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COMPARISON OF ANTIOXIDANT ACTIVITY, CONTENT OF POLYPHENOLS AND FLAVONOIDS IN LITURGICAL AND COMMON WINES

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ABSTRACT

This article deals with the comparison of biologically active substances (antioxidant capacity, content of polyphenols and flavonoids) in samples of common and liturgical wines. For determination were chosen these varieties Pinot Noir, Red Traminer and Chardonnay. The total content of polyphenols and flavonoids were found by visible diode array spectrophotometer. For determination of total antioxidant capacity was used the DPPH test. Results of this paper did not prove a general difference between liturgical and common wines, although between individual samples a statistically significant difference was found. Furthermore, the results show considerably higher values of biologically active substances in red wines Pinot Noir against white wine Red Traminer and Chardonnay – the total antioxidant capacity was considerably in excess of values up to 30 times, the total content of polyphenols up to 50 times and the total content of flavonoids up to 50 times. From the content of biologically active substances point of view, the red wine is recommended for human health.

Keywords: altar wine; kosher wine; antioxidant activity; polyphenols; flavonoids

INTRODUCTION

Wine is a very popular beverage over the world. The traditional production of wine is already known several thousands of years. For example, the ancient Romans knew wine and popularised wine consumption for its health benefits **(Lukacs, 2012)**. Since time immemorial, wine is also used at various religious ceremonials to pay tribute to gods at rituals of corresponding church. In Christian religion wine symbolizes Jesus Christ blood **(Bible, 1991)**. The production of altar wine using as „blood of the Lord“ at Eucharist is followed the rules, that are set down by Czech Bishop Conference for the Czech Republic **(Koudelka, 2010)**. According to instructions of **Congregation for Worship and the Discipline of the Sacraments (2004)** the altar wine must be natural from grapevine, without other additives and chemically treated. First of all, Czech Bishop Conference requires that wine was made only from grapes coming from Bohemia and Moravia. It is possible to give another rule, that used grapes must have 20 degrees of sugar content at least **(Koudelka, 2010)**.

Not only in Christianity, but also in other religions the liturgical wine, which is used for religious purposes, must meet the requirements of relevant religion and must be approved by a relevant religious authority. For example, at Jewish religious ceremonies wine is used much more than in Christian religion and rules for its production are more stringent. In Jewish religion Tóra looks at wine and bread as at „good things of life“ and „things for pleasure of gods and people“ **(Torah, 2012a,b,c; Divecký, 2005; Bondyová and Sliva, 2008)**. Wine is divided into several groups **(Mlček et al., 2018)**. Apart from boiled wine, non-Jew cannot touch kosher wine or open it due to the maintenance of kosher quality. Strict rules, that are apply to the manipulation with wine, are valid even for its growing and processing. For example, according to the commandment in the third book of Moses it is necessary to let a vineyard fallow and rest every seventh year (sabbatical year).

The wine grape is a basis for the production of grape wine. During processing of wine, the large amount of substances convenient for human health arise or get directly into final beverage from grapes. That is why wine is a significant source of biologically active substances such as antioxidants, polyphenols, flavonoids or mineral substances **(Mlček et al., 2018)**. The content of biologically active substances in grapes and wine depends on the variety of wine, locality, climate conditions, used agrotechnology and technology of processing and storage. Important biologically active substances are antioxidants, which prevent or reduce the oxidative destruction of substances, in which are contained in small amount. The substances with high antioxidant capacity have especially plant origin. Wine is the rich source of substances with antioxidant capacity, that can prevent damaging of DNA, peroxidation of lipids and formation of free radicals. So, it can be one of prophylaxis instruments before lifestyle diseases, especially cardiovascular **(Anastasiadi et al., 2010; Snopek et al., 2018b)**. Another biologically active substances in wine are polyphenols, that arise as secondary metabolites. They have an important role in reducing the risk of cardiovascular diseases similarly to antioxidants **(Snopek et al., 2018a)**. Flavonoids are classified to group of polyphenols according to type of reaction. They are very reactive and affect the wine oxidation. In case of gentle processing of grapes and of careful pressing the polyphenols content ranges under 200 mg.L^{-1} in white wine. In red wine the polyphenols content is 3 – 10 times higher. The total daily intake of polyphenols is estimated at 1 g **(Pavloušek, 2010)**. Due to positive effects of biologically active substances in wine on human health it is necessary to observe their content **(Valášek et al., 2014)**. Due to stricter conditions at the production of liturgical wine it can come about the variability of these biologically active substances content and difference from common wines.

Scientific hypothesis

Scientific hypothesis is: The total antioxidant capacity and the content of polyphenols and flavonoids in Czech liturgical and common wines are differed.

