Scientific Papers. Series B, Horticulture. Vol. LIX, 2015
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE INFLUENCE OF THE VINE CULTIVATION TECHNOLOGY ON THE PHENOLIC COMPOSITION OF RED GRAPES

Victoria ARTEM[1,2], Arina Oana ANTOCE[1], Ioan NAMOLOSANU[1], Aurora RANCA[2], Anamaria PETRESCU[1,2]

[1]University of Agronomical Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, Department of Bioengineering of Horti-Viticultural Systems, 59, Mărăşti Ave., Sector 1, 011464 Bucharest, Romania.
[2]Research Station for Viticulture and Oenology Murfatlar, Calea Bucuresti str., no. 2, 905100, Murfatlar, Constanta

Corresponding author email: arina.antoce@horticultura-bucuresti.ro

Abstract

The biosynthesis of phenolic compounds and their accumulation during the grape berry ripening is influenced by various factors such as the genotype, climatic conditions and agricultural practices. The phenolic maturity desired at harvest time refers not only to the total phenolic compound concentration, but also to their quality, which is related to their structure and extractability during the winemaking process. The goal of this work was to improve the total concentration of phenolic compounds of the black cultivars Feteasca neagra and Cabernet Sauvignon by applying various viticultural practices such as organic and conventional growing, no cluster thinning and 30% cluster thinning during summer, before veraison. The work was performed during the period of 2013-2014 and the phenolic compounds were evaluated by the standard Glories method, which provided results for total anthocyanins (ApH 1), extractable anthocyanins (ApH 3,2), the percentage of the extractable anthocyanins (%AE), maturity of the seeds (MS) and total phenols (PT). The statistical analysis of the results showed that the main factor that influences the phenolic composition of the grapes and their extractability is the grape variety, but for the same cultivar the cluster thinning and the agrotechnical practices also induced certain significant differences.

Key words: *phenolic composition, organic, cluster thinning.*

INTRODUCTION

Phenolic compounds are the most important group of chemical substances in grapes, after sugars and acids. They have an important contribution in defining visual, olfactory and gustative sensory characteristics of grapes and wines (Antoce, 2007; Gil-Munoz *et al*., 2009).
Phenolic compounds (anthocyanins, tannins, etc.) accumulates in the solid parts of the grape skin, seeds, rachis; their content is variable, depending on the variety, the maturation degree of grapes, the climatic conditions of the area, farming practices and last but not least the applied winemaking technology (Jackson *et al*., 1993; Downey *et al.,* 2006). Agricultural practices that influence the expression of phenolic composition during ripening process of grapes, reported in various journals are: the system of trunk formation (Zoeckelein *et al.,* 2008), row spacing, pruning, thinning grapes, removing buds and leaves, irrigation management and fertilization (Gonzales-Neves *et al*., 2002; Delgado *et al*., 2004; Poni *et al*., 2007; Antoce, 2007) and so on.
Phenolic compounds in grapes are secondary metabolites, which constitute a category of natural bioactive compounds which show remarkable health benefits for the body (Del Rio *et al*., 2013), due to their antioxidant or rather due to redox signalling actions.
Of these the most important are anthocyanins, the colored pigments of grapes, and tannins from skin and seeds, that are responsible for the red wine astringency and bitterness (Cheynier *et al.,* 2006). The assessment of the

phenolic maturity of the grapes may be useful for their classification at the time of winemaking in accordance with the quality level, even for choosing optimal method based on the extractive phenolic compounds (Zamora, 2002).

