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Improved Chromatic and Sensory Characteristics of Plavac Mali Wines – Efficiency of Maceration Enzymes

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Abstract

Alpeza I., Kovačević Ganić K., Vanzo A., Herjavec S. (2017): Improved chromatic and sensory characteristics of Plavac Mali wines – efficiency of maceration enzymes. Czech J. Food Sci., 35: 236–245.

Two commercial enzyme preparations were used in the production of wine from the Croatian autochthonous red grape variety Plavac Mali in order to improve the extraction of polyphenolic components from grapes, chromatic parameters, and sensory quality. During two vintages, the conventional maceration without enzymes was compared with the maceration using products with different characteristics: pectinase with additional cellulase and hemicellulase activity and pectinase with inactive yeast cells. Both products affected polyphenolic extraction and colour parameters: intensity and hue, and ratio between the yellow, red, and blue colour in young wines (2 months after fermentation) and at the moment of bottling (9 months after fermentation). The correlation between anthocyanins and colour intensity was very strong. The expected reduction of quantitative chromatic parameters during aging was confirmed. Significantly better results were observed in wines produced with pectinase, in relation to all analysed physical and chemical parameters. The sensory analysis showed that wines produced with pure pectolytic enzymes were significantly better than those produced without the enzymes. A product of the combination of pectolytic enzymes and inactive yeast cells had a partial influence on the improvement of the phenolic and sensory quality. The overall quality was significantly more expressed in wines produced with pectolytic enzymes, especially in young wines.

Keywords: insufficient grape quality; exogenous enzymes; anthocyanins; wine colour; sensory quality

The phenolic composition of red wines is a primary factor of their sensory quality; their visual identity and their harmony, which primarily depends on the balance between bitterness and sourness in relation to other wine ingredients. In the traditional wine quality perception, very colourful wines rich in extracts and full taste are valued. This implies ensuring the conditions which enable maximum extraction of phenolic compounds from grapes in order to provide a high colour intensity and stability during the aging period. There is a total of 2–11 g/kg of phenols in red grapes, but the level of phenols in wine rarely reaches 50% of the grape level. Furthermore, phenols make only 1% to 5% of the chemical composition of wine, i.e. between 0.6 and 4 g/l (Kennedy 2006). The polyphenol composition, which also includes anthocyanins as the most important pigments responsible for the colour of red grapes and wine, depends on numerous factors. The use of the pectolytic enzymes in maceration is an acknowledged oenological procedure and its purpose is the enhanced extraction of phenol compounds, especially anthocyanins, and improvement of colour characteristics (Ough & Berg 1974). The enzymes can affect the wine complexity, they enable to improve characteristics of young wines, stability of colour during aging and taste and

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structure of wine due to the extraction of tannins. These preparations are mixtures of pectin lyase (PL), pectin methylesterase (PE), polygalacturonase (PG), hemicellulase, cellulose, and protease. Their activity during maceration is present in breaking down the cellular structure of the grape skin releasing anthocyanins and copigmentation cofactors. Various results of their implementation are presented in the literature (Watson *et al*. 1999; van Rensburg & Pretorius 2000; Canal-Lauberes & Pouns 2002; Revilla & González-Sanjose 2002; Bautista-Ortin *et al*. 2005; Kelebek *et al*. 2007; Gil-Muñoz *et al*. 2009; Ortega-Heras *et al*. 2012; Rio Segade *et al*. 2015). The efficiency of the enzyme use depends on the conditions of maceration, as well as on the characteristics of commercial preparations, their impurity, and potential contamination with other enzymes, beta-glucosidase or phenol esterases, which can have a negative side-effect (van Rensburg & Pretorius 2000).

Plavac Mali is a Croatian red, autochthonous, and very important grape variety, and is known as a variety with the great potential on a limited top locality, but it can also be a high yielding variety with average grape quality and average colour quality at the same time (Mirošević *et al*. 2008). Experiments reported in this article were initiated with *Plavac mali* to determine whether different commercial enzyme products would affect better chromatic properties and sensory quality of wines at the time of bottling, and because no experiments of this type have been reported with Plavac Mali grapes. Wines produced with two different macerating enzymes were compared with wines produced with classical maceration, without exogenous enzymes.

