COPIGMENTATION AND COLOR OF BRAZILIAN MERLOT WINES I. INFLUENCE OF SKIN CONTACT TIME

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Abstract

Aim: To evaluate the impact of maceration time on color, polyphenols and copigmentation of Merlot wines in order to better understand the differences between two important Brazilian viticultural regions, Campanha Gaúcha and Serra Gaúcha, which have not been extensively studied.

Methods and results: Merlot grapes (red *Vitis vinifera* variety) from four vineyards located in the Campanha Gaúcha (n = 2) and Serra Gaúcha (n = 2) regions were harvested, crushed and fermented at the same temperature, with three different maceration times (4, 8 and 15 days). Wines from the 2009 and 2010 harvest were analyzed by spectrometry for phenolic composition and color. The results showed that the origin of the grapes had a higher influence on phenolic composition and color than the winemaking process treatments (i. e., maceration time). Maceration time did not affect copigmentation phenomena, and polymeric anthocyanins had the greatest correlation with color intensity.

Conclusion: The maceration time had different effects according to the wines studied. However, more studies will be necessary to identify the characteristics of the grapes and to understand the differences between these two viticultural regions.

Significance and impact of the study: One of the regions studied, Campanha Gaúcha, is a new Brazilian viticultural region. There are only few studies on this wine region. So, the results presented here will provide some guidance for the selection of the most appropriate oenological practices for this region.

Key words: Merlot, maceration time, copigmentation, polyphenols

Résumé

Objectif: Vérifier l'impact du temps de macération sur la couleur, les polyphénols et la copigmentation de vins issus de cépages Merlot afin de mieux comprendre les différences qui existent entre deux grandes régions viticoles brésiliennes, Campanha Gaúcha et Serra Gaúcha, pour lesquelles il existe peu d'études.

Méthodes et résultats: Des raisins de la variété rouge *Vitis vinifera* Merlot cultivés dans quatre vignobles de la région de Campanha Gaúcha (n = 2) et Serra Gaúcha (n = 2) ont été récoltés, broyés et fermentés à la même température, avec trois temps de macération différents : 4, 8 et 15 jours. La composition phénolique et la couleur des vins des récoltes 2009 et 2010 ont été analysées par spectrométrie. Les résultats montrent que les caractéristiques des raisins sont plus importantes pour la couleur et la composition phénolique des vins que les techniques de vinification utilisées (temps de macération). Le temps de macération n'a eu aucun effet sur la copigmentation et les anthocyanes combinées étaient les plus fortement corrélées à la couleur.

Conclusion: Le temps de macération a des effets différents suivant les vins étudiés. Par conséquent, d'autres études seront nécessaires afin d'identifier les caractéristiques des raisins et de mieux comprendre les différences entre ces deux régions viticoles.

Signification et impact de l'étude: Une des régions étudiées, Campanha Gaúcha, est une nouvelle région viticole brésilienne. Il existe, à ce jour, peu d'études sur les vins de cette région. Les résultats obtenus aideront au conseil de pratiques œnologiques plus adaptées à la région.

Mots clés: Merlot, temps de macération, copigmentation, polyphénols

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INTRODUCTION

Nowadays, the wine industry efforts are focused on the production of deeply colored, full bodied red wines, as this type of wine has become highly preferred by consumers. Color is one of the most important sensory attributes of red wines and is related to anthocyanin extraction from grape skins during the winemaking process. For this reason, many winemaking techniques have been developed to increase color extraction, allowing the production of wines meeting the market demands (Puertas *et al*., 2008).

The concentration and composition of anthocyanins are affected by skin contact time, fermentation and aging conditions, and they affect the color of wine (Mazza *et al*., 1999).

The evolution of grape anthocyanin content is highly influenced by the climatic conditions during the growing season and thus the differences between seasons for a single variety are greater than the differences between varieties within a season (Cacho *et al*., 1992).