The (poly)phenolic compounds of grapes have a variable "extractibility", depending on the conditions during ripening and biological potential of varieties. The extractability of anthocyanins depends also on the state of maturity that controls the decomposition of the grape skins. Although high concentrations of anthocyanins in the skin is necessary to obtain an intensely colored wine, this is not sufficient for the color extraction and color stability. In order to make these molecules easily to extract, cell walls must be decomposed mildly but sufficiently, by non-aggressive technological methods. Upon reaching phenolic maturity, grapes have both an increased pigmentation potential and the ability to release these substances in wine. The grape skin and seeds are considered key factors in the process of red wines vinification because they represent the main source of phenolic compounds, extracted during the process of fermentation and maceration. Anthocyanins, the molecules responsible for the color of wine, gradually accumulate in grapes during grape maturation (Canals *et al.,* 2008). The anthocyanins are not always easy to extract from the skin grapes, the low level of extraction causing light colored wines, even if the concentration of anthocyanins in grapes is sufficient. The anthocyanin extractability is one of the main factors affecting their concentration of the final product (Glories *et al*., 1993). In recent years there have been proposed and developed several methods for measuring the phenolic maturity (Segade *et al.,* 2008), direct measurement of the color absorption of extracts from the grape skins (Celotti *et al.,* 2007) and even multispectral high resolution image analysis (Lamb *et al.,* 2004). These methods, however, remain experimental and are not routinely used in winemaking. The methods usually applied are based on obtaining grape extracts by maceration in different solvents (Iland *et al.,* 2004). Among them, Glories method (Glories, 1984) and ITV method (*http://www.vignevin-sudouest.com*) are the most commonly used. Although is more laborious, Glories method used in this paper provides comprehensive information not only about the content of anthocyanins and polyphenols in grape, but also about the anthocyanins extractability and the maturity of seeds.

MATERIALS AND METHODS

The aim of this paper consisted of improving the polyphenolic potential of Feteasca Neagra and Cabernet Sauvignon grapes varieties, applying different vineyard practices such as organic and conventional culture system with and without additional works to reduce grapes on the vine by 30%, in the climatic conditions of the 2013 and 2014 years. The study was conducted in the viticultural center Murfatlar at the Research Center for Viticulture and Enology Murfatlar and the experimental design regarding the studied plots and variants are presented in Table 1.

Table 1. The characteristics of the studied plots

The system of culture	Variety	Variant	Year of planting	Planting distance	Density vine/ha	Exposi-tion	Slope
Organic	Feteasca neagra	Mt (control)	2007	2.2×1.1	4132	N-S	3-5%
		R30% (30% cluster thinning)					
	Cabernet Sauvignon	Mt (control)	2009	2.2×1.1	4132	N-S	3-5%
		R30% (30% cluster thinning)					
Conventional	Feteasca neagra	Mt (control)	2001	2.4×1.2	3472	E-V	1-2%
		R30% (30% cluster thinning)					
	Cabernet Sauvignon	Mt (control)	2001	2.4×1.2	3472	E-V	1-2%
		R30% (30% cluster thinning)					

For each of the eight variants about 200 berries/ three repetitions were collected, then packed and labeled in plastic bags and carefully transported to the laboratory to avoid crushing. Analyses were performed on the same day of sampling.

The polyphenolic potential of grapes has been evaluated by the Glories method, which consists in extracting anthocyanins from the grape skin, first under mild conditions and then under severe conditions necessary for facilitating the diffusion conditions. Acid medium, used as a facilitator factor of extraction, ruptures the proteo-phospholipid membrane, destroys the protein binding and releases the content of vacuoles. Two different aqueous solutions are used: a solution of pH 1 (0.1 n HCl solution prepared with 8.33 ml of 37% HCl in 1000 ml distilled water) and a solution of pH 3.2 (prepared by dissolving 5 g of tartaric acid in 800 ml of distilled water, adding 22.2 ml NaOH 1n and water up to 1000 ml in a volumetric flask). The method involves extraction of 50 g mashed grape skin with 50 ml of pH 1, while another 50 g of the mashed grape skin is subjected to extraction with pH3.2 solution. Both samples were manually stirred and allowed to rest for 4 hours at the room temperature. Samples are then filtered, resulting 2 solutions identified as "pH 1" and "pH3.2". Analysis of anthocyanins is performed for both solutions, using the differential pH method (Ribereau-Gayon, 1976), the determinations being performed in 1 cm optical path length glass cuvettes, by using a Helios Alpha UV-VIS spectro-photometer, produced by ThermoScientific, USA. The total content of phenols is determined only for the second solution, the pH3.2 at 280 nm, the determinations being spectrophotometrically performed by using 1 cm path length quartz cuvettes. These results allow for simple analysis of the potential total anthocyanins (A_{pH1}) potential extractable anthocyanins ($A_{pH3.2}$), the extractability index of anthocyanins expressed by the equation: $E(\%) = [(A_{pH1}-A_{pH3.2})/A_{pH1}] \times 100$; seed maturity index: $MP (\%) = [(280\text{-dpell})/280] \times 100$, skin grape tannin content, $dpell = (A_{pH3,2}x40)/ 1000$, the tannin content of seeds dTpep = A280-dpell and the content of total polyphenols PT (A280). Statistical calculation was performed using SPSS Statistics 17.0, by applying Duncan test for SD 5%.

