grape seeds was weighed into a porcelain crucible. The crucible was inserted into the cold furnace and crucible contents were incinerated at 500 °C for 5 h. After cooling the crucible ash was moistened with 1 mL distilled water and then 10 mL of hot 1% HCl was added. The mixture was left to leach for 15 min. Then, it was filtered through a medium thick filter to a 50 mL volumetric flask. The insoluble residue was quantitatively transferred to filter paper and washed 3 times with hot distilled water. After cooling the solution was filled up to the mark with distilled water.

#### 2.5.2. Determination of phosphorus in the digest

0.5 mL of sample was pipetted into a 25 mL volumetric flask and phosphorus reagent added to the mark (0.22 g of ammonium metavanadate + 4.40 g ammonium molybdate tetrahydrate was dissolved in 200 mL of distilled water, 15 mL of concentrated sulphuric acid p.a. added and diluted to 1 L with redistilled water). After mixing, the solution was kept at least 30 min to colouring. The colour was stable for about 24 h. The absorbance of the yellow complex of heteropolyacids Mo-VP was measured on the UV–vis spectrophotometer Spectronic Heλios γ (Thermo Spectronic, Cambridge, Great Britain) at $\lambda = 400$ nm against the blank test. The P in the sample was determined from the calibration curve in the range 0–300 μg P. Stock solution of phosphorus concentration 1 mg/mL was prepared by dissolving 3.7138 g of diammonium phosphate p.a. in redistilled water and adjusting to 1 litre volume. Ammonium metavanade, ammonium molybdate tetrahydrate and diammonium phosphate were purchased from Lach-ner, Ltd. (Neratovice, Czech Republic). Results were expressed as mean values of three replicates.

### 2.6. Statistical analysis

Obtained results for three vine varieties (Pinot Noir, Müller Thurgau and Zweigeltrebe) from three vine-growing areas (Prague-Grébovka, Karlštejn and Velké Bílovice) were statistically evaluated by the two-way ANOVA method of analysis of variance; the SAS computer program, version 9.1.3 at the level of significance $P < 0.05$ was used. Tukey's HSD test, where the first factor was vine variety and the second factor vine-growing area was used for all analytes (P, Ca, Mg, Na, K, Fe, Zn, Cu, Mn, vitamin E and TP). Results are given in Table 5. In Fig. 1 for the TP levels in the seeds of white and red vine analysed samples the one-way ANOVA analysis of variance was used.

## 3. Results and discussion

### 3.1. Content of tocols in grape seeds

In the grape seeds we determined three tocotrienols (α-, γ- and δ-) and two tocopherols (α- and γ-). γ-Tocotrienol was the most abundant (46.31 ± 13.37 mg/kg DM), followed by α-tocotrienol

**Table 2**
Tocols in the grape seeds of white and red varieties grown in different vine-growing areas after vinification process [means ± SD ($n = 3$) in mg/kg DM].

| Sample | α-Tcph | γ-Tcph | α-Tct | γ-Tct | δ-Tct | Σ Tct | Σ Tcph | Σ Tcph + Tct |
|---|---|---|---|---|---|---|---|---|
| 1 | 3.595 ± 0.160 | 1.947 ± 0.104 | 26.77 ± 1.036 | 53.73 ± 2.011 | 1.257 ± 0.031 | 81.75 ± 3.074 | 5.542 ± 0.264 | 87.29 ± 3.317 |
| 2 | 15.22 ± 0.204 | 3.723 ± 0.171 | 19.65 ± 0.233 | 54.35 ± 0.219 | 0.654 ± 0.066 | 74.66 ± 0.389 | 18.94 ± 0.375 | 93.59 ± 0.424 |
| 3 | 10.53 ± 0.312 | 2.359 ± 0.044 | 25.43 ± 0.484 | 52.72 ± 1.594 | 0.554 ± 0.010 | 78.71 ± 2.021 | 12.88 ± 0.356 | 91.60 ± 2.304 |
| 4 | 14.50 ± 0.915 | 4.460 ± 0.158 | 21.49 ± 0.618 | 69.73 ± 1.932 | 0.897 ± 0.086 | 92.12 ± 2.618 | 18.96 ± 1.073 | 111.1 ± 3.592 |
| 5 | 8.712 ± 0.696 | 3.322 ± 0.336 | 21.85 ± 1.082 | 74.99 ± 2.224 | 0.766 ± 0.085 | 97.60 ± 3.356 | 9.408 ± 1.032 | 109.6 ± 3.867 |
| 6 | 9.698 ± 0.780 | 4.479 ± 0.325 | 18.94 ± 0.812 | 56.59 ± 2.284 | 0.591 ± 0.009 | 76.11 ± 2.953 | 14.18 ± 1.105 | 90.29 ± 3.857 |
| 7 | 20.56 ± 1.192 | 5.525 ± 0.325 | 38.39 ± 1.067 | 60.52 ± 0.885 | 0.870 ± 0.049 | 99.78 ± 1.995 | 26.09 ± 1.517 | 125.9 ± 1.365 |
| 8 | 16.80 ± 0.530 | 4.623 ± 0.597 | 34.09 ± 0.160 | 37.25 ± 0.641 | 0.609 ± 0.058 | 71.95 ± 0.743 | 21.42 ± 1.127 | 93.35 ± 1.852 |
| 9 | 19.31 ± 0.787 | 5.958 ± 0.350 | 12.15 ± 0.613 | 35.73 ± 1.342 | 0.531 ± 0.013 | 48.41 ± 1.929 | 25.27 ± 1.137 | 73.68 ± 3.057 |
| 10 | 18.44 ± 0.878 | 6.364 ± 0.723 | 29.99 ± 1.810 | 29.24 ± 1.921 | 0.504 ± 0.025 | 59.73 ± 3.753 | 24.80 ± 1.601 | 84.54 ± 5.301 |
| 11 | 11.51 ± 0.977 | 3.967 ± 0.528 | 25.78 ± 2.034 | 41.51 ± 3.106 | 0.319 ± 0.033 | 67.61 ± 5.166 | 15.48 ± 1.505 | 83.09 ± 6.609 |
| 12 | 17.15 ± 0.771 | 5.064 ± 0.197 | 17.84 ± 0.822 | 54.67 ± 0.745 | 0.593 ± 0.037 | 73.10 ± 1.541 | 22.21 ± 0.968 | 95.32 ± 2.404 |
| 13 | 11.82 ± 1.114 | 4.030 ± 0.124 | 24.09 ± 0.975 | 55.61 ± 2.134 | 0.608 ± 0.070 | 80.31 ± 3.171 | 15.85 ± 1.241 | 96.15 ± 4.362 |
| 14 | 8.407 ± 0.568 | 2.097 ± 0.345 | 24.77 ± 1.146 | 62.88 ± 3.592 | 0.606 ± 0.061 | 88.26 ± 4.750 | 10.50 ± 0.913 | 98.76 ± 5.638 |
| 15 | 13.94 ± 0.722 | 3.263 ± 0.113 | 8.627 ± 0.245 | 29.99 ± 0.369 | 0.432 ± 0.022 | 39.05 ± 0.485 | 17.20 ± 0.835 | 56.26 ± 0.446 |
| 16 | 10.92 ± 1.506 | 4.174 ± 0.391 | 15.14 ± 2.817 | 50.21 ± 4.996 | 0.676 ± 0.057 | 66.02 ± 7.868 | 15.09 ± 1.897 | 81.12 ± 9.741 |
| 17 | 22.80 ± 0.990 | 3.276 ± 0.215 | 24.17 ± 0.480 | 48.40 ± 1.096 | 0.759 ± 0.025 | 73.32 ± 1.565 | 26.08 ± 1.897 | 99.40 ± 0.682 |
| 18 | 9.943 ± 0.370 | 7.241 ± 0.150 | 16.18 ± 0.351 | 40.07 ± 0.349 | 0.547 ± 0.014 | 56.80 ± 0.634 | 17.18 ± 0.520 | 73.99 ± 0.169 |
| 19 | 14.37 ± 2.924 | 3.826 ± 0.333 | 11.59 ± 0.511 | 38.42 ± 1.979 | 0.674 ± 0.054 | 50.69 ± 2.465 | 18.20 ± 3.257 | 68.88 ± 5.709 |
| 20 | 5.062 ± 0.416 | 3.395 ± 0.101 | 19.26 ± 0.743 | 44.89 ± 1.444 | 0.803 ± 0.037 | 64.95 ± 2.180 | 8.457 ± 0.517 | 73.41 ± 2.687 |
| 21 | 12.79 ± 1.033 | 3.795 ± 0.134 | 13.55 ± 0.306 | 47.71 ± 0.898 | 0.699 ± 0.021 | 61.95 ± 0.715 | 16.59 ± 1.167 | 78.54 ± 1.273 |
| 22 | 12.38 ± 1.294 | 3.831 ± 0.224 | 12.52 ± 0.531 | 46.49 ± 1.342 | 0.741 ± 0.025 | 59.75 ± 1.804 | 13.67 ± 1.518 | 75.96 ± 3.304 |
| 23 | 12.20 ± 0.518 | 11.57 ± 0.432 | 15.51 ± 0.578 | 47.63 ± 2.243 | 0.628 ± 0.059 | 63.76 ± 2.719 | 23.77 ± 0.950 | 87.53 ± 3.333 |