Gao *et al*. (1997), who worked with Pinot noir wines produced by different vinification processes, concluded that the presence of total or individual polymeric anthocyanins is directly related to wine color intensity, and that all the polymeric anthocyanins are important to wine color. Fermentation conditions has a deep influence on monomeric and polymeric anthocyanins, however, fermentation temperature was found to be the critical factor in the generation of polymeric anthocyanins. A fermentation temperature of 30 ºC favors both the formation of polymeric anthocyanins and a dark-colored wine. After fermentation, the content of individual monomeric anthocyanins declines gradually while that of polymeric anthocyanins increases. At the time of bottling, residual monomeric anthocyanins are only a fraction of the initial concentration, and the total polymeric anthocyanin content reaches the highest levels (Gao *et al*., 1997).

The color of red wines is positively influenced by the copigmentation phenomenon, which is due to molecular associations between pigments and other (usually non-colored) organic molecules in solutions (Levengood and Boulton, 2004 ; Boulton, 2001). These associations cause the pigments to exhibit a greater color than would be expected from those of the fermentation (Boulton, 2001). Moreover, it is suggested that copigmentation may have an important role in the subsequent evolution of color during red wine aging, affecting the speed of polymerization reactions and protecting the anthocyanin pigments from oxidative processes (Boulton, 2001). Copigmentation may also have implications in the sensory perception of tactile sensation (bitterness) attributed to polyphenols (Boulton, 2001). Finally, copigmentation depends on many factors : type and concentration of anthocyanins, type and concentration of copigments, pH, temperature and, in particular, the type of solvents (Ribéreau-Gayon *et al*., 2006).

Table 1 - Location of vineyards for wine production.

Wine	Vineyard
1 and 3	Vineyard 1, Campanha Gaúcha
2	Vineyard 2, Campanha Gaúcha
4	Vineyard 3, Serra Gaúcha
5	Vineyard 4, Serra Gaúcha

Campanha Gaúcha is a new viticultural area in the southwest of the state of Rio Grande do Sul, Brazil, latitude 31º south, along the border with Uruguay. The moderate climate, with warm and dry summers, makes it a good viticultural area. However, it has been observed that grapes reach « technological maturity » (analysis of sugars, acidity and pH based mainly on optimum total solid content) before « phenolic maturity ». Daudt and Polenta (1999), working with *Vitis vinifera* grapes from Campanha Gaúcha and Serra Gaúcha (the traditional Brazilian wine region), showed that these two areas differ with respect to the amount of phenols, anthocyanins and tannins and color intensity of their wines. However, the same authors emphasize that the reasons for these differences need more complex studies.

Today, consumers are increasingly demanding highly colored red wines, with a good structure. To achieve this, it is necessary that polyphenol extraction from grapes be as complete as possible to ensure color intensity and stability during aging. The main aim of this work was to evaluate the impact of skin contact time on color, other phenols and copigmentation of Merlot wines in order to understand the differences between these two Brazilian regions.

MATERIALS AND METHODS

1. Studied area and winemaking process

Merlot grapes from four commercial vineyards located in two different regions of Rio Grande do Sul (Brazil) were harvested in two seasons, 2009 and 2010, and used for the production of five wines (table 1).

The grapes were harvested at technological maturity, according to ºBrix, acidity and pH parameters, and vinified. For each wine, three vinifications were made using different maceration times (4, 8 and 15 days), with three replications each. For all vinifications, dry active yeast (*Saccharomyces cerevisiae*, Blastocel Grand cru, Perdomini) was added (20 mg. L^{-1}) after the addition of 30 mg. L^{-1} of sulfur dioxide. Fermentation was performed at 28 ºC, and at the end, sulfur dioxide was added again (20 mg. L^{-1}). The wines were bottled after two months of vatting.

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Table 2 - Alcohol (%vol.), acidity (g% tartaric acid) and pH in Merlot wines, harvest 2009 and2010.

	2009		2010		
	Wine 1	Wine 2	Wine 3	Wine 4	Wine 5
Alcohol	14,0	12,5	11,7	12,5	12,8
Acidity	0,63	0,56	0,62	0,69	0,71
pH	3,40	3,80	3,65	3,53	3,66

Average of all treatments.

2. Spectrometric analysis

All the measurements were carried out on a UV 11-000 (Pro-Analise, Brazil) spectrophotometer using 10 mm or 1 mm pathlength quartz and glass cells.

