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**RESEARCH REGARDING THE PHENOLIC MATURITY OF THE RED  
WINE VARIETIES IN THE DEALU BUJORULUI VINEYARD**

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The aim of this research was to determine ecoclimatic conditions and also to determine the phenolic maturity of Fetească neagră and Merlot from Dealu Bujorului vineyard in the conditions of the 2016 year of culture. Under the ecoclimatic conditions of 2016, the grapes entered in the ripening process prematurely, and full maturity was achieved very early. The results showed the suitability of ecoclimatic conditions and the proper growth and development of the tested varieties for obtaining wines with superior's quality. The variation of the phenolic characteristic represents a strong marker for wines geographical traceability.

**Keywords:** glories, grapes, phenolic maturity, technological maturity

### **Introduction**

Phenolic compounds, extractable from grape skins and seeds, have a notable influence on the sensorial properties of red wines, especially their chromatic characteristics, bitterness and astringency (Arnold *et al.*, 1980; Robichaud *et al.*, 1990). The phenolic compounds, together with the aroma precursors are the main factors that affect wine quality. Consequently, they have been studied extensively in grapes and wine (Atasanova *et al.*, 2002).

The evaluation of the sugar content and acid profile alone do not fully express the real oenological potential of grapes. Knowing the polyphenolic characteristics of the grapes allow the maceration and winemaking process to be planned so as to allow the winemakers to fully exploit the potentiality that the grapes reach in the vineyard (Gonzales-Neves *et al.*, 2004).

Many studies have been conducted to define the best method to evaluate the polyphenolic compounds in grapes (Margheri *et al.*, 1985; Gunata *et al.*, 1987). Glories and Augustine (1993) used the term "grape phenolic maturity" to indicate the concentration of phenolic compounds in grapes, and the ease with which they are released. This definition encompasses the anthocyanins concentration in the

skin, their degree of extractability, the flavanol concentration in the seeds and skin and their degree of polymerization. The method proposed by Glories consists in extracting the phenolic compounds from the whole berries liquidized under two different concentrations, determining the concentration and subsequently comparing data.

The first stage of the procedure attempts to extract nearly all of the phenolic content using a very low pH (~1) which favours the complete degradation of the cell membrane (Glories *et al.*, 2000). The second stage repeats the extraction under normal maceration conditions using a buffer (pH 3.2) which does not cause any further degradation of the cell membrane other than normally reached during ripening. The smaller the difference in the parameters between pH 1 and pH 3.2, the greater the level of phenolic maturation.

Many compounds are involved in the evolution process of the maturation of the grape, so the definition of phenolic maturity cannot be represented by a few parameters and some confusion can arise when the data are interpreted (Venencie *et al.*, 1998).

The aim of our research was therefore to (1) establishment the ecoclimatic conditions from Dealu Bujorului vineyard 2016 year of culture, (2) determination of phenolic maturity of red wine varieties, (3) establishing some Pearson correlation coefficient between phenolic maturity and (4) determination of wine geographical traceability based on the phenolic maturity.

## Materials and methods

### *Ecoclimatic data from Dealu Bujorului vineyard*

#### *Ecoclimatic data*

The weather data used in this research was recorded at the weather forecasting center and also at the Agro Expert system of RDSVV Bujoru. Based on this data some ecoclimatic indicators for the growth and fructification of the grapevine were determined as follow: global thermal balance ( $\Sigma t^0g$ ) are the sum of all positive daily temperature from active period; active thermal balance ( $\Sigma t^0a$ ) are the sum of all daily mean temperature  $\leq 10$  °C; beneficial thermal balance ( $\Sigma t^0u$ ) are the sum of all daily mean temperature above  $\leq 10$  °C; thermal coefficient (Ct); amount of monthly and annual precipitation; amount of hours with sun ( $\Sigma ir$ ) and real insolation coefficient (Ci). Ct is given by ration of the overall balance ( $\Sigma t^0g$ ) and number of days from the active period; Ci is given by the ration between the hours with sun and the growing season days. Cp is given by the ration between the rainfall from the growing season (mm) and the number of days of the growing season (Bora *et al.*, 2016). In order to get a clearer image about how ecoclimatic factors influence the growth and fructification of grapevine, some interactions of climatic factors were calculated: the real Heliothermal index ( $HI_r$ ), the hydrothermal coefficient (CH), the bioclimatic vineyard index ( $I_{bcv}$ ), annual aridity index Martonne ( $I_{ar-DM}$ ) (Martonne 1926), the Huglin index (HI) (Huglin 1978), enoclimatic skills index ( $IAO_e$ ) and cooling night index (CI).

*Heliothermal index (HI<sub>r</sub>)*

The hydrothermic index of Branas, Bernon, and Levadoux (BBLI) (Branas *et al.*, 1946) takes into 155 accounts the influence of both temperature and precipitation on grape yield and wine quality. This 156 index is the sum of the products of monthly mean temperature (T<sub>mean</sub>, in °C) and monthly 157 accumulated precipitation amount (P<sub>amount</sub>, in mm) during the April-to-August season.

$$\text{BBLI (HI}_r) = \sum_{1 \text{ April}}^{31 \text{ August}} T_{\text{mean}} P_{\text{amount}}$$

*Hydrothermal coefficient (CH)*

Hydrothermal coefficient (CH) expressing binary interaction of temperature and humidity as the ration of the amount of precipitation ( $\sum pp$  mm) and active temperature ( $\sum t^a$  (°C)) multiplied by 10.

$$\text{CH} = \sum pp \text{ (mm)} / (\sum t^a \text{ (}^\circ\text{C)}) \cdot 10$$

*Bioclimatic vineyard index (I<sub>bcv</sub>)*

Bioclimatic vineyard index (I<sub>bcv</sub>) expresses ternary interaction between temperature, insolation and humidity.

$$I_{bcv} = C_t \cdot C_i / C_p : 10$$

C<sub>t</sub> - thermic coefficient; C<sub>p</sub> - precipitation coefficient; C<sub>i</sub> - insolation coefficient.