1: Hibernal, Prague-Grébovka, 2: Pinot Gris, Karlštejn, 3: Pinot Gris, Prague-Grébovka, 4: Müller Thurgau, Karlštejn, 5: Müller Thurgau, Mělník, 6: Müller Thurgau, Prague-Grébovka, 7: Müller Thurgau, Velké Bílovice, 8: Chardonnay, Hustopeče, 9: Traminer Red, Karlštejn, 10: Welschriesling, Hustopeče, 11: Welschriesling, Mělník, 12: Pinot Noir, Karlštejn, 13: Pinot Noir, Prague-Grébovka, 14: Pinot Noir, Velké Bílovice, 15: Cabernet Sauvignon, Velké Bílovice, 16: Zweigeltrebe, Hustopeče, 17: Zweigeltrebe, Karlštejn, 18: Zweigeltrebe, Prague-Grébovka, 19: Zweigeltrebe, Velké Bílovice, 20: Laurot, Lednice, 21: Saint Laurent, Karlštejn, 22: Saint Laurent, Velké Bílovice, 23: Neronet, Prague-Grébovka.

(20.00 ± 7.81 mg/kg DM) and α-tocopherol (12.45 ± 4.85 mg/kg DM). In lesser amounts γ-tocopherol (4.37 ± 1.83 mg/kg DM) and δ-tocotrienol (0.64 ± 0.20 mg/kg DM) were present. Our levels determined in grape seeds after the vinification process were 15 times lower than those reported in grape seed oil, where in addition β-tocotrienol and δ-tocopherol in small or trace amounts have been found (Fernandes et al., 2012). Crews et al. (2006) also observed that γ-tocotrienol was the highest tocol-like compound, which is in line with what was reported in the present study. The highest contents of tocotrienols (ΣTct) and total tocols [Σ(Tcph + Tct)] were found in the seeds of the Müller Thurgau variety from three growing-areas (Karlštejn, Mělník and Velké Bílovice) and Pinot Noir from the growing-area Velké Bílovice (Table 2). High ΣTct was also determined in var. Hibernal (Prague-Grébovka) and Σ(Tcph + Tct) in var. Zweigeltrebe (Karlštejn). Among tocols the highest contents were shown by γ-Tct ranging from 29.24 ± 1.921 mg/kg

