THE STUDY OF ANTIOXIDANT COMPONENTS IN GRAPEVINE SEEDS

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ABSTRACT

The wine pomace is a biodegradable waste that cannot be disposed in landfills used for the municipal waste. The content of antioxidant components that occur in this waste material is rather interesting. This study deals with contents of individual antioxidant components, polyphenolic compounds and with the antioxidant activity of wine pomace.

The antioxidant activity was determined spectrometrically by using two principally different methods, viz. the DPPH antioxidant assay and the FRAP method. The content of total polyphenolic compounds was determined by means of the Folin-Ciocalteu method. The content of resveratrol, rutin, quercetin, gallic acid, coumaric acid, catechin and epicatechin was determined by means of the RP HPLC-UV/VIS detection method.

According to the results obtained it can be concluded that grapevine (*Vitis vinifera)* seeds contain a very high amounts of antioxidant components that show a beneficial effect on the human health. The content of polyphenolic compounds was 6.529 $mg.Lt^{-1}$. The antioxidant activities estimated by means of DPPH and FRAP methods were 5.849 $mg.Lt^{-1}$ and 1.762 $mg.Lt^{-1}$, respectively. Catechin was the most frequent antioxidant and its content was 478 $mg.Lt^{-1}$.
Keywords: biodegradable waste, grapevine seeds, antioxidant components, polyphenolic compounds, antioxidant activity

1. Introduction

Worldwide, the grapevine (*Vitis vinifera)* is one of the most frequently cultivated fruit species (Vrsic *et al*., 2011). In Europe, the total acreage of vineyards is approximately 4.5 million hectares (45,000 sq. km.) and the total volume of produced pomace is about 8 million tons. Recently, the attention of many researchers was focused on the development of new technologies enabling a purposeful and efficient utilisation of this waste product.
At present, the potential spectrum of grapevine pomace use is relatively wide. So, for example, this waste may be used as a feedstuff for farm animals (Besharati and Taghizadeh, 2009), to produce dietetic, top-quality grape seed oil (Shinagawa et al., 2015) and production of energy (Valente *et al*., 2015). The pomace may be also composted for application in horticulture (Dominguez *et al.*, 2014), for grappa production (Da Porto, 2012), and also for other purposes.
Grapevine seeds contain great amounts of biologically active components (Pascoa et al., 2015). This fact was proved in many scientific studies dealing with the antioxidant potential of this waste material. It is also recommended to use grapevine seeds and derived products as a preventive means protecting humans against many diseases. Grape seed extract in particular has been reported to possess a broad spectrum of pharmacological and therapeutic effects such as antioxidative, anti-inflammatory, and antimicrobial activities, as well as having cardioprotective, hepatoprotective, and neuroprotective effects (Nassiri-Asl and Hosseinzadeh, 2009). Because of a synergism effect, natural extracts from these seeds are more efficient than isolates obtained from other identical substances.

Our study was focused on the research of some interesting substances contained in seeds of *Vitis vinifera*. We tried to highlight high content of healthy and beneficial compounds and to demonstrate that they may be used as health beneficial additives both in human and animal nutrition.

2. Material and methods

2.1. Biological material

This experimental study was performed with seeds of grapevine (*Vitis vinifera* L.) cultivar „Marlen“. The experimental material originated from the Department of Viticulture and Oenology, Faculty of Horticulture in Lednice na Moravě (Czech Republic).

2.2. Chemicals

Chemicals used in this study were supplied by the firm Sigma Aldrich (Germany). Antioxidant standards were products of the company Extrasynthese (France). Chemicals: Deionised water, stable free radical DPPH$^{•}$, TPTZ (2,4,6-tripyridyl-s-triazin), hydrochloric acid, $FeCl_3$, methanol, acetic acid (0.2%), liquid nitrogen, acetate buffer, sodium acetate, and Folin-Ciocalteu reagent. The following standard antioxidants were used in this study: resveratrol, rutin, quercetin, gallic acid, coumaric acid, catechin, and epicatechin

2.3. Method of sample preparation

The experimental material originated from grape pomace. To eliminate undesirable water residues, grapevine seeds were screened and purified, placed into the philosophiser CoolSafeBASIC 55-4, (manufacturer Merci, Francie) and stored at the temperature of -55 °C for a period of 48 hours. Thereafter, the seeds were crushed in a mortar together with the liquid nitrogen. Subsequently, 10 g of the homogenate were quantitatively transferred into a volumetric flask. The extraction was performed in a dark and cool environment with 100 ml of 75% methanol using the shaker IKA KS 260 Basic (manufacturer Merci, France) for a period of 5 days. Final extracts were centrifuged for 30 minutes at 16,400 rpm·min^{-1} and transferred into vials and micro test tubes (manufacturer Eppendorf, Germany).