*Annual aridity index Martonne (I<sub>ar-DM</sub>)*

Annual aridity index Martonne (I<sub>ar-DM</sub>) shows the degree of dryness to a certain area (Martonne 1926). This index is calculated annually, for the corresponding period of growing season.

$$(I_{ar-DM}) = P / T + 10$$

P = annual average of precipitations; T = annual average of temperature.

*The Huglin index (HI)*

The Huglin index (HI) was calculated using formula:

$$\text{HI} = \sum (30 \text{ September} / 1 \text{ April}) \times \frac{1}{2} \times [(T_{\text{mean}} - 10)] + (T_{\text{max}} - 10) \times d \quad (1)$$

In the Northern hemisphere in the above formula, T = the mean air temperature (°C), T<sub>x</sub> = maximum air temperature (°C), d = length of day coefficient, ranging from 1.02 to 1.02 between 40° and 50° of latitude. From Romania d = 1.04.

*Enoclimatic skills index (IAO<sub>e</sub>)*

Enoclimatic skills index (IAO<sub>e</sub>) was used to determine the favourable climate of the area and also to determine the synthesis of anthocyanins in grapes. Enoclimatic skills index (IAO<sub>e</sub>) was calculated using formula:

$$\text{IAO}_e = T + I - (P - 250) \quad (2)$$

In the above: T = the amount of active temperature from 01.IV – 30.IX = amount of hours of insolation in the same period, and P = the amount of precipitation in the same period of time.

*Cool night index (CI)*

The determination of the cool night index (CI) is done as given further (Tonietto 1999): In northern hemisphere CI = minimum air temperature in month of September (mean of minim), in °C.

The last one was night coolness variable which takes into account the mean minimum night temperature during the month when ripening usually occurs beyond the ripening period. The purpose of this index was to improve the

assessment of the qualitative of wine-growing regions, notably in relation to secondary metabolites (polyphenols and also aromas) in grape. The ecoclimatic factors are important as regards grape and wine colours and aromas (Tomana *et al.*, 1979).

### ***Phenolic maturity of the red wine varieties in Dealu Bujorului vineyard***

#### *Raw material*

Different grape (*Vitis vinifera*) varieties Feteasca neagra (FN) and Merlot (M) from different vineyard parcels were studied over six weeks (from August to September) to monitor phenolic and technological maturity over grape harvest time in the climatic conditions of 2016. For each sampling 1.200 grape berries were randomly picked with pedicels attached. Vine varieties were collected at different times during grape ripening: A = 16 VIII; B = 22 VIII; C = 29 VIII; D = 05 IX; E = 12 IX; F = 19 IX and also with and different culture system AA = 28 (buds); BB = 20 (buds) and CC = 36 (buds). The last sample for each varieties corresponds to the grape harvest date.

#### *The weight of 100 berries*

To determine the mass of 100 berries, was cut of 100 berries on bunches and was placed in a known glass bottle, was weighing and the results were reported at 100 berries.

#### *pH*

The ionic or real acidity of the wine, designated by pH expresses the concentration of the free hydrogen ions  $[H^+]$  from must or wine. Unlike the total acidity that expresses the titratable acidity, pH is a physico-chemical index that expresses the degree of ionization of its acids and acidic salts. There is no proportionality between total acidity an ionic acidity. In this case the pH was measured with WTW inoLab pH 7110.

#### *Total acidity (titratable acidity)*

Total acidity is defined as the total substances with reaction present in wine, which can be titrated with an alkaline solution in presence of an indicator. Total acidity (g/L  $H_2SO_4$ ) was determined by titrimetric method. The principle of this method lies in the titration or neutralization of the acids from the sample to be analyzed with a sodium hydroxide solution with known normality and factor, in the presence of phenolphthalein as an indicator, after the removal of carbon dioxide. Results were calculated using the formula:

$$\text{Total acidity (in } H_2SO_4) = \frac{V \cdot 0.0049 \cdot 1000}{10} = 0.49 \cdot V \text{ (g/L)}$$

V = volume of Na OH used in titrations (mL);

0.0049 = the amount of sulfuric acid with corresponding to 1 mL of Na OH 0.1 N (in g).

#### *Sugar content (g/L)*

Determination of sugar from fresh must was made with refractometer (refractometer Optronic HRT 32). The method principle was reading of the percentage of soluble solids content in the must, correction of temperature readings and deduction of sugar content from must samples.

**Total anthocyanins potential (mg/L)***Sample preparation for Glories (soluble solids content)*

A representative sampling of the grapes was made during harvesting. Three samples (ca. 400 berries) from all parts of the vineyard were gathered. The technological parameters and the anthocyanin profile of the grapes were determined on half of the berries from each sample. The remaining berries were used to determine the phenolic maturity parameters.

50 g of the resulting grape juice were introduced in a 250 mL Erlenmeyer flask to apply the ITV method. Another 100 g were placed in two Erlenmeyer flasks (50 g of sample in each) to apply the Glories method.

*Glories method*

50 mL of aqueous solution at pH 3.2 were added to the first 50 g of sample. The pH 3.2 solution was prepared by adding 5 g of tartaric acid in to 1 L water with a pH adjustment to 3.2 by NaOH. 50 mL of aqueous solution pH 1 (37% HCl in distilled water with pH adjusted to 1) were added to the second 50 g of sample. Samples were macerated for 4 h at 20 °C then were filtered through glass wool. Anthocyanins and total phenolic contents were estimated.

The dosage of anthocyanins is based on the principle of anthocyanin discoloration by SO<sub>2</sub>. 1 mL of each filtrate (pH 1 or pH 3.2) was added to 1 mL of ethanol 0.1% HCl and 20 mL of concentrated 2% HCl. 10 mL of the mixture and 4 mL of distilled water introduced in a first tube while 10 mL of the mixture and 4 mL of sodium bisulfite (15 %) were introduced in the second tube. Bleaching is practically instantaneous. After 30 min the optical density at 520 nm was measured against distilled water for both tubes.

