THE STUDY OF POLYPHENOLS IN DEALCOHOLISED WINE

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This study is about contents of antioxidant components in some selected samples of dealcoholised wine. Some important antioxidants are identified, i. e. gallic acid, caftaric acid, catechin, epicatechin, cis-resveratrol, rutin, quercitrin, quercetin and others. Furthermore antioxidant activity, content of total polyphenols, flavanols, and hydroxycinnamic acid were investigated. Contents of polyphenolic compounds ranged from 178 to 1159 mg/l, values of antioxidant activity from 49 to 548 mg/l. Based on obtained results, it was possible to conclude that contents of antioxidant components in samples of dealcoholised and "normal wine" were similar.
Keywords: antioxidant activities, dealcoholised wine, HPLC, polyphenolic compounds

Polyphenolgehalte in entalkoholisiertem Wein. Diese Arbeit beschäftigt sich mit den Gehalten antioxidativer Verbindungen in einigen ausgewählten Proben von entalkoholisiertem Wein. Einige wichtige Antioxidantien werden identifiziert: Gallussäure, Caftarinsäure, Catechin, Epicatechin, Cis-Resveratrol, Rutin, Quercitrin, Quercetin und andere. Weiters wurden die antioxidative Aktivität und die Gehalte an Gesamtpolyphenolen, Flavanolen und Hydroxyzimtsäure untersucht. Der Gehalt an Polyphenolen reichte von 178 bis 1159 mg/l, die Werte der antioxidativen Aktivität von 49 bis 548 mg/l. Die Ergebnisse zeigen, dass die Gehalte an antioxidativen Verbindungen in den Proben von entalkoholisiertem und "normalem" Wein ähnlich waren.
Schlagwörter: antioxidative Aktivität, entalkoholisierter Wein, HPLC, Polyphenole

In recent years, consumers have changed their requirements concerning the current assortment of beverages supplied to and available on the market. In general, the demand for light fruit juices containing lower amounts of alcohol has increased and this change forced the wine industry to diversify production and to develop new types of beverages. Due to this change in the demand for dealcoholised wine, it has already become one of those products more and more frequently available on the market of these beverages.

Epidemiological studies on the demand for alcoholic beverages indicate that a moderate consumption of wine is associated with a reduced risk of the occurrence of ischaemic cardiovascular disease and/or *Diabetes mellitus* (Ronksley et al., 2011). In a prospective study Wannamethee et al. (2003) mentioned that the association of a moderate consumption of alcohol with a reduced risk of the occurrence of these diseases is manifested at most in the group of those patients who consumed predominantly wine. This means that some of the performed clinical studies document that the consumption of wine (and, especially, of red wines) could reduce the risk of the occurrence of cardiovascular diseases (Fremont, 2000; Goldberg et al., 1996; Valsa et al., 1995; Xu et al., 1998). The problem, if just the content of alcohol in wine shows positive effects on the human organism and the health condition of people, is being widely discussed nowadays. On the one hand, some studies (Di Castelnuovo, 2009; Rimm et al., 1999) highlight its positive effects on the health state of people, whereas some others (Corrao, 2004; Corrao, 2000; Di Castelnuovo et al., 2002) emphasise the negative ones on the other hand. Conventional wine, however, cannot be enjoyed by everyone just because it contains alcohol.

These are the main reasons why bioactive substances contained usually in food in low concentrations are studied so intensively; of them, just phenolic compounds represent the most important group (Bouchenak and Lamri-Senhadji, 2013). Phenolic compounds show above all unique antioxidant effects (Rop et al., 2011b; Sochor et al., 2014). Wines containing higher amounts of these substances and showing positive effects on human health are mentioned at most (De Nisco et al., 2013; Ginjom et al., 2013).

The aim of this study was to determine contents of some antioxidants in samples of dealcoholised wine. The authors tried to highlight a comparable (or even a higher) biological value and positive effects of dealcoholised wine on humans and their health.

