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Effect of maceration on the making of Fetească neagră wines

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Abstract

The main sensory parameter for the quality of red wines is the colour. Red wine is the result of red grape must fermentation and the parallel extraction of various polyphenolic compounds from the grape berry skin. The aim of this work was to evaluate the influence of maceration conditions on the chromatic, chemical and sensorial characteristics of the Fetească neagră wine. The maximum of polyphenolic and anthocyanins content was achieved when the maceration was done by using rotative tanks (Variant V2). In terms of anthocyanin composition the nine anthocyanins were identified. The malvidin (Mv-3-gl) represented the major participant to the colour composition. Wines made by maceration-fermentation in rotating tanks (Variant V2) and grape cryomaceration before fermentation (Variant V4) showed the highest values of glycerol content (8.8 to 9.0 g/l). From sensorial point of view the wine produced by variant V2 presented superior characteristics compared to variants V1, V3 and V4. The chromatic, chemical and sensorial characteristics of the Fetească neagră wines are significantly influenced by the method of grape maceration. The concentrations of Mv-3-gl, Mv-3-gl-ac and Mv-3-gl-pcum represented more than 50% of total anthocyanins in all variants of maceration. The wine obtained by variant V2 was more appreciated from chemical and sensorial point of view.

Key words: Red wine, polyphenols, anthocyanins, Fetească neagră wines.

Introduction

Consumption of red wines in Romanian diet has a special place. A number of epidemiological studies demonstrate the protective role of dietary polyphenol content against cardiovascular and cancer diseases [1]. The beneficial role of polyphenol content for human health gave reason for assessment of their composition in food and beverages. Red wines are a very rich source of polyphenols components with high antioxidant potential [2]. Polyphenol content possess radical scavenger activity and metal-chelating ability [3]. Polyphenol composition of red wines includes anthocyanins, catechins and flavonols. Red wine is the result of red grape must fermentation and the parallel extraction of various compounds from the grape berry skin [4-6]. Grape skin maceration results in the extraction of all the colour compounds relevant for wine structure, its body, colour, bouquet and aroma perception, and of various substances from polyphenolic to nitrogen compounds, polysaccharides, pectins, mineral substances, terpenes etc. [7]. The solid-liquid phase contact between the grape skin and must during the maceration fermentation period is the most important phase for the final wine quality. In the production of red wines various vinification technologies may be used [8]. They are mostly distinguished by their different treatment of the maceration [9]. Fetească neagră is a Romanian autochthonous red variety of *Vitis vinifera*, which acquires its superior quality in the Murfatlar vineyard where wines with a protected geographic origin are produced. This grape variety is very important in production of high-quality red wines in Romania.

The objective of this study was to determine the effect of maceration conditions on the chromatic, chemical and sensorial characteristics of the Fetească neagră wine.

Materials and Methods

Winemaking: Experiments were done on Fetească neagră grapes obtained in Murfatlar vineyard, Constanta, Romania, in the climatic conditions of the year 2011. Grapes have been harvested at phenolic maturity, and then were transported to the experimental winery in plastic boxes with a capacity of 10 kg. After destemming and crushing of grapes, the obtained mash was sulphited using 100 mg SO_2/l and was transferred to the maceration-fermentation vessels.

In Table 1 experimental variants and applied technology of Fetească neagră wines are presented. For each variant, the experiments were repeated two times. Each experiment was performed with homogeneous mash derived from grapes from a single area.

Anthocyanins separation and quantification: Anthocyanin separation and quantification was done by using a method described by Budic Leto *et al.* [10] with small modifications. Analysis of anthocyanins were performed in a Surveyor Plus high-performance liquid chromatograph equipped with a Diode Array Detector. The injected sample volume was 10 µl. Separation of anthocyanins was carried out with the column AQUASIL C18 (4.6 mm x 250 mm, 5 µm) equipped with a pre-column AQUASIL C18 (4 mm x 10 mm, 5 µm). The chromatographic method conditions were as follows: mobile phase ow rate 0.50 ml/min; DAD detection in the visible at 518 nm; mobile phase A water/formic acid/acetonytril 87:10:3 (v/v/v); mobile phase B water/formic acid/acetonytril 40:10:50 (v/v/v), temperature 40°C. Anthocyanin compounds were eluted under the following conditions: elution with linear gradients from 6% to 30% B in 15 min, from 30% to 50% B in 30 min, from 50% to

Table 1. Technological variants used for experiments.

