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Evolution of polyphenolic compounds during maturation of Cabernet Sauvignon grapes from Dealu Mare vineyard

Liliana DRĂGHICI[a*], Gabriela RÂPEANU[b] and Traian HOPULELE[b]

[a]*Colegiul Tehnic de Industrie Alimentara "Dumitru Moţoc", 16 Spătaru Preda Str., Bucureşti, Romania*
[b] *Dunarea de Jos University of Galaţi, Faculty of Food Science and Engineering, 111 Domneasca Str., 800201, Galati, Romania*

Abstract. The total polyphenols and anthocyanins content of the Cabernet Sauvignon grape variety from Dealu Mare vineyard were determined in order to establish the grapes full maturity. Total phenol content was determined according to the Folin-Ciocalteu colorimetric method and anthocyanins by Poussant-Leon method with small modifications. During the grapes ripening the amount of total polyphenols increased from veraison to full maturity stage and after this a slight decrease was registered. Also, an increase in anthocyanins content of grapes after the grape veraison in years with favorable climatic conditions was noticed and this increase was extended until the grape harvest. The quantity of anthocyanins compounds enhances the possibility to obtain the very good quality red wines in the wine-making process.

Keywords: grapes, red wine, anthocyanins, polyphenols

1. Introduction

During the last years, consumers demand wines with deep red color, full body, soft tannins and fruit scents. Those characteristics can be only achieved with grapes that have reached a high phenolic maturity. Very well-ripe grapes have a high concentration of anthocyanins that proportionate wines with deep red color [1]. The full ripen grapes present low astringency and bitterness probably due to the diminution of the proportion of seed proanthocyanidins which are very galloylated. On the contrary, unripe grapes can lead to wines with poor color as also high levels of bitterness and astringency [2].

The phenolic composition of red wine is important for several organoleptic properties of the wine, including wine color, mouthfeel properties, bitterness, and flavor [3, 4]. The color of red wine is an important quality parameter of the wine, and the color intensity of red wine has been reported to correlate with wine quality grading. It is the phenolic compounds in red wine that are responsible for the wine's color properties, and obviously these phenolics to a large extent originate from the grapes.

However, the relation between the phenolic composition of grapes and the phenolic composition and color attributes of red wine is complex.

The extraction of grape skin phenolic compounds and their transfer into must is an essential part of red wine processing. The evolution of catechins and proanthocyanidins in bunch stems during berry development has been the object of many studies. Monomeric and polymeric phenolic compounds have been found to decrease during grape maturation both in the skins and in the seeds [2, 5, 6].

The maturity of skins and seeds is considered as a key factor for red winemaking because they are the major source of phenolic compounds which are dissolved into the wine during maceration process [7, 8]. Anthocyanins, the molecules responsible of red wine color, accumulate gradually in the skins during ripening [9-11]. However, anthocyanins are not always easily extracted from skins, and low extraction levels can lead to poorly colored wines, even though if the anthocyanins concentration in the original grapes is sufficient [12]. Therefore, the extractability of anthocyanins is also one of the main factors affecting their future concentration in wine

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[13,14]. Moreover, the extractability of anthocyanins increases throughout grape ripening.

Changes that phenolic compounds undergo during fruit ripening are essential for their extractability and transfer from the solid parts of grapes into wine. On the other hand, the chemical structure of phenolic compounds, degree of polymerization and galloylation of proantho-cyanidins influence their affinity to interact with proteins and their pigmentation and co-pigmentation properties [15].

The aim of this study was to assess the phenolic compounds extracted from grapes skins depending on different stages of grape maturation.

2. Experimental

Grape samples.

Grapes Cabernet Sauvignon variety were obtained from private vineyards in Dealu Mare region during the period 2006-2010. A quantity of 2 kg grapes has been harvested periodically, at time intervals of 5 days during the ripeness stage, full maturity and respectively, technological maturity. All grapes were manually destemed and randomly grouped and used immediately for standard and phenolic maturity measurements.

For ripening characterisation the physico-chemical analysis of main composition characteristics of grapes (sugar content, titrable acidity, the weight of 100 berries, anthocyanins content and total polyphenols content) was carried out.