MATERIAL AND METHODS

BIOLOGICAL MATERIAL

Three different wine samples were tested experimentally:

A – white wine made of the variety 'Müller-Thurgau'; B - rosé wine made of the variety 'Zweigeltrebe'; C - red wine made also of the variety 'Zweigeltrebe'. Wine samples originated from the winery Vinselekt Michlovský a.s. (Rakvice, Czech Republic).

Dealcoholisation was performed by vacuum evaporation technology from the Australian company Flavourtech (Griffith, Australia); each wine was dealcoholised to the alcohol contents of 0.1 % and 5 %, respectively. This technology works by the principle of vacuum evaporation at a temperature of 30 °C. Wine aroma is collected in the initial phase of dealcoholisation, so that in the final phase it can be re-added unaltered in original condition. There is a partial dealcoholisation in this initial phase, because most of the volatile aromatic substances are chemically alcohol. Therefore, it is not possible to re-add all the wine aroma, which has been removed in the first phase, because the definition of "non-alcoholic" is not to exceed 0.5 % alcohol.

SPECTROPHOTOMETRIC ANALYSES

Total contents of phenols, anthocyanins and flavonols in wine were determined by spectrophotometry and antioxidant activity by the DPPH method. Samples were analysed in a HELIOS Gama spectrophotometer (Thermo Scientific, Waltham, MA, USA).

DETERMINATION OF THE ANTIOXIDANT ACTIVITY

Spectrophotometric measurements of the antioxidant activity were carried out using the BS-400 automated chemical analyser (Mindray, Shenzhen City, China). Transfer of samples and reagents was provided by a robotic arm equipped with a dosing needle (error of dosage max. ± 5 %vol.). Immediately after addition of reagents or samples, cuvette contents were mixed in an automatic mixer including a stirrer. This method was published by Sochor et al. (2010). A 150 µl volume of the reagent (0.095 mM 2,2-diphenyl-1-picrylhydrazyl - DPPH·) was incubated with altogether 15 µl of the sample. The absorbency was measured at 505 nm for 10 min. The method was calibrated using the phenolic compound gallic acid as a standard and the obtained results were expressed as equivalents of gallic acid in mg/l.

DETERMINATION OF THE TOTAL CONTENT OF POLYPHENOLS

The Folin-Ciocalteu method, based on the reduction of a phosphotungsten-phosphomolybdate complex by phenols to blue reaction products was used to determine the content of phenolic compounds. The sample (0.5 ml) was pipetted into a cuvette and diluted with ACS water (1.5 ml). Subsequently, 50 µl of the Folin-Ciocalteu reagent were added and the solution was incubated at 22 °C for 30 min. The absorbency was measured using a dual-beam SPECORD 210 spectrophotometer (Carl Zeiss, Jena, Germany) at a wavelength λ = 760 nm against a blank (containing all chemicals without a sample or gallic acid) (Sochor et al., 2011). The absorbency was measured three times.

DETERMINATION OF TOTAL FLAVANOLS

Total flavanols were determined using the p-dimethylaminocinnamaldehyde (DMACA) method (Li et al., 1996; McMurrough et al., 1996; Vivas et al., 1994). As compared with a widely used vanillin method, one of the great advantages of this method was that there was no interference from the side of anthocyanins. Furthermore, the sensitivity and specificity of this method were better. A wine sample of 20 µl was poured into a 1.5 ml Eppendorf tube and 980 µl of DMACA solution (0.1 % in 1 M HCl in MeOH) were added. The mixture was vortexed and allowed to react at room temperature for 12 min. The absorbency at 640 nm was then read against a blank sample prepared in a similar way but without DMACA. The concentration of total flavanols was then estimated from a calibration curve and constructed by plotting known solutions of catechin (1 to 16 mg/l) against A_{640} (r = 0.998). Results were expressed as mg/l of catechin equivalents.

