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Study of the maturation of grapes (*Vitis vinifera* L.) grown in Dois Vizinhos, Paraná

Evolução da maturação de uvas finas (*Vitis vinifera* L.), no município de Dois Vizinhos – Paraná¹

Gener Augusto Penso^{2*}; Idemir Citadin³; Luana Aparecida Castilho Maro³; Silvia Scariotto⁴; Alyne Chicoki⁵; Rodrigo Ambrosio⁵

Abstract

The edaphoclimatic conditions in Southwest Parana differ from those in traditional wine-growing regions and have a major influence on the organoleptic characteristics of the grapes. Studies of grape maturation under these conditions may enable us to time the supply of raw materials for the elaboration of differentiated wines. This study aimed to quantify the ripening components of the grape cultivars Cabernet Sauvignon, Merlot, Tempranillo, and Sangiovese grapes grown in Dois Vizinhos, PR, during the harvest seasons of 2008/2009 and 2011/2012. The grapevines were performed according to the espalier system and grafted on rootstock R110 ('Cabernet Sauvignon' and 'Merlot') and Paulsen 1103 ('Tempranillo' and 'Sangiovese'). The ripening of the grapes was monitored every ten days from the beginning of ripening (*verasion*) until harvest. The following variables were evaluated: mass and diameter of berries, total soluble solids (TSS), pH, titratable acidity (TTA), total sugars, estimated alcohol content, anthocyanins, and flavanols. Sangiovese berries showed the greatest weight and diameter in the two years. The TSS was higher in the second year of evaluation for Cabernet Sauvignon and Tempranillo grapes. The pH decreased from the first year to the second for all cultivars. There were no significant differences in ATT among the cultivars. A higher content of flavanols was observed during the first growing season of study than during the second. Tempranillo grapes showed the highest concentration of anthocyanins during both growing seasons. Not all cultivars had enough acidity to permit wine stability and guard time. None of the cultivars required the chaptalization of must, presenting good potential of alcohol production.

Key words: Wine, European grapes, *terroir*

Resumo

As particularidades edafoclimáticas do Sudoeste paranaense diferem das tradicionais regiões de cultivo da videira e exercem grande influência nas características organolépticas das bagas. Estudos sobre a evolução da maturação de uvas nessas condições podem viabilizar o fornecimento de matéria-prima para a elaboração de vinhos diferenciados. Objetivou-se quantificar os componentes da maturação de uvas das cultivares Cabernet Sauvignon, Merlot, Tempranillo e Sangiovese cultivadas no município de Dois Vizinhos – PR, nas safras de 2008/2009 e 2011/2012. As videiras foram conduzidas em espaldeira e enxertadas sobre o porta-enxerto R110 ('Cabernet Sauvignon' e 'Merlot') e Paulsen 1103

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(‘Tempranillo’ e ‘Sangiovese’). A evolução da maturação das uvas foi acompanhada a cada dez dias a partir do início da maturação (*verasion*) até a colheita. As seguintes variáveis foram avaliadas: massa e diâmetro médio das bagas, teor de sólidos solúveis totais (SST), pH, acidez total titulável (ATT), açúcares totais, álcool provável, antocianinas e flavonóis. Bagas da cv. Sangiovese apresentaram maior massa e diâmetro médio nos anos avaliados. Os teores de SST foram maiores no segundo ano de avaliação para as cvs. Cabernet Sauvignon e Tempranillo. O pH sofreu redução do primeiro ano para o segundo de avaliação, para todas as cultivares. Não houve diferenças significativas na ATT entre as cvs. A maior concentração de flavonóis foi obtida no primeiro ciclo de estudo. A cv. Tempranillo apresentou concentração mais elevada de antocianinas nos dois anos avaliados. Todas as cultivares não apresentam acidez total suficiente para permitir ao vinho estabilidade e tempo de guarda. Todas as cvs. dispensam a chaptalização do mosto tendo boa produção de álcool provável.

Palavras-chave: Vinho, uvas europeias, *terroir*

Introduction

Viticulture has high socioeconomic importance in the Brazilian fruit-production sector. It generates a large number of jobs and relevant business opportunities with regard to the production of table grapes and grapes for use in the processing industries. In 2012, approximately 1.45 million tons of grapes were produced across different regions of Brazil. However, the greatest contribution was from the South of Brazil, which accounted for 68% of the total grapes produced (IBGE, 2013).