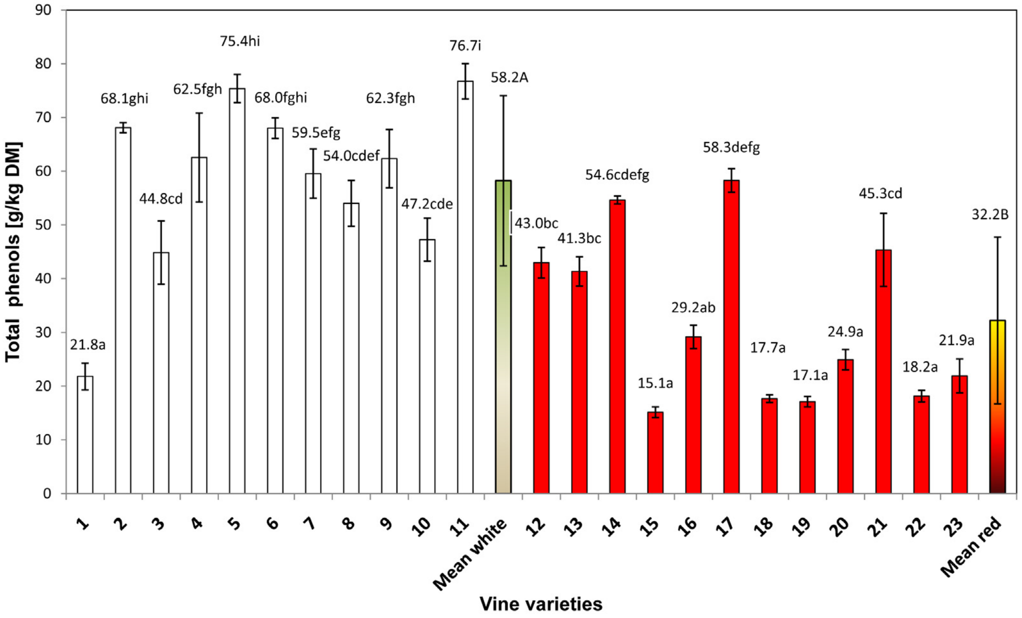

**Fig. 1.** Total phenolic content in the grape seeds of white and red varieties grown in different vine- growing areas after the vinification [g/kg DM].
1: Hibernal, Prague-Grébovka, 2: Pinot Gris, Karlštejn, 3: Pinot Gris, Prague-Grébovka, 4: Müller Thurgau, Karlštejn, 5: Müller Thurgau, Mělník, 6: Müller Thurgau, Prague-Grébovka, 7: Müller Thurgau, Velké Bílovice, 8: Chardonnay, Hustopeče, 9: Traminer Red, Karlštejn, 10: Welschriesling, Hustopeče, 11: Welschriesling, Mělník, 12: Pinot Noir, Karlštejn, 13: Pinot Noir, Prague-Grébovka, 14: Pinot Noir, Velké Bílovice, 15: Cabernet Sauvignon, Velké Bílovice, 16: Zweigeltrebe, Hustopeče, 17: Zweigeltrebe, Karlštejn, 18: Zweigeltrebe, Prague-Grébovka, 19: Zweigeltrebe, Velké Bílovice, 20: Laurot, Lednice, 21: Saint Laurent, Karlštejn, 22: Saint Laurent, Velké Bílovice, 23: Neronet, Prague-Grébovka. Different letters in columns indicate the significant differences between analysed samples at $P < 0.05$; different capitals indicate the significant differences between mean values of white and red vine varieties at $P < 0.001$.

**Table 3**
Selected essential macroelements in grape seeds of white and red varieties grown in different vine-growing areas after the vinification [means ± SD ($n = 3$) in g/kg DM].

| Sample | K | Na | Ca | Mg | P |
|---|---|---|---|---|---|
| 1 | 6.310 ± 0.895 | 0.189 ± 0.036 | 3.965 ± 0.383 | 1.263 ± 0.133 | 3.305 ± 0.405 |
| 2 | 9.524 ± 0.883 | 0.038 ± 0.011 | 5.692 ± 0.203 | 1.714 ± 0.090 | 5.030 ± 0.170 |
| 3 | 3.642 ± 2.530 | 0.200 ± 0.089 | 5.366 ± 0.357 | 0.721 ± 0.050 | 4.575 ± 0.345 |
| 4 | 7.180 ± 0.653 | 0.229 ± 0.065 | 4.401 ± 0.380 | 1.191 ± 0.128 | 4.170 ± 0.040 |
| 5 | 5.775 ± 0.335 | 0.220 ± 0.008 | 5.225 ± 0.443 | 1.281 ± 0.086 | 3.980 ± 0.075 |
| 6 | 5.706 ± 0.668 | 0.169 ± 0.018 | 5.536 ± 0.230 | 1.355 ± 0.090 | 3.870 ± 0.250 |
| 7 | 5.303 ± 0.807 | 0.196 ± 0.006 | 5.346 ± 0.352 | 1.285 ± 0.390 | 2.355 ± 0.075 |
| 8 | 4.201 ± 0.326 | 0.156 ± 0.053 | 6.078 ± 0.295 | 1.334 ± 0.100 | 4.210 ± 0.190 |
| 9 | 7.577 ± 0.210 | 0.040 ± 0.003 | 3.246 ± 0.137 | 1.037 ± 0.071 | 4.015 ± 0.095 |
| 10 | 4.074 ± 0.507 | 0.162 ± 0.038 | 4.683 ± 0.802 | 1.133 ± 0.243 | 2.960 ± 0.080 |
| 11 | 7.488 ± 0.053 | 0.335 ± 0.050 | 5.992 ± 0.490 | 1.385 ± 0.129 | 4.015 ± 0.455 |
| 12 | 7.247 ± 1.080 | 0.248 ± 0.044 | 5.109 ± 0.133 | 1.233 ± 0.171 | 4.425 ± 0.175 |
| 13 | 5.525 ± 0.205 | 0.261 ± 0.075 | 5.443 ± 0.065 | 1.392 ± 0.016 | 3.635 ± 0.085 |
| 14 | 5.531 ± 0.319 | 0.195 ± 0.012 | 5.443 ± 0.537 | 1.482 ± 0.111 | 3.270 ± 0.090 |
| 15 | 6.714 ± 1.105 | 0.297 ± 0.036 | 5.280 ± 0.254 | 1.006 ± 0.029 | 3.775 ± 0.115 |
| 16 | 3.562 ± 0.217 | 0.112 ± 0.014 | 5.958 ± 0.167 | 1.248 ± 0.108 | 4.260 ± 0.350 |
| 17 | 5.083 ± 0.183 | 0.131 ± 0.008 | 5.768 ± 0.332 | 1.484 ± 0.060 | 3.860 ± 0.110 |
| 18 | 5.851 ± 1.500 | 0.255 ± 0.076 | 3.413 ± 1.138 | 0.959 ± 0.357 | 3.180 ± 0.100 |
| 19 | 4.392 ± 0.467 | 0.211 ± 0.047 | 5.122 ± 0.415 | 1.155 ± 0.112 | 3.540 ± 0.120 |
| 20 | 5.386 ± 0.668 | 0.238 ± 0.017 | 6.162 ± 0.055 | 1.450 ± 0.020 | 3.155 ± 0.095 |
| 21 | 6.108 ± 1.005 | 0.169 ± 0.027 | 5.932 ± 1.152 | 1.364 ± 0.310 | 3.515 ± 0.075 |
| 22 | 6.667 ± 2.579 | 0.282 ± 0.080 | 5.485 ± 0.660 | 1.448 ± 0.120 | 2.715 ± 0.275 |
| 23 | 6.922 ± 2.774 | 0.089 ± 0.006 | 5.127 ± 0.470 | 1.298 ± 0.035 | 4.105 ± 0.145 |