*Anthocyanin extractability (AE)*

Anthocyanin extractability (AE) or cell maturity index was calculated as follows (Rajha et al., 2017):

$$AE = \frac{A_{pH1} - A_{pH3.2}}{A_{pH1}} \times 100$$

*Percentage of extractable anthocyanins (PEA)*

The percentage of extractable anthocyanins (PEA) was calculated as follows:

$$PEA = \frac{A_{pH3.2}}{A_{pH1}} \times 100$$

*Total phenolic richness (RPT)*

To estimate the total phenolic richness (RPT) in the extracts macerated at pH 3.2, a dilution to 1/100 was conducted and the optical density was measured at 280 nm against distilled water. The overall estimation of total phenolic compounds was calculated as follows:

$$RPT = 2 \times OD_{280} \times 100$$

*Total content of tannins of skin*

Skin tannins (ST) were calculated as follows:

$$ST = A_{pH3.2} \times 40 / 1000$$

*Total content of tannins of seed (ST)*

Seed tannins (ST) were calculated as follows:

$$ST = RPT - \text{skin polyphenols} = RPT - \frac{ApH3.2 \times 40}{1000}$$

Phenolic maturity of seeds (SM) was calculated as follows:

$$SM = \frac{ST}{RPT} \times 100$$

### **Statistical analysis**

The statistical interpretation of the results was performed using the DUNCAN test, using the SPSS, version 24 (SPSS Inc. Chicago, IL, USA). The statistical processing of the results was primarily made to calculate the following statistical parameters: arithmetic average, standard deviation, average error, using the SPSS version 24 (SPSS Inc. Chicago, IL, USA). In order to determine whether the main quality parameters of wine can influence each other, the correlation coefficient was calculated using SPSS version 23 Pearson (SPSS Inc., Chicago, IL., USA). Linear discriminant analysis (LDA) was performed in order to separate the wines by region and to identify the markers with a significant discrimination value (variables with Wilk's lambda near zero,  $p$  values  $<0.005$  and higher F coefficients). Linear discriminant analysis (LDA) was performed using Microsoft Excel 2016 and XLSTAT Addinsoft version 15.5.03.3707.

## **Results and discussion**

### **Study of ecoclimatic conditions**

The duration of the growing season is within its normal limits over 170 days for the culture of vine (Pop, 2010), but in 2016 this limit was exceeded: 190 days were recorded for Dealu Bujorului Vineyard, Bujoru Wine Centre. Comparing these values (190 days) with the multiannual average (188 days) it can be observe a decrease of the vegetation period.

For this experimental year of 2016, the thermal balance values obtained are much lower than multiannual average: global thermal balance ( $\Sigma t^0g$ ) was 3538 °C and active thermal balance ( $\Sigma t^0a$ ) was 3358 °C. In the case of the useful thermal balance, the multiannual average ( $\Sigma t^0u$  1679) was also much higher than useful thermal balance of 2016 ( $\Sigma t^0u$  1610).

Regarding the number of days with a maximum temperature of over 30 °C, the year 2016 had an interval of 52 days, which is an increase comparing these values to the multiannual average 48 days. The precipitation quantity in 2016 was higher (690.4 mm) than the average of the last ten years (479.7 mm). During the growing season, the recorded precipitation values were 319 mm, much higher than the multiannual average of 287 mm for Bujoru Wine Centre.

The insolation measured by number of hours of sunshine was higher than normal in the months during the growing season, 1500 hours over the normal of 1292 hours (multiannual average). The insolation coefficient ( $C_i$ ) recorded the value of 7.63, and this shows an increase compared to the multiannual average (6.80).

In the climatic conditions of 2016, the real Heliothermal index (Hir) values were 2.33 falling within the limits described in the scientific literature (1.35 and 2.70), which shows an increase in the heliothermal resources and optimal conditions for the ripening of late maturing variety (Bora et al., 2016). Compared with the

multiannual average (2.46), in 2016 this parameter can be observed to show an increase.

The hydrothermal coefficient (CH) had a very low of 0.94 compared to the normal limits for our country, between 0.7 and 1.8 indicating that the humidity was insufficient, with recommendation for irrigation, for both table and wine grapes varieties. The viticultural bioclimatic index (Ibcv) with a value 8.4 for 2016 shows that the heliothermal resources recorded high values due to low hydrous resources for Bujoru wine center (multiannual average 10.26).

The Oenoclimatic suitability (IAOe) had a value of 4721 indicating an area with favorable conditions for growth of red varieties for wine, and also for the white wines. The Martonne aridity index had a value of 8.32 during the growing season, indicating a semiarid forest steppe climate. The heliothermal Huglin index provide useful information regarding the thermal potential for the culture of grape, both for table and wine, with different periods of ripening. Compared to other heliothermal indices, it displays a close link with the sugar from the must. The sum of the Huglin index during the growing season was 2238 (multiannual average was 2350). The cooling night index (CI) was calculated only for September and the obtained value was 9.8, a value that was lower than multiannual average 10.8.

The ecoclimatic conditions of Dealu Bujorului vineyard highlighted the exceptional viticultural characteristics of the Dealu Bujorului vineyard. These characteristics were found in the authenticity and specificity of a wide assortment of wine obtained in the studied area. In this context it was expected that, in qualitative terms, the 2 varieties tested until now present a good adaptability and therefore the results of the phenolic maturity indicate the production of quality wines.

