EVALUATION OF QUALITATIVE PARAMETERS OF TABLE GRAPES VARIETIES IN ECOPEDOCLIMATIC CONDITIONS OF THE EXPERIMENTAL FIELD U.S.A.M.V. BUCHAREST

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Abstract

In the last years, there has been a higher and higher growth of consumers' interest towards the nutritive value of fruits and vegetables, in the context of a correct alimentation, which has a major impact on the health state of the human organism. Within the healthy alimentation framework, grapes represent genuine sources of micronutrients with antioxidant effect, due to their high content of whole polyphenols, flavonoids, and anthocians with a varying qualitative and quantitative distribution in the grape. The purpose of this study is to assess and compare the quantity of these beneficial substances, accumulated in the skins and the seeds, upon full maturation, for the most grown and known table grapes varieties in the varietals conveyor in Romania. From the achieved results, as expected, the total phenolic content of the red grape is higher when compared to white grape.

Key words: flavonoids, grape table, phenolic compounds, maturity, varieties

INTRODUCTION

The crop quality at the grape vine mainly depends on its metabolites. The diversity of grape vine varieties is greatly due to secondary metabolites. A large range of specific chemical compounds represents these and they form part of different groups, such as phenols, terpenes, antibiotics, volatile oils, resins, glycosides, sterols, alkaloids, saponins of which many proved to be very valuable for the pharmaceuticals, agrochemical, food and cosmetics (Zhang et al., 2001). Phenols are a large and complex group of secondary metabolites, which contributes especially to the features of the red grapes and vines. Phenolic compounds are known especially for their contribution to the pigmentation of different parts of the plant, but also due to the role, they play in the plant's resistance to biotic and antibiotic stress. In addition, they have an important role in determining the food quality of grapes. Phenolic composition of grapes depends not only on their degree of ripening but also on the growth parameters of the grape vine such as soil and weather conditions, with applied viticultural together the agrotechnics (pruning, fertilisation or irrigation). In the recent years, consumer's interest has grown more and more on the nutritive value of fruits and vegetable, in the context of the right feeding which has a major impact upon the health condition of the human body. Grapes represent real sources of nutrients with antioxidative effect, these compounds having a diverse spread in the different parts of its berries. Important representatives of phenols, flavonoids can be found, in the skin and seeds of the grapes berry, having a wide spectrum of pharmaceutical, antiallergenic, anticancerigenic, properties etc. Anthocyanins, as red pigments, can be found in the skin of berries (in few cases also in their pulp), as redcoloured heterosides (at low values of pH) or blue (at high values of pH) and they are represented mostly by malvidin, delphinidin, peonidin and petunidin. Anthocyanins gathering in grapes starts at the beginning of the ripening stage, but the content in anthocyanin pigments occasionally drops to the end of the ripening, especially in the areas with warm climate (Fournand et al., 2006). The regular consumption of fruits, vegetables, wine, jam, jellies with a high content of anthocyanins is associated with the decrease of risk of developing chronic diseases, such as cancer, cardiovascular diseases, Alzheimer's diseases (Kroon, 2005). Flavonoids, together with other substances assimilated from the daily diet, such as vitamin C, vitamin E and carotenoids, protect the tissues of the human body against "oxidative stresses" by their action upon free radicals often associated with cancer, cardiovascular diseases and inflammations (Manach et al., 2004, Di Lorenzo et al., Milella et al., 2013). Their beneficial effects upon health depend greatly on the administrated quantity and on their bio-disponibility. It is thereof obvious how important is studies thoroughness identification. the evaluation and on quantification of these compounds with therapeutic role on one hand and energy suppliers on the other hand, in the main varieties of table grapes grown in Romania.