1: Hibernal, Prague-Grébovka, 2: Pinot Gris, Karlštejn, 3: Pinot Gris, Prague-Grébovka, 4: Müller Thurgau, Karlštejn, 5: Müller Thurgau, Mělník, 6: Müller Thurgau, Prague-Grébovka, 7: Müller Thurgau, Velké Bílovice, 8: Chardonnay, Hustopeče, 9: Traminer Red, Karlštejn, 10: Welschriesling, Hustopeče, 11: Welschriesling, Mělník, 12: Pinot Noir, Karlštejn, 13: Pinot Noir, Prague-Grébovka, 14: Pinot Noir, Velké Bílovice, 15: Cabernet Sauvignon, Velké Bílovice, 16: Zweigeltrebe, Hustopeče, 17: Zweigeltrebe, Karlštejn, 18: Zweigeltrebe, Prague-Grébovka, 19: Zweigeltrebe, Velké Bílovice, 20: Laurot, Lednice, 21: Saint Laurent, Karlštejn, 22: Saint Laurent, Velké Bílovice, 23: Neronet, Prague-Grébovka.

DM in Laurot (Lednice) to 74.99 ± 2.224 mg/kg DM in Müller Thurgau (Mělník) and $\alpha$-Tct from 8.627 ± 0.245 mg/kg DM in Cabernet Sauvignon (Velké Bílovice) to 38.39 ± 1.067 mg/kg DM in Műller Thurgau (Velké Bílovice). The lowest levels were found for $\delta$-Tct – ranging from 0.319 ± 0.033 mg/kg DM in Welschriesling (Mělník) to 1.257 ± 0.031 mg/kg DM in var. Hibernal (Prague-Grébovka). Among six vine varieties grown in the growing-area Prague-Grébovka there were significant differences in var. Hibernal with high $\Sigma$Tct and low $\Sigma$Tcph contents and higher $\delta$-Tct content compared to the other five varieties (Zweigeltrebe, Pinot Gris, Müller Thurgau, Pinot Noir and Neronet). Between six varieties grown in the vine growing-area Karlštejn there were significant differences in the Müller Thurgau variety with high contents of $\gamma$-Tct (69.73 mg/kg DM) and $\Sigma$Tct (92.12 mg/kg DM) compared to the other five grown varieties (Red Traminer, Saint Laurent, Pinot Gris, Pinot Noir and Zweigeltrebe). Zweigeltrebe was characterised by high $\alpha$-Tct content (24.17 mg/kg) and $\alpha$-Tcph (22.80 mg/kg). Red Traminer differed by high $\gamma$-Tcph (5.958 mg/kg DM). Similar results have also been observed also in the Moravian vine-growing area Velké Bílovice, where var. Müller Thurgau was marked by high $\Sigma$Tcph (26.08 mg/kg DM), $\alpha$-Tct (38.39 mg/kg DM) and $\gamma$-Tcph (5.524 mg/kg DM). Content of tocopherols ranged from 5.542 to 26.09 mg/kg DM and tocotrienols from 39.05 to 99.78 mg/kg DM. Horvath et al. (2006b) found that in 90-day old seeds 7.4–54 mg/kg DM Tct and Tcph ranged from 20 to 100 mg/kg DM. However, Choi and Lee (2009) reported that grape seeds contain mainly tocotrienols ($\alpha$-Tct 40 mg/kg and $\gamma$-Tct 70 mg/kg, and 120 mg/kg total, respectively) and these findings confirm the results in this study. In the hexane-soluble fraction their contents increased (460 mg/kg $\gamma$-Tct, 200 mg/kg $\alpha$-Tct and 70 mg/kg $\alpha$-Tcph, and 750 mg/kg total tocols, respectively); in the methanol-soluble fraction an increase has also been found (8610 mg/kg $\gamma$-Tct, 160 mg/kg $\gamma$-Tcph, 2.870 mg/kg $\gamma$-Tcph, 860 mg/kg $\alpha$-Tcph, and 12,500 mg/kg total tocols). Tangolar et al. (2011) determined tocopherol content in grape seeds and found as major $\alpha$-Tcph (mean 15.43 mg/kg DM) followed by $\gamma$-Tcph (mean 1.85 mg/kg DM); though two others were detected in trace concentrations.

Statistical analysis showed significant differences between the Müller Thurgau and Zweigeltrebe varieties (in $\alpha$-Tct, $\gamma$-Tct, $\delta$-Tct, $\Sigma$Tct, and $\Sigma$Tc, respectively). Statistically significant differences in levels of $\gamma$-Tct, $\delta$-Tct, $\alpha$-Tcph, $\Sigma$Tcph and $\Sigma$Tc between the vine-growing areas Prague-Grébovka and Karlštejn were found (Table 5).

### 3.2. *Content of total polyphenols (TP) in grape seeds*

After the wine production process it was found that white and red vine varieties differed in the content of total polyphenols remaining in the seeds after wine production (Fig. 1). Remaining TP levels were affected by different ways of white and red grape winemaking. White varieties were fermented immediately after harvest, while red varieties after harvest were first macerated and mashed and only after this procedure they were step fermented (both till the end of the year 2011). White varieties contained higher TP levels in seeds (on average 58.23 ± 3.978 g/kg DM) in comparison with red varieties (32.22 ± 2.197 g/kg DM). Among white varieties the highest TP levels were shown in Welschriesling (76.72 ± 3.311 g/kg DM, Mělník), Müller Thurgau (75.36 ± 2.636 g/kg DM, Mělník), Pinot Gris (68.06 ± 0.947 g/kg DM, Karlštejn) and Müller Thurgau (68.00 ± 1.908 g/kg DM, Prague-Grébovka). Among red varieties only Zweigeltrebe (58.28 ± 2.208 g/kg DM, Karlštejn) achieved the average value of white varieties; others promising TP sources also proved to be Pinot Noir (54.64 ± 0.744 g/kg DM, Velké Bílovice), Saint Laurent (45.33 ± 6.810 g/kg DM, Karlštejn) and Pinot Noir (42.96 ± 2.842 g/kg DM, Karlštejn).

