Extraction of Antioxidants from Fresh Wine Grapes Marc.

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EXTRACTION OF ANTIOXIDANTS FROM FRESH WINE GRAPES MARC

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Abstract

Many agricultural wastes comprise residues of plants or fruits, which contain valued biologically active substances. Some of them represent antioxidants of phenolic nature, vitamins or pigments. As such waste marc is considered, arising during grapes pressing.
Samples of fresh marc from red and white grape varieties (Kobylí region, South Moravia) in original and ground form were processed. Extraction using Soxhlet apparatus for 1-5 hours was carried out with ethanol as a solvent.
The research was focused on determination of content of the resveratrol and antioxidant compounds with respect to individual grape varieties.
The resveratrol content was determined by U-HPLC/MS (Acquity UPLC – Synapt G2 MS) technique for individual extracts. Grinding of raw material caused a significant decrease of resveratrol content. It is evident that this mechanical operation can lead to its oxidation during mild thermal treatment.
Simultaneously, an antioxidant activity was tested by three methods (DPPH, ABTS and Folin-Ciocalteu). In this case, grinding of input biomass had positive effect on its antioxidant activity, which was practically doubled.

Introduction

For the isolation of valuable substances from specific parts of plants, it is necessary to find suitable extraction conditions, the plant material pre-treatment, the selection of a suitable solvent system with sufficient selectivity to extract the desired ingredients from the raw biomaterial, etc. Regarding to the subsequent application of the substances obtained in areas beneficial to human health, it is also necessary to choose a solvent that is harmless to health or has no negative impact on the environment. Furthermore, it is desirable to determine the optimum operating ratio of the plant drug to the extraction agent similarly as the operating temperature and to design the process technological process, i.e. to determine the conditions of the solid phase interaction and the extraction agent.
Last but not least, it is necessary to experimentally determine the needful time for the operation in relation to the yield of separation as well to assess its selectivity in the case of separation of the various components from the input material. It is also important to suggest the subsequent treatment of both the liquid extract and the extracted solid phase.

Materials and methods

Sample preparation and processing

Fresh marc from red and white grape varieties (Kobylí region, South Moravia) was processed. *Vinis vinifera*, VIVC classification: mixture of *Blaufränkisch* and *Zweigeltrebe Blau* (red; dry biomass 40 %) and *Veltliner Frührot* (white; dry biomass 32 %) were selected. Mentioned materials in original and ground form were extracted using Soxhlet apparatus for 1/3/5 hours by ethanol in the absence of light and oxygen. Biomass/solvent ratio 1/25 (w/v counted to dry biomass) was applied.

Antioxidant activity evaluation

The antioxidant activity was determined using three methods: ABTS, DPPH and Folin-Ciocalteu.
ABTS method is reported[1] as a decolourisation assay applicable to both lipophilic and hydrophilic antioxidants (e.g. flavonoids, hydroxycinnamates, carotenoids and plasma antioxidants). The pre-formed radical monocation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) is generated by oxidation of ABTS with potassium persulfate and is reduced in the presence of such hydrogen-donating antioxidants. The extent of decolourisation as percentage inhibition of the $ABTS^{\bullet+}$ radical cation is determined as a function of concentration and time and calculated relative to the reactivity of Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) as a standard, under the same conditions.
DPPH method consist[2] in reaction of stable free radical 1, 1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl) with electron (hydrogen) donor. In order to evaluate the antioxidant potential through free

radical scavenging by the test samples, the change of optical density of DPPH radicals is monitored. Decolourisation of the reaction mixture (violet to yellow) takes place. As a previous case Trolox is used as an activity reference.

The total phenolic content, more specifically the total reduction capacity of the sample, was determined spectrophotometrically using the ***Folin–Ciocalteu*** colorimetric ***method***[3]. The testing system is the mixture of tungstate and molybdate in highly basic medium (aqueous Na_2CO_3). Phenolics are energetically oxidized in basic medium resulting in the formation of $O_2^{\bullet-}$, which in turn, reacts with molybdate with formation of molybdenum oxide, MoO^{4+}. Complex tungsten-molybdenum blue $[(PMoW_{11}O_4)^{4-}]$ (very intensive absorbance near 750 nm) arises by reduction. The results are expressed as equivalent of gallic acid (GAE).

Resveratrol content was determined by U-HPLC/MS (Acquity UPLC – Synapt G2 MS) technique at following conditions: column BEH C18 (100x2.1 mm; 1.7 µm), mobile phase A – 0.1% formic acid in water, B - 0.1% formic acid in methanol, injection 3 µl, ionisation ESI, mode negative.

All analyses were performed at the Department of Food Analysis and Nutrition of the University of Chemistry and Technology Prague.

Results

From the previous experiments, it is apparent that substances with antioxidant activity can be obtained by extraction in an ethanol solvent agent.

Figures 1 and 2 illustrate the antioxidant activity of the studied samples of red and white wine grape marc. It is evident that the antioxidant activity of the prepared samples increases with longer extraction time, however, the increase is not so significant. For a mixture of red wine marcs (Figure 1) , grinding represents a positive pretreatment increasing the antioxidant activity. The magnified interfacial surface between the marc and the extraction agent in this case supported the extraction efficiency.

