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ОПРЕДЕЛЕНИЕ АНТИОКСИДАНТНОЙ АКТИВНОСТИ И[1] СОДЕРЖАНИЯ РЕСВЕРАТРОЛА В БОЛГОРСКИХ ВИНАХ ИЗ МЕСТНЫХ СОРТОВ ЛОЗ

DETERMINATION OF ANTIOXIDANT ACTIVITY AND RESVERATROL CONTENT IN BULGARIAN WINES FROM LOCAL GRAPE VARIETIES

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Аннотоция. Сделано изучение антиоксидантных и антирадикальных свойств, содержания фенольных компонентов (общие фенольные соединения, общие мономерные антоцианы, общие флавоноиды, катехин, эпикатехин, сиринговая кислота, ванилиновая кислота) и транс-ресвератрола в трех болгарских винах, полученных из местных винных сортов Димят (белый), Памид (красный) и Гымза (красный). Сорта выращиваны в районе г. Плевена, Центральная Северная Болгария. Установлено, что белое вино Димят отличается значительно меньшим содержанием фенольных веществ, ресвератрола и антиоксидантными свойствами по отношению к красным винам Памид и Гымза. Вино Памид содержит меньшее количество фенольных компонентов и соответственно имеет более низкую антиоксидантную активность по сравнению с Гымзой. Из анализированных фенольных кислот, проба Гымза содержит большее количество сиринговой кислоты и меньше ванилиновой кислоты чем Памид. Математически доказана очень хорошо обеспеченная разница в данных анализированных фенольных компонентов и антиоксидантной активности между исследуемыми винами. Не наблюдается зависимости между содержанием

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Summary. Study was carried out about the antioxidant and antiradical properties, phenolic components content (total phenolic compounds, total monomeric anthocyanins, total flavonoids, catechin, epicatechin, syringic acid, vanillic acid) and trans-resveratrol in three Bulgarian wines made from local wine varieties Dimyat (white) Pamid (red) and Gamza (red). The varieties were grown in the region of the town of Pleven, Central Northern Bulgaria. It was found that the white wine Dimyat had significantly lower content of phenolic components, resveratrol and antioxidant properties compared to the red wines Pamid and Gamza. Pamid wine contained smaller amount of phenolic components and respectively had a lower antioxidant activity compared to Gamza. From the analyzed phenolic acids Gamza sample contained higher amount of syringic acid and less vanillic acid in comparison with Pamid. Very well secured difference was mathematically proven in the data of the analyzed phenolic components and antioxidant activity between the studied wines. There was no correlation between the phenolic compounds content, the antioxidant properties and the concentration of trans-resveratrol in the experimental samples. The difference in the amount of resveratrol in the studied red wines was

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фенольных соединений, антиоксидантными свойствами и конценрацией транс-ресвератрола в опытных пробах. Разница в количестве ресвератрола в исследованных красных винах незначительна, в пользу пробы Памид.

Ключевые слова: вино, фенольные соединения, антиоксидантная активность, транс-ресвератрол.

insignificant in favour of Pamid.

Keywords: wine, phenolic compounds, antioxidant activity, trans-resveratrol

Introduction. Grapes growing and wine making on the Balkan Peninsula dated back to the ancient times, due to the favorable soil and climatic conditions for vine cultivation. In the situation of accelerating globalization, increased competition on the international wine markets and higher consumer requirements to wine quality the wine producers from the Balkan countries should focus on growing local wine varieties. Combining the natural resources of the region and the varieties potential under these conditions shall turn out to be an essential prerequisite for the production of wines of unique, individual nature.

Chemical composition of grapes and respectively of the produced wines is complex and diverse. It depends on the variety, soil and climatic conditions, the applied growing practices, maturity and the winemaking technology. Due to the large number of phenolic compounds wines, especially red ones are referred to beverages having a high concentration of natural antioxidants - catechins, procyanidins, anthocyanins, gallic acid, etc. (Valkova et al., 2004; Mandic et al., 2009; Savova, 2013). Almost all groups of phenolic substances have the ability to bind with the free radicals and dispose the active oxygen particles in the human body. The amount of antioxidants is one of the most important factors determining health qualities of foods and beverages (Joubert and Beer, 2006).