Statistical analysis showed significant differences in TP amounts (Zweigeltrebe, Pinot Noir and Müller Thurgau), but significant differences were not determined among growing areas (Table 5). Comparison of TP levels in the white and red grape samples has shown significant statistical differences between them related to different vinification process (Fig. 1).

Obtained TP levels in the seeds of assessed varieties ranged from 15.13 g/kg DM (Cabernet Sauvignon, Velké Bílovice) to 76.72 g/kg DM (Welschriesling, Mělník) and are comparable with data reported previously in the study of polyphenol content in the seeds of red grape varieties originating from Turkish vineyards (Bozan et al., 2008), where total phenolic content ranged from 79.2 to 154.6 g/kg DM. These levels are higher as compared with our results, because our seeds were obtained as a by-product after vinification. In the seeds of four Greek varieties relatively high total polyphenols content has been recently determined ranging between 8.26 and 33.14 g/kg DM and seeds were particularly rich in momomeric flavan-3-ols and dimeric procyanidins (Anastasiadi et al., 2010). For TP extraction we have used as the optimal extraction solvent 80% methanol; Makris et al. (2007) used as optimal 57% ethanol with phenolic recovery from the seeds ranging between 79.57 and 133.1 g/kg DM. In grape red pomace from vinification of four Brazilian varieties the lowest TP content was found in the range of 32.32 g/kg DM (Isabel) to 74.75 g/kg DM (Cabernet Sauvignon) (Rockenbach et al., 2011). In our samples we did not detect trans-resveratrol by HPLC-DAD similarly as was recently reported in seeds of Pinot Noir and Cabernet Sauvignon (Rockenbach et al., 2011).

### 3.3. Content of selected nutritional essential macroelements (K, Na, Ca, Mg and P) in grape seeds

In the grape seeds of vine varieties selected nutritional macroelements (K, Na, Ca, Mg and P) were determined (Table 3). Grape seeds contained on average 5.904 ± 1.432 K g/kg DM, 0.192 ± 0.087 Na g/kg DM, 5.208 ± 0.793 Ca g/kg DM, 1.385 ± 0.209 Mg g/kg DM and 3.736 ± 0.654 g P/kg DM.

Red varieties contained on average higher levels of all analysed macroelements of metal characters (6.042 ± 1.471 K g/kg DM, 0.217 ± 0.077 Na g/kg DM, 5.280 ± 0.707 Ca g/kg DM, 1.293 ± 0.180 Mg g/kg DM) by comparison with white vine varieties (5.957 ± 1.241 K g/kg DM, 0.176 ± 0.083 Na g/kg DM, 5.048 ± 0.883 Ca g/kg DM, 1.245 ± 0.244 Mg g/kg DM); the opposite were P levels – in white varieties 3.862 ± 0.769 g/kg DM and in red varieties 3.647 ± 0.507 g/kg DM.

Among red vines rich sources of these macroelements were Saint Laurent seeds from the growing areas Karlštejn (5.932 ± 1.152 Ca g/kg DM) and Velké Bílovice (0.282 ± 0.080 Na g/kg DM, 1.448 ± 0.120 Mg g/kg DM, respectively), Zweigeltrebe from different growing areas (Hustopeče: 5.958 ± 0.167 Ca g/kg DM, 4.260 ± 0.350 P g/kg DM, Karlštejn: 1.484 ± 0.060 Mg g/kg DM, Prague-Grébovka: 0.255 ± 0.076 Na g/kg DM), Pinot Noir from Prague-Grébovka (0.261 ± 0.075 Na g/kg DM), Karlštejn (7.247 ± 1.080 K g/kg DM), and Velké Bílovice (1.482 ± 0.111 Mg g/kg DM). The varieties Cabernet Sauvignon (Velké Bílovice: 0.297 ± 0.036 Na mg/kg DM) and Laurot (Lednice: 6.162 ± 0.055 Ca g/kg DM) showed higher levels of these metals. Among white vines high levels contained Pinot Gris from the growing areas Karlštejn and Prague-Grébovka (Karlštejn: 9.524 ± 0.883 K g/kg DM, 1.714 ± 0.090 Mg g/kg DM, 5.030 ± 0.170 P g/kg DM and Prague-Grébovka 4.575 ± 0.345 P g/kg DM, respectively), Welschriesling from the growing area Mělník (7.488 ± 0.053 K g/kg DM, 0.335 ± 0.050 Na g/kg DM and 5.992 ± 0.490 Ca g/kg DM), Chardonnay from the growing area Hustopeče (6.078 ± 0.295 Ca g/kg DM and 4.210 ± 0.190 P g/kg DM), and Müller Thurgau from the growing area Karlštejn (7.180 ± 0.653 K g/kg DM).

Two-way ANOVA analysis of variance demonstrated no statistical differences between varieties in levels of phosphorus, magnesium, calcium and potassium (Table 5). The vine growing areas Karlštejn and Velké Bílovice only statistically differed in P and K contents.

It has been shown that grape and wine multielemental composition is strongly affected by the solubility of inorganic compounds of the soil (Taylor et al., 2003; Vrček et al., 2011). In agreement with Vrček et al. (2011), who with regards to macroelements found in the investigated wines the most abundant Ca, Mg and K, our results confirmed high levels of these macrometals in the grape seeds. However, their contents could also be influenced by other factors, such as climatic changes and the vinification process. Likewise, Ca contents of grape seeds of Turkish grape varieties were found to be higher than that of K and Mg, ranging from 0.48% and 0.70% (Tangolar et al., 2009). The Mg levels of grape seeds were found to be between 0.13% and 0.17% and P between 0.29% and 0.44%. According to results of Ozcan (2010), Ca, K, Mg, Na, P and S contents of Turkish grape seed samples were generally found to be very high. Ca content ranged between 2.723 g/kg and 10.108 g/kg and Mg from 1.012 g/kg to 1.797 g/kg K was higher in most cases and ranged from 3.489 g/kg to 9.343 g/kg. Na contents of the seeds were found in similarly small percentages in all analysed seeds ranging from 0.424 g/kg to 0.602 g/kg. P was determined in significantly higher amounts, varying between 2.600 g/kg and 4.633 g/kg.