2.4. Determination of antioxidant activity by the DPPH test

This procedure for the determination was published earlier (Sochor *et al*., 2010a). A 150 µl volume of the reagent (0.095 mM 2,2-diphenyl-1-picrylhydrazyl – DPPH$^{•}$) was incubated with 15 µl of the sample. The absorbency was measured at 505 nm for 10 minutes.

2.5. Determination of antioxidant activity by means of the FRAP method

This determination procedure was also published earlier (Sochor *et al*., 2010b). A 150 µl volume of the reagent was injected into a plastic cuvette and, subsequently, 3 µl of the sample were added. The absorbency was measured at 605 nm for 10 minutes.

2.6. Determination of the content of total polyphenols

The total level of polyphenols was determined using the Folin-Ciocalteau method, in which a 0.5 ml sample was diluted with 1.5 ml ACS water containing 0.05 ml of Folin-Ciocalteau reagent (manufacturer Sigma Aldrich, USA). The absorbency was measured after 30 minutes (at 22 °C) using a double-beam spectrophotometer SPEKOL 210 (manufacturer Carl Zeiss Jena, Germany) with a wavelength $\lambda = 640$ nm. The results were expressed as equivalents of gallic acid in mg·Lt^{-1}.

2.7. Assessment of antioxidant components by HPLC

For the determination of the HPLC profiles of the individual antioxidants, the high performance liquid chromatography (HPLC) method with UV-VIS detection was used. This method is described in detail by Zítka et al. (Zitka *et al*., 2011).

3. Results

Grapevine seeds contain great amounts of antioxidant substances. Above all, valuable are anthocyans and their content is very high. In our experiment attention was paid to studies on (i) the content of total polyphenols and the total antioxidant power of seeds; (ii) individual compounds because their pronounced antioxidant properties were very interesting. All estimations and studies on contents of individual polyphenolic substances were performed in three replications.

3.1.Determination of the antioxidant activity and of the content of polyphenolic compounds
There are many different polyphenolic compounds in grapevine seeds and their total contents may be estimated by means of the Folin-Ciocalteu method. This is a spectrophotometric procedure that enables to estimate total contents of polyphenols in a sample on the base of colour changes (Cicco *et al.*, 2009).

The total content of antioxidant compounds can be determined by the method of estimation of the "antioxidant activity". The principle of this method consists in the determination of quenching or neutralisation of free radicals by antioxidants contained in the biological matrix. The principle of the DPPH assay consist in the capability of a stable free radical 2,2-diphenyl-1-picrilhydrazyl to react with donors of hydrogen. The principle of the FRAP (Ferric Reducing Antioxidant Power) method consists in the reduction of the TPTZ (2,4,6-tripyridyl-S-triazine) iron reagent powder with ferric chloride ($FeCl_3$). The TPZT iron reagent powder is nearly colourless (or lightly brownish) and after the reduction the colour of the iron complex is blue (Dudonne *et al.*, 2009). Resulting values of the antioxidant activity are thereafter expressed as equivalents of a standard antioxidant. In our experiments, equivalents of gallic acid were used (GAE).

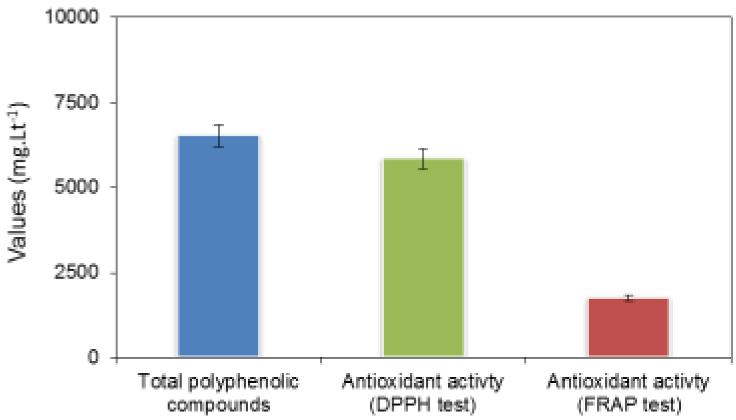

Figure 1: Contents of total polyphenols, values of antioxidant activity by DPPH assay (mg GAE.Lt^{-1}) and the FRAP method (mg GAE.Lt^{-1}).