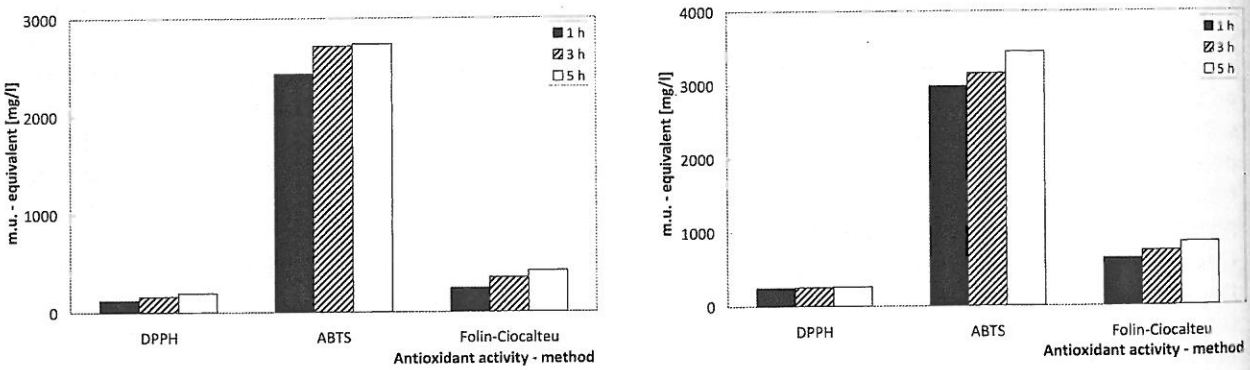

Figure 1. Antioxidant activity of red grape varieties mixture, a) without pretreatment, b) ground DPPH, ABTS: equivalent of Trolox, Folin–Ciocalteu: equivalent of gallic acid

The white variety *Veltliner Frührot* (Figure 2) shows a similar trend, the ground sample has the comparable tendency, but the total antioxidant activity is roughly half that the unprocessed one.

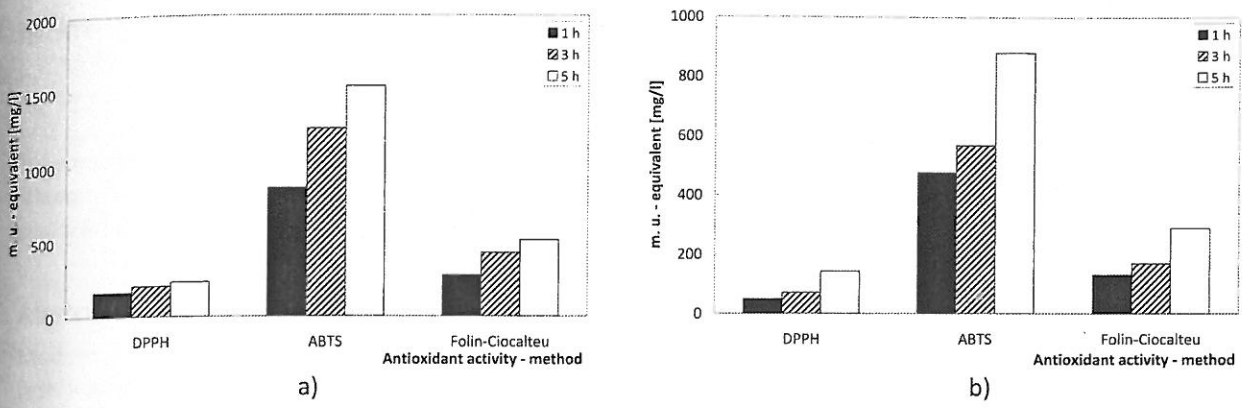

Figure 2. Antioxidant activity of white grape variety, a) without pretreatment, b) ground
DPPH, ABTS: equivalent of Trolox, Folin–Ciocalteu: equivalent of gallic acid

Table I shows that the extraction of phenolic resveratrol requires a polar extraction agent - ethanol, but its concentration in the extract is considerably low. Its quantity relative to the raw material - marc is also small. Its concentration is usually in the range of 4-40 mg/kg in the fresh biomaterial[4].

Table I
Quantification of resveratrol in marc extracts

Biomass *(VIVC classification)*	Extraction time [h]	Resveratrol [mg/$l_{extract}$]
Blaufränkisch + Zweigeltrebe Blau	1	<LOQ
	3	2.84
	5	3.00
Blaufränkisch + Zweigeltrebe Blau ground	1	1.49
	3	1.23
	5	1.64
Veltliner Frührot	1	1.08
	3	1.14
	5	1.17
Veltliner Frührot ground	1	<LOQ
	3	<LOQ
	5	<LOQ

LOQ = 1 mg/l

Conclusions

Two fresh marcs of red and white grape varieties in original and ground form were successfully studied in the Soxhlet extraction apparatus with ethanol as a solvent. Resveratrol content and antioxidant activity were determined by DPPH, ABTS and Folin-Ciocalteu methods.
Although the antioxidant activity of the extraction time and grinding increased, the mechanical operation negatively affected the content of resveratrol in extracts.

Acknowledgement

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