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EXAMINATION OF THE CONTENT OF SOME ANTIOXIDANT SUBSTANCES IN SOME AUTOCHTHONOUS GRAPEVINE VARIETIES (*VITIS VINIFERA L.*) IN R.N. MACEDONIA

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ABSTRACT
In this paper, some bioactive antioxidants have been tested in several grape varieties (*Vitis vinifera L*.) present in R.N. Macedonia. Grapes are a source of various nutrients that are beneficial to the human body in many ways. A grape, as a raw material and its final processing - wine is a complex of many chemical substances and compounds that are responsible for many visible and sensory characteristics that are manifested in varieties (phenotypic). Those chemical compounds are; carbohydrates, organic acids, alcohols, aldehydes, esters, vitamins, polyphenols, minerals, nitrogen compounds, etc. Specifically, in our case, vitamin C (ascorbic acid) and phenolic compounds (total phenols, anthocyanins, flavan-3-ol and antioxidant activity) were tested in several autochthonous grape varieties and one standard variety (Belo zimsko, Stanushina, Crn valandovski drenok, Crven valandovski drenok and Palieri as standard). The tested chemicals are of great importance in their consumption in the human diet. They have antioxidant, anti-inflammatory, antimicrobial, anticancer, anti-mutation effects and reduce the risk of cardiovascular, degenerative and other chronic diseases. The most important for this group of compounds is that they determine the color, astringency, bitterness and some organoleptic and sensory characteristics of grapes and wine.

Key words: vitamin C, phenolic compounds, autochthonous varieties, antioxidant activity.

INTRODUCTION
In the fast pace and urban lifestyle, and especially in the last period of immunodeficiency in the human body, the consumption of fruits, vegetables and grapes has a great advantage in maintaining human health. Therefore, we decided to do a study in the project; Antioxidant activity of fruits of indigenous varieties and populations of fruits, vegetables and grapes, where in a comparative way we will make an analysis of fruits of several fruit species, types of vegetables and grape varieties that are present in R.S. Macedonia.

In this case, the research is about the analysis of vitamin C (ascorbic acid) and phenolic compounds (total phenols, anthocyanins, flavan-3-ol and antioxidant activity) in several autochthonous grape varieties and one standard variety – Belo zimsko, Stanushina, Crn valandovski drenok, Crven valandovski drenok and Palieri as standard (Agius F, *et al.* 2003) Vitamin C is obtained during a certain phenolic activity in plants and it is responsible for certain physiological and biochemical processes that affect the health of the organism of man by increasing immunity, then participates in the binding of certain mineral ions, has antioxidant function and the like. (Juanjuan Liu, *et al* 2016).

Phenolic compounds are responsible for the quality, certain characteristics, color of grapes and wine (as pigments), variety, sensory properties (Chakraborthy, A. *et al.* 2014), and most of all for their antioxidant action (sometimes called oxygen tanks, Ian Hornsey, 2007, Coombe. B.G, *et al* 1992). They are divided into two main groups; non-flavonoids (hydroxybenzoates and hydroxycimetic acids, their derivatives and main groups, stylenes - resveratol, phytoalexin) and flavonoids (flavantriols, anthocyanins, flavonols, flavonoids and flavanones, V. Ivanova *et al.*

2010). Each of these compounds is responsible for one or more of the above characteristics. For example, flavonols play a protective role in grapes by absorbing UV rays, anthocyanins are red pigments in grapes and wine - in grapes they are found in the skin, and in dyes they are also found in grape juice and the like.

Antioxidant activity depends on phenolic compounds, especially flavonoids. The activity of flavonoids is expressed by the formation of less active flavonoid radicals and the chelation of metal ions. Specifically in grapes, the content and chemical composition of phenolic compounds depend on; variety or genotype, phenophase, climate, exposure, ampelotechnic practice, etc. Also, during the processing of grapes into wine, phenolic compounds are extracted differently, depending on the winemaking practices and techniques and the time of their application (ie in wine as a final product, phenolics can be reduced or increased, at times disappear, yes appear or create new ones, etc.)