Variant	Used technology
Variant 1 (V1)	Crushed grapes sulphited with 100 mg SO_2/l, maceration-fermentation in classic wooden vessels with capacity of 100 litres
Variant 2 (V2)	Crushed grapes sulphited with 100 mg SO_2/l, maceration-fermentation in metallic rotatory tanks with capacity of 5000 litres
Variant 3 (V3)	Crushed grapes sulphited with 100 mg SO_2/l, thermo maceration at temperature of 70°C for 15 min, before fermentation in vertical tanks
Variant 4 (V4)	Crushed grapes sulphited with 100 mg SO_2/l, cryomaceration at temperature of -20°C for 24 hours, before fermentation in vertical tanks

100% B in 45 min, followed by washing and reconditioning of the column. The UV-visible spectra (scanning from 200 nm to 600 nm) were recorded for all peaks. Identication of anthocyanins was obtained using authentic standards and by comparing the retention times and spectra with those found in the literature.

Wine analysis: Wine characterisation was done according to the analytical methods recommended by the OIV [11]. Sugar concentration was measured by using the clarified wine reaction with a specific quantity of an alkaline copper salt solution and the excess copper ions determined iodometrically. The titratable acidity was measured by titrimetry using NaOH 0.1 N and bromothymol blue as indicator. Volatile acidity was measured by removing and collecting the volatile acids from the sample by steam distillation, using Parnas-Wagner installation. The collected sample was titrated using NaOH 0.1 N and phenolphtalein as indicator. Alcoholic degree was measured by simple distillation and after the determination of density by pycnometer. Free sulfur dioxide was determined by direct titration with iodine in the presence of starch as indicator. The combined sulfur dioxide was subsequently determined by iodometric titration after alkaline hydrolysis. When added to the free sulfur dioxide, it gives the total sulfur dioxide. Glycerol was measured by using an enzymatic kit (Free Glycerol Determination Kit-Sigma).

The total phenol content was determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method [12] 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/l). The results in every assay were obtained from three parallel determinations. The total anthocyanins content in wines was determined using the method of Budic Leto *et al.* [10] on the basis of maximum absorbance in the visible range (536-540 nm) in acid medium. Colour intensity and nuance (hue) were estimated by measuring absorbance at 420, 520 and 620 nm on the basis of the method reported by EU regulations (1990) [13].

Statistical analyses: The statistical significance of the effect of the enzyme treatment on free and bound volatiles analyzed in triplicate were done by one-way ANOVA using Statistica 8 (StatSoft, Inc.). Means between control and treated samples were compared at $P < 0.001$ by Fisher´s least significant difference test.

Sensorial analysis: Sensorial analysis of wine was conducted by a panel of 8 panelists (6 men and 2 women), all persons being certified as authorized wine tasters.

For red wines the following descriptors were chosen for sensorial analysis: color intensity, olfactive intensity, olfactive quality, gustative intensity, gustative quality, astringency, bitterness and roundness. The maximum score of 5 points was awarded for excellent, 4 points for very good, 3 points for good, 2 points for less good and 1 point for poorly.

Results and Discussion

Chromatic characteristics of wines: Regarding the total polyphenolic content, it can be seen that for grape Fetească neagră variety the maximum of phenolic compounds was observed when the maceration was done by using rotative tanks (Variant V2). This confirms again that this method of maceration for extraction is the most recommended in order to obtain maximum content of phenolic compounds from grapes (Table 2). The minimum values of the same phenolic compounds were achieved for the variant V4 when cryomaceration of the grapes was done. Anthocyanin content is different depending on the grape variety, the conditions of the harvest and maceration type used [14, 15].

Anthocyanin content varied from 45.5 mg/l in V4 to the maximum values above 300 mg/l for V2 when maceration fermentation takes place in rotative metal tanks. Similar results were reported by Kelebek *et al.* [16] for the Boğazkere and Öküzgözü wines that reached the total amount of anthocyanins in a maximum values of 308.7 mg/l and 180.2 mg/l, respectively, on the skin maceration time of 6 days. For the variants where the fermentative process takes place along with maceration (V1 and V2), the extraction of anthocyanins increased because of the favourable action of alcohol, temperature, pH etc. In experiments conducted by the first two variants (V1 and V2) the maceration was developed concurrently with fermentation, and in the other variants (V3 and V4) maceration process took place before alcoholic fermentation.