#### ***Phenolic maturity of red wine varieties in Dealu Bujorului vineyard***

Regarding the weight of 100 berries (g), the highest values were recorded by Merlot variety [135.76±0.64 g (28)(29.VIII)]; [130.31±0.81 g (36)(16.VIII)]; and [124.45±0.64 g (28)(22.VIII)]. The lowest values were recorded by Merlot variety in all three forms of culture system in 19.IX [101.34±0.11 (28); 106.23±0.09 (20); 103.23±0.07 (36)]. Among the variants analyzed there are very significant differences ( $F = 864.751$ ;  $p \leq 0.000$ ). It can be seen that in this case data of sampling ( $F = 38.618$ ;  $p \leq 0.000$ ) but also the interaction between data of sampling and culture system ( $F = 4.000$ ;  $p \leq 0.000$ ) had a very significant influence on the weight of 100 berries. While culture system ( $F = 6.953$ ;  $p \leq 0.035$ ) has a significant influence on this parameter. The results are comparable with those reported by Mota *et al.*, 2011 [112.00±0.30 g (Merlot), 108.00±0.20 g (Cabernet Sauvignon)], and also comparable with those reported by Bora *et al.*, 2014 [129.77±2.65 g (Feteasca alba), 121.24±4.04 g (Italian Riesling)]

Table 1. Ecoclimatic conditions in Dealu Bujorului

| Area               | Climate conditions             | Multia<br>annual  |                        | Specific values |       | Vine<br>breakpoint<br>ts |               |
|--------------------|--------------------------------|---|------------------------|-----------------|-------|--------------------------|---------------|
|                    |                                | 2006-<br>2015   | 2016                   | Min.            | Max.  |                          |               |
| Dealu<br>Bujorului | The vegetation period          | Days  | 188                    | 190             | 188   | 190                      | 150-170       |
|                    |                                | Global ( $\Sigma t^{\circ}g$ )                          | 3538                   | 3431            | 3254  | 3568                     | 2700-<br>3600 |
|                    |                                | Active ( $\Sigma t^{\circ}a$ )                          | 3358                   | 3370            | 3154  | 3451                     | 2600-<br>3500 |
|                    |                                | Beneficial ( $\Sigma t^{\circ}b$ )                      | 1679                   | 1610            | 1578  | 1684                     | 1000-<br>1700 |
|                    | Thermal balance (°C)           | Thermic Coefficient (C <sub>t</sub> )                   | 17.7                   | 17.4            | 16.8  | 18.2                     | 16-19         |
|                    |                                | Minimum absolute air temp.                              | -25.2                  | -17.4           | -23.0 | -                        | -             |
|                    |                                | Maximum absolute temp.                                  | 41.5                   | 35.6            | -     | 37.3                     | -             |
|                    |                                | Avg. max. temp. August                                  | 30                     | 30              | -     | -                        | -             |
|                    |                                | Avg. temp. decade I and II June                         | 21                     | 20              | -     | -                        | -             |
|                    |                                | No. of days max temp. > 30                              | 48                     | 52              | -     | -                        | -             |
|                    |                                | Real ( $\Sigma t^{\circ}r$ )                            | 2087                   | 1862            | 1657  | 1732                     | 1200-<br>1600 |
|                    |                                | $\Sigma$ hours of insolation in the<br>growing season   | 1292                   | 1500            | -     | -                        | -             |
|                    |                                | Insolation Coefficient (C <sub>i</sub> )                | 6.80                   | 7.63            | 6.68  | 8.75                     | 7-9           |
|                    |                                | $\Sigma$ precipitations in the growing<br>season        | 287                    | 319             | -     | -                        | -             |
|                    |                                | Precipitations (mm)                                     | Annual ( $\Sigma pp$ ) | 479.7           | 690.4 | 439.1                    | 631.5         |
| Bujoru<br>Vineyard |                                | Precipitation Coefficient (C <sub>p</sub> )             | 1.5                    | 1.7             | 1.02  | 2.04                     | 0.9-2.7       |
|                    |                                | Real Heliothermal index (HI <sub>r</sub> )              | 2.46                   | 2.33            | 2.05  | 2.61                     | 1.35-2.70     |
|                    |                                | Hydrothermal coefficient (CH)                           | 0.84                   | 0.94            | 0.81  | 1.51                     | 0.6-1.8       |
|                    |                                | Bioclimatic vineyard index<br>(I <sub>bcv</sub> )       | 10.26                  | 8.4             | 6.5   | 8.1                      | 4-15          |
|                    |                                | Oenoclimatic skills index<br>(IAOe)                     | 4843                   | 4721            | -     | -                        | -             |
|                    | Interaction of climate factors | Annual aridity index Martonne<br>(I <sub>ar-DM</sub> )  | 22.3                   | 32.5            | -     | -                        | -             |
|                    |                                | Heliothermal Huglin index (HI)<br>in the growing season | 2350                   | 2238            | -     | -                        | -             |
|                    |                                | Cooling nights index (CI)                               | 10.8                   | 9.8             | -     | -                        | -             |

pH shows values between 3.12 and 3.57 with an average value of 3.31. The highest values were recorded by Merlot variety [3.57±0.28 (28)(05.IX)] and Feteasca neagra variety [3.42±0.03 (28)(29.VIII)]; [3.43±0.05 (20)(29.VIII)]; [3.47±0.08 (36)(29.VIII)]. The lowest values were recorded by Feteasca neagra variety [3.17±0.07 (28) (16.VIII)]; [3.15±0.04 (20)(16.VIII)]; [3.15±0.05 (36)(16.VIII)] and Merlot variety [2.98±0.04 (28)(16.VIII)]; [3.12±0.12 (20)(16.VIII)]; [2.97±0.12 (36)(16.VIII)]. The results are comparable with those reported by Bora et al., 2016 (3.47±0.10 [Muscat Ottonel], 3.32±0.17 [Şarba], 3.54±0.17 [Sauvignon blanc], 3.46±0.01 [Italian Riesling]), and also with those reported by Bora et al., 2016b (3.30±0.01 [Merlot]).