The main goal of this paper is to prove the authenticity of the rich diversity of vines, i.e. grapes as a source of many useful compounds for human health. Autochthonous varieties are a rich source of biologically active substances that provide quality, nutritional and health characteristics. In specific tests, our indigenous varieties have a satisfactory, significant content and can be considered a significant and stable source of vitamin C and antioxidants. The examined table varieties have a solid content of the previously mentioned bioactive substances (Jiang Bao *et al*. 2011), while in the wine varieties that content may be higher, but also increase during the processing (vinification) of grapes in wine.

MATERIAL AND METHODS

Some bioactive antioxidant chemicals have been tested in grapes of several autochthonous grape varieties in the R.N. Macedonia and they are compared with a standard introduced variety.

Belo zimsko - autochthonous variety, very late table variety, belongs to the ecological-geographical group of eastern varieties (orientalis), subgroup antasiatica. In our examination, samples will be taken from vineyards from localities in the Gevgelija-Valandovo vine district. **Stanushina** – autochthonous, domestic variety, wine variety, belongs to the ecological-geographical group Black Sea varieties (pontica), subgroup balcanica. In our examination, samples will be taken from vineyards from localities in the Tikvesh vine district.

Crven valandovski drenok - autochthonous variety or transferred and domesticated during Ottoman Empire, very late table variety, belongs to the ecological-geographical group of eastern varieties (orientalis), subgroup antasiatica. In our examination, samples will be taken from vineyards from localities in the Gevgelija-Valandovo vine district.

Crn valandovski drenok - autochthonous variety or transferred and domesticated during Ottoman Empire, and is often thought to be a variety of red Valandovo dogwood, a very late table variety, belongs to the ecological-geographical group of eastern varieties (orientalis), subgroup antasiatica. In our examination, samples will be taken from vineyards from localities in the Gevgelija-Valandovo vine district.

Palieri (Michele Palieri) introduced a table variety in R.N. Macedonia. It is a medium-late table variety, belongs to the ecological-geographical group of western varieties (occidentalis), subgroup gallica. In our examination, samples will be taken from vineyards from localities in the Gevgelija-Valandovo vine district.

In the determination of bioactive antioxidants in grapes, several standard methods were used to determine their presence and to compare them with other substances.

The content of vitamin C (mg%) in grapes, some fruits and tomatoes is determined by the method of Muri, and the filtrate is titrated with 2,6 dihlor phenol indofenol. The filtrate is titrated to a pale pink color. For those filters that are very colored before treatment, titration with 50 mg of activated carbon is performed until complete decolorization. HPLC methods (according to Kall and Andersen) are also used to test for vitamin C in grapes.

Method for determination of the content of total phenols, anthocyanins and flavan-3-ol - spectrophotometric method, expressed in mg/kg FW. In our case, the content of total phenols, anthocyanins and flavan-3-ol was determined with the Agilent 8453 UV-VIS spectrophotometer.

Before starting their determination, the samples are prepared. Take approximately 5 g of the homogenized sample and transfer to a zucchini, add to the zucchini 20 ml of pre-prepared extraction solution (methanol: water: hydrochloric acid in a ratio of 70 : 30 : 0,1) and place in an ultrasonic bath for time of 15 min and 30 min on a magnetic mixer. The solution is centrifuged, and the resulting clear liquid is transferred to a 25 ml zucchini and made up to the mark with the same solution. These methods are considerably suitable for routine analysis, are rapid, and can be used to monitor changes in polyphenols during fruit ripening.

Determination of total phenols. Total phenols in the samples were determined according to the Folin-Ciocalteu method (Singelton & Slinkar, 1999). In parallel with the samples, a blank sample is prepared, where distilled water is used instead of the examined sample, and the other reagents remain the same. Determination of total anthocyanins.

The content of total anthocyanins was performed according to the Acid ethanol method (Somers *et al.*, 1977). Ethanol chloride solution is used as a blank test. Anthocyanins were read on a spectrophotometer at a wavelength of 550 nm.

Determination of total flavan-3-ol. P-dimethylaminocinnamaldehyde (p-DMACA) was used to quantify total flavan-3-ols in the samples (Di Stefano *et al.*, 1989). Methanol was used for the control sample. The absorbance was measured at a wavelength of 640 nm.