DETERMINATION OF TOTAL CONTENTS OF HYDROXYCINNAMIC ACIDS

These measurements were performed using well-established spectrophotometric methods (Somers and Evans, 1977; Zoecklein, 1990). The wine sample was placed into a 0.2 cm path-length quartz cuvette; 200 µl of the sample and 1.8 ml of 1.1 M HCl were added and the resulting solution was thoroughly mixed and kept for a period of 180 min at room temperature.
A 0.22 M solution of $K_2S_2O_5$ was used as a blank. The absorbency was read at 320 nm (A_{320}^{HCl}) for anthocyanins. Concentrations (mg/l) were calculated as follows:

$$\text{Total content of hydroxycinnamic acids (mg/l)} = 10 \times \text{dilution} \times 12.387 \times A_{320}^{HCl}$$

DETERMINATION OF IMPORTANT ANTIOXIDANTS BY HPLC

For the determination of HPLC profiles the method of High Performance Liquid Chromatography combined with the electrochemical and the UV-VIS detection was used. The system consisted of two Model 582 ESA chromatographic pumps (ESA Inc., Chelmsford, MA, USA) with a working range of 0.001 to 9.999 ml/min and a Zorbax SB C18 (150 × 4.6) column; the size of particles was 5 µm (Agilent Technologies, Santa Clara, CA, USA) reverse phase chromatographic column. For the UV detection, a Model 528 ESA UV detector (ESA Inc., Chelmsford, MA, USA) was used. The electrochemical detection was performed in a twelve-channel CoulArray detector (ESA). Samples were injected automatically by an auto-sampler (Model 542, ESA), which had incorporated a thermostatic space for a column.

STATISTICAL ANALYSIS OF MEASURED DATA

Statistical analyses were performed using the mathematical software MATLAB, version R2010a (The MathWorks, Natick, MA, USA), and EXCEL (MS office 2010, Microsoft Corporation, Redmond, WA, USA).

RESULTS

Altogether nine samples, i. e. three of white, three of rosé and three of red dealcoholised wine and normal wine, were used for analyses. The samples contained either 0.1 %, 5 %, or 12 % of alcohol. In this study, wine samples containing 0.1 %, 5 % and 12 % of alcohol are labelled as "alcohol-free", "light wine", and "normal wine", respectively. The following basic parameters were determined: total polyphenols, flavanols, hydroxycinnamic acids, and antioxidant activity. The determination of the antioxidant activity was carried out by a method that expressed the strength of all antioxidants present in the given sample. Last, but not least, important antioxidants were also mapped on the basis of their antioxidant activity.

DETERMINATION OF THE ANTIOXIDANT ACTIVITY

There are many analytical methods available that enable the determination of the antioxidant activity in fruit, food or beverages (Pohanka et al., 2012; Sochor et al., 2013; Sochor et al., 2010). In this study, the DPPH˙ method was used; this method is based on the principle of quenching of free radicals contained in the analysed sample. This method is simple, cheap, and easily interpretable (Jurikova et al., 2012).
In the white and rosé wine samples, values of the antioxidant activity reached very similar levels (i. e. 52 and 59 mg/l EGA). In red wine, these values were more than six times higher while those of alcohol-free wine samples were lower (409 mg/l EGA) than in light wine (558 mg/l EGA) and normal wine (690 mg/l EGA) (Fig. 1).

DETERMINATION OF TOTAL POLYPHENOLS

Polyphenols contained in wine are an important group of secondary metabolites. The content of polyphenolic