The thermomaceration method applied (Variant V3) consisted in heating at the temperature of 70°C for 15 min the partially separated grape pomace from free run must for extraction of grape skin colour. In this situation the phase of maceration is before the alcoholic fermentation. The heating of the crushed grapes followed by assembling them with the unheated must which was separate in advance present some advantages as follows: first inactivates polyphenoloxidases located mainly in the pulp and solid components, in the separated free run must are kept an important part of natural pectolytic enzymes. Keeping a quantity of free run must in grape pomace (25-50%) is required to maintain the fluidity,

Table 2. Chromatic characteristics of wines.

Characteristics	Variant 1 (V1)	Variant 2 (V2)	Variant 3 (V3)	Variant 4 (V4)
Total polyphenols, g/l	2.11 ± 0.132^{a}	4.52 ± 0.145^{b}	2.22 ± 0.025^{a}	1.24 ± 0.210^{a}
Anthocyanins, mg/l	234 ± 0.204^{b}	312 ± 0.110^{ab}	256 ± 0.025^{b}	45.5 ± 0.051^{c}
Color intensity (OD_{420nm}+ OD_{520nm}+ OD_{620nm})	1.60 ± 0.204^{a}	2.70 ± 0.400^{ab}	2.3 ± 0.005^{c}	1.1 ± 0.005^{b}
Nuance (OD_{420nm}/ OD_{520nm})	0.52 ± 0.068^{ns}	0.45 ± 0.310^{ns}	0.55 ± 0.022^{ns}	0.60 ± 0.010^{ns}

a,b,c different superscripts in the same row indicate significant differences at the $P < 0.001$ level. ns not significant.

while forming the medium where the components of solid parts are dissolved. Results showed that by heating the pulp at the temperature of 70°C for 15 min positively influence the anthocyanins extraction (Table 2). On the other hand, temperature and retention time should not be exceeded because it can present a negative effect, irreversible for the new wine by the thermal degradation of sugars. By thermomaceration the total anthocyanin content of wines was 256 mg/l in the case of Fetească neagră variety. The thermomaceration using the temperature of 70°C for 15 min can be used for grapes from damaged crops in years with unfavourable ripening conditions.

By cryomaceration (Variant V4) lower amounts of anthocyanins of 45.5 mg/l were extracted, compared with the above procedures (V1, V2 or V3), which represents approximately 20% of the amount obtained by the classic maceration procedure (Variant V1). The data obtained shows that maceration-fermentation variants involving the use of low temperatures are not recommended for production of red wines with a high phenolic content. The anthocyanin content was lower than that reported by Gómez-Míguez *et al.* [17] for Syrah wines elaborated with pre fermentative cold maceration. These recommendations are supported by low values of total polyphenolic content, anthocyanins and colour parameters obtained by this technology. A high efficiency in extraction of phenolic compounds has the classical maceration and maceration in rotating tanks. The colour intensity and nuance reflect the important information regarding the wine age and maceration condition. The highest value of colour intensity was achieved for variant V2 (rotative tanks maceration) and the lowest for variant V4 (cryomaceration). These results are in line with ones reported by others [16, 18].

The anthocyanins in red wines bring their several qualities highly valued by red wines consumers. In anthocyanin composition the nine anthocyanins were identified as follows: five 3-monoglucosides, delphinidin (Dp-3-gl), cyanidin (Cy-3-gl), petunidin (Pt-3gl), peonidin (Pn-3-gl) and malvidin (Mv-3-gl); two aceticacid-acylated 3-monoglucosides, peonidin (Pn-3-gl-ac) and malvidin (Mv-3-gl-ac) and two p-coumaric acid esters of the 3-monoglucosides, peonidin (Pn3-gl-pcum) and malvidin (Mv-3-gl-pcum). Table 3 shows the concentrations of the above mentioned anthocyanins (mg/l), together with standard deviations. The measured differences between the maceration treatments proved to be statistically signicant when analyzed by the ANOVA for p level lower then 0.05. These results are in agreement with the literature for most *Vitis vinifera* L. varieties[19-21].