Determination of antioxidant activity. Performed as anti-radical activity against stable product DPPH (2,2-diphenyl-1-picrylhydrazil). Ascorbic acid was used as standard, preparing a series of standard solutions. The reading was performed spectrophotometrically, at a wavelength of 517 nm. The absorbents of all the extracts are measured individually, and then from the obtained results for the absorbents the antioxidant activities expressed in percent of inhibition are calculated.

Determination of color, intensity and shade. The color of the grapes and its intensity depend on the composition and concentration of the anthocyanins. It is defined as the sum of absorbers at different wavelengths (420, 520 and 620 nm). To determine the color, it is necessary to obtain juice from the sample and then centrifuge it to obtain a clear liquid. Place the centrifuged sample in a 2 mm cuvette and then directly determine the absorbent values at the specified wavelengths. The obtained values are used to determine the color intensity (CI) and shades (H), red - (% R), blue - (% B) and yellow - (% Y).

1. Color intensity calculation (CI):

$(CI) = A420 + A520 + A620$

2. Shade calculation (H):

$H = A420 / A520$

3. Calculation of the composition of the color:

$\% Y = A420 / CI \times 100$

$\% R = A520 / CI \times 100$

$\% B = A620 / CI \times 100$

Where:

A420 - absorption at 420 nm

A520 - absorption at 520 nm

A620 - absorption at 620 nm

Figure 1 shows the examined varieties in the following order: Belo zimsko, Stanusina, Crn valandovski drenok, Crven valandovski drenok and Palieri (standard).

Figure 1. Examined varieties: Belo zimsko, Stanusina, Crn valandovski drenok, Crven valandovski drenok and Palieri (standard)

RESULTS AND DISCUSSION

In our examinations of bioactive antioxidants in the tested grape varieties the following results were obtained. The content of vitamin C in the autochthonous grape varieties is on average – 9,0 mg%, the highest content is registered in the standard variety Palieri - 12,83 mg%, and the lowest content in the variety Belo zimsko – 7,00 mg%. The content of total phenols in the autochthonous grape varieties is on average – 1037,19 mg/kg fresh weight (FW), the highest content is in the variety Stanushina – 1358,54 mg/kg fresh weight, and the lowest content in the variety Belo zimsko – 643,46 mg/kg fresh weight. The content of anthocyanins in the autochthonous grape varieties is on average – 206,96 mg/kg fresh weight, the highest content is in the standard variety Palieri – 416,73 mg/kg fresh weight and the lowest content in the variety Belo zimsko – 38,93 mg/kg fresh weight. The content of flavan 3-ol in the autochthonous grape varieties is on average – 130,23 mg/kg fresh weight, the highest content is in the standard variety Palieri – 186,407 mg/kg fresh weight and the lowest content in the variety Crven valandovski drenok – 69,698 mg/kg fresh weight. The antioxidant activity of the indigenous grape varieties is on average – 40,86 mg/kg fresh weight, the highest is in the standard variety Palieri – 42,5 mg/kg fresh weight and the lowest in the Belo zimsko variety – 38,3 mg/kg fresh weight.

From the results it can be said that the antioxidative activity of the varieties depends mostly on the content of total phenols in grapes and its percentage is highest in those varieties with higher content of total phenols. The examined parameters and the obtained results are in correlation with the obtained results from several authors listed in the discussion in the following text.

From the results obtained from the examination of the color, shade and intensity of the grape varieties, the following can be said; the color shade of the grapes is average – 1883,40 AU, with the most intense shade of the Crn valandovski drenok - 2,030 AU, and the smallest shade of the White Winter variety - 1,805 AU. The color intensity of the grapes is on average – 0,303 AU, with the strongest intensity in the Palieri standard – 0,404 AU, and with the weakest intensity in the White Winter variety – 0,228 AU. The highest percentage of yellow color is in the variety Crn valandovski drenok – 56,94%, the highest percentage of red color is in the variety Stanushina – 29,38% and the highest percentage of blue color is again in the variety Crn valandovski drenok – 18,41%.

According to Rodríguez-Delgado *et al*. 2002, the concentration of polyphenols depends on the nature of the raw material - ie the grape variety and genotype, phenophase, degree of maturity and soil-climatic conditions, and a similar conclusion was presented by the authors (Fernandez-Mar *et al.*, 2012), who in their research has concluded that the percentage of polyphenolic components depends on the grape variety, the application of viticultural practices as well as soil and climatic conditions.