One hundred grape berries were weighted and used for determining the sugar content, the titratable acidity according with the analytical methods recommended by the OIV. Sugar concentration was measured using a refractometer. The titratable acidity was measured by titrimetry using NaOH 0.1 N and Bromothymol blue as indicator.

The determination of grape anthocyanins content was done by Poussant-Leon method with small modifications [16]. Skins of 50 red berries are mixed with fine quartz sand (previously washed with 1% HCl solution) and after was transferred into Erlenmayer flask (with glass stopper preferably) by using 50 ml HCl solution 1%. Extraction is done at room temperature for 24 hours.

Extraction is repeated 2-3 times by using 1% HCl solution, separating the supernatant each time, until the grape skins acquires a pink color. The colored liquid extract is collected in a 200 mL flask with glass stopper and is used like anthocyanins extract. The absorbance was measured at 520 nm, and anthocyanins content was expressed as:

$$\text{Anthocyanins, mg/kg grapes} = \frac{OD_{520nm} \cdot 22.76 \cdot 0.4}{G} \cdot 1000$$

where:
OD_{520nm} – optical density at 520 nm;
G – the weigh of 100 berries;
22.76 and 0.4 – coeficients.

The total phenol content in grape skins samples was determined spectrophotometrically according to the Folin–Ciocalteu colorimetric method [17] using gallic acid as a standard polyphenol: 0.1 mL of grape skin extract was mixed with 7.9 mL distilled water and 0.5 mL of Folin–Ciocalteu reagent. After 1 min, 1.5 mL of 20% Na_2CO_3 was added. The absorbance was measured after 120 min at 760 nm. The concentration of the total phenolic compounds was expressed as gallic acid equivalents (g/kg). The results in every assay were obtained from three parallel determinations.

3. Results and Discussions

In the year 2006, the adverse climatic conditions have led to an advanced contamination gray mold on grape (30%). During the grapes ripening period was an excess of precipitation (224.3 mm), which caused a real grapes depreciation. The obtained must was due to gray mold contamination with a lower content of polyphenolic compounds and anthocyanins. At grapes full maturity (15.09) grapes had a sugar content of 184 g/L, a total acidity of 6.7 g/L H_2SO_4, the weight of 100 berries of 110 g, total polyphenol content was 4.3 g/kg and anthocyanin content in skins of 1210 mg/kg.

The year 2007 was not a favorable year for the polyphenolic compounds accumulation in order to obtain suitable quality red wines. Due to abundant rainfall of 450.2 mm during the growing season, the grapes have attained full maturation on 20.09. Sugar content at harvest was 201 g/L equivalent to a potential alcohol of 12% v/v, anthocyanins content of 1211 mg/kg, and total polyphenols of 3.24 g/kg.

Years 2008 and 2009 were most favorable for achieving high quality red wines. Time of grapes full maturity was reached at 15.09 (2008) and 10.09 (2009), sugar content was close for both years, 198 g/L and respectively 192 g/L.

Total polyphenols content was 4.84 g/kg (2008) and 4.90 g/kg (2009) and the anthocyanins conten was 1096 mg/kg and 996 mg/kg.

Finally, the year 2010 was very dry, during the vegetation period fell only 202.9 mm rainfall leading to poor development of the grapes.

There was a forced grapes ripening and their full maturity was reached at 25.08 with a sugar content of 176 g/L, acidity 7.1 g/L H_2SO_4, 91 g weight of 100 berries, the total polyphenol content was 4.92 g/kg and anthocyanins content was 962 mg/kg.

After the full maturity reaching, the total polyphenols content decreased slightly, while the anthocyanins content increased throughout the ripening period, except the harvest of 2006 year, when, after grape full maturity anthocyans content decreased due to gray mold contamination (the presence of oxidative enzymes).

The grapes harvesting coincided with the maturity period that has performed between 25.09 and 5.10. In **Table 1.** are presented the values of grape physico-chemical characteristics during the five years at harvest. In 2006 at harvest, the grapes had a sugar content of 224 g/L, acidity 5.1 g/L H_2SO_4, 101 g weight of 100 berries, the total polyphenols content 3.24 g/kg and anthocyanins content 1120 mg / kg (Table 1).