The major participant to colour composition is malvidin (Mv-3-gl) which represents approximately 60.2% (V1), 48.9% (V2) 53.1% (V3) and 84.1 % (V4). Petunidin (Pt-3gl) and peonidin (Pn-3-gl), that give the red colour of wines, are in percentages of 2.0 to 13.0% and 4.3 to 13.5% for the studied variants. The maximum values were found for variant V2. Similarly, delphinidin (Dp-3-gl) represents from 0.9 to 9.5% of total anthocyanin compounds, its presence in red wines is very important because of its antioxidant and antiinflammatory properties. The results are in concordance with Sun *et al.* [22] for the Tinta Miúda red wines. Cyanidin Cy-3-gl, that gives red wine the purple colour, is found only in a proportion of 0.4 to 3.4% for the four variants studied. As previously reported cyanidin-3-glucoside is the anthocyanin present in the lowest concentrations in *Vitis vinifera* L., with the exception of some varieties [23]. Other researchers also pointed out that cyanidin is considered to be the precursor of the other anthocyanidins in the metabolism of the vine [24].

To complete the anthocyanin evaluation also the acetyl and *p*-coumaroyl derivatives were quantified. Acylated forms are found in very small quantities and are a feature of each variety. The values of these acetylated forms differentiated depending on the technology applied. Because during the wine storage acetylated forms are more stable and resistant to condensation it is important to establish the anthocyanin fingerprint of red wines by using the sum of acetyl and *p*-coumaroyl derivatives and the ratio from acetyl and *p*-coumaroyl derivatives. Results showed that Fetească neagră variety is characterized by a higher amount of *p*-coumaroyl anthocyanins, making the ratio of acylated anthocyanins/*p*-coumaroyl anthocyanins to be subunitar. These findings are in accordance with previous studies for the wines made from *Vitis vinifera* cv. Tinto Fino [25], Tannat, Cabernet Sauvignon, and Merlot [24], Boğazkere and Öküzgözü [26].

When grape variety authentication is desired by using the ratio from acylated and cumaroyl anthocyanins the way how maceration process was done should be taken into account because it influences the ratio from acylated and cumarylated anthocyanins. The acylated peonidin (Pn-3-gl-ac) and malvidin (Mv-3-gl-ac) oscillates between 0.4 and 2.0% and between 4.0 and 7.6%, the differences were due to the specific conditions of maceration. The *p*-coumaroyl derivatives presented similar values which varied between 0.4 and 1.8% for the p-coumaric acid ester of the peonidin (Pn3-gl-pcum) and between 1.5 and 4.8% for the p-coumaric acid ester of the malvidin (Mv-3-gl-pcum). To know the percentage of the acylated derivatives which contribute to the wine anthocyanin profile their sum was calculated and ranged between 1.0 and 9.5%.

When the total amount of anthocyanin compounds of Fetească neagră was compared with the wines produced from other varieties under similar conditions it can be seen that the wines presented

Table 3. Anthocyanin profile of wines (mg/l).

Anthocyanins, mg/l	Variant 1 (V1)	Variant 2 (V2)	Variant 3 (V3)	Variant 4 (V4)
Cyanidin (Cy-3-gl)	3.6 ± 0.002^{a}	9.7 ± 0.011^{b}	4.6 ± 0.021^{c}	0.2 ± 0.001^{a}
Delphinidin (Dp-3-gl)	22.2 ± 0.019^{a}	26.5 ± 0.059^{a}	24.1 ± 1.012^{c}	0.4 ± 0.901^{a}
Petunidin (Pt-3gl)	23.4 ± 0.012^{a}	30.4 ± 0.202^{b}	31.2 ± 0.711^{a}	0.9 ± 0.331^{a}
Peonidin (Pn-3-gl)	25.7 ± 0.142^{c}	37.2 ± 0.042^{c}	29.1 ± 0.092^{c}	1.9 ± 0.716^{a}
Malvidin (Mv-3-gl)	140.6 ± 1.110^{a}	139.4 ± 2.512^{ab}	125.5 ± 5.001^{ab}	37.1 ± 3.442^{b}
Peonidin (Pn-3-gl-ac)	1.1 ± 0.162^{ns}	5.4 ± 0.094^{ns}	2.1 ± 0.730^{ns}	0.9 ± 0.642^{ns}
Malvidin (Mv-3-gl-ac)	1.3 ± 0.014^{ns}	21.6 ± 0.117^{ns}	6.9 ± 0.604^{ns}	1.8 ± 0.691^{ns}
Peonidin (Pn3-gl-pcum)	4.2 ± 0.084^{a}	4.1 ± 0.605^{ab}	3.5 ± 0.346^{b}	0.2 ± 0.260^{b}
Malvidin (Mv-3-gl-pcum)	11.3 ± 0.413^{ab}	10.2 ± 0.238^{c}	9.1 ± 0.032^{ac}	0.7 ± 0.900^{d}
Total anthocyanins, mg/l	233.4 ± 12.114^{a}	284.5 ± 9.032^{ab}	236.1 ± 6.079^{ab}	44.1 ± 9.086^{ab}