The phenolic composition of grapes becomes a useful parameter for the selection of the varieties, which should take into account their natural nutraceutical qualities.. apart from the grapes appearance (size, colour, berry shape), related to marketing (sales). Generally, in the worldwide viticulture practice, to determine the ideal moment of the table grapes consumption, are used parameters as sugar concentration (g/l) and total acidity (g/l tartaric acid) as well as the implicitly gluco-acidometric index in which the evolution of the first two parameters, during grapes ripening, being in inverted correlation. Despite all that, these quality parameters seem to be insufficient, in approaching the quality term used in the case of varieties of table grapes, since their nutritional and therapeutic quality is also given by the concentration of phenolic compounds (over 500 compounds in Vitis vinifera). The phenolic content of the grape berry is distributed as follows: 1% in solid pressed pulp; 5% in juice; 50% in the skin of red grapes or 25% in the skin of white grapes; and the remaining 46 to 69% in seeds. As result, the determination of the content in phenolic compounds (polyphenols, flavonoids,

anthocyanins) gets a significant importance and it is useful in the study of table grapes varieties, offering clues about reaching full ripeness as well as about their nutritional value.

MATERIALS AND METHODS

Plant material.

In the present paper, there have been analysed 6 genetically related table grape varieties, grown in Romania. 'Bicane' variety has been used as common maternal genitor for varieties 'Xenia' and 'Tamina': in Italia. 'Muscat Hamburg' variety for the same varieties represented the paternal form; 'Muscat d'Adda' variety has been obtained by self-pollination of 'Muscat Hamburg' variety. The study has been approached starting from two reasons: for the compositional rating and evaluation of the above-mentioned compounds and for studying their conveyance to descendants. Grape vine varieties are located in the experimental field of collection the ampelographic from the University of Agronomic Sciences and Veterinary Medicine of Bucharest. They have been conducted on the semi-stalk; the type of pruning in the prior year was Guyot on semistem, with a load of 42 buds/vine. During the developing of the seasonal phenological stages, there were performed measurements and ratings to evaluate the fertility and productivity elements, and on the date of harvesting, on an average sample of 10 harvested grapes from 10 shoots. There were made physical-carpometric and chemical ratings: fertility indexes, the number of grapes per vine, the average weights of one grape, the average weight of 100 berries, production/vine, sugar (g/l), total acidity (g/l tartaric acid), gluco-acidometric index, as well as the content of total polyphenols, flavonoids, anthocyanins, present in the skin and in the seeds. The harvesting of the grapes samples were performed upon their technological maturity.

Preparation of grape skin and seed extracts. After harvest, the grapes samples were processed immediately, separating skin, pulp and seeds from 10 grapes berry / replicate and the phenolic compounds were obtained with an ethanol: water: hydrochloric acid as extractant (70: 29: 1, v/v/v), 20 ml per sample. The extracts were centrifuged for 20 min at 6000 rpm, using a centrifuge EBA 20, stored in a refrigerator (4° C) and analyzed in the short-term from the extraction.

Total phenolic assey

The determination of the total phenolic content was made using the Folin-Ciocâlteu method, (Singleton and Rossi, 1965). In brief, an aliquot (0.5 ml) of the appropriate diluted wine was added to a 10 ml volumetric flask, containing 2.5 ml of distilled water. Then, 0.5 ml of Folin-Ciocâlteu reagent was added and the contents mixed. After 3 min, was added 2 ml Na₂CO₃ solution of concentration 10 g/l and made up to a total volume of 10 ml distilled water. After keeping the samples at 50°C (water bath) for 16 min in sealed flasks and subsequent cooling, their absorbance were read at 700 nm against distilled water as the blank. A calibration curve was constructed using gallic acid standard solutions (0÷100 mg/l). The concentration of total phenolic is expressed as the gallic acid equivalent per litter of extract. All samples were prepared in triplicate.

Total flavonoids assay. Total flavonoids content was evaluated according to а colorimetric assay with aluminium chloride. For the determination of the total flavonoids, a colorimetric method using AlCl₃ was applied for the analysis of the fruit pulp and skin extracts (Zhishen et al., 1999). This method is based on the formation of stable complexes with the C-4 keto group and either the C-3 or the C-5 hydroxyl group of flavones and flavonols, which exhibit maximum absorbance at 510 nm. A 1 ml aliquot of wine (appropriately diluted) was added to a 10 ml volumetric flask containing 4 ml of distilled water, followed by the addition of 0.3 ml of solution of NaNO₂ (0.5 g/l). After 5 min, 0.3 ml of a 1 g/l solution of AlCl₃ was added and 6 min later, 2 ml of NaOH (1 mol/l) was added to the mixture. The total volume was made up to 10 ml with distilled water, the solution was mixed and the absorbance was measured at 510 nm against water blank. Catechin was used as the standard for the construction of a calibration curve and the concentrations are expressed as catechin equivalents (mg/l).