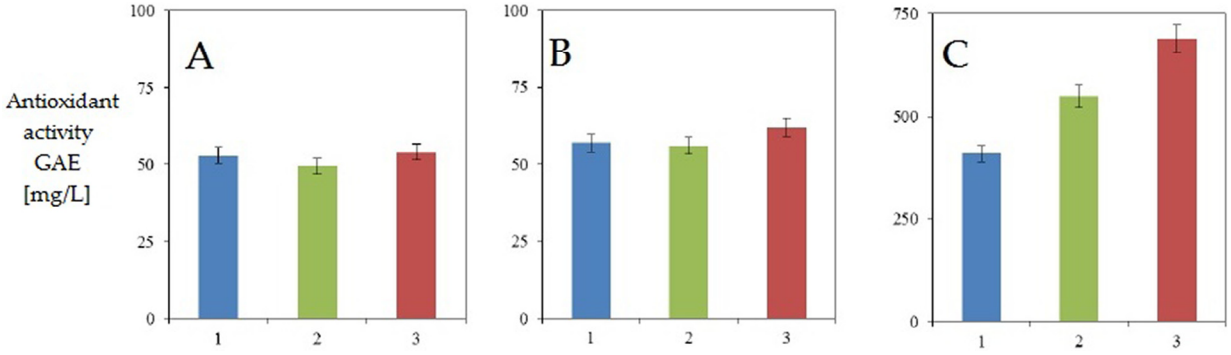

Fig. 1: Values of the antioxidant activity in samples of dealcoholised wine. A) white wine, B) rosé wine, C) red wine (1 - alcohol-free wine 0.1 %; 2 - light wine 5 %; 3 - normal wine 12 %)

compounds is dependent partly on the grapevine variety and partly on the winemaking technology (Garrido and Borges, 2013), and just the content of total polyphenolic compounds is responsible for the total antioxidant activity of not only grape wine (Kurin et al., 2012) but also of other kinds of fruit wine (Rop et al., 2010). Similarly as in the case of values of the antioxidant activity, the content of total polyphenolic compounds in white and rosé wines is rather similar (determined values ranged from 179 to 218 mg/l; in the normal rosé wine it was 261 mg/l). The content of alcohol itself, however, did not show any principal effect on the content of polyphenolic compounds in wine. However, a different situation existed in samples of red wine: in this case, contents of polyphenolic compounds ranged from 824 mg/l (in alcohol-free wine) to 1,379 mg/l (in light wine). The maximum content of polyphenols was in normal red wine (1,490 mg/l) (Fig. 2).

DETERMINATION OF TOTAL FLAVANOLS

Quercetin, rutin, myricetin, and kaempferol are the most important flavonols occurring in wine. In our experiments, the concentration of total flavanols was determined by a method based on their reaction with p-dimethylaminocinnamaldehyde (Li et al., 1996). Because in this case there is no interference with anthocyanins, this method ensures a higher sensitivity and also a higher selectivity than a widely used method that is based on the reaction of flavonols with vanillin.
The highest content of flavanols was recorded in red wine (354 and 379 mg/l in samples of alcohol-free wine and light wine, respectively), comparable to normal wine (385 mg/l). In rosé and white wine, contents of flavanols were rather similar (i. e. 50 to 59 mg/l) while in samples of light white wine it was only 25 mg/l; the higher content was in normal white wine (76 mg/l) (Fig. 3).

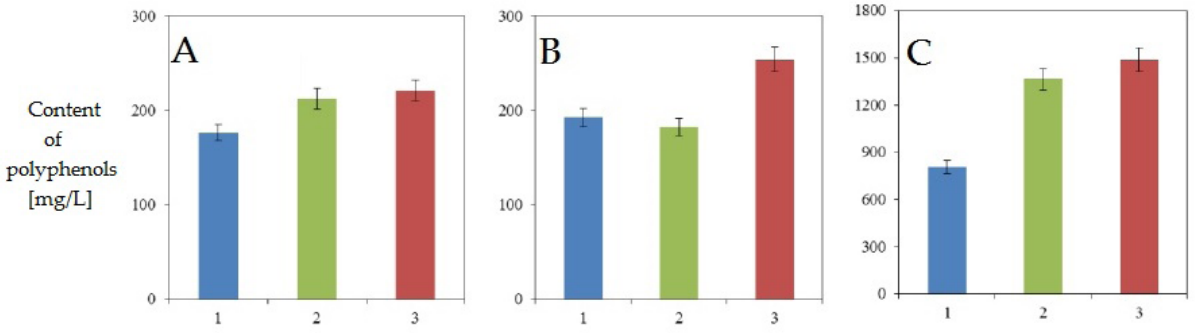

Fig. 2: Values of the polyphenols in samples of dealcoholised wine. A) white wine, B) rosé wine, C) red wine (1 - alcohol-free wine 0.1 %; 2 - light wine 5 %; 3 - normal wine 12 %)

