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ORIGINAL RESEARCH PAPER

COLOR ENHANCEMENT OF FETEASCĂ NEAGRĂ WINES BY USING PECTOLYTIC ENZYMES DURING MACERATION

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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 pectolytic enzymes addition during maceration fermentation on the chromatic characteristics of the Fetească neagră wine.
An increase in concentrations of anthocyanins was observed when pectolytic enzymes were used during maceration fermentation process.

Keywords: wine color, anthocyanins, enzymes, grape skins

Introduction

Phenolic compounds are a large group of very important molecules of red wines involved in the formation of color, flavor, body and structural characteristics (Monages *et al.,* 2006). Their evolution is influenced on the one hand by the rate of biosynthesis and on the other hand by the rate of transformation taking place under the influence of different factors (genetic, climate, soil, relief, etc.).
During the winemaking process from grape skins only a small fraction of phenolic compounds (30-50%) is extracted. This depends on the degree of the grapes maturation and the process of maceration. The total content of phenolic compounds in red wines is very variable ranging from 127-988 mg/l (Zou *et al*., 2002).
Wine polyphenols are divided into two main groups: flavonoids (anthocyanins, catechins, tannins and flavanols) and the non-flavonoids (benzoic acids, stilbenes and phenolic alcohols). Anthocyanins and compounds which are derived from them are mainly responsible for the color of the wines (Kelebek *et al.*, 2007).
Phenolic compounds present a high chemical reactivity and are involved in wine into many processes like: oxidation, condensation, polymerization and co-polymerization. The phenolic compounds are able to consume the oxygen in wine

and thus protect the wine from oxidation. Oxidation of phenolic compounds leads to their polymerization, a phenomenon that occurs during storage and aging of wine (Ribereau-Gayon, 1982).
In grapes, the anthocyanins are accumulated in different amounts, depending on the biological variety, degree of grapes maturation and ecological conditions of the vineyard. The anthocyanins content is expressed as the total amount of anthocyanins that is accumulated in grape berry and their degree of extractability from grape skins.
The winemaking techniques greatly influence the extraction of anthocyanins from which the skin contact time, temperature and enzyme preparation addition have the highest influence on the extraction of polyphenols during winemaking (Canal-Llauberes, 2000; Ribereau-Gayon *et al*., 2006).
The aim of this study was to study the effects of using enzymes with pectolytic activity on the extraction of anthocyanins in wines made from Fetească neagră grapes.

Materials and methods

Experiments were done on Fetească neagră grapes from Murfatlar vineyard, Constanta, Romania in climatic conditions of the year 2010.
To highlight how the addition of enzyme preparations with pectolytic activity influences the evolution of fermentation maceration at micro-winemaking level the experiment was done in polstif recipients with 10 liter capacity.
Fetească neagră wines obtaining are presented in Table 1 experimental variants and applied technology of.

Table 1. Technological variants used for experiments

Variants	Used biotechnology
Variant 1 (V1)	Destemmed and crushed grapes sulphited (100 mg/l SO_2)
Variant 2 (V2)	Destemmed and crushed grapes sulphited (100 mg/l SO_2) with the addition of pectolytic enzymes Endozym Ruby (1.5 g/100 kg grapes)
Variant 3 (V3)	Destemmed and crushed grapes sulphited (100 mg/l SO_2) with the addition of pectolytic enzymes Endozym Ruby (3.0 g/100 kg grapes)

All variants have in common the addition of sulfur dioxide SO_2 by using a concentration of 100 mg/l applied directly after destemming and crushing. For each variant, the experiments were repeated two times.
For enzymatic maceration, a pectolytic enzyme preparation was used. Endozym Ruby preparation produced by AEB Group, Spindal France presented good results in our previous research. The temperature during maceration fermentation was kept at 25°C.

Anthocyanins separation and quantification

Anthocyanins separation and quantification was done by using a method described by Budic Leto *et al.,* 2006.
The analyses 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. The separation of anthocyanins was carried out with the column AQUASIL C18 (4.6 x 250 mm, 5 µm) equipped with a pre-column AQUASIL C18 (4 x 10 mm, 5 µm).
The chromatographic method conditions were as follows: mobile phase flow 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 with three successive linear gradient mobile phases, as follows: from 6% to 30% B in 15 min, from 30% to 50% B in 15 min, from 50% to 100% B in 25 min, followed by washing and reconditioning of the column.
Identification of anthocyanins was obtained using authentic standards and by comparing the retention times and spectra with those found in the literature. All determinations were done in triplicate and the relative SDs were less than ±1%.
The total anthocyanins content in wines was determined using the method of Rigo *et al.* (2000) 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).