Total anthocyanins assay. The samples were diluted with a solution consisting of 70/29/1 (v/v/v) ethanol/water/HCl (concentrated) and the absorbance was measured at 540 nm. Due

to the lack of a malvidin-3-glucoside standard, the total anthocyanins contents are expressed as malvidin-3-glucoside equivalents and calculated using the following equation purposed by Di Stefano and Cravero, 1991

TA (mg/L) = A540 nm x 16.7 x d

Where A540 nm is the absorbance at 540 nm and d is the dilution.

Total acidity and *sugar* content were made according to the official methods of O.I.V. (1990).

RESULTS AND DISCUSSIONS

In Tables 1, 2 and 3 are presented obtained results with the most representative Romanian table grape varieties in the agro-climatic conditions of the 2013 year. Assigning the same load of buds per grapevine to all six varieties taken to study shows a differential behaviour of these, with regard to yield quality and quantity.

To facilitate the evaluation of the achieved results, the data interpretation was made taking as point of reference the 'Muscat Hamburg' variety (Fig. 1). From the presented data (Table 1), there can be noticed that the control variety 'Muscat Hamburg' achieved, for a load of 42 buds/vine, the highest values of the fertility indexes, this having as direct result the obtaining of a greater number of grapes per vine (32 bunch) as compared to the other varieties. In opposition, somewhat expected, carpometric parameters have had the lowest values (Table 2).



Figure 1. 'Muscat Hamburg' variety

Thus, the reference variety recorded more reduced sizes of berries and bunch as compared to the other varieties (2.75g - average weight of a berry, 220g - average weight of a bunch as compared to the 'Tamina' variety (Fig. 3). which recorded 6.2g - average weight of a

berry and respectively, 460g – average weights of a bunch). These values are reflected in the vield per hectare, relatively low production for a table grape variety. At maturity, the reference variety 'Muscat Hamburg' gathers an impressive amount of sugar, together with an acidity which confers to it a balanced glucoacidometric index, content which surpasses by far the normal limits of the variety group which it forms part of (Table 3). With regard to the analysed parameters, the other varieties behave differently, recording fertility indexes with low values.

A low number of grapes per grapevine, but an average weight of 100 berries and of a cluster obviously higher as compared to those recorded at the reference variety so that, these varieties have entailed better productions, even providing a surplus of 4 kg/grapevine (for instance, 'Tamina' variety).

With regard to the amount of gathered sugar, it was noticed that only the varieties 'Muscat d'Adda' with 214 g/kg (Fig. 2) and 'Xenia' (Fig. 5), 188 g/kg of grapes are closer to the performance reached by the control variety – 'Muscat Hamburg' (228g /kg of grapes).

The table grape varieties can be harvested before the full maturity, practically at the consumption maturity, based on the glucoacidometric index. Usually, this index has values between 2.5÷4.5. For the tested varieties, it was between 2.88 and 6.88, the highest values being recorded for the varieties 'Muscat Hamburg' and 'Muscat d'Adda' (6.83; 6.88 respectively). Although the varieties 'Italia', 'Bicane', 'Xenia', 'Tamina' usually reach their full ripeness later than the reference variety, since they belong to the Vth-VIth maturity ages, in the conditions of the 2013 year they have reached the optimum level of harvesting simultaneously with the variety 'Muscat Hamburg' (September 12th, IVth age of maturity).

The above-analysed qualitative parameters seem to be insufficient in approaching the quality term used in the case of table grape varieties, since their nutraceutical feature is due to the phenolic potential gathered both in the skin and in the grape berries. The level of gathering of phenols is different depending upon the genotypes, culture range, (soil, climatology), fertilisation, eye load left on the grapevine and, in general, upon the applied technology.



Figure 2. 'Muscat d'Adda' variety

The analysis of the recorded data (Table 3) has indicated a higher phenolic potential of the varieties with red grapes. 'Muscat Hamburg' (intense red to black), 'Muscat d'Adda' (very dark red) and 'Tamina' (red-violet) - as compared to the varieties of which berries are coloured in green-yellow ('Xenia'), golden yellow ('Bicane') and green yellow grapes – 'Italia', (Figure 4, 6).