RESULTS AND DISCUSSIONS

The synthesis of phenolic compounds in grapes was influenced by the different climate conditions of the two years studied; both years are considered as favorable, with for proper maturation conditions in July, August and September, although the average temperature of the air was with 2,7-4,7 ^{0}C higher than the average calculated over the last 20 years, and the precipitation were quantitatively significantly above the multi-annual average for Murfatlar vineyard ecosystem (Fig 1).

It is found that the significant amount of precipitations and the increase of the average air temperature have contributed to the inhibition of phenolic compound synthesis in 2014, while higher anthocyanin values were recorded in 2013, this phenomenon being consistant with the findings of other authors (Mori *et al.,* 2007; Cohen *et al.,* 2008).

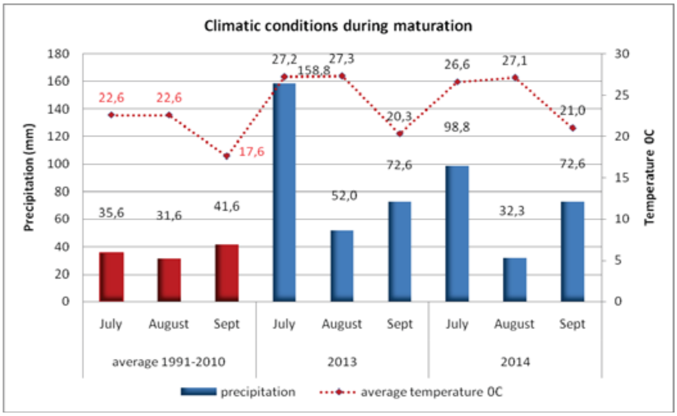

Fig. 1. The precipitation and the average temperatures during grape maturation in 2013 and 2014 years as compared to the averages of 1990-2010

The potential total anthocyanins (ApH1), and the potential extractable anthocyanins (ApH3,2) presented in Fig 2 (a and b) were influenced both by climatic conditions that led to a decrease in 2014 as compared to 2013, but also by the viticultural practices applied (organic and conventional culture system and special pruning techniques by suppression of the clusters) that led to a significant increase in anthocyanins. In the case of Feteasca Neagra variety the accumulation of total anthocyanins decreased in 2014 as compared to 2013, the recorded values for the ecological and conventional system being 27.4% and 11.2%, respectively. For Cabernet Sauvignon the decreasing trend is maintained for the 2014, with reduction of 26.4% for ecological and 31.1% for the conventional system. In case of the grapes thinning, this green harvest operation leads to an increase in the total anthocyanin content in the case of ecological system as compared to conventional one, regardless the year of harvest. The same trend is kept for extractable anthocyanins, the differences being significant between years and the applied viticultural practices.