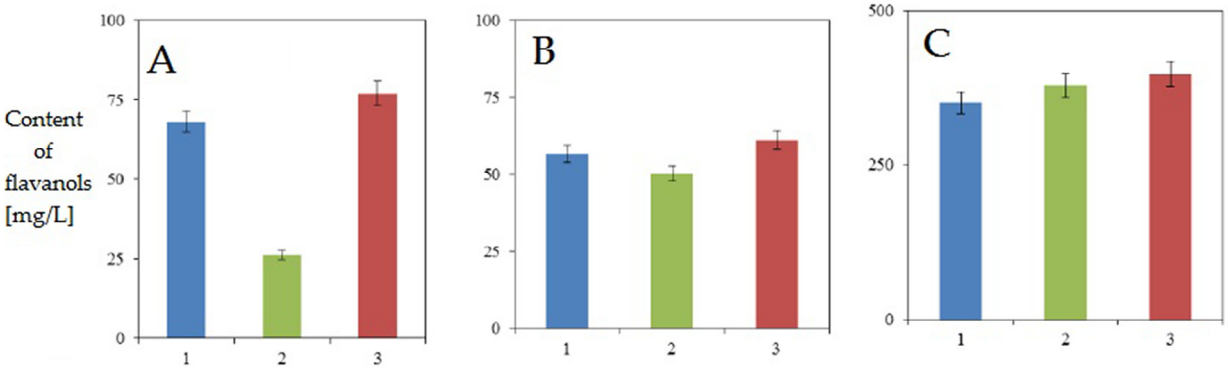

Fig. 3: Contents of flavanols in samples of dealcoholised wine. A) white wine, B) rosé wine, C) red wine (1 - alcohol-free wine 0.1 %; 2 - light wine 5 %; 3 - normal wine 12 %)

DETERMINATION OF THE TOTAL HYDROXYCINNAMIC ACID CONTENTS

These compounds are hydroxy-derivatives of cinnamic acid. In grapevine, they occur above all in the pulp of berries. Their content is rather independent of the winemaking technology. The most important and also the most frequently studied are the following ones: caffeic, p-coumaric, and ferulic acids as well as their corresponding tartaric acid esters (caftaric, coutaric, and fertaric acids) (Goncalves et al., 2013; Rodriguez-Diaz et al., 2006).

Hydroxycinnamic acid contents in samples of white and rosé wine ranged from 6 to 8.5 mg/l, only in normal rosé wine the content was higher (24 mg/l). In red wine, however, the content of this compound was markedly different. In samples of alcohol-free and light red wine, the recorded values were 20 and 34 mg/l, respectively, in normal red wine 44 mg/l (Fig. 4).

DETERMINATION OF THE TOTAL CONTENT OF SOME SELECTED ANTIOXIDANTS

The method of HPLC seems to be ideal for the determination of antioxidants occurring in not only in wine but also in grapes and fruit (Dobes et al., 2013; Rop et al., 2011a; Sochor et al., 2014). Studied were some important antioxidants that typically occur in wine, viz. gallic acid, vanillic acid, syringic acid, caftaric acid, caffeic acid, p-coumaric acid, fertaric acid, ferulic acid, catechin, epicatechin, cis-resveratrol, rutin, quercitrin, quercetin, and tyrosol. Contents of these compounds are presented in Table 1.

Of all antioxidants under study, the highest content was recorded for catechin (i. e. 88.4 mg/l in light red wine); high contents of gallic and caffeic acid were monitored as well. Based on obtained results it is possible to conclude that higher contents of antioxidants under study were found in samples of red wine than in those of white and rosé wine. Comparing samples of light wine with samples of alcohol-free wine, tyrosol and quercetin contents were higher in light wine.

Our results indicated that the content of alcohol did not show a significant effect on contents of polyphenolic compounds; this was demonstrated also in other studies (Ivanova-Petropulos et al., 2015; Xu et al., 2014) and it can be concluded that, above all, both the technology of wine making and the variety are responsible for contents of antioxidant components in wine (Balik et al., 2008; He et al., 2014).