MATERIAL AND METHODS

Grape processing. Plavac Mali grapes used in the experiment originated from the central part of the Pelješac peninsula. In two vintage years, the grapes (400 kg) were picked at technological maturity and transported in 15 kg boxes to the Experimental Winery of the Faculty of Agriculture in Zagreb. The grapes were crushed and destemmed, and distributed into 9 suitable containers, each treatment in triplicate. All samples were equally treated with 15 g/kg potassium metabisulphite. The selected dry yeast, Lalvin EC-1118 (Lallemand, Canada), was used in alcoholic fermentation of all samples in the amount of 20 g/ 100 l must. The Fermaid E yeast nutrient (Lallemand, Canada) was added in the amount of 25 g/100 l must. The alcoholic fermentation was regularly monitored by measuring the sugar content and temperature, and the must temperature was kept at about 20°C during the maceration and fermentation process. After seven-day fermentation and maceration with plunging twice a day all wines were separated from skins on a wine press providing free-run and pressings, then the wines were homogenised and stored at a cellar temperature. The wines were racked twice: for the first time one month after fermentation and then in February. Potassium metabisulphite was added each time (10 g/hl). The wines were bottled after the second decanting cycle.

Enzymatic treatment. Two different commercial pectolytic enzyme preparations were used in this experiment; Lallzyme EX-V (Lallemand, France) and Red Style (Lallemand, Estonia). The preparations were used in the recommended doses: Lallzyme EX-V: 2 g/100 kg of grapes (E1), and Red Style in a dose of 30 g/100 kg of grapes (E2), dissolved in water at a 1 : 10 ratio. The E1 preparation is a pectinase preparation with hemicellulase and cellulase considered as secondary activities. Its standard composition includes 4000 Pgu/g (PG, EC 3.2.1.15), 1000 Peu/g (PE, EC 3.1.1.11), and 120 Plu/g (PL, EC 4.2.2.10). It is extracted from *Aspergillus niger*, as a pectinase with a specific impact on the improvement of colour intensity and stability, and phenol extraction. The E2 preparation comprises pectinases and inactive *Saccharomyces cerevisiae* yeast cells. Thanks to the synergy of compounds, such a combination should ensure improved efficiency of the enzymes during maceration and phenol quality of the future wine. The enzymes were added to homogenised crushed and destemmed grapes at the beginning of maceration. The control sample (C) underwent a common 7-day fermentation and maceration process in the same conditions, but without the addition of enzymes.

Analysis of colour parameters and sensory evaluation. The main parameters of the must and wine quality were analysed by OIV methods (2006). Absorbance (A) measurements were performed with a Perkin Elmer UV/VIS, Lambda 20 spectrophotometer, with 0.2 cm path length glass cells. Colour intensity (CI) was calculated as the sum of absorbances at 420, 520, and 620 nm (Glories 1984), and hue (CH) as the ratio between absorbance at 420 nm and absorbance at 520 nm. The percentages of yellow (A_{420}/CI × 100), red (A_{520}/CI × 100), and blue (A_{620}/CI × 100)

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pigments were calculated as well (Glories 1984). Specific anthocyanins were extracted and identified by the high performance liquid chromatography (Agilent HPLC 1100), with DAD detector and NDS ChemStation (Agilent Technologies, USA) according to the method reported by Vanzo *et al.* (2008). Sensory evaluation was organised in controlled conditions according to ISO 8589 standard:2007. Seven oenologists who are professional wine tasters and were also granted their competence in the knowledge of the *Plavac mali* variety were assessors. Samples were stored for two days in the refrigerator at 16°C before sensory analyses. The comparative methods that were used included ranking and paired comparison test (Amerine & Rossler 1976). Each of the methods was used for the assessment of colour parameters, quality and intensity, and overall quality.

Statistical analysis. The data were subjected to an analysis of variance to test the effect of pectolytic enzymes. For comparison of mean values, Duncan's multiple range test was applied. The statistical analyses were performed using the Statistica V.7 software (Statsoft Inc., USA).