By analyzing the period 2006-2010 it was observed at harvest the grapes had a sugar content between 201-224 g/L, the highest value being reached in 2009 (**Fig. 1**).

The accumulation of sugar during grape maturation period was also observed by others [18, 19].

The total acidity of the grapes at harvest ranged from 5.1 to 5.5 g/L H_2SO_4, the lowest value was recorded in 2006 and the highest in 2008 (**Fig. 2**).

The grapes acidity depends on the grape variety, soil and climatic conditions and its value is correlated with the grapes ripeness [20].

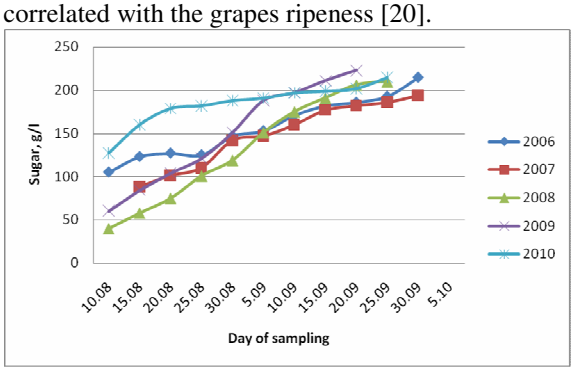

Fig. 1. Evolution of sugar content during grapes maturation (2006-2010)

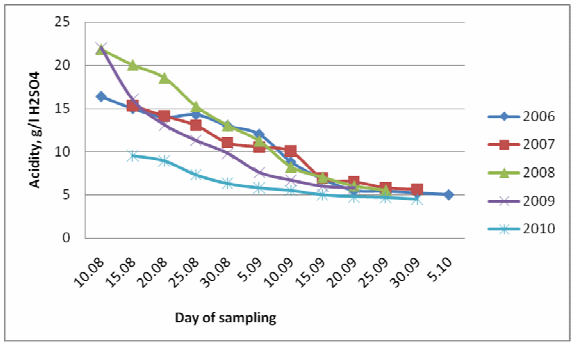

Fig. 2. Evolution of acidity during grapes maturation (2006-2010)

The decrease in acidity during ripening is due to the mobilization of bases to neutralize acids from fruit, the combustion processes and especially by the phenomenon of dilution [21].

Table 1. Physico-chemical characteristics Cabernet Sauvignon grapes at harvest (2006-2010)

Year	Harvesting day	Productivity t/ha	Physico-chemical characteristics				
			Sugar, g/l	Total acidity, g/l H_2SO_4	Weight of 100 berries, g	Anthocyanins, mg/kg	Total polyphenols, g/kg
2006	30.09	7,2	221	5,1	101	1120	3,24
2007	25.09	6,8	201	5,4	130	1211	3,41
2008	20.09	8,2	214	5,5	124	1184	4,23
2009	15.09	9,5	224	5,2	132	1241	4,53
2010	5.10	6,4	212	5,4	87	1114	3,76

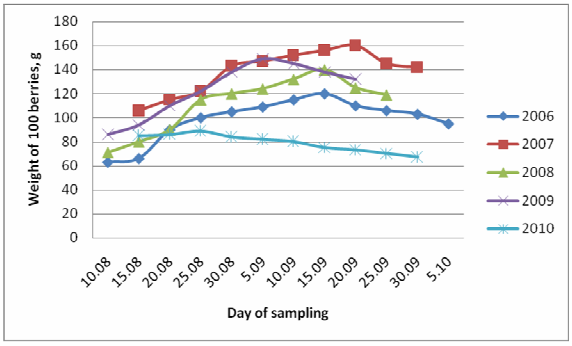

Fig. 3. The weight of 100 berries evolution during grapes maturation (2006-2010)

According to Calo et al. [22], the degradation suffered by some acid forms is produced primarily by temperature. Changes in weight of 100 berries values are shown in **figure 3.**

During the harvest period weight of 100 berries ranged from 87 to 131 g, the lowest value was obtained in 2010 and the highest values were reached in 2009.

These results are in line with Esteban et al. [23], who indicate that the largest berry size is directly related to dilution of the different compounds of grapes as a result of a test conducted with different grape varieties where they conclude that the variety Monastrell has a fast response to greater water availability.