The average content of total polyphenols was 6,529 mg.$Lt^{-1}$and values of antioxidant activity determined by the DPPH assay and the FRAP method were and 5,849 and 1,762 mg of GAE.Lt^{-1} respectively. Values of the antioxidant activity fluctuated in dependence on the type of phenolic compounds present in the biological matrix (the reason of this fluctuation was the fact that tome types of these phenolic compounds show a higher antioxidant activity than others. It is supposed that the protective effect is caused by the capability of some plant polyphenols to quench those reactive oxygen radicals that are able to generate highly reactive hydroxyl radicals.

3.2. Determination of some interesting antioxidants by means of HPLC
Chromatography is a laboratory technique enabling a simultaneous separation and identification of compounds occurring in a mixture. It can be also use for the estimation of individual components on the base of the comparison of measured values with the so-called standard values. Individual zones of separated components of the sample are transferred into the detection system where the emitted signals are recorded and evaluated. This method is very suitable for the detection of polyphenolic compounds (Zitka *et al*., 2011). As far as the grapevine seeds are concerned, they contain a profile of seven phenolic compounds the show significant antioxidant properties. In these experiments, contents of resveratrol, rutin, quercetin, gallic acid, coumaric acid, catechin and epicatechin were determined and recorded.

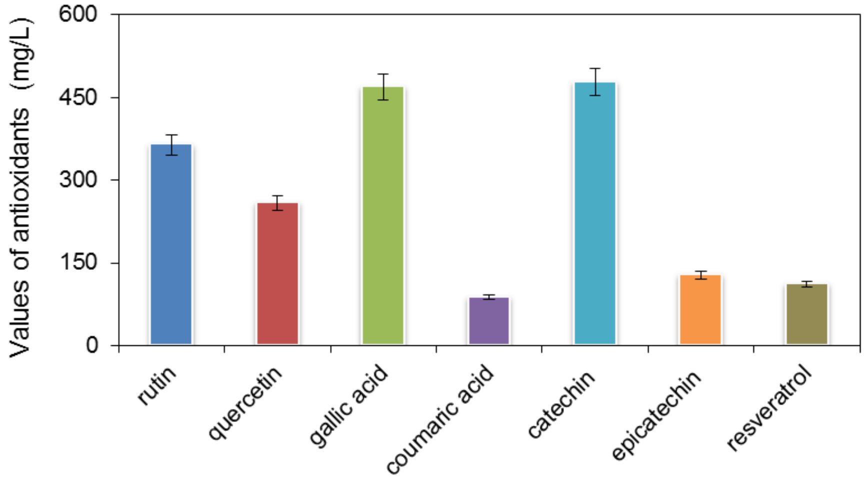

Figure 2: Contents of individual antioxidant compounds estimated by HPLC method.

Our experimental results indicate that of the compounds under study the content of catechin was the highest (478 mg.Lt^{-1}) and that of gallic acid occupied the second place (470 mg.Lt^{-1}). The content of coumaric acid was the lowest (88 mg.Lt^{-1}). As compared with other studies dealing with the determination of contents of antioxidants in different fruit species, the contents of all compounds under study were very high.

4. Conclusions

Production of oil is one of the most important methods of the utilisation of grapevine seeds. This product is popular not only in the domain of gastronomy but also in the field of aromatherapy and in the cosmetic practice. After pressing, the oil cake may be pulverized and used as flour. Another possibility is to use this material for production of wine vinegar.

In our experiments the following parameters were determined: antioxidant activity, content of total polyphenols, and contents of those antioxidant that show interesting strong antioxidant properties. Presented results corroborated that contents of antioxidant components in grapevine seeds were very high. It is well known that they show positive health effects and that the consumption of products made of grapevine seeds shows beneficial effect on humans.

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