Concerning the total acidity (g/L H<sub>2</sub>SO<sub>4</sub>), Feteasca neagra [10.41±0.13 g/L H<sub>2</sub>SO<sub>4</sub> (20)(16 VIII); 10.76±0.06 g/L H<sub>2</sub>SO<sub>4</sub> (36)(16 VIII)] and Merlot [11.88±0.22g/L H<sub>2</sub>SO<sub>4</sub> (20)(16 VIII)] recorded the highest values. A decrease in total acidity can be observed at Merlot variety [5.08±0.03 g/L H<sub>2</sub>SO<sub>4</sub> (28)(05 IX); 4.61±0.11 g/L H<sub>2</sub>SO<sub>4</sub> (20)(05 IX); 4.43±0.10 g/L H<sub>2</sub>SO<sub>4</sub> (36)(05 IX)], that gradual decrease of acidity we observe again at the Merlot variety in (19 IX) [4.31±0.13 g/L H<sub>2</sub>SO<sub>4</sub> (28)(19 IX); 4.21±0.09 g/L H<sub>2</sub>SO<sub>4</sub> (20)(19 IX); 4.31±0.12 g/L H<sub>2</sub>SO<sub>4</sub> (36)(19 IX)]. The results are comparable with those reported by Bora et al., 2014 [4.83±0.13 g/L H<sub>2</sub>SO<sub>4</sub> (Feteasca alba), 6.11±0.56 g/L H<sub>2</sub>SO<sub>4</sub> (Feteasca regala), 5.38±0.15 g/L H<sub>2</sub>SO<sub>4</sub> (Italian Riesling)] and also with those reported by Bonilla et al., 2015 [7.92±0.99 g/L H<sub>2</sub>SO<sub>4</sub> (Tempranillo)].

Physiologically, the acid sensation of wine is exerted by free hydrogen ions and increases with their concentration [H<sup>+</sup>]. The acid sensation persists in the oral cavity because wine is a strong buffered solution and opposes the acid neutralization action of salivary alkalinity. All the organic acids in wine act in the same way on the acid sensation, at the same values and buffering power. The only acid that distinguishes itself from the other acids is the lactic acid, its presence in wine being perceived only by taste (Țârdea, 2007).

In the beginning, the accumulation of sugar in berries is slow and occurs by mobilizing the starch from the vineyard deposited as reserve substance. Gradually, the sugar accumulation increases on the photosynthesis process of the leaves. The grape berries act as a receptor, in the sense of the increased influx of sugars, which also corresponds to a quantity of water to reach an osmotic balance throughout the vine. The lowest amount of sugar was recorded in (16.VIII) by Feteasca neagra [160.41±1.95 g/L (28); 171.16±0.99 g/L (20); 160.20±0.90 g/L (36)] and Merlot [171.14±0.23 g/L (28); 149.37±0.73 g/L (20); 160.40±0.66 g/L (36)]. The highest amount of sugar was recorded in (05.IX) by Feteasca neagra [260.39±0.08 g/L (28); 258.34±0.10 g/L (20); 260.35±0.15 g/L (36)] and in (19. IX) by Merlot [295.46±0.15 g/L (28); 298.21±0.07 g/L (20); 295.34±0.07 g/L (39)]. The results are comparable with those reported by Donici et al., 2016 [213.66±0.67 g/L (Bujoru), 215.30±0.67 g/L (Babeasca gri) and 203.00±1.00 g/L (Feteasca regala)] and also with those reported by Bunea et al., 2014 [178.60 (Radames), 192.30 (Rubin), 195.70 (Brumariu)].

Anthocyanins constitute a very large family of polyphenols in plants and are responsible for many of fruit and floral colours observed in nature (Nile *et al.*, 2014). They are pigments dissolved in the vacuolar sap of the epidermal tissues of flowers and fruit which impart red, pink, blue or purple colours (Mazza *et al.*, 1993). Grapes are among the fruits containing the highest content of phenolic substances, which are partially extracted during the winemaking process and brewing (Revilla *et al.*, 2002).

Regarding the total anthocyanins potential (mg/L), the highest values were recorded by Merlot in (05.IX) [2177.97±16.15 mg/L (20); 2025.36±38.80 mg/L (36)] and also in (29.VIII) by Feteasca neagra variety [1924.48±41.06 mg/L (36)]. The lowest concentration of total anthocyanins potential was recorded in wine from Merlot variety in (16.VIII) [855.15±9.72 mg/L (20); 833.94±3.91 mg/L (36)]. The difference between the analyzed variants is statistically assured ( $F = 1137.893$ ;  $p \leq 0.000$ ) as a significant influence was between them. Based on the polyfactorial analysis, we can see that the total anthocyanins potential was significantly influenced by the data of sampling factor ( $F = 142.344$ ;  $p \leq 0.000$ ), while the rest of the factors did not have any influence on the accumulation of total anthocyanins. The results are comparable with those reported by Artem *et al.*, 2016 [1875.00±45.00 mg/L (Cabernet Sauvignon), 1741.00±40.00 mg/L (Feteasca neagra), 1652.00±26.00 mg/L (Merlot) 3134.00±43.00 mg/L Pinot noir, 622.00±19.00 mg/L (Mamaia)].

Feteasca neagra in (29.VIII) [663.22±2.37 mg/L (28); 679.52±1.95 mg/L (20); 660.12±1.19 mg/L (36)] and Merlot [665.72±1.34 mg/L (20)] recorded the highest values to extractable anthocyanins potential, compared to Merlot variety in (12. IX) [196.07±2.49 mg/L (28); 197.88±33.76 mg/L (20)] and Merlot from (19. IX) [133.99±3.91 mg/L (20)], varieties that recorded the lowest values for extractable anthocyanins potential.

In the case of extractable anthocyanins potential (AE %), Merlot variety in (12. IX) [84.78±0.42 AE% (28); 87.23±2.17 AE% (20)] and in (19. IX) [89.43±0.34 AE% (20)] recorded the highest values, compared with Feteasca neagra in (16. VIII) [46.09±1.64 AE% (28); 58.61±0.53 AE% (20); 59.79±0.48 AE% (36)] and Merlot neagra in (16. VIII) [65.46±0.57 AE% (28); 62.73±0.55 AE% (20); 60.79±0.12 AE% (36)]. The lower the AE%, the higher the degree of extractability of anthocyanins in the grapes, and the wine will be more intensely colored. The results are comparable with those reported by Artem *et al.*, 2016 [69.70±5.00 % (Cabernet Sauvignon), 60.30±5.00 % (Feteasca neagra), 66.20±4.50 % (Merlot) 59.40±4.40 % Pinot noir, 61.20±3.70 % (Mamaia)].