RESULTS AND DISCUSSION

The basic wine quality parameters are shown in Table 1. The wines were dry (residual sugars less than 2 g/l) with volatile acidity from 0.3 to 0.4 g/l (acetic acid) and total SO_2 from 111 to 126 mg/l. No significant differences were determined in the basic parameters. Since in both years the grapes used in the experiment were an excellent representative of the 'mass', i.e. they were of the average quality regularly used for the production of Plavac Mali quality wine and since the grape vines suffered from a mild form of powdery mildew and botrytis, the basic quality parameter data were consistent with official wine standards for the Plavac Mali wines of the same origin.

Anthocyanins. Both wines produced by maceration with enzymatic treatments were richer in anthocyanins compared to maceration without enzymes (Table 2 and 3). A significant difference was observed between the control wines and the wines produced by maceration with pectolytic enzymes (E1) in both vintages. The difference in the concentration of anthocyanins between the control sample and E2 treatment samples was significant in one vintage, but E2 treatment samples had a higher anthocyanin concentration in both vintages. In all wines, regardless of the treatment, the anthocyanin profile was dominated by malvidin-3 monoglucoside, which is usual for *Vitis vinifera* varieties (Wulf & Nigel 1978). A substantially higher concentration of anthocyanin and its forms was obtained in treatment samples than in the control sample. In both experimental years, the exogenous enzymatic activity largely influenced the increase of anthocyanin monoglucosides, monoglucoside para-coumarates, whereas the increased concentration of acetate forms of anthocyanin was statistically inconsistent, despite larger absolute values. It is important to emphasise that the analyses of anthocyanins were carried out on samples which had been frozen, which could be a cause of variations in results. Therefore, it is necessary to pay a special attention to the unfreezing process and preparation of samples for the analysis in order to ensure consistency and redissolving of tartrates.

Wine colour. A higher colour intensity was obtained in samples with both enzymatic treatment in comparison with the control samples (Table 4) in

Table 1. Basic quality parameters of *Plavac mali* wines (means of three replications)

Parameter	C/V1	E1/V1	E2/V1	C/V2	E1/V2	E2/V2
Alcohol (vol%)	11.2 ± 0.1	11.2 ± 0.1	11.0 ± 0.1	10.6 ± 0.1	10.7 ± 0.1	10.7 ± 0.1
Reducing sugars (g/l)	1.1 ± 0.3	1.1 ± 0.2	1.6 ± 0.3	1.7 ± 0.4	1.7 ± 0.4	1.8 ± 0.3
Total dry extract (g/l)	18.7 ± 0.8	19.1 ± 0.6	18.4 ± 0.7	22.2 ± 0.7	21.3 ± 0.9	21.1 ± 0.9
Ash (g/l)	2.1	2.1	2.1	3.1	3.0	3.0
Total acidity (g/l, tartaric acid)	5.8 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	6.3 ± 0.1	6.1 ± 0.1	6.1 ± 0.1
pH	3.4	3.4	3.4	3.8	3.8	3.8
Volatile acidity (g/l, acetic acid)	0.3	0.3	0.3	0.4	0.4	0.4
Total SO_2 (mg/l)	116 ± 8	119 ± 9	123 ± 8	126 ± 11	111 ± 9	116 ± 12

C – maceration without enzyme addition; V1 and V2 – vintages; E1 – maceration with pectinase preparation; E2 – maceration with pectinase and inactive yeast cell preparation

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Table 2. Composition of individual anthocyanins in *Plavac mali* wines (mg/l)