Statistical analyses

The statistical significance of the effect of the enzyme treatment on anthocyanins extraction was done by one way ANOVA using Statistica 8 (StatSoft, Inc.). The means between control and treated samples were compared at $P < 0.05$ by Fisher's least significant difference test.

Results and discussion

In Figure 1 is shown the amount of anthocyanins extracted expressed in mg/l for the three studied variants.
At variants sulfited with 100 mg SO_2/l, the presence of enzyme preparations with pectolytic activity is found to accelerate the effect of anthocyanin extraction. During maceration, anthocyanin extraction rate is faster on the first day with dose of enzyme preparation used is more highly visible for variant V3.
At the end of maceration-fermentation, the extraction of anthocyanin compounds in the case of variant V3 recorded the highst value of 479 mg/l.

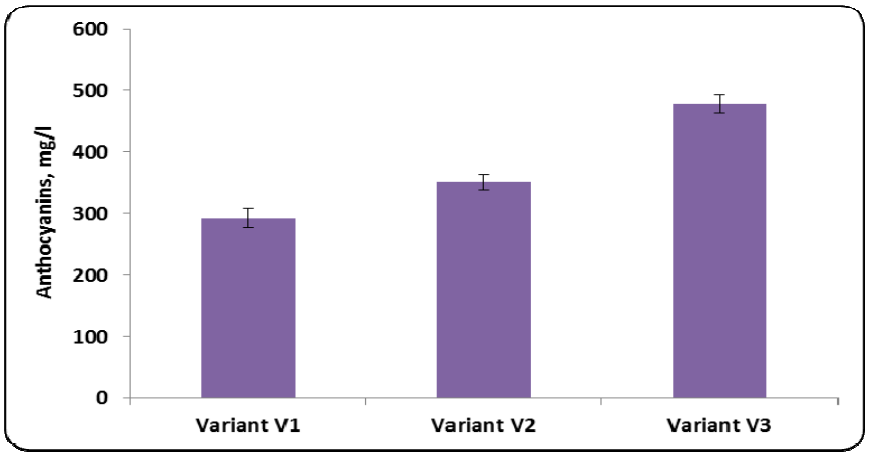

Figure 1. *Anthocyanins content of enzymatically treated and untreated variants*

For the studied variants during alcoholic fermentation the color intensity presented values ranging from 2.76 to 3.12 depending on the dose of enzyme preparation used (Figure 2). Under these conditions, the effectiveness of pectolytic enzyme preparations action is even higher as color intensity values are higher (this parameter is given by the sum of the optical densities measured for yellow, red and violet color).

The color intensity in the case of variant V3 was increased approximately by 11.5%, compared to variant V1 and by 4.8% compared to the variant V2.

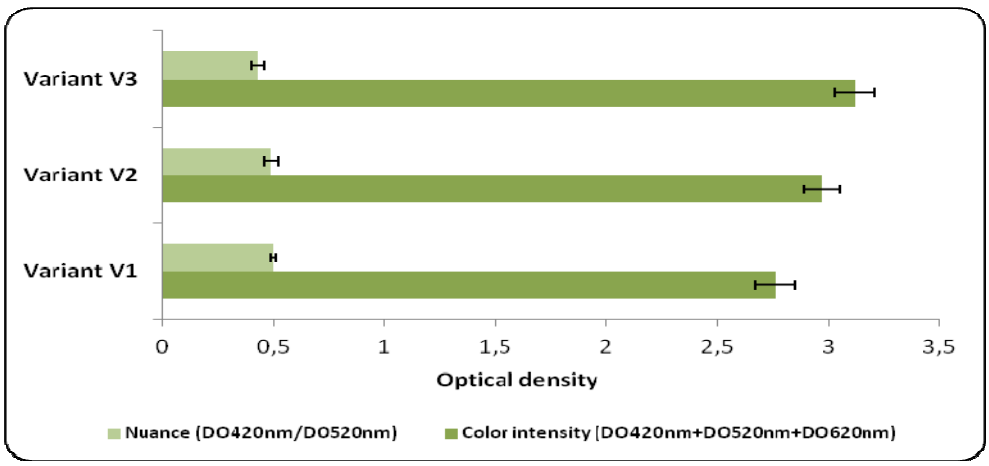

Figure 2. Color intensity and nuance of enzymatically treated and untreated variants

