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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

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) Prague-Grébovka (B)
Müller Thurgau	White	1941	CHE Thurgau	Karlštejn (B) Mělník (B) 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) Mělník (B)
Pinot Noir	Red	1941	FRA Burgundy	Karlštejn (B) Prague-Grébovka (B) Velké Bílovice (M)
Cabernet Sauvignon Cabernet Moravia	Red	1980/2001	FRA Bordeaux Cabernet Moravia Zweigeltrebe × Cabernet Franc CZE	Velké Bílovice (M)
Zweigeltrebe	Red	1980	AUT Klosterneuburg	Hustopeče (M) Karlštejn (B) Prague-Grébovka (B) Velké Bílovice (M)
Laurot	Red	2004	CZE Velké Bílovice	Lednice (M)
Saint Laurent	Red	1941	FRA Alsace	Karlštejn (B) 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