Anthocyanin	C/V1	E1/V1	E2/V1	C/V2	E1/V2	E2/V2
Delphinidin 3-monoglucoside	5.60 ± 0.41	7.01 ± 0.28	6.43 ± 0.86	11.30 ± 0.19	12.05 ± 0.82	11.05 ± 0.56
Cyanidin 3-monoglucoside	0.512 ± 0.03	0.63 ± 0.04	0.69 ± 0.18	1.97 ± 0.21	2.46 ± 0.22	2.36 ± 0.16
Petunidin 3-monoglucoside	11.57 ± 0.27	13.56 ± 0.16	12.47 ± 0.74	22.63 ± 0.12	24.95 ± 0.28	23.18 ± 0.15
Peonidin 3-monoglucoside	6.20 ± 0.49	6.89 ± 0.11	6.66 ± 0.39	8.93 ± 0.15	8.67 ± 0.45	8.76 ± 0.21
Malvidin 3-monoglucoside	96.48 ± 0.59	106.48 ± 2.37	100.26 ± 0.94	141.44 ± 0.57	147.00 ± 1.72	148.16 ± 2.25
Delphindin 3-monoglucoside acetate	0.39 ± 0.10	0.37 ± 0.01	0.35 ± 0.13	1.84 ± 0.25	2.21 ± 0.20	1.65 ± 0.04
Cyanidin 3-monoglucoside acetate	0.10 ± 0.02	0.13 ± 0.04	0.12 ± 0.05	1.43 ± 0.35	1.24 ± 0.32	0.81 ± 0.15
Petunidin 3-monoglucoside acetate	0.39 ± 0.01	0.42 ± 0.01	0.43 ± 0.09	1.71 ± 0.16	2.04 ± 0.04	1.56 ± 0.02
Peonidin 3-monoglucoside acetate	0.84 ± 0.09	2.30 ± 0.02	1.73 ± 0.38	4.71 ± 0.78	5.00 ± 0.73	3.55 ± 0.11
Malvidin 3-monoglucoside acetate	4.55 ± 0.03	4.91 ± 0.19	4.53 ± 0.34	6.91 ± 0.36	7.38 ± 0.04	7.19 ± 0.22
Malvidin 3-(6-*O*-caffeoyl)-glucoside	1.41 ± 0.02	1.57 ± 0.05	1.48 ± 0.09	3.09 ± 0.02	3.90 ± 0.02	2.90 ± 0.18
Delphinidin 3-monoglucoside *p*-coumarate	0.77 ± 0.01	0.81 ± 0.04	0.57 ± 0.16	3.60 ± 0.12	4.00 ± 0.08	3.84 ± 0.20
Cyanidin 3-monoglucoside *p*-coumarate	0.02 ± 0.00	0.02 ± 0.00	0.08 ± 0.03	1.77 ± 0.29	2.17 ± 0.16	1.81 ± 0.15
Petunidin 3-monoglucoside *p*-coumarate	0.82 ± 0.01	0.95 ± 0.04	0.82 ± 0.10	2.84 ± 0.12	3.64 ± 0.04	3.04 ± 0.01
Peonidin 3-monoglucoside *p*-coumarate	1.58 ± 0.11	1.81 ± 0.02	1.65 ± 0.10	4.77 ± 0.06	5.26 ± 0.21	4.94 ± 0.20
Malvidin 3-monoglucoside *p*-coumarate	10.60 ± 0.16	12.00 ± 0.44	11.16 ± 0.29	22.11 ± 0.49	23.36 ± 2.11	24.45 ± 0.22

C – maceration without enzyme addition; V1 and V2 – vintages; E1 – maceration with pectinase preparation; E2 – maceration with pectinase and inactive yeast cell preparation

both vintages, immediately after fermentation and pressing. A significant difference was established between the samples treated with pure pectolytic enzymes (E1) and control samples, whereas there was no significant difference between the control samples and samples treated with a combination of pectolytic enzymes and inactive yeast cells (E2). After the first racking, the colour intensity of wine samples with both treatments was higher than the colour intensity of control samples, despite the decrease and significantly lower values in all young wine samples and regardless of the treatment and vintage. There was a statistically significant difference between the control sample and E1 treatment sample in both vintages. E2 treatment sample significantly differed from the control sample in the first vintage, whereas this was not the case in the second vintage. The aging process additionally reduced the initial differences. At the time of bottling, the absolute values were higher in samples with both treatments in comparison with

Table 3. Content and forms of specific anthocyanin compounds (mg/l)