Phenolic compounds have long been considered to be basic components of wines and over 200 compounds have been identified. The concentration of total phenolic compounds in commercially available red wines is rarely above 2.5 g/L (Singleton *et al.*, 1982). Two primary classes of phenolic that occur in grapes and also in wine are flavonoids and nonflavonoids.

Total polyphenols (RPT) is the amount of tannins from skin and tannins from seeds. The highest values of the total polyphenols (RPT) were recorded by Merlot variety in (05. IX) [30.20±1.83 total polyphenols (RPT) (11.32±0.01 tannins from

skin and  $18.88 \pm 1.82$  tannins from seeds) (20)]; [ $27.13 \pm 1.42$  total polyphenols (RPT) ( $11.45 \pm 0.93$  tannins from skin and  $15.68 \pm 1.93$  tannins from seeds)]. While Merlot variety from (16. VIII) [ $13.40 \pm 0.60$  total polyphenols (RPT) ( $7.33 \pm 0.02$  tannins from skin and  $6.07 \pm 0.61$  from seeds (28));  $12.53 \pm 0.50$  total polyphenols (RPT) ( $6.37 \pm 0.04$  tannins from skin and  $6.16 \pm 0.52$  tannins from seeds (20));  $11.13 \pm 0.61$  total polyphenols (RPT) ( $6.54 \pm 0.05$  tannins from skin and  $4.59 \pm 0.57$  tannins from seeds) (36)] recorded the lowest values for total polyphenols (RPT). The results are comparable with those reported by Odăgeriu *et al.*, 2007 [ $33.98$  (Feteasca neagra),  $30.56$  (Babeasca gri)].

The maturity of the seeds (%) shows an exponential growth, Merlot variety from (29. VIII) [ $40.65 \pm 0.90$  (28);  $35.21 \pm 2.76$  (20);  $40.8 \pm 2.19$  (36)] recorded the lowest values while Merlot variety from (19. IX) [ $82.74 \pm 0.39$  (28);  $84.74 \pm 1.62$  (20);  $80.70 \pm 0.60$  (36)] recorded the highest values. The results are comparable with those reported by Artem *et al.*, 2016 [ $62.20 \pm 6.10$  % (Cabernet Sauvignon),  $61.50 \pm 4.90$  % (Feteasca neagra),  $68.20 \pm 5.50$  % (Merlot)  $57.70 \pm 4.70$  % Pinot noir,  $88.70 \pm 20.20$  % (Mamaia)].

#### ***The Pearson correlation between the grape maturity indexes***

In order to determine whether the grape maturity index can influence each other, the Pearson correlation coefficient was calculated for each studied parameter as it shown in Table 3.

A Pearson correlation coefficient value higher than 0.5 shows a strong correlation between the analysed varieties, a positive correlation between the two parameters shows that both parameters increased, a negative correlation indicates that a parameter increased while the second one decreased and vice-versa. These provide a large number of both positive and negative correlations between the main parameters of the analysed wines.

There are some relevant examples: S Cont. & pH ( $r^2 = 0.730$  \*\*); S Cont. & Tot. A ( $r^2 = -0.835$  \*\*); P. Extr & S Cont. ( $r^2 = 0.695$  \*\*); Tot. cont t Seeds & S Cont. ( $r^2 = 0.563$  \*\*); M Seeds & S Cont. ( $r^2 = 0.688$  \*\*); A. Extr & Tot. Ap ( $r^2 = 0.630$  \*\*); Tot. Phen rich & Tot. Ap ( $r^2 = 0.805$  \*\*); Tot. cont t Skin & Tot. Ap ( $r^2 = 0.630$  \*\*); Tot. cont t Skin & Tot. Ap ( $r^2 = 0.999$  \*\*); Tot. cont t Seeds & P. Extr ( $r^2 = 0.701$  \*\*); M Seeds & P. Extr ( $r^2 = 0.709$  \*\*); Tot. cont t Skin Tot. & Tot. cont t Seeds ( $r^2 = 0.742$  \*\*); M Seeds & Tot. cont t Skin ( $r^2 = 0.767$  \*\*); M Seeds & Tot. cont t Seeds ( $r^2 = 0.808$  \*\*).

Regarding negative correlations it can be observed that in all the analyzed cases there was a weak negative correlation Tot. A & pH ( $r^2 = -0.731$  \*\*); Tot. Ap & Tot. A ( $r^2 = -0.465$  \*\*); P. Extr & Tot. A ( $r^2 = -0.666$  \*\*); Tot. Phen rich & Tot. A ( $r^2 = -0.528$  \*\*); M Seeds & Tot. A ( $r^2 = -0.575$  \*\*); P. Extr & A. Extr ( $r^2 = -0.716$  \*\*); M Seeds & Tot. Ap ( $r^2 = -0.767$  \*\*); Tot. cont t Skin & P. Extr ( $r^2 = -0.716$  \*\*); (Table 3).

Based on the previous Pearson correlation index, through this present research have been shown that the grape maturity index have an influence on each other; in other words, the phenolic maturity of the red wine varieties from Dealu Bujorului are directly contingent on all these parameters.