### 3.4. Content of selected nutritional essential microelements (Fe, Cu, Zn and Mn) in grape seeds

Contents of analysed microelements are summarised in Table 4. Average Fe, Cu, Zn and Mn levels were 51.63 ± 17.56, 7.524 ± 1.467, 11.27 ± 5.708 and 14.94 ± 5.708 mg/kg DM, respectively. Red vine varieties contained on average higher Fe, Cu and Zn levels (45.48 ± 18.30, 6.740 ± 1.053 and 11.00 ± 3.334 mg/kg DM), respectively as compared to white vine varieties – 57.26 ± 15.48, 8.243 ± 1.456 and 11.53 ± 0.907 mg/kg DM, respectively. Mn content was relatively equal; slightly higher has been found in white varieties (15.38 ± 7.946 mg/kg DM) in comparison with red varieties (14.53 ± 2.721 mg/kg DM).

Among red varieties high microelement levels were found in the seeds of Laurot grown in the vine-growing area Lednice (74.96 ± 5.763 Fe mg/kg DM, 10.14 ± 1.731 Cu mg/kg DM, and 12.89 ± 0.857 Zn mg/kg DM), Pinot Noir from the growing areas Karlštejn and Velké Bílovice (Karlštejn: 88.53 ± 7.282 Fe mg/kg DM, Velké Bílovice: 66.87 ± 0.893 Fe mg/kg DM and 10.11 ± 0.143 Cu mg/kg DM), and similarly Cabernet Sauvignon (Velké Bílovice: 9.047 ± 0.464 Cu mg/kg DM), Zweigeltrebe (Hustopeče: 9.042 ± 0.233 Cu mg/kg DM and 13.28 ± 0.578 Zn mg/kg DM), and Saint Laurent (Velké Bílovice: 64.60 ± 6.594 Fe mg/kg DM and 9.561 ± 0.447 Cu mg/kg DM), respectively. Between white vine varieties those characterised by higher Fe level were Müller Thurgau (Hustopeče: 83.262 ± 8.145 mg/kg) and Zn level Pinot Gris (Karlštejn: 14.18 ± 0.400 mg/kg), Műller Thurgau (Mělník: 12.32 ± 0.850 mg/kg DM) and Welschriesling (Mělník: 18.08 ± 1.441 mg/kg DM). Varieties characterised by higher levels of manganese were equally balanced between white and red varieties: Welschriesling (Hustopeče: 34.57 ± 7.201 mg/kg DM), Chardonnay (Hustopeče: 23.24 ± 0.112 mg/kg), Müller Thurgau (Velké Bílovice: 19.21 ± 0.487 mg/kg DM), Pinot Noir (Velké Bílovice: 17.90 ± 0.507 mg/kg) and Zweigeltrebe (Velké Bílovice: 17.63 ± 0.335 mg/kg DM).

Statistical analysis showed significant differences between Pinot Noir and Müller Thurgau Zn and Cu contents and Müller Thurgau and Zweigeltrebe in Fe content. Grape seeds from the growing areas Karlštejn and Velké Bílovice differed significantly in Cu and Mn contents, while from Karlštejn and Prague-Grébovka in Zn content. Levels of essential elements were affected rather by the vine-growing area, whereas TP and tocols by the vine variety. This is in agreement the findings of many studies which have indicated that trace elements can be used to fingerprint regional wines (Coetzee et al., 2005; Angus et al., 2006; Miskelly et al., 2006; Galgano et al., 2008). Also among microelements in wines Vrček et al. (2011) found the most abundant to be Al, Mn, Fe, Cu and Zn, which corresponds with our results of analysed microelements in grape seeds and in both, seeds and wine, the microelements

**Table 4**
Selected essential microelements in grape seeds of white and red varieties grown in different vine-growing areas after vinification process [means ± SD ($n = 3$) in mg/kg DM].

| Sample | Fe | Cu | Zn | Mn |
|---|---|---|---|---|
| 1 | 31.902 ± 1.316 | 6.358 ± 0.214 | 11.440 ± 0.732 | 14.147 ± 0.287 |
| 2 | 55.685 ± 3.929 | 7.271 ± 0.235 | 14.175 ± 0.400 | 13.277 ± 0.562 |
| 3 | 44.991 ± 2.106 | 7.175 ± 0.093 | 11.096 ± 1.004 | 14.212 ± 0.339 |
| 4 | 52.070 ± 5.012 | 6.381 ± 0.938 | 7.918 ± 2. 007 | 8.385 ± 1.582 |
| 5 | 27.034 ± 0.718 | 5.511 ± 1.382 | 12.319 ± 0.850 | 7.001 ± 1.109 |
| 6 | 64.134 ± 2.737 | 6.062 ± 0.313 | 11.385 ± 1.969 | 13.632 ± 0.543 |
| 7 | 83.262 ± 8.145 | 6.612 ± 1.995 | 9.106 ± 0.552 | 19.212 ± 0.487 |
| 8 | 26.781 ± 2.910 | 4.990 ± 1.209 | 5.502 ± 1.100 | 23.236 ± 0.112 |
| 9 | 53.077 ± 3.929 | 7.090 ± 0.518 | 8.668 ± 0.518 | 13.396 ± 0.789 |
| 10 | 25.382 ± 5.472 | 8.413 ± 0.706 | 11.291 ± 1.682 | 34.573 ± 7.201 |
| 11 | 35.999 ± 2.013 | 8.279 ± 0.422 | 18.081 ± 1.441 | 8.093 ± 1.316 |
| 12 | 88.532 ± 7.282 | 6.757 ± 1.140 | 10.784 ± 0.987 | 14.066 ± 0.439 |
| 13 | 43.606 ± 2.687 | 7.046 ± 0.807 | 11.437 ± 0.748 | 14.022 ± 0.681 |
| 14 | 66.871 ± 0.893 | 10.11 ± 0.143 | 11.834 ± 0.555 | 17.903 ± 0.507 |
| 15 | 62.294 ± 2.784 | 9.047 ± 0.464 | 11.559 ± 0.601 | 17.229 ± 0.374 |
| 16 | 53.148 ± 4.884 | 9.042 ± 0.233 | 13.280 ± 0.578 | 17.381 ± 0.826 |
| 17 | 37.948 ± 1.041 | 6.376 ± 0.430 | 9.922 ± 0.749 | 11.445 ± 0.255 |
| 18 | 52.988 ± 1.952 | 8.411 ± 0.917 | 10.889 ± 0.787 | 11.461 ± 1.840 |
| 19 | 43.560 ± 2.778 | 8.348 ± 0.670 | 11.121 ± 0.347 | 17.634 ± 0.335 |
| 20 | 74.962 ± 5.763 | 10.14 ± 1.731 | 12.891 ± 0.857 | 12.566 ± 0.206 |
| 21 | 37.832 ± 1.020 | 8.291 ± 1.341 | 11.737 ± 2.238 | 10.356 ± 0.172 |
| 22 | 64.597 ± 6.594 | 9.561 ± 0.447 | 11.786 ± 0.658 | 16.490 ± 2.056 |
| 23 | 60.778 ± 3.082 | 5.785 ± 1.951 | 11.087 ± 1.269 | 13.848 ± 2.236 |