Anthocyanins	C/V1	E1/V1	E2/V1	C/V2	E1/V2	E2/V2
Delphinidin	6.76^{a}	8.19^{a}	7.34^{a}	16.74^{b}	18.16^{a}	16.53^{b}
Cyanidin	0.64^{b}	0.78^{b}	0.92^{a}	5.16^{a}	5.87^{a}	4.98^{a}
Petunidin	12.79^{b}	14.91^{a}	13.71^{a}	27.18^{b}	30.64^{a}	27.77^{b}
Peonidin	8.61^{b}	10.99^{a}	10.03^{a}	18.42^{a}	18.92^{a}	17.26^{a}
Malvidin	113.04^{b}	125.00^{a}	117.43^{a}	173.59^{b}	181.75^{a}	182.70^{a}
Monoglucosides	120.37^{c}	134.55^{a}	126.49^{b}	186.26^{b}	195.13^{a}	193.49^{a}
Acetates	6.26^{b}	8.14^{a}	7.16^{a}	16.59^{a}	17.87^{a}	14.77^{b}
Monoglucoside p-coumarates	13.78^{b}	15.58^{a}	14.31^{a}	34.58^{b}	38.44^{a}	37.14^{a}
Total	141.84	159.86	149.65	241.03	255.33	249.23

C – maceration without enzyme addition; V1 and V2 – vintages; E1 – maceration with pectinase preparation; E2 – maceration with pectinase and inactive yeast cell preparation; $^{a–b}$mean values within a single line marked by different symbols significantly differ according to Duncan's test ($P < 0.05$)

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the control sample, but without any significant difference. The analysis of the relative relationships in colour intensity between the treatments and aging shows that the highest decrease in colour intensity occurred in the most efficient treatment. However, the relative decrease in relation to pressing/bottling in wines with standard maceration amounted to 39% in the first vintage and 45% in the second vintage. In E2 treatment samples, the decrease amounted to 44% in the first vintage and 49% in the second vintage. The highest relative decrease of 54 and 50% was recorded in E1 treatment samples.

The colour hue refers to colour changes according to orange tones which come along in wine during the aging process. After the initial difference which was significant, upon fermentation and racking, as expected, a significant increase of the colour hue was observed in wines after the second racking. Although the lowest intensity of colour was observed in the control sample wines in all phases of testing and analysis, it was to expect that the values of the colour hue were the highest in such wines, but the results did not confirm that (Table 4). Contrary to the expectations, in both research years, the colour hue of such wines was the lowest right after pressing. It is to assume that specific enzymatic interactions, caused by the additional activities of contaminating foreign enzymes, occurred besides the additional enzymatic activity of exogenous enzymes. Moreover, the amount of haze in wine is very high right after pressing and it affects the value of relative absorbance. Therefore, the colour hue does not logically follow the colour intensity, as expected. In other two phases of sampling, the highest hue level was measured in standard wine samples, which confirmed the assumptions, since the impact of the unwanted enzymes of *Botrytis cinerea* and *Uncinula necator* fungi was lost during the aging process and the wines cleared on their own.

An increase of the colour hue value was observed in all young wines regardless of their treatment and vintage (Table 4). In both wines produced with enzymatic treatment, the measured values were smaller than in the control wines, with a statistically significant difference between the control sample and E1 treatment sample, but only in one vintage. No significant difference between the control sample and E2 treatment sample was observed in the young wines. The aging of the wine affected a further increase of the colour hue value. Although the relative values were better in wines with enzymatic treatment in terms of their quality, the statistical analysis did not confirm that. The significant difference was recorded only between E1 treatment sample and control sample in one research year.

Taking into consideration its domination, the red colour (% 520) has the most important place when it comes to the mutual relationship among individual colours, which was confirmed by the results (Table 4). The optimum ratio of colour percent-