In recent years there has been an increased interest in resveratrol (3,5,4-trihydroxystilbene) - a natural antioxidant contained in the grape berries skins and from there passing to wines during maceration and fermentation (Fartsov et al., 2012). Its amount in wine varies depending on the variety and the growing area. The highest content has been found in Pinot Noir, Cabernet Sauvignon and Merlot. The richest in resveratrol are Burgundy and Chilean wines (Videnova and Fartsov, 2012).

In studies on the antioxidant activity and the content of resveratrol in Bulgarian wines it has been found that white wines contained significantly smaller amounts compared to red ones, due to the winemaking technology. Red wines are characterized by a higher content of compounds possessing antioxidant properties, as the ranking is as follows: Cabernet Sauvignon > Pinot Noir > Merlot > Gamza > Pamid (Ivanov et al., 2008; Yoncheva et al.,

2010).

However, red wines are not always preferred beverage because of the high content of tannins. The change of the consumer tastes towards greater demand of white wines has determined the need of better knowing their composition and antioxidant properties.

The objective of the study was to determine the phenolic composition, antioxidant activity and resveratrol content in Bulgarian wines produced from the local wine varieties Dimyat, Pamid and Gamza.

Material and Methods. The study was carried out at the Institute of Viticulture and Enology (IVE) – Pleven, Bulgaria and at the Institute of Viticulture (IV) - Tekirdag, Turkey. The study was focused on wines, vintage 2014, made from the local wine varieties Dymiat, Pamid and Gamza spread in Bulgaria and most of the Balkan region.

The varieties were grown at the Experimental base of IVE – Pleven (Central Northern Bulgaria). Upon reaching technological maturity, grapes were picked up and processed at the Experimental winery under the conditions of micro-vinification. The classical technology for making white and red dry wines were applied (Amerine et al., 1972):

- White wine making - crushing, draining, pressing, sulphating (50 mg/dm^3 SO_2), must clarification, adding pure culture lyophilized wine yeast *Saccharomyces cerevisiae* (*Vitilevure B+C)* in the amount of 20 g/hl, fermentation temperature 20^{o}C.
- Red wine making - removing the berries, crushing, sulphating (50 mg/kg SO_2), adding pure culture lyophilized wine yeasts *Saccharomyces cerevisiae* (*Vitilevure CSM)* in the amount of 20 g/hl, fermentation temperature 28^{o}C.

The course of the alcoholic fermentation was monitored by the change in the dry matter, measured daily with Abbe refractometer to a constant value. After the completion of the process the samples were decanted (white wines) or separated from solid particles, decanted (red wines) and further sulphated to 30 mg/dm^3 free SO_2.

The basic indicators of wine chemical composition were analyzed in the laboratories of IVE – Pleven by conventional methods used in the winemaking practice (Ivanov et al., 1979; Chobanova, 2007):

- sugars, g/dm^3 – Schoorl's method;
- alcohol, vol. % - distillation method, Gibertini apparatus with densitometry of the distillate density;
- total extract (TE), g/dm^3 - Gibertini apparatus with densitometry, density of alcohol-free sample;
- sugar-free extract (SFE), g/dm^3 - calculation method (the difference between TE and sugars);

- titratable acids (TA), g/dm^3 - titration with NaOH;
- color intensity I [abs. units] - method of Somers;
- pH - pH meter.

The indicators concerning the phenol complex of the wines, the antioxidant activity and trans-resveratrol content were analyzed in the laboratories of IV - Tekirdag. The following methods were used:

