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Influence of Microoxygenation Treatment on the Phenolic Composition of the Plavac Mali Wine

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Abstract

Microoxygenation allows the addition of small, continues and controlled amounts of oxygen, in order to improve wine quality. The objective of this study was to assess the influence of microoxygenation technique on the colour and phenolics composition of a Plavac mali wine (*Vitis vinifera* L.) when microoxygenation treatment was applied before malolactic fermentation. Colour and phenolics were determined by UV-VIS spectrophotometry and high-performance liquid chromatography coupled with diode array detection. The results showed that the microoxygenation treatment changed anthocyanins composition of wines. In microoxygenated wine the amount of monomeric anthocyanins decreased whereas the amount of polymeric anthocyanins increased. Comparison between the control and microoxygenated wines demonstrated changes in colour characteristics.

Key words: colour, microoxygenation, phenols, red wine

Utjecaj procesa mikrooksigenacije na fenolni sastav vina Plavac mali

Sažetak

Proces mikrooksigenacije podrazumijeva dodatak malih, kontinuiranih i kontroliranih udjela kisika u vino s ciljem poboljšanja njegove kakvoće. Cilj ovoga rada bio je odrediti utjecaj mikrooksigenacije, koja je provedena prije jabučno-mliječne fermentacije, na boju i fenolni sastav vina Plavac mali ((*Vitis vinifera* L.). Boja i fenolni sastav određeni su pomoću UV-VIS spektrofotometrije i tekućinske kromatografije visoke djelotvornosti. Rezultati su pokazali da primjena mikrooksigenacije utječe na sastav antocijana vina. Kod vina koje je podvrgnuto mikrooksigenaciji udjel monomernih antocijana se smanjio dok se udjel polimernih antocijana povećao. Usporedbom rezultata kontrolnog i mikrooksigeniranog vina utvđeno je da mikrooksigenacija utječe na boju vina.

Klučne riječi: boja, mikrooksigenacija, fenolni spojevi, crno vino

Introduction

Microoxygenation is a process during which measured amounts of oxygen is introduced to wines with the aim of bringing desirable changes. Some of these include enhanced colour stability and intensity, softening of astringent tannins and decreased reductive and vegetative aromas (Parish et al., 2000). Oxygen play an important role in different physicochemical and microbiological processes that take place during

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fermentation and aging of wines. This is due to the important role that oxygen plays in oxidation, condensation and polymerization reactions in which different compounds (mainly phenolic compounds) are involved (Perez-Magarino et. al., 2007). Traditionally, oxygen supply takes place indirectly, through different processes such as pump over and racking. However, the amount of oxygen provided by these treatments is difficult to control. Microoxygenation is a technique developed in France (Madeiran) by Patrick Ducournau and Michael Moutounet in the early nineties. Pure oxygen was slowly diffused through a ceramic membrane placed close to the bottom of stainless steel tank. This diffuse allowed a slow and constant flow rate of few milliliters of oxygen per liter of wine per month, permitting the wine phenols to consume the oxygen without acquiring oxidative characteristics (Roig and Yerle, 2003). Addition of oxygen must be controlled since an excess of oxygen can give rise to negative effects, such as higher astringency, appearance of anomalous characters, phenol oxidation and negative microbial activities (Parish et al., 2000). The rate of oxygenation and total oxygen added depend on volatile sulfide, anthocyanin and tannin concentrations, and also ability of wine to consume this oxygen. Therefore, rate cannot be determined "a priori", and is in general indirectly related to the relative concentration of polyphenols and determined by tasting. This treatment requires continuous control of wine evolution in order to avoid negative results on wine quality. The purpose of the present work is therefore, to evaluate the effect of microoxygenation before malolactic fermentation on red wine colour and phenolics composition.