Table 4. Percentages of colour intensity in Plavac Mali wines

Parameters	After fermentation			Young wine			Bottling		
	C	E1	E2	C	E1	E2	C	E1	E2
$I(A_{420} + A_{520} + A_{620})$, V1	0.781^{b}	0.982^{a}	0.871^{a}	0.633^{b}	0.685^{a}	0.668^{a}	0.474^{a}	0.485^{a}	0.486^{a}
$I (A_{420} + A_{520} + A_{620})$, V2	0.713^{b}	0.865^{a}	0.823^{a}	0.419^{b}	0.453^{a}	0.427^{b}	0.395^{a}	0.401^{a}	0.420^{a}
$H (A_{420}/A_{520})$, V1	0.676^{b}	0.774^{a}	0.795^{a}	0.700^{a}	0.706^{a}	0.669^{a}	0.941^{a}	0.924^{b}	0.957^{a}
$H (A_{420}/A_{520})$, V2	0.787^{a}	0.816^{a}	0.801^{a}	1.006^{a}	0.898^{b}	0.983^{a}	0.929^{a}	0.897^{a}	0.961^{a}
% 420 (yellow), V1	34.44^{a}	31.36^{b}	34.33^{a}	35.55^{a}	35.77^{a}	34.28^{b}	40.42^{a}	40^{a}	40.7^{a}
% 420 (yellow), V2	36.2^{a}	35.3^{b}	34.8^{b}	40.81^{a}	38.85^{c}	40.05^{b}	39.75^{b}	38.9^{c}	40.95^{a}
% 520 (red), V1	50.96^{a}	40.53^{b}	43.17^{b}	50.87^{a}	50.36^{a}	51.35^{a}	42.95^{a}	43.3^{a}	42.59^{a}
% 520 (red), V2	46.0^{a}	43.2^{b}	43.4^{b}	40.57^{b}	43.27^{a}	40.75^{b}	42.8^{a}	43.39^{a}	42.62^{a}
% 620 (blue), V1	14.6^{b}	20.7^{a}	22.5^{a}	13.59^{b}	14.01^{a}	14.37^{a}	16.6^{a}	16.7^{a}	16.67^{a}
% 620 (blue), V2	17.8^{b}	21.5^{a}	21.8^{a}	18.6^{a}	17.9^{b}	19.2^{a}	17.47^{a}	17.71^{a}	16.83^{b}

C – maceration without enzyme addition; V1 and V2 – vintages; E1 – maceration with pectinase preparation; E2 – maceration with pectinase and inactive yeast cell preparation; $^{a–b}$mean values within a single line marked by different symbols significantly differ according to Duncan's test ($P < 0.05$)

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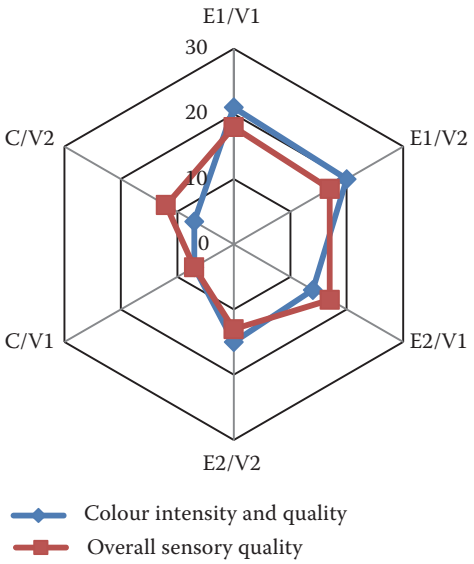

Figure 1. Sensory quality of Plavac Mali wines before bottling, ranking method

ages would be as follows: yellow – 35%, red – 55%, and blue – 10% (Glories 1984). In the first testing year, both wines with enzymatic treatment had a significantly higher share of the colour after pressing; E1 treatment samples had a 10% higher share of the red colour, and E2 treatment samples had an 8% higher share of the red colour compared to the wine produced by standard maceration. Besides the higher percentage of the red colour, a higher percentage of the blue colour was also observed. The lower percentage of yellow was measured in E1 samples with enzymatic treatment. After the first racking, the wines produced by maceration with the addition of pectolytic enzymes (E1) had the best red/blue/yellow ratio in favour of the red and blue colour. The ratio was close to the ideal according to Glories (1984). This trend continued even after the second racking. Despite small differences, the lowest percentage of the blue and the highest percentage of the yellow colour were observed in the control wines. When it comes to the wines with enzymatic treatments, the colour ratio was better in E1 treatment samples in comparison with the wines produced by maceration with a combination of enzymes and inactive yeast cells. In the second vintage, E1 treatment samples had a significantly higher percentage of the blue colour compared to the other treated samples and E2 treatment samples had the lowest percentage of the yellow colour. Although the highest percentage of the red colour was observed in wines produced by the standard maceration, in both wines produced by maceration with enzymatic treatment this difference was minor because of the increased percentage of the blue colour in the other two samples with enzymatic treatment (Table 4). These differences were reduced by aging, and the ratio between the red and blue colour in all wine samples with enzymatic treatment was equable and without any significant differences, with a significantly lower percentage of the yellow colour in the E1 treatment samples after the first racking. Despite the increased percentage of the yellow colour in all wine samples with enzymatic treatment, except E1 treatment samples, such a ratio was preserved after the second racking. However, it must be noted that the lowest percentage of the yellow colour after the second racking was repeatedly observed in the wines produced by maceration with pectolytic enzymes.