CONCLUSION

Some clinical studies indicate that both juice or wine with lowered contents of alcohol may show a more posi-

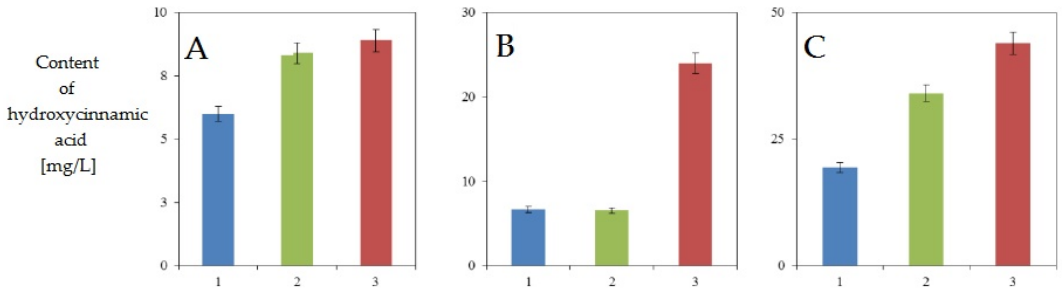

Fig. 4: Contents of hydroxycinnamic acid in samples of dealcoholised wine. A) white wine, B) rosé wine, C) red wine (1 - alcohol-free wine 0.1 %; 2 - light wine 5 %; 3 - normal wine 12 %)

Table 1: Contents of some antioxidant components in wine samples. WF = alcohol-free white wine, WL = light white wine, W N = white alcoholic wine, Ro F = alcohol-free rosé wine, Ro L = light rosé wine, Ro N = rosé alcoholic wine, Re F = alcohol free red wine, Re L= light red wine, Re N = red alcoholic wine.

Component	W F	W L	W N	Ro F	Ro L	Ro N	Re F	Re L	Re N
Gallic acid	5.10	4.85	6.8	3.79	4.28	5.41	40.7	51.9	88.9
Vanillic acid	1.24	1.63	1.91	1.75	1.73	1.85	5.63	3.54	5.40
Syringic acid	0.50	0.37	0.66	0.51	0.54	0.71	6.09	8.05	9.8
Caftaric acid	28.1	45.4	55.9	38.1	26.0	62.7	46.1	32.8	71.2
Caffeic acid	39.0	54.7	55.8	50.1	37.4	49.7	63.1	45.2	81.7
p-Coumaric acid	1.13	2.26	2.11	1.20	1.21	1.13	5.30	5.08	5.18
Fertaric acid	2.02	2.11	2.23	2.05	1.64	2.08	2.90	2.10	2.79
Ferulic acid	2.62	3.20	4.12	2.47	2.06	2.58	3.45	3.71	3.58
Catechin	27.9	7.20	32.52	24.0	10.0	28.9	63.1	88.4	88.7
Epicatechin	14.1	4.50	19.7	8.80	10.0	12.2	35.2	38.5	37.9
Cis-resveratrol	0.06	0.07	0.07	0.12	0.16	0.15	3.28	2.82	3.15
Rutin	0.77	1.47	1.54	0.84	0.12	0.98	12.4	3.53	13.7
Quercitrin	0.05	0.18	0.21	0.04	0.04	0.04	0.43	2.03	2.12
Quercetin	0.04	0.11	0.15	0.09	0.02	0.09	0.26	2.96	3.11
Tyrosol	8.54	17.20	17.82	9.13	10.26	11.42	20.2	26.5	25.9

tive effect on human health than alcoholic wine (Fantinelli et al., 2005; Vinson et al., 2001). This means, that in future dealcoholised wine might become an important substitute/alternative for contemporary conventional alcoholic wine. These products are ideal not only for drivers, sportsmen, people preferring a healthy life style, and/or pregnant women, but also for all oenophiles who cannot enjoy 'normal' wine because of higher alcohol contents. Unfortunately, for the time being, these wine products are not well-known and popular among consumers.

Our results demonstrated that contents of antioxidants contained in dealcoholised wine were comparable and nearly the same as those occurring in normal, "conventional", alcohol-containing wine. Positive effects of this beverage on the human body mean that their benefits should be highlighted and for that reason it should be recommended for consumption in amounts comparable to those of aforementioned normal, alcoholic wines.

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