The aim of the study was a comparison of the antioxidant capacity and the content of polyphenols and flavonoid in Czech liturgical and common wines. Wine samples were selected with respect to their comparability of vintage, subregion and attribute.

MATERIAL AND METHODOLOGY

Wine samples

For analysis of total antioxidant capacity and the total content of polyphenols and flavonoids in sacramental wines were selected wines Pinot Noir and Red Traminer, Chardonnay was used for comparison in kosher wines. For each variety, 2 samples of common wine and 2 samples of liturgical wine were tested. Samples were chosen to ensure the highest comparability possible (vintage, sub-area and attribute), but different producers. Due to the difficulty of acquiring the comparable samples, their gathering took 15 months.

Samples were bought gradually in the common market, specialized wine shops and directly from the producers. Two bottles of each wine were bought and analysed. The samples in Table 1 were tested.

Table 1 Wine origin and category.

Sample	Category	Vintage	Sub-area, village, track	Quality
			Pinot Noir	
PN1	M	2012	Znojemská, Stošíkovice na louce, U tří dubů	VB
PN2	M	2012	Velkopavlovická, Havraníky, Staré vinice	VH
PN3	B	2012	Znojemská, Miroslavské Knínice, Stará hora	VH
PN4	B	2012	Velkopavlovická, Velké Bílovice	VH
			Red Traminer	
TR1	M	2013	Velkopavlovická	VH
TR2	M	2013	Znojemská, Stošíkovice na louce, U tří dubů	VH
TR3	B	2013	Znojemská, Bzenec	PS
TR4	B	2013	Znojemská, Sedlec, Nad Nesytem	PS
			Chardonnay	
CH1	K	2010	Izrael, Samson	Q
CH2	K	2011	Slovácká, Hýsly / Moštěnsko	PS
CH3	B	2010	Mikulovská, Perná, Purmice	PS
CH4	B	2011	Znojemská, Bzenec	VH

Note: K – kosher wine, M – communion wine, B – common wine, VB – special selection of berries, VH – special selection of grapes, PS – Late harvest, Q – quality.

Table 2 Results of the total antioxidant activity, the content of polyphenols and flavonoids in czech liturgical and common wines of varieties Pinot Noir (RM), Red Traminer (TR) and Chardonnay (CH).

Wine	Use	Total antioxidant activity [mg.L^{-1} AAE]		Total content of polyphenols [mg.L^{-1} GAE]		Total content of flavonoids [mg.L^{-1}]	
		M	SD	M	SD	M	SD
RM1	Liturgical	7160.2	57.0	12118.8	274.3	8689.3	476.1
RM2	Liturgical	2684.7	99.6	6349.1	291.4	5273.9	452.8
RM3	Common	4704.2	79.2	8146.7	393.3	5907.9	686.7
RM4	Common	3750.0	81.3	8246.7	806.7	6526.3	706.7
TR1	Liturgical	228.5	2.9	228.0	2.4	148.7	11.0
TR2	Liturgical	775.0	50.0	416.7	4.4	612.0	395.6
TR3	Common	894.0	5.7	440.2	6.9	250.0	163.3
TR4	Common	683.3	37.5	325.0	41.7	377.6	237.2
CH1	Kosher	359.2	40.5	298.6	8.8	591.8	290.8
CH2	Kosher	750.0	158.3	395.8	62.5	357.1	7.7
CH3	Common	890.0	10.0	453.4	11.8	906.7	442.1
CH4	Common	375.0	262.5	304.2	12.2	349.0	11.0

Total antioxidant capacity assay

To determine total antioxidant capacity (TAC) DPPH (1,1- diphenyl -2 picrylhydrazyl) assay was used according to the study by **Brand-Williams et al. (1995)**. The stock solution was prepared by dissolving 24 mg of DPPH with 100 mL of methanol and then stored at -20 °C until needed. The absorbance of DPPH radical without wine was measured daily. The sample solution was obtained by mixing 10 mL of the stock solution with 45 mL of methanol to obtain the absorbance of 1.1 ±0.02 units at 515 nm using the spectrophotometer LIBRA S6 (Biochrom, Cambridge, UK). The wine (210 µL) was allowed to react with 4 mL DPPH solution for 1 hour in the dark. Then, the absorbance was taken at 515 nm. Antioxidant capacity was calculated as a decrease of the absorbance value using the formula:

Antioxidant capacity (%) = (A0 - Ai/A0) x 100%, where A0 is the absorbance of a blank (without the sample) and Ai is the absorbance of the mixture containing the sample. Calculated antioxidant capacity was converted using a calibration curve of the standard and expressed in ascorbic acid equivalents (AAE) **(Rupasinghe, Jayasankar and Lay, 2006)**.