- total phenolic content was determined using the Folin-Ciocalteu's colorimetric assay (Waterhouse, 2002) and results were expressed as gallic acid equivalents (mg GAE/l);
- DPPH (1,1-diphenyl-2-picrylhydrazil) Radical Scavenging Activity assay was used based on the methods of Brand-Williams et al. (1995), as modified by Xu and Chang (2002). The free radical scavenging activity of wines was expressed as an equivalent of Trolox (μmol TEAC/ml) using the calibration curve of Trolox. Linearity range of the calibration curve was 20 to 1000 μM;
- ABTS [2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid)] Radical Scavenging Activity was determined according to the method described by Re et al. (1999). The calibration curve between % inhibition and known solutions (0.5, 1.0, 1.5, 2.0 mM) of Trolox was then established. The radical-scavenging activity of the wines were expressed as trolox equivalent antioxidant capacity (μmol TEAC/ml);
- total monomeric anthocyanin content was determined by the pH differential method as described by Giusti and Wrolstad (2001) and results were expressed as malvidin-3-glucoside equivalents (mg/l);
- total flavonoid content of the samples was determined according to the method described by Zhishen et al. (1999). The results were calculated and expressed as catechin equivalents (mg CAE/l) using the calibration curve of catechin;
- catechin, epicatechin, syringic acid,vanillic acid and trans-resveratrol levels (mg/l) in wine samples were measured by a HPLC system (Shimadzu LC 20 A). This system combined with a fluorescence detector in an Inertsil ODS-3(C18) column (5μm, 4.6 × 250 mm). Mobile phase A: 0.2% Formic acid in Water, mobile phase B: 0.2% Formic acid in Acetonitrile. For separation to following gradient; B Conc. 23 % (5 min), 26 % (12 min),40 % (14 min), 100% (14.01-18 min), 23 % (22 min); the flow rate was 1,5 ml/min. Column temperature was 30°C. The fluorescence detector was set at λex 278 nm and λem 360 nm for catechin, epicatechin, syringic acid and vanillic acid, λex 300 nm and λem

386 nm for trans-resveratrol. Samples of 5 μl of standard or wine were directly injected. The wine samples, standard solutions were filtered by a 0.45 μm pore size PTFE syringe filter.

The presented experimental results are the average values of three independent repetitions from the measurement of each analyzed indicator.

Data were mathematically processed by analysis of variance at confidence levels of the differences (Student criteria) p=5.0 %, p=1.0 %, p=0.1 % (Dimova and Marinkov, 1999).

Results and discussion. The chemical composition, the phenolic complex, the antioxidant activity and resveratrol content in one white and two red Bulgarian wines made from the local varieties Dimyat, Pamid and Gamza were determined.

The chemical composition of the experimental samples is presented in Table 1. The results did not show any deviations of the wines from the normal rates of the studied indicators. They were within the specific range for each variety, according to its varietal characteristics and potential. The alcoholic fermentation in the samples was complete, with sugars fermented in full and maximum alcohol accumulation. That was evidenced by the residual sugars content.

The amount of sugar-free extract and titratable acids, determining respectively the density and freshness of flavour are of special significance for the wine taste. In the red wines the rates of these indicators were lower in Pamid sample, which was a varietal characteristic. That was also applied with regard to the colour intensity.

Table 1.

Chemical composition of the studied experimental wines

Indicators Wine	Alcohol vol. %	Sugar g/l	Total extract g/l	Sugar-free extract g/l	Titratable acids g/l	Colour intensity [abs. un.]	pH
White wine							
Dimyat	12.44	1.45	21.00	19.55	6.70	0.16	3.26
Red wine							
Pamid	12.17	1.06	21.80	20.74	4.71	7.75	3.10
Gamza	12.32	1.47	23.73	22.26	5.51	9.66	3.17

The phenolic compounds contained in wine had also a significant effect on the sensory characteristics - color, aroma, taste, astringency. As a result of their interaction with proteins, polysaccharides or other phenolic compounds, they had an important role in the aging process and the physicochemical stability of wines (Frankel et al., 1995). They were also determining with regard to the antioxidant properties of wine.

Data on the content of total phenolic compounds, anthocyanins and phenolic fractions in the studied wines are presented in Table 2.

The analysis data of the wine phenolic complex had confirmed the results obtained by other authors, namely that red wines were distinguished for higher concentration of phenolic compounds (Valkova et al., 2004; Mandic et al., 2009; Savova, 2013). The difference in the total phenolic content of the samples was determined by the influence of a number of technological factors, but mainly from the grapes varietal specificity and its phenol contents (Ghiselli et al., 1998). Because of that, the wine from Gamza variety contained 3-fold greater amount of total phenols, total monomeric anthocyanins and total flavonoids than Pamid. Catechin and epicatechin concentration was also higher. Concerning the analyzed phenolic acids in the samples, Gamza wine had higher amount of syringic acid and less vanillic acid, compared to Pamid wine. In the three studied samples it was found higher content of catechin in comparison with epicatechin. All experimental wines contained vanillic acid in various concentrations, depending on the variety. Syringic acid in white wine Dimyat was not found while in Gamza wine its quantity was 7 times higher than in Pamid wine.