The size will affect the extraction of anthocyanins and tannins in the wine through its impact on the proportions of skin / juice.

Anthocyanins were accumulated starting with the veraison period and anthocyanin content ranged from 1114-1241 g/kg, the highest value was recorded for the 2009 harvest (**Fig. 4**).

The behavior of the total anthocyanin content in grape samples correspond with the evolution of anthocyanins obtained by Glories [24] in the skin, according to this author, the anthocyanin content increased during ripening and after reaching its maximum value, it suffer a decline.

Fernandez-Lopez et al. [25] showed that the pigment in the Monastrell grape develops rapidly in the second week after veraison when grape color is almost dark blue, although it should be noted that the accumulation and distribution of anthocyanins in the skin can vary from year to year depending on climatic conditions [26, 27].

At veraison, the quantity of total phenolic compounds is mainly influenced by the content of seeds tannins. During the maturation period, these tannins are polymerized, while the skin is an increase in the content of anthocyanins and tannins that are less polymerized than seed [24, 28].

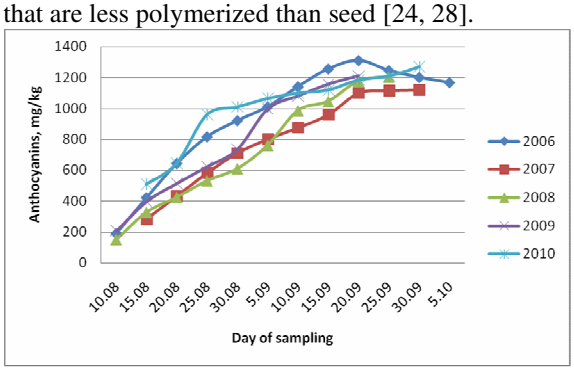

Fig. 4. The anthocyanins content evolution during grapes maturation (2006-2010)

Dynamics of total polyphenols content is shown in **Fig. 5**. The total polyphenol content ranged from 3.24 g/kg to 4.56 g/kg, the highest value being obtained for harvest in 2009, namely 4.53 g/kg gallic acid.

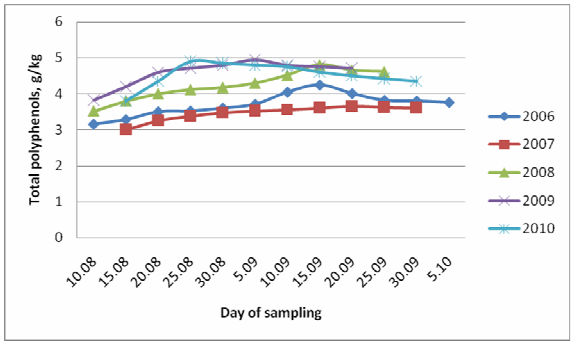

Fig. 5. The total polyphenols content evolution during grapes maturation (2006-2010)

4. Conclusions

The full maturity of the Cabernet Sauvignon grapes from Dealu Mare vineyard (2006-2010) was reached during the period 5th of September to 20 th of September, except the year 2010 when grapes full

maturity was forced (25.08) because of the dry period.

During the grapes ripening the amount of total polyphenols increased from veraison to full maturity and after a slight decrease was registered.

An increased anthocyanins content of grapes after the grape veraison in years with favorable climatic conditions was noticed, this increase is extended until the harvest.

In years where the gray mold contamination is present it was observed a decrease in anthocyanins content after reaching the full maturation of the grapes.

Climatic conditions influence the amount of polyphenols from grapes, because the rain, cold and wet weather are leading to slow accumulation of anthocyanins compounds in grape skins.

In favorable conditions the contamination with gray mould a decrease in content of phenolic compounds, especially anthocyanins occurs as a result of mold activity.

The grapevintage have to be made when the quantity of anthocyanins reached the highest values before gray mold contamination which can induce an anthocyanins degradation.

The variation in the phenolic compounds of grape skins, the rate of their decrease during ripening period, the different extractability during wine fermentation, and the wine colour characteristic have to be considered when grapes in different maturity stages are used in winemaking. The pomace contact with different duration may be used in relation to grape maturity and the desired wine character.

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6. References

* E-mail address: lilidraghici87@yahoo.com

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