The North of Paraná has recognized importance in the production of table grapes and wines with strong aromas characteristic from American cultivars (*Vitis labrusca*). The grape production in Southwest of Paraná is primarily intended for the elaboration of table wine and juices, among other products (SATO; ROBERTO, 2008). This emerging grape-producing region has a plantation area of approximately 1,000 hectares, totaling approximately 12,000 tons, according to data from SEAB-DERAL (2011). The expansion of viticulture in the Southwest region of Paraná may help to meet the increasing demand for high-quality Brazilian wines.

The study of grape maturity is extremely important to determine the quality of the wines produced. It is during this period that the synthesis, degradation, and/or translocation of desirable compounds occurs in the grapes, and these compounds include sugars, terpenes, anthocyanins, tannins, and organic acids (CONDE et al., 2007).

Grape composition is defined by the interaction

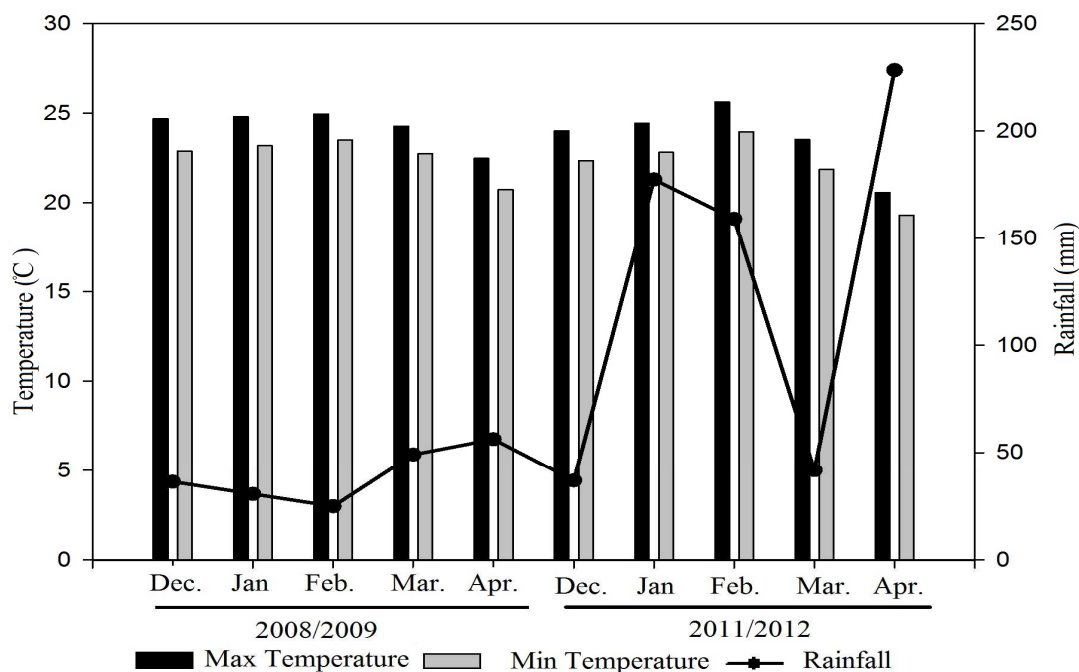
between climate, soil, rootstock, cultivar, and other agronomic parameters, which together determine the *terroir* of a wine-growing region (ZSÓFI et al., 2009). Thus, the knowledge of the factors involved in the production and quality of each cultivars will help define strategies that promote higher expression of desirable characteristics. Such characteristics may confer differentiation and originality to the wines elaborated from grapes produced in the Southwest region of Paraná.

The aim of this study was to quantify the different components during the maturation process of Cabernet Sauvignon, Merlot, Tempranillo, and Sangiovese grapes grown in Dois Vizinhos, PR, from the beginning of maturation until harvest to verify whether they meet the requirements for the production of high-quality wines.

Materials and Methods

This study was carried out in a commercial vineyard located in Dois Vizinhos, Paraná (25°51' S, 53°06' W with an average altitude of 568 m). The climate in this region is humid subtropical, and the average annual rainfall is 1,800–2,000 mm (CAVIGLIONE et al., 2000). The soil is an Umbric Distroferic Red Nitosoil, clayey texture, subtropical forest evergreen phase, wavy relief, associated with Cambisoil (BHERING et al., 2008). The rainfall and temperature in this region were monitored during the study period to determine the influence of these parameters on grape quality and consequently on the wine produced (Figure 1).

Figure 1. Mean maximum and minimum temperatures and rainfall from December to April in 2008/2009 and 2011/2012. UTFPR Câmpus Pato Branco, 2012.



Source: Elaboration of the authors.