Ratiu *et al.* 2020 in their research evaluated the effects of different climatic conditions on the production of phenolic compounds. They have shown that low temperatures and lack of water increase the synthesis of phenolic compounds, especially the accumulation of anthocyanins. The same authors concluded in their study that soils with „nutritional stress" or soils rich in sucrose affect the synthesis of phenolic compounds.

A group of authors (García-Falcó *et al.,* 2007; López-Roca *et al.,* 2007) have published several studies describing the factors that influence the greater extrusion of phenolic compounds during the vinification process.

According to Kennedy, 2008 maceration has a major impact on the concentration of anthocyanins, the tannins present in the sample. The maceration process is influenced by a number of factors, including time and temperature.

Polyphenols are a large group of secondary metabolites, with a very complex structure that are essential for quality (Von Baer *et al.,* 2008). Phenolic compounds are cyclic benzoic compounds having one or more hydroxyl groups directly attached to the aromatic ring (Flamini *et al.*, 2013). Precursors for polyphenols are derived from carbohydrate metabolism (Winkle-Shirley, 2001) Flavan- 3-ol in grapes are synthesized mainly in seeds and stalks (60% in seeds, 20% in stalks, and in the husk about 15% (Bourzeix *et al.*, 1986; Downey *et al.*, 2003).

Typical flavan-3-ol in grapes are: (+) catechin, (-) epicatechin, (-) epicatechin-gallate and very rarely (-) epicatechin-3-O-gallate (Pineiro *et al.*, 2013).

The tannins located in the skin are extracted in the initial stages of fermentation, and as the maceration time increases, the concentration of tannins derived from the seeds increases (Peyrot des Gachons & Kennedy, 2003).

Scientists De Santis & Frangipane, 2010, recorded an increased concentration of catechins, epicatechins and tannins when the maceration process is prolonged.

Singleton *et al,* 1999, describes the procedure for the determination of total polyphenols.

Maceration time has a great influence on the antioxidant activity of wines. During short maceration due to insufficient extraction of phenolic compounds and low alcohol content at the beginning of vinification, wines with lower antioxidant activity are obtained (Sanja Kostadinovic *et al.*, 2012), while during prolonged maceration due to increased concentration of procyanidins and the reduced content of anthocyanins (Kovac *et al.*, 1992), are obtained wines that show high antioxidant activity.

The examined parameters and the obtained results are in accordance with the obtained results from several authors listed in the discussion.

Table 1 gives the results of the examination of the content of vitamin C, total phenols, anthocyanins and flavan-3-ol and according to them determination of antioxidant activity in the examined varieties: Table 2 gives the results of determining the color, intensity and hue of the examined varieties, figure 2 graphically shows the antioxidant activity and figure 3 graphically shows the color, intensity and shades of grapes.

Table 1. Vitamin C, total phenols, anthocyanins and flavan-3-ol and antioxidant activity

	Variety	Vitamin C *(mg%)	Total phenols (mg/kg *FW)	Anthocyanins (mg/kg FW, fresh weight)	Flavan - 3 ols (mg/kg FW)	Antioxidant activity (% inhibition)
1.	Belo zimsko	7,0	643,46	38,93	98,198	38,3
2.	Stanusina	10,0	1358,54	343,07	135,059	42,3
3.	Crn valandovski drenok	8,0	1202,99	154,03	161,768	41,3
4.	Crven valandovski drenok	8,0	843,42	82,03	69,698	39,9
5.	Palieri (st.)	12,0	1137,54	416,73	186,407	42,5
	Average	9,00	1037,19	206,96	130,23	40,86
	sd	2,00	288,70	165,21	47,07	1,76
	CV%	22,22	27,84	79,83	36,15	4,31
	Xmin	7,0	643,46	38,93	69,698	38,3
	Xmax	12,0	1358,54	416,73	186,407	42,5
	Variation Width VW	5,0	715,08	377,80	116,709	4,20