The mathematical processing of the test results proved well (total monomeric anthocyanins) and very well secured difference in the data of the rest analyzed indicators among the studied wines from the different varieties (Table 2).

Table 2.

Content of total phenolic compounds, anthocyanins and phenolic components in the studied experimental wines. Analysis of variance

Indicators / Wine	Total Phenolic, mg GAE/L	Total Monomeric Anthocyanin Content, mg/l	Total Flavonoid Content mg CAE/l	Catechin, mg/l	Epicatechin, mg/l	Syringic acid, mg/l	Vanillic acid, mg/l
White wine							
Dimyat	315.90 +++	-	54.24 +++	3.02 +++	0.70 +++	N.D.	0.09 +++
Red wine							
Pamid	819.50 +++	23.63 ++	301.71 +++	20.42 +++	9.47 +++	0.74 +++	2.95 +++
Gamza	2402.00 ++++	61.86 ++	902.32 +++	34.12 +++	17.60 +++	5.20 +++	2.37 +++
	GD(5.0%) =73.769 GD(1.0%) =122.346 GD(0.1%) =228.800	GD(5.0%) =14.410 GD(1.0%) =33.237 GD(0.1%) =105.815	GD(5.0%) =15.620 GD(1.0%) =25.906 GD(0.1%) =48.447	GD(5.0%) =0.623 GD(1.0%) =1.033 GD(0.1%) =1.931	GD(5.0%) =0.493 GD(1.0%) =0.818 GD(0.1%) =1.529	GD(5.0%) =0.050 GD(1.0%) =0.115 GD(0.1%) =0.365	GD(5.0%) =0.052 GD(1.0%) =0.087 GD(0.1%) =0.162

N.D. – not detected; (+) – the difference is significant; (++) – the differences is well secured; (+++) – the difference is very well secured; N.S. – the difference is not significant

The higher content of phenolic compounds in the analyzed red wines had meant respectively higher antioxidant activity. Therefore, it was determined and proven their antioxidant effect through the use of two analytical tests - DPPH and ABTS. The results are presented in Table 3.

Table 3.

Antioxidant activity of the studied experimental wines. Analysis of variance

Wine	Radical Scavenging Activity, μmol TEAC/ml			
	DPPH		ABTS	
White wine				
Dimyat	0.41	+++	3.52	+++
Red wine				
Pamid	0.98	+++	6.56	+++
Gamza	1.91	+++	23.70	+++
	GD(5.0%) =0.145 GD(1.0%) =0.241 GD(0.1%) =0.451		GD(5.0%) =0.324 GD(1.0%) =0,537 GD(0.1%) =1.004	

N.D. – not detected; (+) – the difference is significant; (++) – the differences is well secured; (+++) – the difference is very well secured; N.S. – the difference is not significant

The data showed that higher antioxidant activity was in correlation with

the higher content of phenolic components in the experimental wines composition. Both analytical tests had proved the increase in the antioxidant properties of the studied wines in the following order: Dimyat < Pamid < Gamza.

The red wine Pamid had twice higher antioxidant activity than white wine Dimyat. Significantly higher antioxidant properties were accounted in the Gamza sample (Table 3). The higher antioxidant activity of red wines was due to the higher phenolic content as well as on the different degree of polymerization of procyanidins in white and red wines and the different ratio of individual catechins in the polymer phenols molecule (Valkova et al., 2004).

Resveratrol refers to the group of polyphenolic compounds and in wines it passes from the berries skins during maceration and fermentation (Fartsov et al., 2012). It exists simultaneously as cis and trans isomer, as well as in glycosidic form (Gu et al., 2000). The trans isomer is prevailing in wine.

The results of trans-resveratrol content in the studied experimental wines are presented in Figure 1.