Plants were conducted according to the espalier system, with a spacing of 2.5 m between plants and 3 m between rows with approximately 40 plants per cultivation line for Merlot and Cabernet Sauvignon grapes and 2 m between plants and 3 m between rows with approximately 30 plants per cultivation line for Sangiovese and Tempranillo grapes. Merlot and Cabernet Sauvignon (planted in 2003) are clones of IS5 grafted onto the rootstock R110, whereas Sangiovese and Tempranillo (planted in 2005) are clones of VCR 49 grafted onto the rootstock Paulsen 1103.

The grapes were collected from the *veraison* period until they were completely ripened, which was between 12/16/2008 and 02/12/2009 in the 2008/2009 growing season, and 12/22/2011 and 02/13/2012 during the 2011/2012 growing season, with collections taking place every ten days throughout both cycles. For each cultivar, four cultivation lines were collected, with samples of 100 grapes berries being collected from each line (two

grapes berries per plant). Then, the samples were separated into three groups of 25 grapes berries, in the laboratory discarding $\frac{1}{4}$ of the sample, giving 12 replications per cultivar.

The samples were transported to the Fruticulture Laboratory at the Federal University of Technology – Paraná (UTFPR), Câmpus Pato Branco and the following physical properties were determined: mean grape berries mass was weighed using an analytical balance, expressing the data in grams (g), and the average grape berries diameter was measured using a digital pachymeter and was expressed in millimeters (mm).

The grapes berries used in each experiment were then crushed manually to extract the must, without separating the seeds. The extract was then used to assess the following variables: pH, total soluble solids (TSS) by refractometry and expressed in °Brix, and total titratable acidity (TTA) by titration with 0.1 N sodium hydroxide, with the result being expressed in meq tartaric acid L⁻¹, according to

the guidelines stated by the Adolfo Lutz Institute (2008). The anthocyanins and flavonols were quantified using the process described by Lees and Francis (1972). The results were expressed in mg of anthocyanins and/or flavonols per 100 g⁻¹ of peel.

The expected alcohol content was estimated from the content of total soluble solids according to the methodology proposed by Jaulmes and Espezel (1935).

The experimental design used was a randomized block arranged in a 2 × 4 factorial with factor A formed by the growing seasons (2008/2009 and 2011/2012) and factor B by the cultivars (Cabernet Sauvignon, Merlot, Tempranillo, and Sangiovese).

The data were initially subjected to the Shapiro-Wilk test to verify the normality of the data. When a normal distribution was not found, the data were transformed using $\sqrt{(x+1)}$. Analysis of variance (ANOVA) was calculated, and means were compared using Tukey test ($p = 0.05$). The analyses were performed using the program Genes (CRUZ, 2013).

Results and Discussion

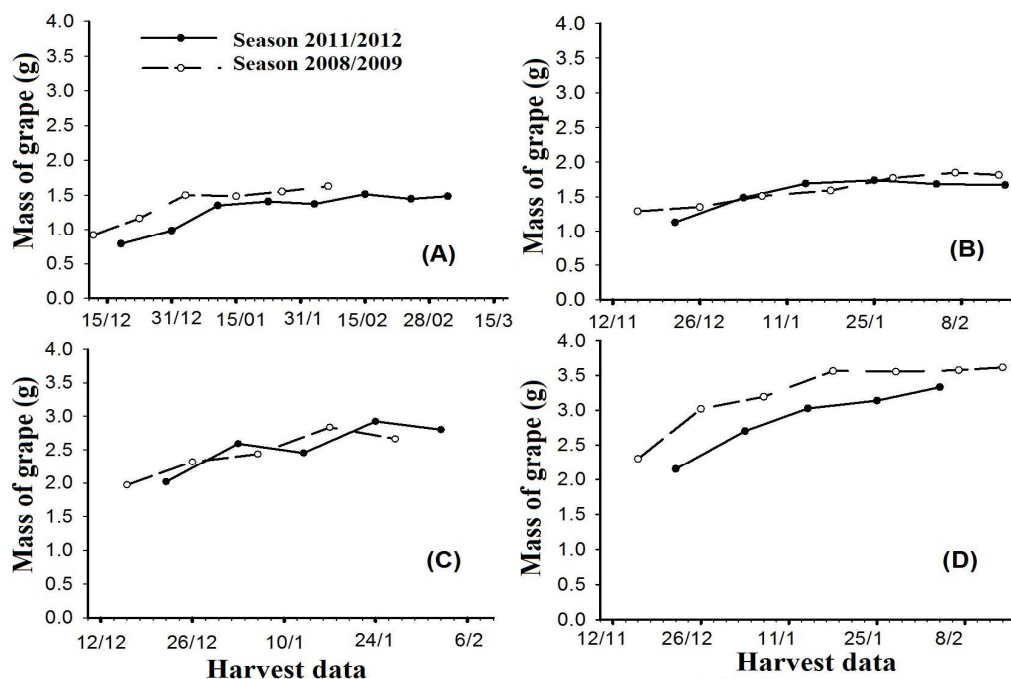
Under the study conditions (Figure 1), there was an increase in the total mass of Merlot, Tempranillo, and Sangiovese grape berries until January 25th during both growing seasons, with little variation during the following samples. However, for Cabernet Sauvignon, this increase in mass occurred until January 29th and January 31st, which may have been due to this cultivar having a delayed cycle