Color characteristics, i. e., intensity (A420 + A520 + A620) and hue (A420/A520), were measured directly on the basis of absorption at 420 and 520 nm in a 1 mm optical path cell. The results were then multiplied by 10 in order to normalize the data to the conventional 10 mm optical path (Ribéreau-Gayon *et al*., 2006).

Monomeric anthocyanins (%M), copigmented anthocyanins (%C) and polymeric anthocyanins (%P) were determined according to the method proposed by Boulton (1996). Briefly, the wines were adjusted to pH 3.6 (for standardization) and membrane filtered (0,45 mm pore size). For the monitoring of anthocyanins, 20 ml of 10 % acetaldehyde solution was added to 2 ml of wine sample. After resting for 45 minutes, the absorbance was measured at 520 nm. Another 100 l of wine sample was mixed with 1 900 l of buffer solution pH 3.6. After a few minutes, the absorbance was read at 520 nm. For the monitoring of the total amount of phenols, a diluted wine sample (1/100) was read directly at 280 nm.

3. Statistical analysis

The Statistica program for Windows release 5.1 (StatSoft Inc., 1998) was used for data processing. Analysis of variance (ANOVA) was used to compare the results among wines; and the means were statistically separated by a Tukey's test at 5 %.

The data corresponding to the five wines and the two years were calculated and the linear correlations between those were analyzed. The correlations between color and phenolic composition of the wines were also calculated.

RESULTS AND DISCUSSION

According to the physico-chemical analysis of wines (table 2), it is possible to observe that the grapes had different degrees of technological maturity. pH was an important factor for this study as it directly affects the balance between the colored and colorless forms of anthocyanins. Indeed, at high pH values (>4,5), the flavylium cation (color) is converted to colorless species (Brouillard *et al*., 1989).

Table 3 shows the percentage of color due to copigmented anthocyanins, free anthocyanins (monomeric) and polymeric anthocyanins. Copigmentation has been shown to account for 30 to 50 % of the color in young wines (Boulton, 2001); however, in this study copigmentation accounted for 15.8 to 41.5 % of color, which is less than expected. Copigmentation is primarily influenced by the levels of several specific, non-colored phenolic components or cofactors (Boulton, 2001). Maceration time did not influence the percentage of copigmented anthocyanins found in wines 2, 4 and 5. However, in wines 1 and 3 (both from the same vineyard but from different years), the percentage of copigmented anthocyanins decreased with 15 days of skin contact. The different levels of cofactor and anthocyanin content in grapes and the winemaking procedures could explain these differences (Darias-Martin *et al*., 2007).

The percentage of monomeric anthocyanins (%M), which represents the free forms of anthocyanins, increased with 15 days of maceration for wines 1 and 3, whereas the other wines did not show any difference. With some exceptions, the percentage of color due to monomeric forms was greater than that due to copigmented anthocyanins.

Polymeric anthocyanins (%P) rarely showed any significant difference among the three maceration times. However, in wines 1 and 2, the percentage of color due polymeric anthocyanins was greater than 50 % with 8 days of skin contact. This could explain why wine 2, although showing a high pH value (table 2), had a high color intensity (figure 1).

As expected, the longer the maceration time, the greater the amount of total phenols (TPI) extracted in wines, especially with 15 days of skin contact. It is assumed that these phenols are originated from seed tannins.

According to several authors, an increase in tannin content, but not in ant hocyanin content, is expected with the increase in maceration time (Sacchi *et al*., 2005). From the analysis of the content of total anthocyanins (units of absorbance) obtained by the addition of acetaldehyde to the sample, it is possible to observe that for Campanha

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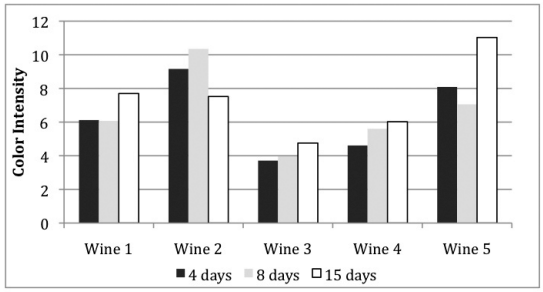


Figure 1 - Color intensity of Merlot wines, harvest 2009 and 2010, with different skin contact times.