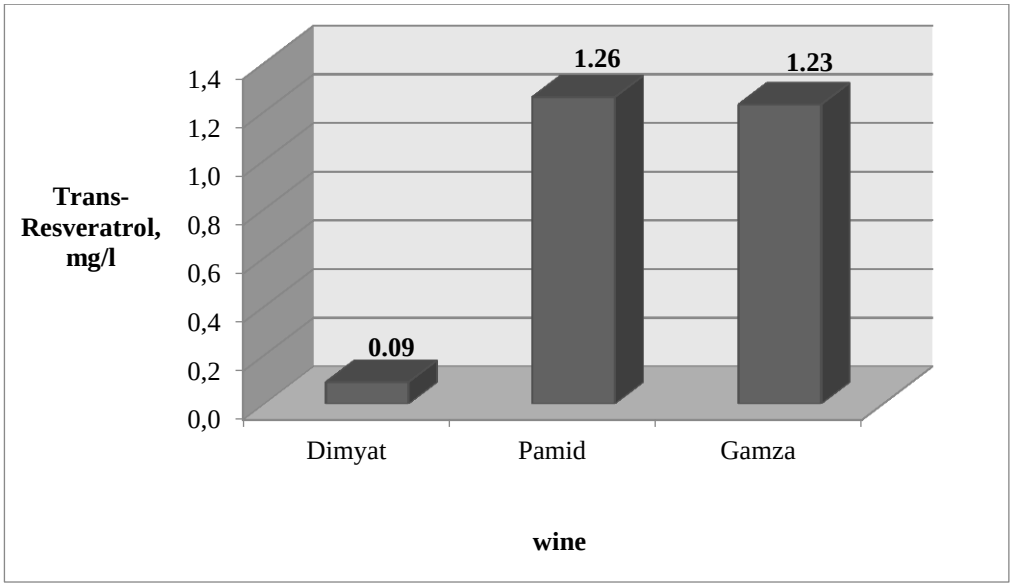

Fig. 1. Trans-resveratrol content in the studied experimental wines

The data had confirmed the findings of other authors that the amount of resveratrol in red wines was significantly more than in white wines (Souto et al., 2001). The obtained results did not reveal a direct correlation between the content of phenolic components, the antioxidant properties of wines and the concentration of resveratrol in the studied experimental samples. Although Pamid wine was characterized by significantly lower rates of antioxidant activity and phenolic compounds, as compared with Gamza wine, the difference in the amount of resveratrol in both wines was insignificant, in

favor of Pamid sample. That evidenced that resveratrol synthesis in vine, passing respectively in grapes and wine, was determined by several factors, the main of which was the variety, its potential and specificity.

The mathematical processing of the results proved very well secured difference in data on the antioxidant activity of the studied wines as defined in both analytical tests (Table 3). For the resveratrol content the difference was very well secured between white wine and red wines, while between the samples of Pamid and Gamza it was statistically insignificant at GD(5.0%)=0.033, GD(1.0%)=0.055, GD(0.1%)=0.102.

Conclusion. On the basis of the obtained data it could be summarized that:

- The white wine Dimyat had significantly lower content of phenolic compounds, resveratrol and antioxidant properties compared to the red wines Pamid and Gamza.
- From the studied red wines Pamid contained less phenolic components and respectively it had lower antioxidant activity compared to Gamza. From the analyzed phenolic acids, Gamza wine had a greater amount of syringic acid and less vanillic acid in comparison with Pamid wine.
- It had not been found direct correlation between the content of phenolic components, the antioxidant properties of wine and the resveratrol concentration in the experimental samples. The difference in the amount of resveratrol in the tested red wines was insignificant in favour of Pamid sample.

References

1. Amerine, M.A. Technology of Winemaking/ M.A. Amerine, H.W. Berg, W.V. Cruess - Westport, Connecticut, The Avi Publishing Company, 1972. - 380-392.
2. Brand-Williams, W. Use of a free radical method to evaluate antioxidant activity/ W. Brand-Williams, M.E. Cuvelier, C.L. Berset// LWT-Food science and Technology, 1995. - 28(1) – P. 25-30.
3. Chobanova, D. Textbook for Exercises in Enology/ D. Chobanova - Plovdiv, Academic Publishing House of UFT, 2007. – 139 p.
4. Dimova, D. Experimental work and biometrics/ D. Dimova, E. Marinkov - Plovdiv, Academic Publishing VSI, 1999. - 263 p.
5. Fartsov, K. Factors that Determine the Trans-Resveratrol Content in Wines/ K. Fartsov, D. Toshev, A. Atanasov, R. Videnova, N. Simov, G. Mihaylova, D. Kozareva// Viticulture and Enology Journal (BGN), 2012. - LX, 2 – P. 25-31.
6. Frankel, E.N. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-dwnsity lipoproteins/ E.N. Frankel, A.L. Waterhouse, P.L. Teissedre// Journal of Agricultural and Food Chemistry, 1995. – 43 – P. 890-894.
7. Ghiselli, A. Antioxidant activity of different phenolic fractions separated from Italian red wine/ A. Ghiselli, M. Nardini, A. Baldi, C. Scaccini// Journal of Agricultural and Food Chemistry, 1998. – 46 – P. 361-367.
8. Giusti, M. Characterization and measurement of anthocyanins by UV-