Material and methods

Microoxygenation treatment

The analysed wine was a red wine, made from *Vitis vinifera* var. Plavac mali, vintage 2006. Once alcoholic fermentation was over (nearly complete consumption of reducing sugars; < 2 g/L) and before the malolactic fermentation started the wine was distributed into four stainless steel tanks (5000 L) two tanks were saturated with N_2 (control) and two were microoxygenated. In all tanks the quantity of 5 g/hL ellagitanins (Ellagitan-Chene, AEB S.p.A., Italy) was added. Microoxygenation equipment provided by ECO2 (Oenodev, France) was used. The total amount of oxygen added in microoxygenated wine in 39 days was 40.1 mL O_2/L. After alcoholic fermentation, wines were tasted by professional tasters in order to establish their sensorial characteristics, and the appropriate microoxygenation doses were determined. Initially, the doses of oxygen applied were higher to eliminate some reductive compounds that sometimes appear just after alcoholic fermentation, and the vegetal characters of some wines, allowing a better fruity expression. After that, the flows were reduced to provide colour and tannin stabilization and to complete the structuring phase before malolactic fermentation started. The microoxygenation treatment was stopped when, in wine, the malolactic fermentation were carried out spontaneously. The temperature was controlled and maintained around 15 °C, volatile acidity and SO_2 were checked every two days while microoxygenation treatment was applied.

Analyses of phenolic compounds

Phenolic analyses included: total phenolics, flavonoids, catechins, total anthocyanins content, polymeric anthocyanins content and free anthocyanins.

Total phenols were evaluated as stated by Singleton and Rossi (1965) using Folin-Ciocalteau reagent. The quantification of total phenols was carried out using a calibration curve prepared with known amounts of gallic acid.

The flavonoids content in selected wine samples was determined spectrophotometrically according to the method of Lee et al. (2003).

Catechins were determined by their reaction with vanillin, and were expressed as mg/L (+)-catechin (Di Stefano et al., 1989).

The total anthocyanins content in wines was determined using bisulfite bleaching method (Ribereau-Gayon and Stonestreet, 1965).

Polymeric anthocyanins were determined spectrophotometrically according to the method of Mazza et al. (1999) and were expressed as mg/L malvidin-3-glucoside.

HPLC detection of free anthocyanins

The free anthocyanins content was determined with HPLC according to method of Berente et al. (2000). The wine samples were filtered through a 0.45 μm filter (Nylon Membranes, Supelco, Bellefonte, USA) before the HPLC analysis. Twenty microliters of each sample was injected for HPLC analysis using a Varian Pro Star Solvent Delivery System 230 (Varian, Walnut Creek, USA) and a Photodiode Array detector Varian Pro Star 330 (Varian, Walnut Creek, USA) using a reversed-phase column Pinnacle II C-18 column (Restek, USA) (250x4.6mm, 5μm i.d.). The following mobile phases were used: buffer: 10mM KH_2PO_4+H_3PO_4 to pH 1.6, solvent A: acetonitrile-buffer (5:95), solvent B acetonitrile-buffer (50:50). The oven temperature was 50 °C. Gradient eluation was applied at 1 ml/min flow-rate according to the program which described by Berente et al. (2000). Chromatograms were recorded at 518 nm. Detection was performed with a Photodiode Array Detector by scanning between 200-600 nm, with a resolution of 1.2 nm. Individual anthocyanins were identified by comparing their retention times and visible spectra with those of authentic standards. Quantitative determinations were performed using standard curves of malvidin-3-O-glucoside (Polyphenols, Sandnes, Norway). The data acquisition and treatment were conducted using the Star Chromatography Workstation Version 5 software. All analyses were repeated three times, and the results were expressed as mean values in milligrams per liter of wine ± SD.

Colour measurement

Colour intensity and hue were evaluated and stated by Glories (1984), measuring the optical density (OD) at 420 nm and 520 nm using Unicam Helios β spectrophotometer (Unicam Ltd., United Kingdom).

Results and discussion

Phenolic compounds and colorimetric parameters were analyzed at two stages; just at the end of alcoholic fermentation (initial sampling (T0)), and the same day on which application of the microoxygenation treatment finished (T1) and the malolactic fermentation started.

Table 1. Phenolic compounds and colour characteristics of Plavac mali wine.

Compounds		Control wine	Microoxygenated wine
	T0	T1	T1
Total phenols (mg/L GAE)	3110±7	3240±8	3200±5
Flavonoid phenols (mg/L GAE)	2975±5	3094±6	3067±4
Non flavonoid phenols (mg/L GAE)	134±1	146±2	132±1
Catechins (mg/L (+)-catechin)	2338±6	2421±7	2392±7
Total anthocyanins (mg/L)	296±1	257±2	249±2
Polymeric anthocyanins (mg/L malvidin-3-glucoside)	62±1	46±1	55±1
Colour intensity	1.57±0.15	1.35±0.09	1.42±0.14
Hue	0.71±0.21	0.63±0.06	0.58±0.5

T0-initial sampling; T1- the microoxygenation treatment was finished.