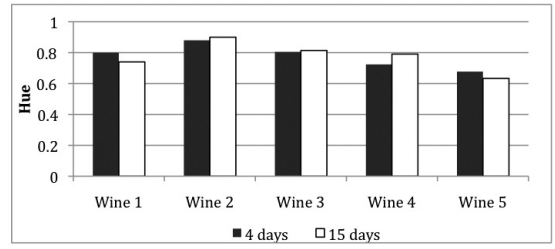


Figure 2 - Color Hue of Merlot wines, harvest 2009 and 2010, with different skin contact times.

Table 3 - Total (ANT), copigmented [C], monomeric [M] and polymeric [P] anthocyanins and total phenolic index (TPI) of Merlot wines, harvest 2009 and 2010, with different times of skin contact.

Wine		Skin contact time		
		4 days	8 days	15 days
Harvest 2009				
1	% [C]	22.1^{ab}	27.5^{a}	16.1^{b}
	% [M]	35.4^{a}	22.4^{b}	39.3^{a}
	% [P]	42.4^{b}	50.1^{a}	44.5^{b}
	ANT	3.36^{b}	3.70^{ab}	3.93^{b}
	TPI*	31.7^{b}	39.1^{a}	40.4^{a}
2	% [C]	20.4^{ns}	22,0	33,3
	% [M]	29.5^{ns}	24,9	27,4
	% [P]	50.1^{ab}	53.1^{a}	39.3^{b}
	ANT	4.76^{ns}	5,15	4,9
	TPI	39.6^{b}	43.3^{b}	50.1^{a}
Harvest 2010				
3	% [C]	36.0^{a}	28.9^{a}	15.8^{b}
	% [M]	31.2^{b}	43.9^{ab}	53.6^{a}
	% [P]	32.7^{ns}	27,2	31,6
	ANT	3.62^{ns}	3,53	3,62
	TPI	35.5^{b}	29.2^{c}	39.6^{a}
4	% [C]	31.9^{ns}	27,3	20,1
	% [M]	39.2^{ns}	47,4	48,5
	% [P]	28.8^{ab}	25.4^{b}	31.2^{a}
	ANT	4.21^{b}	4.73^{a}	4.95^{a}
	TPI	30.0^{b}	37.0^{a}	35.2^{a}
5	% [C]	40.4^{ns}	32,5	41,5
	% [M]	24.6^{ns}	36,4	12,8
	% [P]	35.7^{ns}	31,1	45,8
	ANT	4.66^{b}	6.22^{ab}	6.84^{a}
	TPI	33.8^{b}	44.5^{ab}	46.5^{b}

Means within each row followed by different letters are significantly different ($p < 0,05$). ns = not significant. * TPI: OD 280 mn, adjusted to pH 3.6.

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Table 4 - *F* value and p value of two-way ANOVA of analyzed parameters, considering all the means of the studied wines, harvest 2009 and2010.

	Origin		Skin contact time	
	F value	*P* value*	*F* value	*P* value*
% [C]	3,382	**0,019**	1,320	0,279
% [M]	6,79	**0**	0,76	0,471
% [P]	16,25	**0**	0	0,998
ANT	19,14	**0**	1,29	0,285
TPI	2,73	**0,043**	1,13	0,339
CI	17,41	**0**	0,65	0,528
Hue	9,07	**0**	0,50	0,610

* Bold values indicate significance at p < 0,05, % C – copigmented anthocyanins; % M. – monomeric anthocyanins; % P – polymeric anthocyanins; CI – color intensity; ANT – total anthocyanins; TPI – total phenolic index

Table 5 -Total (ANT), copigmented [C], monomeric [M] and polymeric [P] anthocyanins, total phenolic index (TPI), color intensity (CI) and hue of Merlot wines, harvest 2009 and 2010, with different times of skin contact, considering all the means of the studied wines

	2009		2010		
	wine 1	wine 2	wine 3	wine 4	wine 5
% [C]	21.7a	25.2 ab	27.2 ab	26.4 ab	38.1c
% [M]	32.4 ab	27.3 a	42.2 b	45.1 b	24.5 a
% [P]	45.7 bc	47.52 c	30.4 a	28.5 a	37.4 ab
ANT	3.67 a	4.90 b	3.46 a	4.63 b	5.91 c
TPI	37.1 ab	31.0 a	34.7 ab	34.1 ab	41.62 b
CI	6.62 bc	8.39 cd	4.16 a	5.25 ab	8.52d
Hue	0.79 a	0.87 c	0.82 bc	0.77 ab	0.70 a