Table 2. Grape maturity index of Feteasca neagra and Merlot at harvest in 2016 (mean ± standard deviation) – part 1

| Data of sampling | Variety         | Culture system | The weight of 100 berries (g) | pH        | Total acidity (g/L H <sub>2</sub> SO <sub>4</sub> ) | Sugar content (g/L) | Total anthocyanins potential (mg/L) | Anthocyanin extractability (AE) | Percentage of extractable anthocyanins (PEA %) | Total phenolic richness (RPT) | Total content of tannins in skin | Total content of tannins in seeds (ST) | Maturity of seeds MP (%) |
|------------------|-----------------|----------------|-------------------------------|-----------|---|---------------------|-------------------------------------|---------------------------------|--|-------------------------------|----------------------------------|--|--------------------------|
| 16.08. 2016      | Feteasca neagra | 28             | 123.42±0.79                   | 3.17±0.07 | 9.85±0.09   | 160.41±1.95         | 981.38±9.98                         | 528.97±14.36                    | 46.09±1.64                                     | 18.07±0.61                    | 10.58±0.29                       | 7.49±0.59                              | 41.41±2.15               |
|                  |                 | 20             | 119.29±0.96                   | 3.15±0.04 | 10.41±0.13  | 171.16±0.99         | 1099.33±34.99                       | 454.99±15.55                    | 58.61±0.53                                     | 15.53±0.50                    | 9.10±0.31                        | 6.43±0.38                              | 41.40±1.60               |
|                  |                 | 36             | 117.25±0.95                   | 3.15±0.05 | 10.76±0.06  | 160.20±0.90         | 1220.91±17.92                       | 490.95±5.88                     | 59.79±0.48                                     | 17.00±0.53                    | 9.82±0.12                        | 7.78±0.47                              | 44.18±1.40               |
| 16.08. 2016      | Merlot          | 28             | 125.10±0.39                   | 2.98±0.04 | 9.86±0.26   | 171.14±0.23         | 1060.33±19.53                       | 366.27±0.78                     | 65.46±0.57                                     | 13.40±0.60                    | 7.33±0.02                        | 6.07±0.61                              | 45.26±2.51               |
|                  |                 | 20             | 118.29±0.17                   | 3.12±0.12 | 11.88±0.22  | 149.37±0.73         | 855.15±9.72                         | 318.68±1.95                     | 62.73±0.55                                     | 12.53±0.50                    | 6.37±0.04                        | 6.16±0.52                              | 49.09±2.22               |
|                  |                 | 36             | 130.31±0.81                   | 2.97±0.12 | 9.64±0.15   | 160.40±0.66         | 833.94±3.91                         | 326.95±2.37                     | 60.79±0.12                                     | 11.13±0.61                    | 6.54±0.05                        | 4.59±0.57                              | 41.16±2.78               |
| 22.08. 2016      | Feteasca neagra | 28             | 120.50±0.8                    | 3.21±0.06 | 6.64±0.08   | 210.38±0.27         | 1802.91±17.92                       | 490.17±6.27                     | 72.81±0.60                                     | 26.00±2.00                    | 9.80±0.13                        | 16.20±1.92                             | 62.16±2.61               |
|                  |                 | 20             | 117.48±0.32                   | 3.22±0.09 | 6.66±0.08   | 212.47±0.11         | 1678.75±11.85                       | 499.74±8.10                     | 70.23±0.54                                     | 25.20±1.06                    | 9.99±0.16                        | 15.21±0.97                             | 60.30±1.43               |
|                  |                 | 36             | 114.35±0.23                   | 3.31±0.03 | 6.69±0.10   | 209.33±0.33         | 1474.40±0.00                        | 413.61±1.55                     | 71.95±0.11                                     | 25.33±1.14                    | 8.27±0.03                        | 17.06±1.17                             | 67.30±1.56               |
| 22.08. 2016      | Merlot          | 28             | 124.45±0.64                   | 3.15±0.06 | 6.72±0.45   | 182.48±0.62         | 1363.17±11.85                       | 425.77±0.45                     | 68.77±0.24                                     | 25.20±1.11                    | 8.52±0.01                        | 16.68±1.11                             | 66.16±1.52               |
|                  |                 | 20             | 116.37±0.10                   | 3.15±0.05 | 6.22±0.08   | 174.43±0.93         | 1748.59±11.85                       | 380.50±5.05                     | 78.24±0.22                                     | 24.20±1.71                    | 7.61±0.10                        | 16.59±1.67                             | 68.46±2.06               |
|                  |                 | 36             | 106.37±0.10                   | 3.17±0.04 | 5.72±0.10   | 184.27±0.32         | 1839.12±13.44                       | 402.74±14.78                    | 78.10±0.74                                     | 25.47±1.72                    | 8.05±0.30                        | 17.41±1.45                             | 68.32±1.12               |





**Table 3.** Pearson correlation matrix between the grape maturity index

| Parameters        | pH       | Tot. A   | S Cont.  | Tot. Ap | A. Extr  | P. Extr  | Tot. Phen rich | Tot. cont t Skin | Tot. cont t Seeds | M Seeds |
|-------------------|----------|----------|----------|---------|----------|----------|----------------|------------------|-------------------|---------|
| pH                | 1.000    |          |          |         |          |          |                |                  |                   |         |
| Tot. A            | -0.731** | 1.000    |          |         |          |          |                |                  |                   |         |
| S Cont.           | 0.730**  | -0.835** | 1.000    |         |          |          |                |                  |                   |         |
| Tot. Ap           | 0.360**  | -0.465** | 0.069    | 1.000   |          |          |                |                  |                   |         |
| A. Extr           | -0.001   | 0.128    | -0.397** | 0.630** | 1.000    |          |                |                  |                   |         |
| P. Extr           | 0.408**  | -0.666** | 0.695**  | 0.064   | -0.716** | 1.000    |                |                  |                   |         |
| Tot. Phen rich    | 0.372**  | -0.528** | 0.248*   | 0.805** | 0.442    | 0.155    | 1.000          |                  |                   |         |
| Tot. cont t Skin  | -0.001   | 0.128    | -0.397** | 0.630** | 0.999**  | -0.716** | 0.442**        | 1.000            |                   |         |
| Tot. cont t Seeds | 0.399**  | -0.663   | 0.563**  | 0.392** | -0.273** | 0.701**  | 0.742**        | -0.273**         | 1.000             |         |
| M Seeds           | 0.347**  | -0.575** | 0.688**  | -0.106  | -0.767** | 0.907**  | 0.218*         | -0.767**         | 0.808**           | 1.000   |

Tot. A = Total acidity (g/L H<sub>2</sub>SO<sub>4</sub>); S Cont. = Sugar content (g/L); Tot. Ap = Total anthocyanins potential (mg/L);