Total phenolic content assay

To measure total phenolic content (TPC) Folin-Ciocalteau reagent was used. 0.1 mL of wine was taken and mixed with water in a 50 mL volumetric flask. There-after, 0.5 mL of Folin-Ciocalteau reagent and 1.5 mL of 20% solution of Na_2CO_3 were added. The resulting absorbance was measured by LIBRA S6 spectrophotometer (Biochrom, Cambridge, UK) at the wavelength of 765 nm. Water was used as reference **(Thaipong et al., 2006)**. The results were expressed as grams of gallic acid (GAE) per kg of fresh mass (FM).

Total flavonoid content assay

Total flavonoid content (TFC) was determined by using 0.85 mL of juice mixed with 8.5 mL of 30% ethanol, 0.375 mL of $NaNO_2$ (c = 0.5 mol.dm^{-3}) and 0.375 mL of $AlC1_3.6H_20$ (c = 0.3 mol.dm^{-3}) as is described by **Park et al. (2008)**. The mixture was measured at the wavelength of 506 nm by LIBRA S6 spectrophotometer (Biochrom, Cambridge, UK). Total flavonoid content was calculated from a calibration curve by using rutin as the standard. The results were expressed in mg.kg^{-1} of FM.

Statistic analysis

The data were analysed using Excel 2013 (Microsoft Corporation, USA) and STATISTICA Cz version 12 (StatSoft, USA). Results were expressed by average ± standard deviation. Comparison of the results was performed using by Kruskal-Walllis test (α = 0.05). The samples of individual varieties were compared to each other. Furthermore, all samples of individual varieties of liturgical wines were compared against common wines.

RESULTS AND DISCUSSION

Total Antioxidant Capacity

In the study basic biologically active substances were determined in wine of these varieties – Pinot Noir, Red Traminer and Chardonnay. The basic measured results of the total antioxidant capacity, the content of polyphenols and flavonoids are given in Table 2.

The highest values of total antioxidant capacity were determined for variety Pinot Noir and these ones significantly exceed values of another observed varieties (up to 30x between sample RM1 a TR1). For variety Pinot Noir average values were measured over 2600 mg.L^{-1} AAE for every sample, while for another two varieties Red Traminer and Chardonnay were found below 900 mg.L^{-1} AAE. This finding confirms the fact, that antioxidant capacity is much higher for red wines than for white wines. This is due to the fact that in red wines substances with antioxidant capacity, for example phenolic substances, are in a significantly higher amount. In these more general facts results are in accordance with **Rupasinghe and Clegg (2007)** and **Szajdek and Borowska (2008)**. **Špakovská (2012)** in her study presents the higher antioxidant activity in red wines than in white wines. Average values were measured for each sample for variety Pinot Noir.

Results, presented in Table 2, were used for a comparison between each sample for given wine variety. Results of the comparison of total antioxidant capacity are shown in Table 3. Even though between many compared samples the statistically significant difference (p <0.05) was found out for each variety, the difference between liturgical and common wines was not generally proven. For example, for variety Pinot Noir the statistically significant differences were calculated for all samples.

However, total antioxidant capacity in common and altar wine is in the same range of values (Table 2) so the statistically significant difference between these varieties of wine was not confirmed.

Total Phenolic Content

Similarly, to total antioxidant capacity, the values of polyphenolic substances are significantly higher than for other observed varieties. For varieties Pinot Noir the total phenolic content was above 6300 mg.L^{-1} and for varieties Red Traminer and Chardonnay, average values of total phenolic content were set to 500 mg.L^{-1}. Statistical results of comparison for the total content of polyphenols are shown in Table 4. Again, the statistically significant difference (p <0.05) was found between many samples of each variety, but the difference between liturgical and common wines was not generally proven.

The phenolic substances are mainly contained in a grape peel. White wines are not macerated with peels in the production process, but peels are immediately removed and for this reason white wines contain fewer phenolic substances. In this aspect, the results are consistent with **Rupasinghe and Clegg (2007)** and **Faitova et al. (2004)**. **Špakovská (2012)** presents the content of polyphenols in the range of 299 to 407 mg.L^{-1} for white wines and for red wines in the range of 2130 to 650 mg.L^{-1}, which is consistent with our measured values.

Jančářová et al. (2013) states that the total polyphenol content is gradually decreasing with increasing time. Similarly, **Andjelkovic, Radovanović and Radovanović (2013)** also states, that the total polyphenol content grows during maturation and then decreases, ranging from 74.04 to 315.45 mg GAE.g^{-1}. The result of the above studies is finding, that the total polyphenol content varies with time.