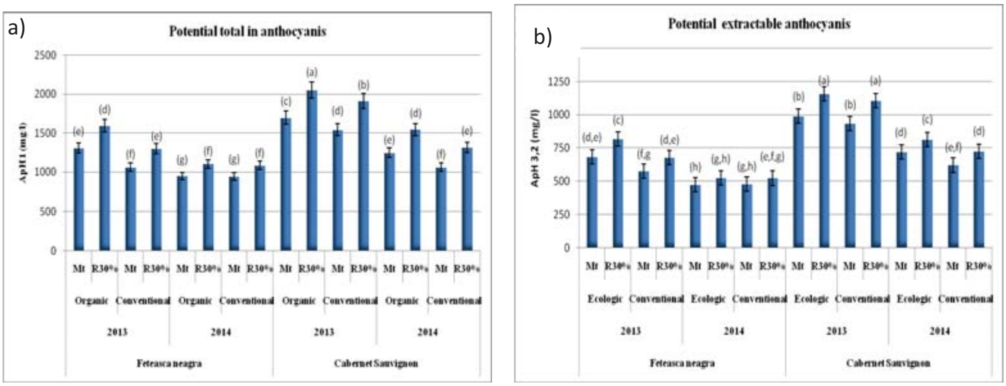

Fig. 2. The influence of climate and viticultural practices on potential total anthocyanins (a) and potential extractable anthocyanins (b). Mean values ± standard deviation (n = 3), letters represent difference of significance at $p < 0.05$ between variants.

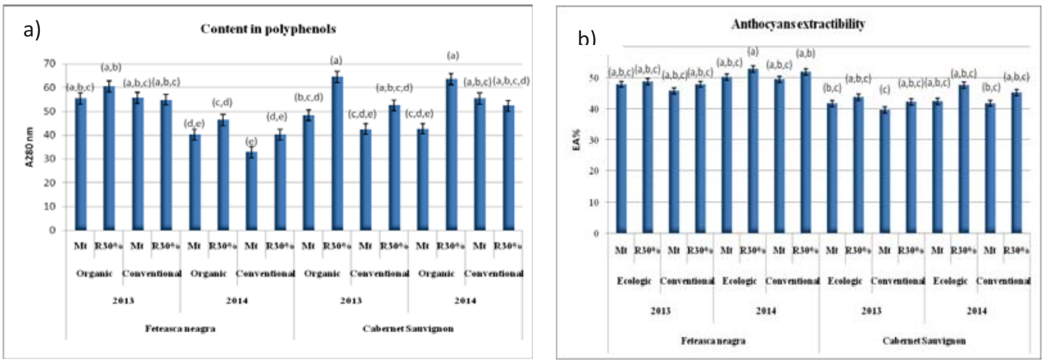

Fig. 3. The influence of climate and viticultural practices on the content of polyphenols (a) and anthocyanins extractability (b). Mean values ± standard deviation (n = 3), letters represent difference of significance at $p < 0.05$ between variants.

In the case of Feteasca Neagra the content in polyphenols (Fig. 3a) was significantly influenced by climatic conditions only, while the influence of thinning of the clusters and of the type of culture system was insignificant. For Cabernet Sauvignon, polyphenol content (Fig. 3a) was significantly influenced by the thinning of the clusters only in the case of ecological system, for both studied years. The percentage of extractability of anthocyanins (% EA) was not statistically influenced by any of the factors presented in this paper, higher values of this index being found for Cabernet Sauvignon variety, showing that extraction is more difficult in this case as compared to Feteasca Neagra. Skin grapes tannin content (dpell), shown in Table 2 was significantly influenced in the case of Feteasca Neagra variety only by the climatic conditions, but not by the viticultural practices applied. For Cabernet Sauvignon, skin grape tannin content showed higher values than Feteasca Neagra, being influenced by the applied viticulture practices (ecological and conventional and thinning of the clusters). Tannin content of seeds (dTpep) values were lower in 2014 as compared to 2013 for both varieties studied. The operation of clusters thinning and the organic and conventional culture system showed significant influences in this index. Maturity seeds showed significant differences in accordance to the variety and insignificant differences due to the climatic conditions and applied viti-cultural practices.