A. Extr = Anthocyanin extractability (AE); P. Extr A = Percentage of extractable anthocyanins (PEA);

B. Tot. Phen rich = Total phenolic richness (RPT); Tot. cont t Skin = Total content of tannins in skin;

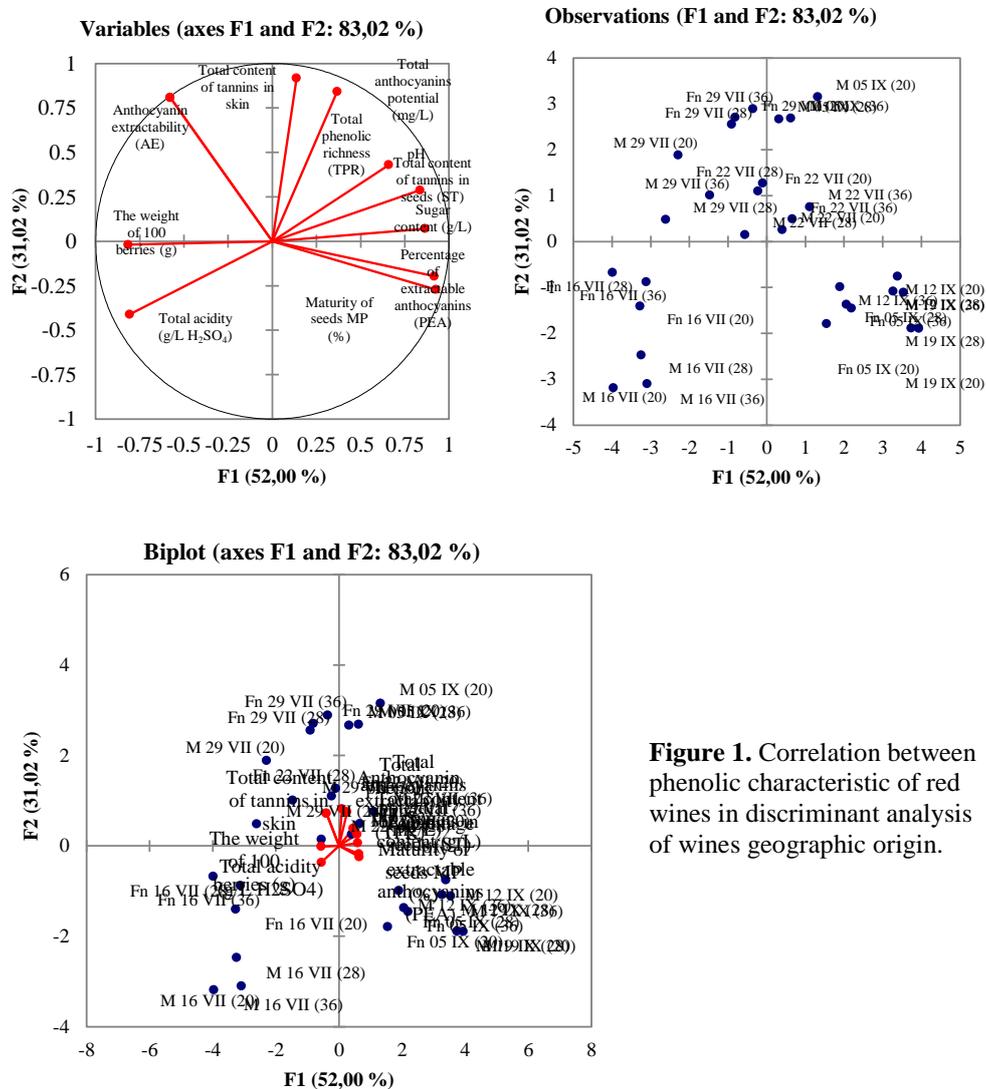
A. Tot. cont t Seeds = Total content of tannins in seeds; M Seeds = Maturity of seeds MP (%).

\*the correlation is significant at  $p < 0.05$  in 95%; \*\* the correlation is highly significant at  $p < 0.01$ , in 99%; N = 90.

### Combining the phenolic characteristics of red wines for wine geographical discrimination

Multivariate chemometric method was applied for the differentiation of wines into groups on the basis of their geographic origin. Stepwise linear discriminant analysis (LDA) was used to identify significant tracers for classification to the geographical discrimination of the wines samples. By cross-validation, we established the optimal number of parameters required to obtain a robust model.

The differentiation of wines according to geographic origin based on the phenolic characteristic of wine, in this case a 83.02% percentage of predicted membership according to the wine geographic origin (F1 = 52.00% and F2 = 31.02%) (Figure 1).



**Figure 1.** Correlation between phenolic characteristic of red wines in discriminant analysis of wines geographic origin.

Based on the phenolic characteristic, a relevant discrimination of wines according to their geographical origin and culture system was performed. The variation of the phenolic characteristic represents a strong geological marker for wines geographical traceability. The proposed methodology allowed an 83.02 % successful classification of wines according to the region of provenance.

### **Conclusions**

The ecoclimatic conditions in the Dealu Bujorului, Bujoru Wine Centre, highlighted the exceptional viticultural value as well as the authenticity encountered in the wide variety of wines produced in the studied areas. Based on the results regarding the qualitative assessment of the tested varieties, they have a very good suitability in the studied areas.

Based on the previous Pearson correlation index, through this present research has been shown that the grape maturity index have an influence on each other; in other words, the phenolic maturity of the red wine varieties from Dealu Bujorului are directly contingent on all these parameters.

The results showed the suitability of ecoclimatic conditions and the proper growth and development of the tested varieties for obtaining wines with superior's quality. Under the ecoclimatic conditions of 2016, the grapes entered in the ripening process prematurely, and full maturity was very early. The dynamics accumulation of the sugars and color compounds until the harvest was alert.

Based on the phenolic characteristic, a relevant discrimination of wines according to their geographical origin and culture system was performed. The variation of the phenolic characteristic represents a strong marker for wines geographical traceability. The proposed methodology allowed an 83.02% successful classification of wines according to the region of provenance.

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