*FW - fresh weight

Table 2. Color, intensity and shade in the examined varieties

	Variety	Color *(AU)	Intensity *IC (AU)	% yellow colour	% red colour	% blue colour
1.	Belo zimsko	1,805	0,228	52,50	28,05	15,01
2.	Stanusina	1,835	0,305	53,92	29,38	16,69
3.	Crn valandovski drenok	2,030	0,342	56,94	29,09	18,41
4.	Crven valandovski drenok	1,839	0,237	53,32	28,99	17,70
5.	Palieri (st.)	1,908	0,404	54,70	28,67	16,63
	Average	1883,40	0,303	54,276	28,836	16,888
	sd	90,22	0,074	1,693	0,507	1,285
	CV%	4,79	24,296	3,120	1,759	7,611
	Xmin	1,805	0,228	52,50	28,05	15,01
	Xmax	2,030	0,404	56,94	29,38	18,41
	Variation Width (VW)	225,00	0,176	4,44	1,33	3,40

*AU - international unit of color and intensity
*IC - color intensity

Group of authors (Landete, J.M. 2011; Alcalde *et al.*, 2014) in their research suggested that the polyphenols influence of the sensorial properties, influence of the stability of vine and possess antioxidant activity, as well (Sanja Kostadinovic *et al.*, 2012).

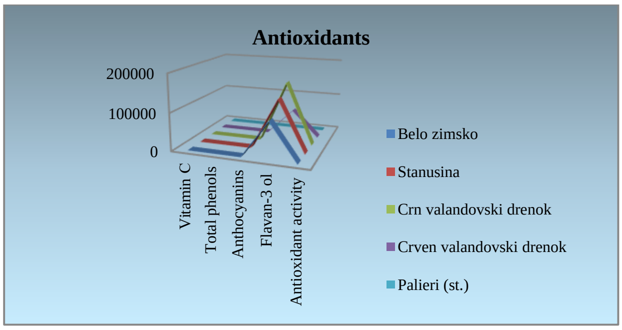

Figure 2. Antioxidants

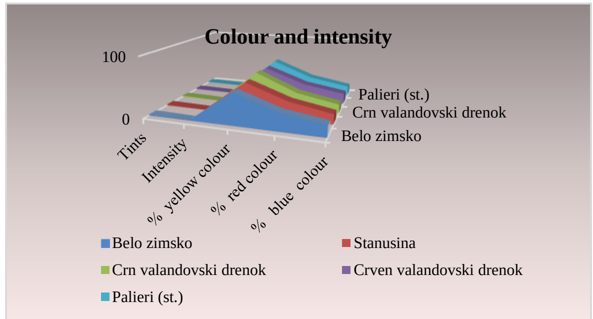

Figure 3. Colour and intensity

CONCLUSIONS

From the obtained results the following can be concluded:

- The oxidative activity of the varieties depends mostly on the content of total phenols in the grapes and its percentage is highest in those varieties with higher content of total phenols.
- The content of vitamin C in the autochthonous grape varieties is on average – 9,0 mg%, the highest content is registered in the standard variety Palieri – 12,83 mg%, and the lowest content in the variety Belo zimsko – 7,00 mg%.
- Studies have shown that the total phenols have a greater impact on the antioxidant activity of varieties compared to vitamin C, but the content of vitamin C in autochthonous varieties is significant.
- The content of total phenols in the autochthonous grape varieties is on average – 1037,19 mg/kg fresh weight (FW), the highest content is in the variety Stanushina – 1358,54 mg/kg fresh weight, and the lowest content in the variety Belo zimsko – 643,46 mg/kg fresh weight.
- Antioxidant activity in the autochthonous grape varieties is on average – 40,86 mg/kg fresh weight, the highest is in the standard variety Palieri – 42,5 mg/kg fresh weight and the lowest in the variety Belo zimsko – 38,3 mg/kg fresh weight.
- The color, its nuances and intensity in the examined varieties mostly depend on the content of anthocyanins in the grapes and it is in many respects a quality trait that depends on the genotype (variety characteristic). The examined autochthonous varieties have a stable content of anthocyanins and even color. They are not pigments and the dyes are found only in the skin, but are not present in the grape juice (pulp).
- As a general conclusion, the examined autochthonous varieties have stable contents of antioxidants and are varieties with high potential for further selection for obtaining other varieties that will have improved hereditary characteristics and will have a higher content of significant bioactive antioxidants.

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