During the fermentation maceration an optimal time of enzymatic hydrolysis action of pecto-cellulosic cell walls is necessary to ensure, equivalent to a reduction in the time of pre-fermentative maceration for a secure and optimized extraction of color compounds.

Using enzyme preparations for maceration-fermentation process allows obtaining red wine with intense and harmonious taste and high amounts of color compounds.
Another aspect that stands out is the change of the ratio solid/liquid after the fermentation maceration operation (Figure 3).
As it can be seen in Figure 3, the addition of enzyme preparation allows to increase the liquid fraction extracted from crushed grapes treated with Endozym ruby enzyme preparation by using a dose of 3 g/100 kg grapes.
The enzymatic treatment by using Endozym ruby enzyme preparation ensures an increase in the total must yield by 6% and 8% when compared to variant V1 and to variant V2.

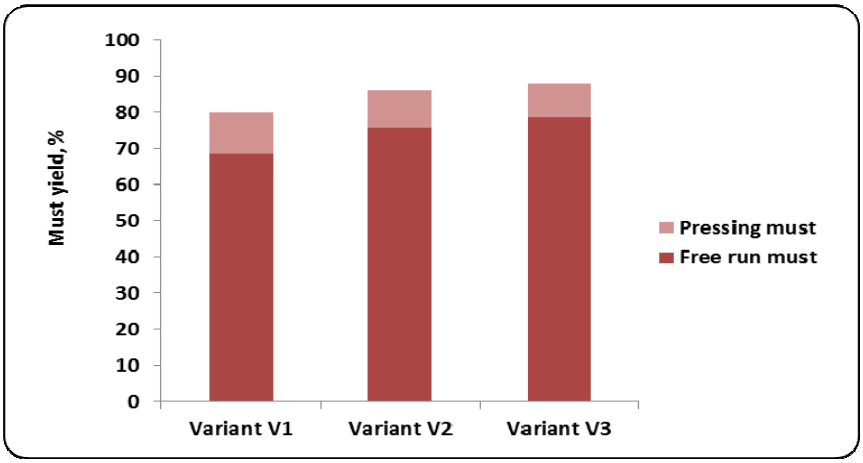

Figure 2. Comparative evolution of must yield after the treatment with Endozym ruby enzyme preparation

In terms of anthocyanins 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), two p-coumaric acid esters of the 3-monoglucosides: peonidin (Pn3-gl-pcum) and malvidin (Mv-3-gl-pcum).
Table 2 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 significant when analyzed by the ANOVA for p level lower than 0.05.
Analyzing this anthocyanins profile, it is found that for all maceration variants studied, malvidin is presented in the highest amount, followed by petunidin and peonidin. The amount of cyanidin is the lowest.
For all the three variants of maceration, although anthocyanins content is quantitatively different (250.2 to 438.9 mg/l), the proportions established between anthocyanins are very close.
The major participant in wine color composition is malvidin which represented

approximately 58.3%, 53.5% and 43.1% for the three all variants studied.