a,b,c different superscripts in the same row indicate significant differences at the $P < 0.001$ level. ns not significant.

higher anthocyanin contents than the Boğazkere and Öküzgözü wines [12], Tinto Fino [21], Tinta Miúda [18], and had lower anthocyanins than the Tannat [22].

Chemical characteristics of wines: The chemical characteristics of wines produced by the four maceration variants are presented in Table 4.

Regarding the alcoholic degree, the difference of 0.3% (v/v) was between thermomaceration (Variant V3) and classical maceration (Variant V1). All wines are fermented in order to make dry wines with levels less than 4 g/l residual reducing sugar. The residual reducing sugar level was between 0.63 to 2.20 g/l for the studied variants.

Comparing all used maceration procedures the lowest total acidity values are given by cryomaceration technology (3.65 g CH_3COOH/l). The opposite situation is the thermomaceration technology (4.00 g CH_3COOH/l). Volatile acidity values are very important because they indicate grape health status and condition of the wine category where they may be included. For red wines with protected geographic origin, volatile acidity shall not exceed the value of 1.2 g CH_3COOH/l. Up to this value volatile acids positively influence the taste of wine. In all wines made from Fetească neagră variety the volatile acidity was between 0.50 to 0.75 g CH_3COOH/l. The pH values of wines obtained with four variants of maceration were in the range from 3.48 to 3.51. By cryomaceration (V4) a decreasing of wine total acidity up to 3.65 g H_2SO_4/l was observed. By thermomacation, although acidity decreased to 3.80 g H_2SO_4/l (V3), the pH value remained at 3.51 indicating the presence of highly ionized acids in wine due to moderate detartration.

Regarding the SO_2 content the wines obtained by maceration methods in rotating tanks (V2) and by cryomaceration (V4) contain only 69.2 to 71.1 mg/l total SO_2, of which 20 to 25 mg/l as free SO_2. The combined amount of SO_2 is quite small.

In general, total extract content of red wines, which are produced in the Romanian vineyards have values between 18 and 30 g/l, depending on the grape variety, specific vineyard conditions, and the winemaking applied. In the case of variants V1 and V2 large quantities of substances that increase total extract were extracted from solid parts of the grape. The value of total extract for the variant V2 was 24.6 g/l. When maceration-fermentation was done in static wooden vessels (Variant V1), the extract decreased to 23.1 g/l. Wines made using thermomaceration (Variant V3) and cryomaceration (Variant V4) had lower extract values ranging between 19.9 to 21.7 g/l.

The results show that because of the less favourable climatic conditions of the year 2011 the obtained wines (V3 and V4) presented the extract values less than 23 g/l, and they cannot be included in wines with protected geographic origin category. Only wines of variants V1 and V2 are suitable for integration into wines with protected geographic origin category. Wines made by maceration-fermentation in rotating tanks (V2) and cryomaceration (V4) showed the highest values of glycerol content (8.8 to 9.0 g/l) due to yeasts that have found favorable conditions during alcoholic fermentation. The variants V1 (classical maceration) and V3 (thermomaceration) obtained the lowest values (7.9 to 8.2 g/l). We consider that the differences are small and Fetească neagră variety wines are rich in glycerol, which contributes favourably on their sensorial properties.

Sensorial analysis of wines: From sensorial point of view, the wine obtained through variant V1 presented a crystalline clarity and a glossy red - intense ruby colour. The primary flavour is predominant, which determined freshness and fruitiness of the wine. The taste is balanced, healthy, less evolved, but not astringent. Wine obtained by variant V2 presented a crystalline clarity, brilliant and intense ruby red colour compared with variant V1. Aroma was pleasant, pregnant floral (flavour specific of grapes flower). The taste is pleasant, healthy, lively and very well balanced. High glycerol content and total extract gives them consistency and smoothness that are easily perceived, giving more quality taste. The wine is well structured with less tannins and less bitter flavour and less astringent. These results are in agreement with other analytical data obtained (Fig. 1).