Effect of enzymes on sensory quality of wines. The sensory testing was performed in standardised conditions (ISO 8589:2007) by seven oenologists who are professional wine tasters and connoisseurs of the *Plavac mali* variety. Two methods were used for assessing the wines: paired comparison test and ranking. The paired comparison test is used when the characteristics of the tested samples are quite similar and it is also a tool for the assessment of wine tasters.

The following parameters were analysed: colour intensity, colour quality, and overall sensory quality. The wines produced by conventional maceration were ranked the lowest, whereas the wines produced by maceration with pectolytic enzymes were ranked the best for quality and intensity of colour (Figure 1). By using the ranking method in three replications, a significant difference in the colour parameter was observed at the level of 1% between the wines produced by conventional maceration and E2 treatment samples. No statistical difference was observed between the control samples and E2 treatment samples. Although no significant difference was observed between them, the wine tasters assessed better the wines produced by maceration with E1 enzyme treatment than the wines produced by maceration with E2 enzyme treatment. The ranking method was also used to assess the overall quality. According to the statistical tables (Amerine & Rossler 1976), a significant difference ($P < 0.01$) was recorded between the control wines and the wines subjected to E1 enzymatic treatment.

The results of the paired comparison test confirmed the results of the ranking method. The wines produced with the addition of pure pectolytic enzymes

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(E1) had a significantly better quality and intensity of colour ($P < 0.05$) compared to the control wine samples in both research years. No significant difference in colour parameters was observed between the wines produced by maceration with pectolytic enzyme (E1) and the wines produced by maceration with the addition of a combination of pectolytic enzymes and inactive yeast cells (E2) neither between the wines produced by conventional maceration and wines from E2 treatment. However, in the absolute sense, more wine tasters observed that the wines produced with enzymes had higher colour intensity than the control wine samples.

DISCUSSION

Numerous authors provide information in their researches about an undisputed effect on the increased extraction of anthocyanins, which is up to 50% higher in values obtained immediately after the completed maceration and fermentation, compared to the maceration without enzymatic treatment (Canal-Lauberes & Pouns 2002; Muñoz *et al*. 2004; Kelebek *et al*. 2007; Ortega-Heras *et al*. 2012; González-Neves *et al*. 2015; Rio Segade *et al*. 2015). However, a substantial decrease of anthocyanins was recorded in all wines already in the first months of the aging process, yet the samples treated with pectolytic enzymes produced better results (Wightman *et al*. 1997; González-Neves *et al*. 2001, 2003; Rosario-Salinas *et al*. 2003). The concentration of anthocyanins depends not only on the extraction during the maceration process but also on the level of degradation, which is related to their hydrolysis and oxidation which occur simultaneously (Monagas *et al*. 2005), and which can also be the cause of the decreased colour intensity. The loss of anthocyanins is also related to the yeast absorption (Morata *et al*. 2003) and settling of coloured tartrates.

When comparing the results of wine colour parameters in the present study with other values, similar results can be noticed. Several researchers obtained the best results in young wines (Bakker *et al*. 1999; Pardo *et al*. 1999; Guerrand & Gervais 2002; Bajard-Sparrow *et al*. 2005; Riponi *et al*. 2005; Sáenz-Navajas *et al*. 2006; González-Neves 2012). Despite the modifications during the aging process, the exogenous enzymatic activity can significantly affect the synthesis of copigments, which are important for the colour of young wines (Boulton 2001) and colour stability during aging (González-Sanjose *et al*. 2003; Guadalupe *et al*. 2007; Ortega-Heras *et al*. 2007; Ducasse *et al*. 2010).

