THE INFLUENCE OF GRAPE PROCESSING AND WINEMAKING TECHNIQUES ON PHENOLIC COMPOUNDS IN WINE PRODUCED FROM MALVERINA WINEGRAPE VARIETY, SOUTH MORAVIA, CZECH REPUBLIC

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ABSTRACT

The study of phenolic compounds in grape and wine shows their positive influence on human health. The polyphenolic content in white wines is usually lower than in red ones. However there are grape processing and winemaking techniques that favor their extraction. Wines from Malverina winegrape variety made by different grape processing and winemaking techniques (kakhetian technology, sur lies technology and reducing – standard technology) were examined for total phenol, total flavanol content as well as antiradical activity and reducing power using spectrophotomety. HPLC analyses were carried out to study the content of individual polyphenolics responsible for antioxidant properties of wine. It was found that kakhetian wine is the best from the standpoint of health. As it has the highest total phenol and total flavonol content as well as antiradical activity and reducing power. The highest content of such strong antioxidants as trans-resveratrol and tyrosol is in the standard wine (4.09 mg/l) and sur lies wine (29.16 mg/l) respectively. According to obtained data it is possible to say that grape processing and winemaking techniques really influence polyphenolic content. Especially long-term maceration with skins, seeds and stalk favors better extraction of polyphenols, thus increasing beneficial effect of white wines.

Key words: polyphenols, white wine, antioxidant activity, Malverina winegrape variety, kakhetian winemaking technology, sur lies.

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INTRODUCTION

Nowadays the study of phenolic compounds and their content in food is of great interest due to their versatile capabilities, and above all their beneficial influence on human health (4). Modern human is affected by permanent stress that can lead to various dysfunctions in organism, therefore one have to investigate various means to prevent that state of beings. According to numerous research works winegrape phenolic compounds are able to decrease the level of cardiovascular diseases, and have bactericidal, fungistatic, antioxidant and vitamin properties (5). They also play an important role in enology. Phenolic compounds are responsible for the differences between white and red wines, such as the color and flavor (1, 13). These molecules come from various parts of the grape bunches and are extracted during winemaking, they also assist winegrape plant in standard development. The polyphenol content in white wines is about 1 - 10 g/l, and tannin content is about 1 - 100 mg/l, that is far less than in red wines (8). A lot of research works have been recently dedicated to trans-resveratrol content, especially in case of red wines, as it is considered to be a strong antioxidant (17, 18). The average content of trans-reveratrol in white wine is about 0,05 - 1,8 mg/l (14). Tyrosol is less known phenolic antioxidant, the principal source is olive oil (15). As an antioxidant, tyrosol can protect against injury due to oxidation. Recently tyrosol present in white wine is also shown to be cardioprotective (16). Due to different investigations the correlation between antiradical activity and reducing power and flavanol (catechin, epicatechin) content was found (19 - 23). However it is known that the grape processing and winemaking technologies can significantly influence polyphenolic content. It was shown that in order to increase polyphenols extraction on should prolong maceration time, thus the contact time with skins, seeds and stalks. It is natural that this process has to be regulated by taking into account the nature of certain phenols in different parts of the bunch, as well as their effect on sensory qualities of wine. One of the ancient winemaking technologies is kakhetian one. It is applied in Kakhetian region, Georgia, using earthwork amphorae “kvevri” dug into the ground, thus providing temperature regulation during maceration and fermentation. The principal feature of this technology is prolonged contact with must and stalk, because of which wine contain more polyphenolic and aromatic compounds. “Sur lies” technology has certain effect as well (13).

The present investigation was undertaken to determine the polyphenolic composition in wine from Malverina winegrape variety using different grape processing and winemaking technologies (kakhetian technology, sur lies and reducing – standard (standard).

MATERIALS AND METHODS

Wines were produced from Malverina winegrape variety that derive from complex interspecific crossing performed by the group of selectionists under guidance of Ing. Miloš Michlovský, CSc. Grape was harvested in September 2008 at the technological stage of maturity. The first sample was produced according to standard white winemaking technology (11, 12) in stainless steel tanks (5000 l), the grapes were destemmed, pressed, clarified, the cultured yeasts were added, the fermentation was made with temperature regulation at 18 ˚C in 10 days, 31.01.09 the second racking with filtration took place, the sulfur dioxide was added in the amount of 40 mg/l. The other two samples were made according to kakhetian winemaking technology. The fermentation with must was carried out in oak barrels on 600 l. The second sample was pressed and left on yeast sediment (so called sur lies technology), the sulfur dioxide was added right before the bottling at the amount of 40 mg/l, the batonnage was made several times a year. The third sample wasn't pressed at the end of fermentation, and must was in wine all the year till bottling, the batonnage was performed several times a year, sulfur dioxide was added right before bottling at the amount of 20 mg/l.