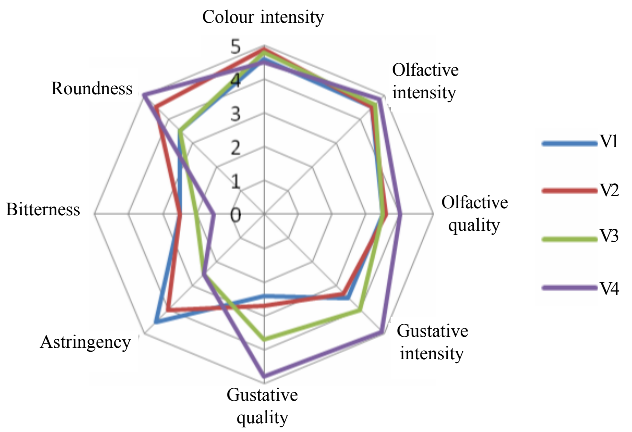

Figure 1. Sensorial profile of wines.

Wine obtained by variant V3 had a crystalline clarity, red - ruby colour as intense as in variant V1. In the smell, the presence of primary flavours is easily perceived, which gives a pronounced fruitiness. The taste was good, but no personality and overall harmony. Acidity, although having a normal value, stands out and gives them harshly and hardness. It seems to be less evolved and is lacking of finesse necessary to Fetească neagră wines.

Table 4. The physico-chemical characteristics of wines.

Characteristics	Variant 1 (V1)	Variant 2 (V2)	Variant 3 (V3)	Variant 4 (V4)
Alcoholic degree, % (v/v)	12.1 ± 0.201^{ns}	12.4 ± 0.115^{ns}	12.6 ± 0.008^{ns}	12.3 ± 0.511^{ns}
Reducing sugar, g/l	1.92 ± 0.500^{a}	0.84 ± 0.204^{b}	0.63 ± 0.111^{ab}	2.20 ± 0.340^{a}
Total acidity, g H_2SO_4/l	3.90 ± 0.301^{b}	4.00 ± 0.201^{ab}	3.80 ± 0.201^{b}	3.65 ± 0.201^{a}
Volatile acidity, g CH_3COOH/l	0.75 ± 0.008^{a}	0.65 ± 0.502^{a}	0.50 ± 0.550^{ab}	0.55 ± 0.400^{c}
pH	3.48 ± 0.006^{ns}	3.50 ± 0.009^{ns}	3.51 ± 0.011^{ns}	3.80 ± 0.028^{ns}
Free SO_2, mg/l	23.4 ± 1.504^{b}	20.1 ± 0.211^{ab}	22.6 ± 0.611^{b}	21.5 ± 1.009^{b}
Total SO_2, mg/l	71.1 ± 3.111^{a}	68.4 ± 1.634^{ac}	$81.3 \pm 3.007a^{bc}$	69.2 ± 5.284^{b}
Total extract, g/l	23.1 ± 1.211^{a}	24.6 ± 2.007^{b}	21.7 ± 1.700^{ab}	19.9 ± 0.551^{b}
Glycerol, g/l	7.9 ± 0.004^{a}	8.8 ± 0.622^{bc}	8.2 ± 0.158^{ab}	9.0 ± 0.044^{b}

a,b,c different superscripts in the same row indicate significant differences at the $P < 0.001$ level. ns not significant.

Wine obtained by variant V4 presented a crystalline limpidity, which confirms to all wines that are stable under chemical and biological aspect. The colour is red-ruby. The smell is quite evolved, pleasant, with a freshness that impress favourably. Taste, however, is short with a minimum of persistence. There is a favourable balance between the main taste components of wine, making it non tipic variety.

Conclusions

The concentrations of total polyphenols, anthocyanins, color intensity and nuance in Fetească neagră wines are significantly influenced by the method of grape maceration. The concentrations of Mv-3-gl, Mv-3-gl-ac and Mv-3-gl-pcum represent more than 50% of total anthocyanins in all variants of maceration. The wine processed by variant V2 presented better chemical and sensorial characteristics.

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