1: Hibernal, Prague-Grébovka, 2: Pinot Gris, Karlštejn, 3: Pinot Gris, Prague-Grébovka, 4: Müller Thurgau, Karlštejn, 5: Müller Thurgau, Mělník, 6: Müller Thurgau, Prague-Grébovka, 7: Müller Thurgau, Velké Bílovice, 8: Chardonnay, Hustopeče, 9: Traminer Red, Karlštejn, 10: Welschriesling, Hustopeče, 11: Welschriesling, Mělník, 12: Pinot Noir, Karlštejn, 13: Pinot Noir, Prague-Grébovka, 14: Pinot Noir, Velké Bílovice, 15: Cabernet Sauvignon, Velké Bílovice, 16: Zweigeltrebe, Hustopeče, 17: Zweigeltrebe, Karlštejn, 18: Zweigeltrebe, Prague-Grébovka, 19: Zweigeltrebe, Velké Bílovice, 20: Laurot, Lednice, 21: Saint Laurent, Karlštejn, 22: Saint Laurent, Velké Bílovice, 23: Neronet, Prague-Grébovka.

could be severely affected by the winemaking process. In a study of levels of trace elements detected in grape seeds of Turkish varieties Fe content ranged between 17.3 mg/kg and 27.0 mg/kg, Zn content between 12.28 mg/kg and 18.97 mg/kg and Mn content between 11.13 mg/kg and 23.86 mg/kg, respectively. Cu levels ranged between 7.54 mg/kg and 13.04 mg/kg. Results of Ozcan (2010) showed higher variation for Mn (3.4–105.1 mg/kg), while Fe and Zn mineral contents of seeds were found partly similar in all the seeds ranging between 5.4 and 43.9 mg Fe/kg, and 6.5 and 25.6 mg Zn/kg. Cu was contained in lesser amounts ranging from 0.87 to 17.80 mg/kg. Fe, Cu, Zn and Mn levels could also be affected by formation of complexes with polyphenols, where good correlations were found and individual correlation of both Zn and Cu with cyanidin-3-O-glucoside in red varieties has been established (Esparza et al., 2004).

In general, the wine-making process for red and white wine is different; the former in commonly made by fermenting crushed berries, whereas the latter is usually made from fermenting grape juice after foregoing separation of pomace. Trace elements present similar tissue-dependent distribution as macroelements, and red and white varieties show great differences in the concentrations of these elements (Yang et al., 2010).

### *3.5. Grape seeds as a by-product – potential sources of nutrients in feed or food additives*

Whole fruit and vegetable wastes could serve as a potent source of antioxidants and other nutrients either as food and feed supplements or isolated compounds with optimisation of extraction parameters for the highest yield of phytochemicals (Luthria, 2012; Wijngaard et al., 2009). Grape seeds as well as grape pomace are a natural source of phenolic compounds with high antioxidant activity (Sáyago-Ayerdi et al., 2009). In their study dietary grape pomace concentrate significantly caused an inhibitory effect on lipid oxidation of raw and cooked breast chicken patties. The inhibition of TBARS values in cooked chicken patties could be due to a protective effect derived from the polyphenols contained in the added grape pomace or seeds, which may act in a similar way to vitamin E on the lipid bilayers and can also spare vitamin E. Our results indicate that promising antioxidant sources are seeds of the white varieties Welschriesling, Müller Thurgau and Pinot Gris containing higher levels of total polyphenols and tocotrienols possessing antioxidant activity and tocopherols with properties of vitamin E. Our results related to variety differences are in agreement with the results of Poudel et al. (2008) who found differences in total

**Table 5**
Two-way ANOVA analysis of variance (Tukey's HSD test vine variety × vine growing-area) of tocols, selected macro- and microelements and total phenols in seeds of three selected grape varieties grown in three selected vine-growing areas.