visible spectroscopy/ M. Giusti, R. Wrolstad// Current protocols in food analytical chemistry, 2001.

9. Gu, X. Analysis of resveratrol in Wine by Capillary Electrophoresis/ X. Gu, Q. Chu, M. O'Dwyer, M. Zeece// Journal of Chromatography A, 2000. – 881 – P. 471-481.

10. Ivanov, S. Influence of Variety Specifics on the Antioxidant Content of Grape/ S. Ivanov, L. Miteva, T. Yoncheva, V. Dimitrova, V. Alexieva// Agricultural Science (BGN), 2008. - XLI, 2 – P. 3-8.

11. Ivanov, T. Practicum in Wine Technology/ T. Ivanov, S. Gerov, A. Yankov, G. Bambalov, T. Tonchev, D. Nachkov, M. Marinov - Plovdiv, Publishing "Hristo G. Danov", 1979. - 531 p.

12. Joubert, E. Antioxidant Activity of South African Red and White Cultivar Wines/ E. Joubert, D. Beer// Wynboer, A Technical Guide for Wine Producers, April, 2006. - http://www.wynboer.co.za/recentarticles/ 200604antioxidant.php3.

13. Mandic, A. Antioxidant Activity of White Grape Seed Extracts on DPPH Radicals/ A. Mandic, S. Djilas, J. Brunet, G. Cetkovic, J. Vulic// Acta Periodica Technologica, 2009. – 40 (1) – P. 53-61.

14. Re, R. Antioxidant activity applying an improved ABTS radical cationdecolorization assay/ R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans// Free radical biology and medicine, 1999. - 26 (9) – P.1231-1237.

15. Savova, S. Catechins in Bulgarian White Wines/ S. Savova// Medical Review (BGN), 2013. - 49, 2 – P. 69-72.

16. Souto, A. Determination of Trans-Resveratrol Concentrations in Brazilian Red Wines by HPLC/ A. Souto, M. Carneiro, M. Seferin, M. Senna, A. Conz, K. Gobbi// Journal of Food Composition and Analysis, 2001. – 14 – P. 441-445.

17. Valkova, T. Antioxidant Properties of Bulgarian White and Red Wines/ T. Valkova, B. Koleva, N. Stoyanov, S. Kemilev, M. Dilcheva// Scientific Papers of the University of Food Technologies - Plovdiv, 2004. - LI, 1 – P. 130-137.

18. Videnova, R. Statistics Investigations for the Trans-Resveratrol Content in Red Wines from Different Varieties and Regions in Bulgaria and in the World/ R. Videnova, K. Fartsov// Viticulture and Enology Journal (BGN), 2012. - LX, 4 – P. 6-10.

19. Waterhouse, A. L. Determination of total phenolics/ A. L. Waterhouse// Current protocols in food analytical chemistry, 2002.

20. Xu, B.J. A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents/ B.J. Xu, S.K.C. Chang// Journal of food science, 2007. - 72 (2) – P. S159-S166.

21. Yoncheva, T. An Antioxidant Capacity of Selected Bulgarian Wines/ T. Yoncheva, L. Miteva, V. Alexieva, P. Kerchev, V. Dimitrova, L. Brankova, E. Shopova, J. Koňa, S. Ivanov// Acta Horticulturae et Regiotecturae, Nitra (Slovaca Universitas Agriculturae Nitriae), 2010. - 3, 1 – P. 5-8.

22. Zhishen, J. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals/ J. Zhishen, T. Mengcheng, W. Jianming// Food chemistry, 1999. - 64 (4) – P. 555-559.