(Figure 2). For Merlot and Tempranillo, the grape berries mass decreased in the final samples during both growing seasons, which may indicate over-ripening. The mass loss is due to the interruption of the transport of nutrients to the berry along with the occurrence of drought causing desiccation and consequent weight loss (GUERRA; ZANUS, 2003).

Cabernet Sauvignon, Merlot, and Tempranillo showed very similar results in both seasons. Sangiovese had a greater average mass of grape berries during both growing seasons (Table 1). The greater grape berries mass is an intrinsic characteristic of this cultivar as described by Gil and Pszczółkowski (2007), who classified the size of the Sangiovese grape berries as medium to large. The seeds are also large and contribute to higher weight of the berries. Cabernet Sauvignon and Merlot grape berries are smaller, and our results are consistent with those obtained by Rizzon and Mielle (2003) in several harvests in the Serra Gaúcha region, who found the grape berries mass to be consistently less than 2 g.

The grape berries mass during maturation is correlated with sugar accumulation, availability of water in the soil and atmosphere, and the number of seeds. Smaller fruits ultimately release larger quantities of minerals including potassium, calcium, and magnesium, which greatly influence pH and total titratable acidity. Smaller grapes berries also affect the organoleptic characteristics of the wine due to the release of high quantities of tannins, which are present in large concentrations in the seeds and may turn the wine astringent (CONDE et al., 2007).

Figure 2. Changes in mass during maturation of Cabernet Sauvignon (A), Merlot (B), Tempranillo (C), and Sangiovese (D) grape berries in Dois Vizinhos, PR during the 2008/2009 and 2011/2012 growing seasons. UTFPR Câmpus Pato Branco, 2012.



Source: Elaboration of the authors.

Table 1. Average mass of grape berries (g), grape berries diameter (mm), pH of must, total soluble solids (°Brix), estimated alcohol content (% v/v), total titratable acidity (meq L⁻¹ of tartaric acid), flavonoids (mg 100 g⁻¹ of peel), anthocyanins (mg 100 g⁻¹ of peel) of *Vitis vinifera* produced in Dois Vizinhos during 2008/2009 and 2011/2012 growing seasons. UTFPR Câmpus Pato Branco, 2012.

VARIABLES	Growin seasons	CROPS				CV.
		Cabernet Sauvignon	Merlot	Tempranillo	Sangiovese	
Grape berries mass (g)	2008/2009	1.63 Ac	1.85 Ac	2.67 Ab	3.56 Aa	5.33
	2011/2012	1.49 Ac	1.65 Ac	2.82 Ab	3.33 Ba	
Grape berries diameter (mm)	2008/2009	13.56 Aa	14.51 Aa	15.81 Aa	17.92 Aa	1.08
	2011/2012	12.70 Bb	13.14 Bb	15.88 Aa	16.52 Bb	
pH of must	2008/2009	3.76 Aa	4.21 Aa	4.04 Aa	4.49 Aa	0.65
	2011/2012	3.54 Ba	3.55 Ba	3.42 Ba	3.39 Ba	
TSS (°Brix)	2008/2009	18.07 Bc	21.03 Aa	19.40 Bb	20.50 Aa	2.42
	2011/2012	18.79 Ab	20.90 Aa	20.62 Aa	18.24 Bb	
Estimated alcohol content (% (v/v))	2008/2009	10.27 Ac	12.22 Aa	11.10 Bb	11.87 Aa	2.65
	2011/2012	10.72 Ab	12.10 Aa	11.92 Aa	10.37 Bb	
Total titratable acidity (TTA) (meq L ⁻¹ of tartaric acid)	2008/2009	49.25 Bb	36.25 Bc	61.00 Ba	44.00 Bb	6.05
	2011/2012	56.66 Ac	69.58 Ab	74.58 Aab	79.51 Aa	
Flavonoids (mg 100 g ⁻¹ of peel)	2008/2009	146.61 Aa	109.10 Abc	127.15 Aab	89.29 Ac	11.88
	2011/2012	55.80 Bb	73.91 Bab	90.99 Bb	68.80 Bab	
Anthocyanins (mg 100 g ⁻¹ of peel)	2008/2009	344.37 Aa	194.94 Aba	388.99 Aa	137.10 Ac	9.89
	2011/2012	133.39 Bb	156.64 Bb	332.63 Bb	151.53 Ab	