| Variable | α-Tct | γ-Tct | δ-Tct | ΣTct | α-Tcph | γ-Tcph | ΣTcph | ΣTc | TP | P | K | Na | Ca | Mg | Zn | Cu | Fe | Mn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pinot Noir | 22.2$^{ab}$ | 57.7$^{a}$ | 0.606$^{a}$ | 80.6$^{a}$ | 12.5$^{a}$ | 3.73$^{a}$ | 16.2$^{a}$ | 96.7$^{a}$ | 46.3$^{b}$ | 3.78$^{a}$ | 6.11$^{a}$ | 0.23$^{a}$ | 4.77$^{a}$ | 1.37$^{a}$ | 11.4$^{b}$ | 7.97$^{b}$ | 66.3$^{a}$ | 15.4$^{a}$ |
| Müller Thurgau | 26.3$^{b}$ | 62.3$^{a}$ | 0.660$^{a}$ | 89.3$^{a}$ | 14.9$^{a}$ | 4.82$^{a}$ | 19.$^{7a}$ | 109$^{a}$ | 63.4$^{c}$ | 3.47$^{a}$ | 6.06$^{a}$ | 0.20$^{a}$ | 5.09$^{a}$ | 1.28$^{a}$ | 9.47$^{a}$ | 6.35$^{a}$ | 66.5$^{a}$ | 13.7$^{a}$ |
| Zweigeltrebe | 17.3$^{a}$ | 42.3$^{b}$ | 0.786$^{b}$ | 60.3$^{b}$ | 15.7$^{a}$ | 4.78$^{a}$ | 20.5$^{a}$ | 80.8$^{b}$ | 31.0$^{a}$ | 3.53$^{a}$ | 5.71$^{a}$ | 0.20$^{a}$ | 5.33$^{a}$ | 1.20$^{a}$ | 10.6$^{ab}$ | 7.71$^{ab}$ | 44.8$^{b}$ | 13.5$^{a}$ |
| Prague-Grébovka | 19.7$^{a}$ | 50.8$^{a}$ | 0.582$^{b}$ | 71.1$^{a}$ | 10.5$^{a}$ | 5.25$^{a}$ | 15.7$^{a}$ | 86.8$^{a}$ | 42.3$^{a}$ | 3.56$^{ab}$ | 5.69$^{ab}$ | 0.23$^{a}$ | 4.80$^{a}$ | 1.24$^{a}$ | 11.2$^{b}$ | 7.17$^{ab}$ | 53.6$^{a}$ | 13.1$^{a}$ |
| Karlštejn | 21.2$^{a}$ | 57.6$^{b}$ | 0.750$^{a}$ | 79.5$^{a}$ | 18.2$^{b}$ | 4.27$^{a}$ | 22.4$^{b}$ | 101.9$^{b}$ | 54.6$^{a}$ | 4.15$^{b}$ | 6.50$^{b}$ | 0.20$^{a}$ | 5.09$^{a}$ | 1.30$^{a}$ | 9.54$^{a}$ | 6.51$^{a}$ | 59.5$^{a}$ | 11.3$^{a}$ |
| Velké Bílovice | 24.9$^{a}$ | 53.9$^{ab}$ | 0.717$^{a}$ | 79.6$^{a}$ | 14.5$^{ab}$ | 3.82$^{a}$ | 18.3$^{ab}$ | 97.8$^{ab}$ | 43.8$^{a}$ | 3.06$^{a}$ | 5.09$^{a}$ | 0.20$^{a}$ | 5.30$^{a}$ | 1.31$^{a}$ | 10.7$^{ab}$ | 8.36$^{b}$ | 64.6$^{a}$ | 18.3$^{b}$ |

Varieties and vine-growing areas in the same columns followed by different letters are significantly different (significance at $P < 0.05$); Tct: tocotrienol; Tcph: tocopherol; ΣTc: total tocols = ΣTct + ΣTcph; TP: total phenols; α-Tct, γ-Tct, δ-Tct, ΣTct, α-Tcph, γ-Tcph, ΣTcph, ΣTc, Zn, Cu, Fe, Mn: mean values in mg per kg DM; P, K, Na, Ca, Mg, TP: mean values in g per kg DM.

polyphenols and procyanidin monomers in grown and wild grape seeds as potential sources of nutraceutical phenolics. The antioxidant properties of the procyanidin-rich extract from grape seeds may also have cardioprotective effects and that why toxicology evaluation of a procyanidin-rich extract from grape seeds and skin was also performed (Lluís et al., 2011). Doses of up to 2 g/kg showed no increase in micronucleated erythrocytes, $LD_{50}$ was higher than 5 g/kg. However, slight mutagenicity to the dose of 5 mg/plate has been indicated. Grape seed proanthocyanidins could be widely consumed dietary supplements with antioangiogenic activity (Huang et al., 2012). Comparison of seeds from grape pomace produced from white and red varieties in Italy and California showed that the red Italian samples had higher organic matter, acid and Cu (Spanghero et al., 2009). In addition, in red samples from California, K ($P < 0.01$), Fe ($P < 0.01$), and Zn ($P < 0.01$) were higher. Italian white samples tended to have higher total extractable phenolics both in seeds and pulp ($P = 0.07$ and $P = 0.10$, respectively). Grape seed and peel are increasingly being used to obtain functional food ingredients such as natural antioxidants and dietary supplements. Goñi et al. (2005) determined the extent of potential digestibility and bioavailability of grape seed dietary fibre, protein and polyphenols and found that the intestinal microflora degraded 95–97% of total polyphenols, 30–32% of dietary fibre and 60–70% of protein. It may be summarised that grape seeds contain a digestible component available in the small intestine, indigestible compounds degraded by intestinal microflora available in the large intestine and an indigestible non-fermented and unavailable fraction. Basalan et al. (2011) recommended particularly pomace from red grapes rich in skin and seed as suitable food for ruminants and to nonruminants with extensive cecal fermentation.

## 4. Conclusion

Remaining levels of nutrients in grape seeds in winemaking were affected by the variety, process of vinification and winemaking, and in a lesser extent by the vine-growing area. The highest total tocols – lipophilic antioxidants tocotrienols and tocopherols (vitamin E) – were found in the seeds of the Müller Thurgau, Pinot Noir and Zweigeltrebe varieties. Vinification and variety have significant impact on the content of total polyphenols with high remaining levels especially in the seeds of white varieties. Unlike total polyphenols content, the seeds of red varieties contained higher levels of macroelements except P, however no statistically significant differences between varieties have been found. The results of mineral analysis showed that the red varieties contained higher amounts of Fe, Cu, Zn, while Mn contents were comparable. Statistical analysis showed that vine varieties have significant impact on the content of individual and total tocotrienols, total phenols and some microelements (Zn, Cu and Fe), while vine-growing areas (vineyards) on tocotrienols and tocopherol levels, essential macroelements (P and K) and microelements (Cu, Mn and Zn).

On the basis of these experimental results, grape seeds or grape seed meal as a by-product of the vinification process were found to be a good source of nutritionally beneficial health-promoting compounds – hydrophilic polyphenols and lipophilic tocols (vitamin E) antioxidants, macroelements (Ca, P, K, Na and Mg) and microelements (Fe, Cu, Zn and Mn) and due to their abundance and nutrition value may be proposed as exploitable resources for use as feed components in the diets of poultry, pigs, rabbits and others.

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