All the chemicals needed for wine analysis such as acetonitrile (ACN) and methanol (MeOH) were supereluents of purity for HPLC. Catechin, epicatechin, vanillic acid, protocatechuic acid, p-hydroxybenzoic acid, gallic acid, syrigic acid, p-coumaric acid, caffeic acid, ferulic acid, trans-resveratrol, p-dimethylaminocinnamaldehyde (DMACA), Folin-Ciocalteu reagent, 2,2-diphenyl-β-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ) and perchloric acid were from Sigma Chemical Co. (St. Louis, MO). The rest chemicals were bought from the local importer (Lachema, Penta).

The standard wine analysis was made according to official OIV methods (1990).

Spectrophotometric analysis (the determination of total phenols, total flavanols, antiradical activity and reducing power) was made according to Arnous et al. (2001).

The determination of individual phenolics by HPLC (phenol acids, catechin, epicatechin, trans-resveratrol and tyrosol), employing high pressure binar system Shimadzu LC-10A, system controller SCL-10Avp, two pumps LC-10ADvp, termostat for Rheodyne: CTO-10ACvp, DAD detektor: SPD-M10Avp, Software: LCsolution. The elution program used was as follows: column Alltech Alltima C18 3 μm; 3 x 150mm with guard column 3 x 7.5mm, columns were maintained at 60 ˚C, the flow rate was 0,6 ml/min, eluent A was 15 mM $HClO_4$, eluent B was 15 mM $HClO_4$, 10 % MeOH, 50 % ACN. The elution program used was as follows: 0,00 min 2 % B; 20,00 min 26 % B; 30,00 min 45 % B; 35,00 min 70 % B; 37,00 min 100 % B; 38,00 min.100 % B; 38,01 min 0 % B; 39,99 min 0 % B; 40,00 min 2 % B; 45,00 min 2 % B. Total run time is 45 min. The determination of individual phenols was made using calibration curves of standard solutions. 200 nm: catechin and epicatechin, 260 nm: vanillic acid, protocatechuic acid, p-hydroxybenzoic acid, 280 nm: gallic acid, syrigic acid, cis-resveratrol, cis-piceid; 310 nm: p-coumaric acid and its derivatives, trans-resveratrol, trans-piceid, 322 nm: caffeic acid and its derivatives, ferulic acid and its derivatives. The derivatives of hydroxycinnamates were calibrate using basic acids.

Differences between means were detrmined using Fischer's least significant difference test (α=0,05).

RESULTS AND DISCUSSION

The results of standard wine analysis are presented in table 1. According to obtained data it is possible to say that maximum alcohol content was in kakhetian wine (13,04 %o). It is possible to explain the raised alcohol content by fermentation of all sugars that are usually eliminated by must separation. The reducing sugars were at their maximum in third sample as well (1,69 g/l). pH of the second and the third samples were significantly higher than pH of the first sample. The highest titratable acidity (5,91 g/l) was in the standard wine. The lower titratable acidity in two other samples is likely due to malo-lactic fermentation (MLF). The maximum level of volatile acidity is in the kakhetian wine (0,79 g/l). In accordance to obtained data MLF didn't take place in standard wine. Minimum level of citric acid was in kakhetian wine, that is the additional confirmation of MLF. The maximum level of glycerol was in sur lies wine (8,93 g/l), and the highest extract was in the first sample (24,2 g/l).

Tab. 1 Standard wine analysis

Indexes	Standard	Sur lies	Kakhetian wine
Alcohol (%)	11,93	12,35	13,04
Reducing sugars, g/l	1,51	1,17	1,69
pH	3,13	3,22	3,23
Titratable acidity (g/l of tartaric acid)	5,91	4,53	4,6
Volatile acidity (g/l acetic acid)	0,24	0,56	0,79
Malic acid, g/l	2,34	0,06	0,08
Lactic acid, g/l	0,74	1,91	1,97
Tartaric acid, g/l	2,34	2,32	2,27
Citric acid, g/l	0,17	0,08	0,01
Density	0,99358	0,99231	0,99178
Glycerol, g/l	8,93	9,24	8,92
Total extract, g/l	24,2	22,2	22,9
Sugar-free extract, g/l	22,7	21	21,2

Data on wine total phenols, total flavanols content, antiradical activity and reducing power are presented in table 2. Total phenols content (255 mg/l), total flavanols content (60,6 mg/l), antiradical activity (61,9 mg/l) and reducing power (37,4 mg/l) is the highest in kakhetian wine, that can be explained by prolonged contact with skins and seeds, that favor extraction.