Table 2. Tannin content in the skin and seeds of grapes

Variety	Culture system and year	Variant	Skin tannin content (dpell)	Tannin content of seeds (dTpep)	Maturity seeds (Mp %)
FETEASCA NEAGRA	Organic 2013	Mt	28.9±3.4 (cde)	26.5±3.4 (ab)	47.8±4.5 (abc)
		R30%	34.3±4.8 (bcd)	26.1±3.9 (ab)	43.2±5.8 (bcde)
	Conventional 2013	Mt	26.1±5.1 (def)	27.5±2.6 (a)	49.5±4.3 (ab)
		R30%	30.6±3.1 (cde)	24.2±1.8 (abc)	44.2±3.6 (bcd)
	Organic 2014	Mt	20.3±4.7 (fg)	19.9±2.2 (bcd)	49.5±4.7 (ab)
		R30%	25.9±3.5 (ef)	20.4±2.6 (bcde)	44.1±3.8 (bcd)
	Conventional 2014	Mt	14.7±2.9 (g)	18.1±3.0 (cde)	55.2±4.2 (a)
		R30%	20.2±3.3 (fg)	20.0±2.6 (bcde)	49.8±5.1 (ab)
CABERNET SAUVIGNON	Organic 2013	Mt	31.8±4.3 (cde)	16.6±3.5 (de)	34.3±6.2 (efg)
		R30%	43.6±5.1 (a)	20.8±4.8 (bcde)	32.3±5.8 (fg)
	Conventional 2013	Mt	26.9±4.5 (def)	15.6±5.1 (e)	36.7±4.6 (defg)
		R30%	37.1±5.6 (abc)	15.4±3.6 (e)	29.3±5.2 (g)
	Organic 2014	Mt	24.6±4.4 (ef)	18.1±3.2 (cde)	42.4±4.6 (bcde)
		R30%	40.3±6.2 (ab)	23.1±4.0 (abcd)	36.5±4.8 (defg)
	Conventional 2014	Mt	29.2±3.1 (cde)	26.2±3.9 (ab)	47.3±5.4 (abc)
		R30%	32.1±3.8 (cde)	20.2±4.3 (bcde)	38.6±5.8 (cdef)
	SD%		0.052-0.145	0.053-1.000	0.055-0.174

Mean values ± standard deviation (n = 3), letters represent difference of significance at p <0.05 between variants. Different letters indicate the existence of statistically significant differences.

Tannin content in the skin and seeds with ApH1, ApH3,2,% EA, come to complete the information that technologist needs to know in order to adapt extraction technique for a sepcific vintage depending on the type of wine that wants to produce and the organo-leptic characteristics that wants to express in the wine. Based on the information obtained from the calculated indices regarding the po-lyphenolic potential the enologist can adjust the maceration-fermentation time adjustment, oxygenation, treatment with enzyme extrac-tion, temperature control, apply special me-thods of crushing and technological opera-tions (pumping, agitation etc.). In this way, the technologist is better equiped to make decisions for the production of high quality wines able to satisfy consumer preferences and needs of all of red wines lovers.

CONCLUSIONS

Based on the statistical analysis of the results it was concluded that the polyphenolic potential of the grapes is significantly influenced primarily by variety, followed by the climatic conditions of the two years studied, by the thinning cluster technique and last, but not least, by the culture system applied (organic and conventional).

All of the parameters obtained by the method Glories (total anthocyanins potential, potential extractable anthocyanins, the anthocyanin extractability index, index of maturity of seeds, skin tannin content, tannin content and seed polyphenol content) provide useful information necessary for the technologist to adapt winemaking techniques to improve the extraction of anthocyanins and tannins, in order to obtain quality wines, with intense color, but also balanced in taste and with distinctive typical varietal aroma, not covered by the agresivity of tannins. Adopting new vineyard practices such as cluster thinning for the ecological cultivation system may lead to an increase of the quality of grapes, especially as regards the polyphenolic compounds attracting in this way more consumers to the ecologically cultivated grapes and ecological wines.

REFERENCES

Antoce Oana-Arina, 2007. Oenologie; Chimie şi analiză senzorială, Editura Universitaria Craiova, ISBN 978-973-742-879-0.

Canals R., Llaudy M.C., Canals J.M., Zamora F., 2008. Influence of elimination and addition of seeds on the color, phenolic composition and astringency of wine. *Eur. Food Res. Technol*. 226 (5).