Table 1 gives the phenolics composition and colorimetric parameters of Plavac mali wine before malolactic fermentation started. The amount of phenolic compounds (flavonoid phenols, non-flavonoid phenols and catechins) in non-microoxygenated control wine and microoxygenated wine during the period of 39 days was slightly increased. The microoxygenated wine showed slightly lower phenolics composition that did non-microoxygenated counterparts.

On the other hand the amount of total anthocyanins in the same period was decreased. Microoxygenated wine showed lower amount of total anthocyanins (249±2 mg/L) than non-microoxygenated wine (257±2 mg/L). The losses of anthocyanins are in agreement with experimental studies carried out by Llaudy et.al.

(2006), who found lower concentrations of anthocyanins in microoxygenated wines than in no-microoxygenated ones.

The results showed higher amount of polymeric anthocyanins in microoxygenated wine than non-microoxygenated wine. These results made clear the influence of the microoxygenation treatment on the polymerization reactions, showing a more important formation in the microoxygenated wine. Castel et al. (2001) also found an increase in polymeric anthocyanins, concluding that the addition of oxygen activated the reactions among free anthocyanins and flavanols, forming new coloured compounds stable to changes of SO2 and pH.

Colour intensity is higher and colour hue is slightly lower in microoxygenated wines. This trend is according to the findings of Sartini et al. (2007). This lower value could be explained by the formation of red polymeric pigments. During microoxygenation some ethyl bridge compounds and condensation products that decrease the colour hue could be formed (Cano-Lopez et al., 2006).

Anthocyanins analysed were delphinidin-3-*O*-glucoside (Df-3-Gl); cyanidin-3-*O*-glucoside (Cy-3-Gl); petunidin-3-*O*-glucoside (Pt-3-Gl); peonidin-3-*O*-glucoside (Pn-3-Gl); malvidin-3-*O*-glucoside (Mv-3-Gl); delphinidin-3-*O*-acetylglucoside (Df-3-Gl-Ac); cyanidin-3-*O*-acetylglucoside (Cy-3-Gl-Ac); peonidin-3-*O*-acetylglucoside (Pn-3-Gl-Ac); malvidin-3-*O*-acetylglucoside (Mv-3-Gl-Ac); peonidin-3-*O*-coumarylglucoside (Pn-3-Gl-Cm); malvidin-3-*O*-coumarylglucoside (Mv-3-Gl-Cm). These compounds were quantified with Mv-3-Gl as standard because this is the most representative anthocyanin in wines.

Table 2. Anthocyanins concentration in Plavac mali wine (mean±standard deviation).

		Control wine	Microoxygenated wine
Anthocyanins (mg/L)	T0	T1	T1
Df-3-Gl	18.08±0.13	14.71±0.12	14.10±0.09
Cy-3-Gl	4.46±0.29	1.93±0.05	1.09±0.12
Pt-3-Gl	25.39±0.11	16.81±0.21	16.75±0.10
Pn-3-Gl	14.12±0.16	12.29±0.13	9.95±0.09
Mv-3-Gl	138.16±0.26	125.13±0.14	119.82±0.22
Df-3-Gl-Ac	7.09±0.09	6.87±0.07	5.84±0.08
Cy-3-Gl-Ac	2.11±0.10	1.54±0.15	0.79±0.05
Pn-3-Gl-Ac	1.84±0.06	1.46±0.10	0.85±0.16
Mv-3-Gl-Ac	10.25±0.23	8.32±0.05	6.98±0.15
Pn-3-Gl-Cm	5.11±0.09	4.59±0.14	3.50±0.19
Mv-3-Gl-Cm	15.31±0.10	12.89±0.22	13.26±0.09
Sum total	241.92±0.11	206.54±0.13	192.68±0.08

T0-initial sampling; T1- the microoxygenation treatment was finished.

Non-microoxygenated wine showed higher values in the monomeric anthocyanin glucosides, acetylated and cinnamylated (Table 2).

The malvidin-3-glucoside was the major anthocyanin in analysed wine and their concentration was higher in the non-microoxygenated wine.

Conclusions

Whereas a strong oxygenation increases wine oxidation, controlled microoxygenation with the same amount of oxygen could stabilize wine colour inducing the formation of blue-red polymeric pigments from the direct condensation of anthocyanins with other flavonoids or from a combination of pigments and flavonoids with ethyl bridges.

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