Tab. 2 Total phenols content, totoal flavanols content, antiradical activity and reducing power in wine

Index, mg/l	Standard	Sur lies	Kakhetian wine
Total phenols (as gallic acid)	162,8	178,7	255
Total flavanols (as catechin)	11,2	11	60,6
Antiradical activity (as gallic acid)	25	32,7	61,9
Reducing power (as gallic acid)	28,5	33,4	37,4

Data on individual polyphenolic compounds are presented in table 3. Among benzoic acids gallic acid has the highest value (61,8 %), it is rather high in the third sample (24,07 mg/l), protocachetuic acid (19,6 %) prevails in the first sample (3,85 mg/l), and vanillic acid (11,7 %) prevails in the third sample as well (2,29 mg/l), syrigic acid (1,8 %) and 4-hydroxybenzoic acid (5,2 %) prevail in the second (0,4 mg/l) and first samples (0,95 mg/l) respectively. Among cinnamic acids caftaric acid prevails (60,2 %), especially high content is in the third sample (43,96 mg/l), caffeic acid (10,9 %), coumaric acid (3,3 %) and ferulic acid (5,2 %) prevail in the third sample (9,72; 3,13 и 4,24 mg/l respectively). Coutaric acid (8,1 %) and ferataric acid (7,8 %) prevail in the second sample (5,4 and 5,44 mg/l respectively). Ethyl caffeate (3,2 %), ethyl coumarate (1,1 %) and ethyl ferulate (0,3 %) prevail on the third sample (3,02; 0,87 and 0,25 mg/l respectively).

Catechin and epicatechin were major flavanoid compounds determined in wine. They reach maximum levels in the third sample (39,8 and 37,1 mg/l respectively).

The maximum levels of trans-resveratrol and tans-piceid are found in the first sample (4,09 and 0,99 mg/l respectively). And the highest concentration of tyrosol was found in the second sample (29,16 mg/l).

Tab. 3 Individual polyphenolic compounds in wine

Index, mg/l	Standard	Sur lies	Kakhetian wine
Gallic acid	1,41	4,35	24,07
Protocatechuic acid	3,85	2,8	2,79
4-hydroxybenzoic acid	0,95	0,78	0,77
Vanillic acid	1,26	2,08	2,29
Syrigic acid	0,18	0,4	0,31
Caffeic acid	5,43	6,41	9,72
Kaftaric acid (as caffeic acid)	35,94	39,39	43,96
Ethyl caffeate (as caffeic acid)	1,52	1,85	3,02
Coumaric acid	1,78	1,67	3,13
Coutaric acid (as coumaric acid)	5,32	5,4	5,29
Ethyl coumarate (as coumaric acid)	0,6	0,73	0,87
Ferulic acid	2,8	3,18	4,24
Ferataric acid (as ferulic acid)	5,27	5,44	4,72
Ethyl ferulate (as ferulic acid)	0,11	0,15	0,25
Trans-reveratrol	4,09	3,35	3.30
Catechin	6,67	8,03	39,8
Epicatechin	0,17	6,23	37,1
Trans-piceid (as free trans-resveratrol)	0,99	0,22	0,05
Tyrosol	25,29	29,16	21,06

CONCLUSIONS

According to our investigation maximum concentration of polyphenols, in particular gallic acid, catechin and epicatechin, that have beneficial influence on human health is in kakhetian wine. It is this wine that has maximum indexes of antiadical activity and reducing power. The indexes of total phenols, total flavanols and antiradical activity were higher in kakhetian wine than in standard wine (57, 441 and 48 % respectively). Such important indexes as catechin and epicatechin content were at their maximum in the third sample, they exceed standard on 497 and 21724 % respectively. The trans-resveratrol content is significantly higher than the average values of white wines. It reaches 4,09 mg/l in case of standard wine, it is more common for red wines. But these values are rather common for Malverina winegrape variety.

The results indicate that grape processing and winemaking technologies really influence polyphenolic composition. Prolonged contact with must favors better extraction of polyphenols that in turn increases beneficial qualities of white wine. The special attention has to be paid to kakhetian winemaking technology, as that wine is the best from the viewpoint of beneficial effect on human health.

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