Figure 3. 'Tamina' variety

Thus, the content of total polyphenols in the skin was framed between 2.074 g of gallic acid/kg of fresh weight-fw (the variety 'Muscat d'Adda') and 0.283 g of gallic acid/kg of fw, ('Bicane' variety).

It was noticed the fact that 'Xenia', a white grapes variety, gathered in the skin a considerable amount of polyphenols, higher to that recorded for the maternal variety, 'Bicane', and also, as compared to the reference variety, 'Muscat Hamburg' (paternal variety). The total content of flavonoid compounds in the skin recorded values between 0.698 g catechin/kg of fw ('Tamina') and 1.27 g catechin/kg of fw ('Muscat d'Adda') - varieties with the skin coloured in different intensities of red - and between 0.166 g catechin/kg of fw ('Xenia') and 0.585 g catechin/kg of fw ('Italia') varieties with yellow grapes.

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% Dif. compared	to the control		2.39	-0.29	1.17	4.00	1	1.99
Production	(kg /vine)		9,43	6,75	8,21	11,04	7,04	9,03
% Dif.	compared	to the control	190	50	45	240	-	80
Average	weight	of a grape (g)	410	270	265	460	220	300
% Dif.	compared to the control		6-	-5		8-	ı	-1
No. of	grapes/	vine	23	27	31	24	32	31
% Dif.	compared to the control		-0.19	-0.2	0.12	-0.02	-	-0.14
Relative	fertility	coefficient	1,09	1,08	1,4	1,26	1,28	1,14
% Dif.	compared to the control		-0.18	-0.29	60.0	-0.06	-	- 0.1
Absolute	fertility	coefficient	0,92	0,81	1,19	1,04	1,10	1,00
Experimental variants	and specification		Italia	Bicane	Xenia	Tamina	Muscat Hamburg (control)	Muscat d'Adda

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Table 2. Carpometric indexes of the studied table grapevine varieties

% Dif. compared to the control	0.485	0.719	0.328	0.622		0.204
Weight of seeds (g)	1,038	1,272	0,881	1,075	0,553	0,757
% Dif. compared to the control	-1	4	<i>L</i> -	-6	-	-4
No. seeds in to 10 berries	22	19	16	17	23	19
% Dif. compared to the control	3.5	4.86	0.55	1.71	-	-0.8
Weight of 10 skins (g)	9,91	11,27	6,96	8,12	6,41	5,61
% Dif. compared to the control	7	18		19		-1
Weight of 10 berries (g)	50	61	42	62	43	42
% Dif. compared to the control	175	245	145	345		70
Weight of 100 berries (g)	450	520	420	620	275	345
Experimental variants and specification	Italia	Bicane	Xenia	Tamina	Muscat Hamburg (control)	Muscat d'Adda

% Dif.	compared	to the	control			-5.07	-5.67	-5.4	-4.25	I	2.75
Total	flavonoids	seeds	50	catechin/	kg f.w. (seeds)	2,57	1,97	2,24	3,39	7,64	10,39
% Dif.	compared	to the	control			-2.79	-5.31	-4.93	-2.79	I.	3.9
Total	polyphenols	g ac. gallic/	kg f.w.	(seeds)		2,98	3,46	3,84	5,98	8,77	12,67
% Dif.	compared	to the	control			-281.0	-281.1	-280	-168.8		482.4
Total	anthocyanins	mg	malvidin-3-	O-glu/	kg f.w. (skins)	6,4	6,3	1,4	112,6	281,4	763,8
% Dif.	compared	to the	control			-0.403	-0.789	-0.822	-0.290	I	0.282
Total	Flavonoids	g catechin/	kg f.w.	(skins)		0,585	0,199	0,166	0,698	0,988	1,270
% Dif.	compared	to the	control			-1.007	-1.444	0.113	869.0-	-	0.347
Total	polyphenols	g gallic	acid/	kg f.w.	(skins)	0,720	0,283	1,864	1,029	1,727	2,074
% Dif.	compared	to the	control	COLLEGE		-3.95	-3.46	-2.04	-3.27	1	0.05
Gluco-	acidometric	index				2.88	3.37	4.79	3.56	6.83	6.88
% Dif.	compared	to the	ontrol	COLLEG		2.48	1.5	0.59	1.37	·	-0.22
Total	acidity		tortorio	ual tat IC	aciu/L	5,81	4,83	3,92	4,70	3,33	3,11
% Dif.	compar	ed to	the	om o	control	-60.1	-64.9	-39.6	-60.1		-13.6
Sugar		(g/kg)))			167,6	162,8	188,1	167,6	227,7	214,2
Experimental	variants and	specification	1			Italia	Bicane	Xenia	Tamina	Muscat Hamburg (control)	Muscat d'Adda