Table 2. *Anthocyanins profile of wines (mg/l)*

Anthocyanins, mg/l	Variant 1 (V1)	Variant 2 (V2)	Variant 3 (V3)
Cyanidin (Cy-3-gl)	2.5 ± 0.122^{a}	3.6 ± 0.342^{b}	7.2 ± 0.048^{a}
Delphinidin (Dp-3-gl)	18.3 ± 0.162^{a}	21.4 ± 0.162^{a}	41.2 ± 0.162^{c}
Petunidin (Pt-3gl)	27.6 ± 0.162^{a}	36.9 ± 0.162^{b}	81.4 ± 0.162^{a}
Peonidin (Pn-3-gl)	25.8 ± 0.162^{a}	39.3 ± 0.162^{c}	56.2 ± 0.162^{c}
Malvidin (Mv-3-gl)	145.9 ± 3.481^{a}	163.1 ± 2.188^{ab}	189.4 ± 2.699^{ab}
Peonidin (Pn-3-gl-ac)	2.4 ± 0.162^{ns}	2.6 ± 0.162^{ns}	4.9 ± 0.162^{ns}
Malvidin (Mv-3-gl-ac)	10.2 ± 0.162^{ns}	15.3 ± 0.162^{ns}	28.9 ± 0.162^{ns}
Peonidin (Pn3-gl-pcum)	5.3 ± 0.162^{a}	6.4 ± 0.162^{ab}	9.6 ± 0.162^{a}
Malvidin (Mv-3-gl-pcum)	12.2 ± 0.162^{ab}	15.7 ± 0.162^{ac}	20.1 ± 0.162^{c}
Total anthocyanins, mg/l	**250.2 ± 0.162^{a}**	**304.3 ± 0.162^{ab}**	**438.9 ± 0.162^{ab}**

ns - no statistically significant difference determined. The values followed by the same letter (a, b, c and d) indicate that they are not significantly different ($p<0.05$)

The peonidin and petunidin, which are responsible for the red color of wine, are in percentages from 12.2 to 15.0% and from 11.0 to 18.5%. The maximum values for petunidin and peonidin were found for variant V3.

Similarly, delphinidin content varied from 7.3 to 9.4% of the total anthocyanin compounds. Its presence in red wines is very important because of its antioxidant and anti-inflammatory properties.

The cyanidin that gives red purple color of wine was found and in a proportion from 1.0 to 1.6% for all the three variants studied.

In order to establish the full profile, anthocyanins also their acetylated and *p*-coumaroyl derivatives were determined (Table 2). Acyl forms are found in very small quantities and are a feature of each variety (Echeverry *et al.*, 2005).

The values of these acyl forms are differentiated depending on the technology used. In this study the acetylated and *p*-coumaroyl forms of peonidin and malvidin were quantified.

Because during maceration of pomace and wine storage period, acylated anthocyanins are more stable and resistant to condensation. In order to establish the anthocyanin fingerprinting of red wines, the sum and ratio of acetylated and *p*-coumaroyl anthocyanins have to be calculated (Kelebek *et al.,* 2007).

Results showed that Fetească neagră variety is characterized by a higher amount of *p*-coumaroyl anthocyanins, making the ratio acylated anthocyanins/*p*-coumaroyl anthocyanins be subunitar.

When the grape variety authentication is desired by using the ratio from acylated and p cumaroyl anthocyanins the way how maceration process was done should be taken into account because it influences the ratio. The acylated peonidin (Pn-3-gl-ac) and malvidin (Mv-3-gl-ac) ranged between 0.85 and 1.11% and of between 0.4 and 6.5%. The differences between values were due to the specific conditions of maceration, in this case the addition of enzyme preparations with pectolytic enzyme activity (Endozym ruby, 3 g/100 kg grapes).

The *p*-coumaroyl derivatives presented similar values which vary between 0.54 and

2.08% for the p-coumaric acid ester of the peonidin (Pn3-gl-pcum) and between 1.85 and 5.51% 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 anthocyanins profile, their sum was calculated and was ranging between 5.50 and 7.71%.

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 higher anthocyanin contents than the Boğazkere and Öküzgözü wines (Kelebek *et al.,* 2007), Tinto Fino (Revilla and González-Sanjose, 2001), Tinta Miúda (Sun *et al.*, 2001), and had lower anthocyanins than the Tannat (Echeverry *et al.*, 2005).

Conclusions

Addition of pectolytic enzymes before maceration fermentation presents an important role for antocyanins content in final wine.

The total content of antocyanins was 293 mg/l for variant V1. Due to the use of pectolytic enzymes, the total antocyanins content increased to 351 mg/l for variant V2 and to 479 mg/l for variant V3.

Analyzing the anthocyanin profile of wines, it was found that for all maceration variants studied, malvidin is found in the highest proportion, followed by petunidin and peonidin, the amount of cyanidin being the lowest.

Thus, regarding the three variants of maceration, although anthocyanins content is quantitatively different, the proportions established between anthocyanins are close.

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