Celotti E., Della Vediva T., Ferrarini R. and Martinand S., 2007. The use of reflectance for monitoring phenolic maturity curves in red grapes. *Ital. J.Food Sci*., 19, 91-100.

Cheynier, V., Dueñas-Paton, M., Salas, E., Maury, C., Souquet, J. M., Sarni-Manchado, P., Fulcrand, H. 2006. Structure and Properties of Wine Pigments and Tannins. *American Journal of Enology and Viticulture* 57 (2): 298-305.

Cohen, S., Tarara, J., Kennedy, J. 2008. Assessing the impact of temperature on grape phenolic metabolism. *Analytica Chimica Acta* 621: 57-67.

Del Rio D., Rodriguez-Mateos A., Spencer J.P.E, Tognolini M, Borges G. and Crozier A. 2013. Dietary (Poly)phenolics in Human Health: Structures, Bioavailability, and Evidence of Protective Effects Against Chronic Diseases. *Antioxid Redox Signal*. 18(14): 1818–1892.

Delgado, R., Martín, P., Del Álamo, M. and González, M.-R. (2004) Changes in the phenolic composition of grape berries during ripening in relation to vineyard nitrogen and potassium fertilisation rates . *Journal of the Science of Food and Agriculture* 84 (7).

Downey, M., Dokoozlian, N., Krstic, M. 2006. Cultural Practice and Environmental Impacts on the Flavonoid Composition of Grapes and Wine : A Review of Recent Research. *American Journal of Enology and Viticulture* 57 (3): 257-268.

Gil-Muñoz, R., Vila-López, R., Fernández- Fernández, J., Martínez-Cutillas, A. 2009. Effects of cluster thinning on anthocyanin extractability and chromatic parameters of Syrah and Tempranillo grapes and wines. *Journal International des Sciences de la Vigne et du Vin* 43 (1) : 45-53.

Glories, Y. (1984). *La couler des vins rouges. Connaisance Vigne Vin, 18(4), 253-271.*

Glories Y. and Augustin M., 1993. Maturité phénolique du raisin, consÉquences technologiques: applications aux millésimes 1991 et 1992. In : *Actes du Colloque Journée Technique du CIVB, CIVB, Bordeaux*.

González-Neves, G., Gil1, G. and Ferrer, M. (2002) Effect of Different Vineyard Treatments on the Phenolic Contents in Tannat (*Vitis vinifera* L.) Grapes and their Respective Wines. *Food Science and Technology International* 8, 315-321.

Iland P, Bruer N., Edwards G., Weeks S., and Wilkes. E. 2004. *Chemical Analysis of Grapes and Wine: Techniques and Concepts.*

Jackson, D., Lombard, P. 1993. Environmental and management practices affecting grape composition and wine quality. A review. *American Journal of Enology and Viticulture* 44: 409-430

Lamb D.W., Weedon M.M, and Bramley R.G.V., 2004 - Using remote sensing to map grape phenolics and coulour in Cabernet Sauvignon vineyard. The impact of image resolution and vine phenology. *Australian J. Grape WineRes.*,10(1).

Mori, K., Goto-Yamamoto, N., Kitayama, M., Hashizume, K. 2007. Loss of anthocyanins in red-wine grape under high temperature. *Journal of Experimental Botany* 58 (8).

Poni, S., Bernizzoni, F., Civardi, S. and Libelli, N. (2009) Effects of pre-bloom leaf removal on growth of berry tissues and must composition in two red *vitis vinifera* L. cultivars. *Australian Journal of Grape and Wine Research* 15 (2), 185-193.

Ribereau – Gayon et al, 1976 – Traite d'oenologie – Sciences et techniques du vin, vol 1 - Dosage des anthocyanes dans le vins rouge, Paris, 494-499.

Segade, S.R., Rolle, L., Gerbi, V. & Orriols, I., 2008. Phenolic ripeness assessment of grape skin by texture analysis. *J. Food Comp. Anal., 21*: 644-649.

Zamora F., 2002. Enologos.

http://www.vignevin-sudouest.com/methode-analyse/Potentiel-polyphenolique-vendange.