Table 3. Physical and chemical characteristics of the grapes belonging to the studied varieties



Figure 4. 'Italia' variety

Anthocyanins in the skin have a similar evolution to that of flavonoids, namely, the red varieties (especially, the variety 'Muscat d'Adda' with 764 mg malvidin-3-O glucosid/kg of fw) distinguished themselves by a high anthocyanin potential, as compared to the three varieties with green vellow grapes to which the gathering was low. Regardind to the content of the seeds of total polyphenols, it is mentioned that the highest concentration in these compounds was recorded for the 'Muscat d'Adda' variety (12.67g gallic acid/kg of fw and the lowest for the 'Bicane' and 'Xenia' varieties (3.46g gallic acid/kg of fw respectively, 3.84g gallic acid/kg of fw). 'Tamina' and 'Italia' varieties recorded almost 6 g gallic acid/kg of fw while the reference variety contented 8.77 gallic acid/kg of fw. The analytical tests revealed a high phenolic potential for the 'Muscat d'Adda' variety, both in skins and in seeds, the values of the polyphenols, flavonoids and anthocyanins surpassing those recorded for the reference variety, 'Muscat Hamburg'.

It could be noticed an exception in the group of studied varieties with green-yellow grapes and namely, 'Xenia' variety gathered an amount of total polyphenols significantly higher than the paternal variety 'Muscat Hamburg', surpassing it by a value of 9.93%.

In addition, the 'Muscat d'Adda' variety surpassed the reference variety by 20.09%, being followed by 'Xenia' variety. By analysing the flavonoids content in the berry skin, it could be noticed a significant difference of the 'Muscat d'Adda' variety which surpasses the reference variety by 28,54%.



Figure 5. 'Xenia' variety

The varieties 'Italia' and 'Tamina' reach a level a little bit beyond the half of the value of 'Muscat Hamburg' variety, (0.585 mg catechin/kg fw and respectively 0.698 mg catechin/kg fw).



Figure 6. 'Bicane' variety

From this point of view, 'Bicane' and 'Italia' varieties recorded values under the limit of the control variety. From the correlation of the recorded data, it could be noticed that on the consumption maturity, which coincided with the full maturity, in the agro-climatic conditions of the 2012-2013 vegetative year, the studied varieties also reached their phenolic maturity, conferring to them special nutritional value.

CONCLUSIONS

Climatic particularities of the vegetative year 2012-2013 caused for the varieties such as

'Italia', 'Bicane', 'Xenia' and 'Tamina' to reach the optimum level of harvesting simultaneously with the reference variety, although usually these ripen later, belonging to the ages of maturity Vth-VIth.

The varieties 'Muscat d'Adda', 'Xenia' and 'Tamina' distinguished them self by a high phenolic content in the skin recording 2.074 g gallic acid/kg of fw, 1.864 g gallic acid/kg of fw and respectively 1.029 g gallic acid/kg of fw, as compared to the reference variety 'Muscat Hamburg', 1.727 g gallic acid/kg of fw. It can be appreciate that the rating of the phenolic compounds (polyphenols, flavonoids, anthocyanins) is also useful in the study of the table grapes varieties, not only of those for wine, the achieved data providing clues about the nutritional and therapeutic value of these varieties.

It is recommend the extension of culture of new varieties, 'Xenia' and 'Tamina', for their organoleptic and nutraceutical qualities which equal those of the genitor varieties, 'Bicane' and 'Muscat Hamburg'.

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