It needs to be pointed out that a high level of linear correlation (0.86 and 0.93) between anthocyanins and wine colour intensity was obtained in the present study, which is in accordance with results of other researchers (Gao *et al*. 1997; Castillo-Sánchez *et al*. 2006; Toit & Visagie 2012). It is to note that the above-mentioned authors also included the wine aging as a factor in testing the relationship between the relevant parameters.

Several researchers presented similar results according to which the wines produced by maceration with enzymes have a higher colour intensity, and a higher percentage of the red and blue colour, with a lower percentage of the yellow colour and lower colour hue, which is also a sign of the higher stability of newly formed pigments which ensure the stability of purple nuances (Revilla & González-Sanjose 2001; González-Sanjose *et al*. 2003; Bautista-Ortin *et al*. 2005; Gambuti *et al*. 2007; Ortega-Heras *et al*. 2007; González-Neves *et al*. 2015). The wine colour plays a very important role as a characteristic of young red wine, which consumers recognise and appreciate (Mercurio *et al*. 2010). Lower chromatic values in all wines due to aging can also be related to the use of sulphur dioxide which has an important role in reducing quinone structures and/or producing colourless compounds (Boulton *et al*. 2001). Therefore, it is important to mention that the measuring was performed in wines after sulphiting.

Despite numerous researches, the scientific knowledge is still insufficient to be able to explain all structural modifications of the polyphenol composition, which occur during the extraction and aging, especially for anthocyanins (He *et al*. 2012). These processes include a large number of different chemical compounds which undergo different modification processes, from hydrolysis, condensation, copigmentation to settling, etc. This is also supported by the fact that the research results are rather inconsistent because of the effect of the added enzymes, their partial activity, up to undoubtedly positive results. The majority of the authors connects this diversity of results with differences between varieties and other technological procedures used in experiments, as well as with differences in the characteristics of commercial enzymatic preparations, their composition and

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activity, as well as with other unwanted effects due to the presence of other enzymes, such as β-glucosidase (Bautista-Ortin *et al*. 2005; Romero-Cascales *et al*. 2008; Romero-Cascales *et al*. 2012). These enzymes can affect the transformation of anthocyanins into less stable aglycones and result in the colour reduction (Sacchi *et al*. 2005).

The following authors also recorded better sensory characteristics in wines produced by maceration with pectolytic enzymes, but a different effect of the used enzymes: Bakker *et al*. (1999), Gambutti *et al*. (2007), Guadalupe *et al*. (2007), and Kelebek *et al*. (2007). Besides the above-mentioned methods, other authors also used the descriptive sensory analysis which enabled them to specify particular descriptive attributes (Guerrand & Gervais 2002; Bautista-Ortin *et al*. 2005; Bucelli *et al*. 2006; Espejo & Armada 2010) and describe the wines produced by maceration with specific enzymes which have similar characteristics like the wines used in this research as not only having better chromatic characteristics, but also less herbal, less sour and bitter, and more balanced.

CONCLUSION

The enzymatic preparations used in this research showed different effects on the quality of Plavac Mali wines in all tested parameters: total and individual anthocyanins, colour hue and intensity, share of the red, yellow and blue colour, and sensory characteristics. The enzymatic preparation with the addition of inactive yeast cells (E2) did not show stability in ensuring the enhanced quality of the tested parameters when compared to the pure enzymatic preparation (E1), whose application resulted in all produced wines having significantly better characteristics than the wines produced without the addition of enzymes. The positive effect of maceration with the addition of pectolytic enzymes has been established, not only in young wines, but also in wines before bottling, at the time when the quality wines of this variety are usually on the market. Although the final quality of the wines was affected by a large number of factors, the exogenous enzymatic activity is an important 'benefactor' of quality, provided that there is respectable knowledge of the characteristics of preparations and grape variety and that the conditions of the particular production year are closely monitored. In that sense, but keeping in mind a large number of types within the same variety, especially in case of the Plavac Mali variety, the attention must be paid to the selection and promotion of those types with a better ratio between the active surface of the skin and the flesh of the grape in order to enhance the efficiency of enzymatic activity.

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