Table 3 Comparison of total antioxidant activity between each samples for each variety. *p*-values were calculated by Kruskal-Walllis test.

Sample	RM1	RM2	RM3	RM4
RM1	-	**0.00**	**0.00**	**0.00**
RM2	**0.00**	-	**0.00**	**0.00**
RM3	**0.00**	**0.00**	-	**0.00**
RM4	**0.00**	**0.00**	**0.00**	-
	TR1	**TR2**	**TR3**	**TR4**
TR1	-	**0.00**	**0.00**	**0.00**
TR2	**0.00**	-	**0.00**	0.05
TR3	**0.00**	**0.00**	-	**0.00**
TR4	**0.00**	0.05	**0.00**	-
	CH1	**CH2**	**CH3**	**CH4**
CH1	-	**0.00**	**0.00**	0.89
CH2	**0.00**	-	0.56	**0.01**
CH3	**0.00**	0.56	-	**0.00**
CH4	0.89	**0.01**	**0.00**	-

Table 4 Comparison of total phenolic content between each samples for observed varieties. *p*-values were calculated using Kruskal-Walllis test.

Sample	RM1	RM2	RM3	RM4
RM1	-	**0.00**	**0.00**	**0.00**
RM2	**0.00**	**-**	**0.00**	**0.00**
RM3	**0.00**	**0.00**	-	0.79
RM4	**0.00**	**0.00**	0.79	-
	TR1	**TR2**	**TR3**	**TR4**
TR1	-	**0.00**	0.00	**0.00**
TR2	**0.00**	-	0.00	**0.00**
TR3	**0.00**	**0.00**	-	**0.00**
TR4	**0.00**	**0.00**	0.00	-
	CH1	**CH2**	**CH3**	**CH4**
CH1	-	**0.00**	**0.00**	0.38
CH2	**0.00**	-	0.51	**0.01**
CH3	**0.00**	0.51	-	**0.00**
CH4	0.38	**0.01**	**0.00**	-

Table 5 Comparison of total flavonoid content between each samples for observed varieties. *p*-values were calculated using Kruskal-Walllis test.

Sample	RM1	RM2	RM3	RM4
RM1	**-**	**0.00**	**0.00**	**0.00**
RM2	**0.00**	-	0.09	**0.00**
RM3	**0.00**	0.09	-	0.16
RM4	**0.00**	**0.00**	0.16	-
	TR1	**TR2**	**TR3**	**TR4**
TR1	-	**0.02**	0.16	**0.04**
TR2	**0.02**	-	0.07	0.24
TR3	0.16	0.07	-	0.30
TR4	**0.04**	0.24	0.30	-
	CH1	**CH2**	**CH3**	**CH4**
CH1	-	0.08	0.18	0.07
CH2	0.08	-	**0.01**	0.17
CH3	0.18	**0.01**	-	**0.01**
CH4	0.07	0.17	**0.01**	-

Due to the method of agrotechnical processing of altar wines, which should have the sugar content 20 °NM and more, later harvesting is supposed. Harvest time may be affected by the amount of polyphenols, their content can be lower.

On the other hand, the content of fragrances can increase, and organoleptic properties can improve.

Total flavonoid content

The last observed group of biologically active substances was group of flavonoids and their total content. Similarly, to the two previous observed parameters, values of total flavonoid content for variety Pinot Noir were significantly higher compared to other varieties.

For variety Pinot Noir the content of flavonoids ranged above 5200 mg.L^{-1}, but for varieties Red Traminer and Chardonnay values were measured below 1000 mg.L^{-1}. Comparison between each sample for observed varieties is given in Table 5. However, one can conclude here (to take account of total values), that the statistically significant difference between liturgical and altar wines cannot be established, but the difference between the individual samples can be established.

As stated by **Rupasinghe and Clegg (2007)**, the nature and concentration of flavonoids in wine samples could influence both the wine variety and damaged grapes during harvest and the differences in processing methods. **Sandler and Pinder (2003)** states, that flavonoid content in red wines can exceed 1200 mg.L^{-1}. This reality is in line with our obtained results. In case of white wines, the content of flavonoids is up to several times lower due to their technological processing.

CONCLUSION

This study deals with the content of biologically active substances in samples of liturgical and common wines and their comparison, which did not show the difference between liturgical and common wines, although the statistically significant difference between the individual samples was found. The total content of polyphenols and flavonoids and the total antioxidant capacity of samples were measured. The biologically active substances are important for the impact on human health. Results documented significantly higher values of biologically active substances in red wine for variety Pinot Noir against white wines for varieties Red Traminer and Chardonnay. The article also mentions the fact, that the content of individual biologically active substances changes and it is necessary to balance these quantities according to the requirements of the producer and the customer.

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