Means within each row followed by different letters are significantly different (p < 0,05). * % C – copigmented anthocyanins; % M. – monomeric anthocyanins; % P – polymeric anhtocyanin; IPT – total phenol index; ANT – total anthocyanin

Gaúcha wines (1, 2 and 3), the increase in total anthocyanin content with the increasing maceration time was less than that found in Serra Gaúcha wines (4 and 5), which increased with skin contact time. It should be noted that since pH has a strong influence on the color of anthocyanins, this determination was performed with wine samples adjusted to pH 3.6. This difference in the extraction of anthocyanins is most likely linked to the maturation degree of the grapes, which should be investigated in future work. The work by González-Neves (2008) reinforces the idea that the characteristics of the grapes have a strong influence on the effect of the winemaking techniques.

Figure 1 shows the color intensity of the wines studied. In general, all wines except wine 2 showed an increase of color intensity with increasing skin contact time, with wine 5 showing the biggest increase in the color intensity parameter. Regarding hue (figure 2), skin contact time did not have any influence.

The p values of two-way ANOVA (table 4) was performed considering all data obtained and shows the effect of grapes' origin (vineyards) and skin contact time for each parameter studied. In general, the origin of the grapes but not skin contact time had a major influence on the parameters. Based on these results, all means were grouped for each wine, with the aim of grouping wines by harvest and by region, regardless of treatments (i. e., skin contact time) (table 5). All wines showed significant differences among them. Wine 5 had the greatest intensity of color, the highest amount of total anthocyanins, the highest percentage of copigmented anthocyanins and the lowest percentage of monomeric anthocyanins.

The use of the correlation coefficient (table 6) was conducted with the purpose of determining the main parameter influencing the color intensity and hue of the wines. For wine 5, there was a negative correlation between color intensity and the percentage of monomeric anthocyanins (-0.86) and a positive correlation with the percentage of polymeric anthocyanins (0.81); wine 2 also showed a positive correlation with the percentage of polymeric anthocyanins (0.72). These data emphasized the importance of polymeric pigments in the color of these wines and that copigmentation did not have a positive

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Table 6 - Correlation coefficients between chemical and color parameters in Merlot wines, harvest 2009 and2010.

	wine 1		wine 2		wine 3		wine 4		wine 5	
	CI*	H	CI	H	CI	H	CI	H	CI	H
% C	-0,68	0,48	-0,29	0,08	**-0,87**	0,4	-0,05	-0,26	0,39	-0,64
% M	0,56	-0,5	-0,35	0,47	**0,77**	-0,28	0,01	0,27	**-0,86**	**0,79**
% P	-0,18	0,36	**0,72**	-0,58	0,07	-0,21	0,07	-0,01	**0,81**	-0,56
TPI	0,45	-0,53	0,55	-0,22	0,62	0,01	**0,71**	0,25	-0,01	0,44
ANT	**0,8**	-0,53	0,62	-0,18	-0,44	-0,35	0,59	-0,1	0,51	-0,04

Bold values correspond to strong correlations. CI – color intensity; H – Hue; % C – copigmented anthocyanins; % M. – monomeric anthocyanins; % P – polymeric anhtocyanins; TPI – total phenolic index; ANT – total anthocyanins

correlation with color intensity in this case; in fact, a negative correlation was observed for wine 3.

Wine 2 had the second highest color intensity and the highest polymeric anthocyanins (table 5), and these two parameters were positively correlated (0.72).

CONCLUSION

The maceration time influenced the phenolic characteristics and color of the wines studied; however, major differences were found when the wines were grouped according to vineyard. Thus, the origin of the grapes seems to have a higher influence on phenolics than the treatments used during the winemaking process. Maceration time did not affect copigmentation phenomena, and polymeric anthocyanins had the greatest correlation with color intensity. However, more studies are necessary to identify the characteristics of the grapes and to understand the differences between these two Brazilian viticultural regions.

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