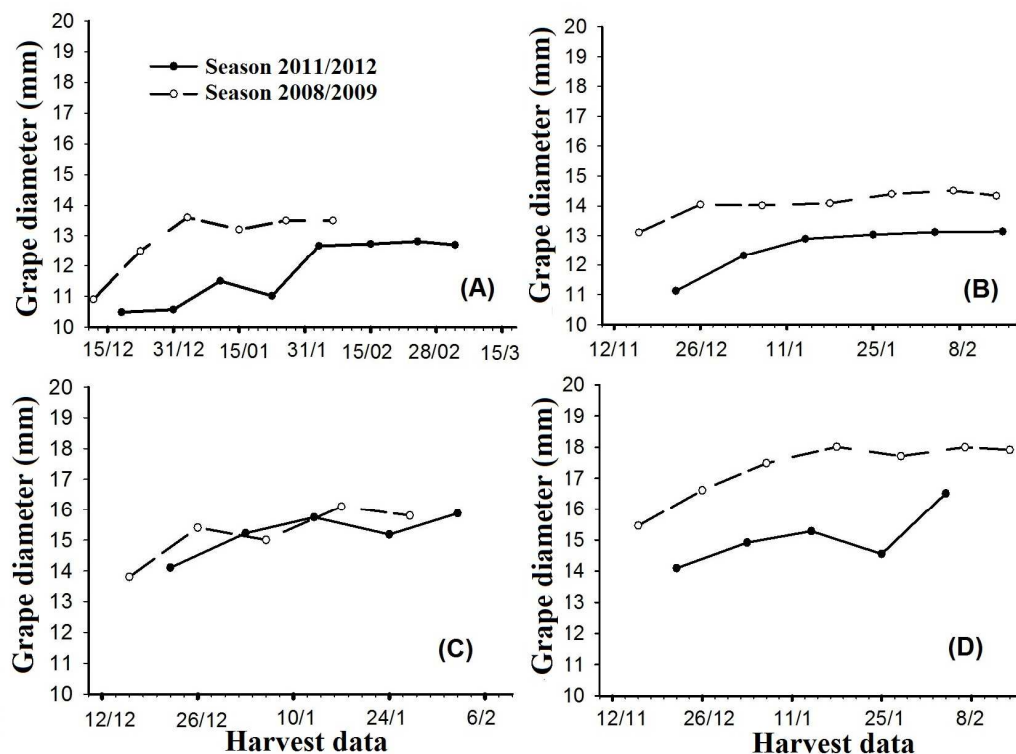
Averages followed by the same uppercase letters in columns and same lowercase letters in rows do not differ significantly by Tukey's test ($p \leq 0.05$). The data for diameter and pH were transformed using $\sqrt{(x+1)}$

Source: Elaboration of the authors.

The mean diameter of grape berries in all cultivars increased from *veraison* until harvest, with the increase being greatest for Cabernet Sauvignon and Tempranillo (Figure 3). For Tempranillo, the results were similar during both growing seasons

(Table 1). For Cabernet Sauvignon and Merlot, there was a significant difference between both growing cycles, with the best results being obtained in the 2008/2009 growing season.

Figure 3. Changes in grape berries diameter during maturation of Cabernet Sauvignon (A), Merlot (B), Tempranillo (C), and Sangiovese (D) grapes in Dois Vizinhos, PR during 2008/2009 and 2011/2012 growing seasons. UTFPR Câmpus Pato Branco, 2012.



Source: Elaboration of the authors.

Grape berries diameter is influenced by several factors including: the genetic characteristics of the cultivar, hormonal equilibrium, the amount of water absorbed by the plant, and the concentration of sugar. According to Dias (2006), temperature influences the migration of nutrients during maturation, which directly increases cell volume; ideal temperatures are close to 20°C. During this study, the average temperature was 23.75°C in the first growing season and 23.55°C in the second (Figure 1). However, during the 2011/12 growing season, the maximum temperatures were often higher than the optimum, which might have caused interference in

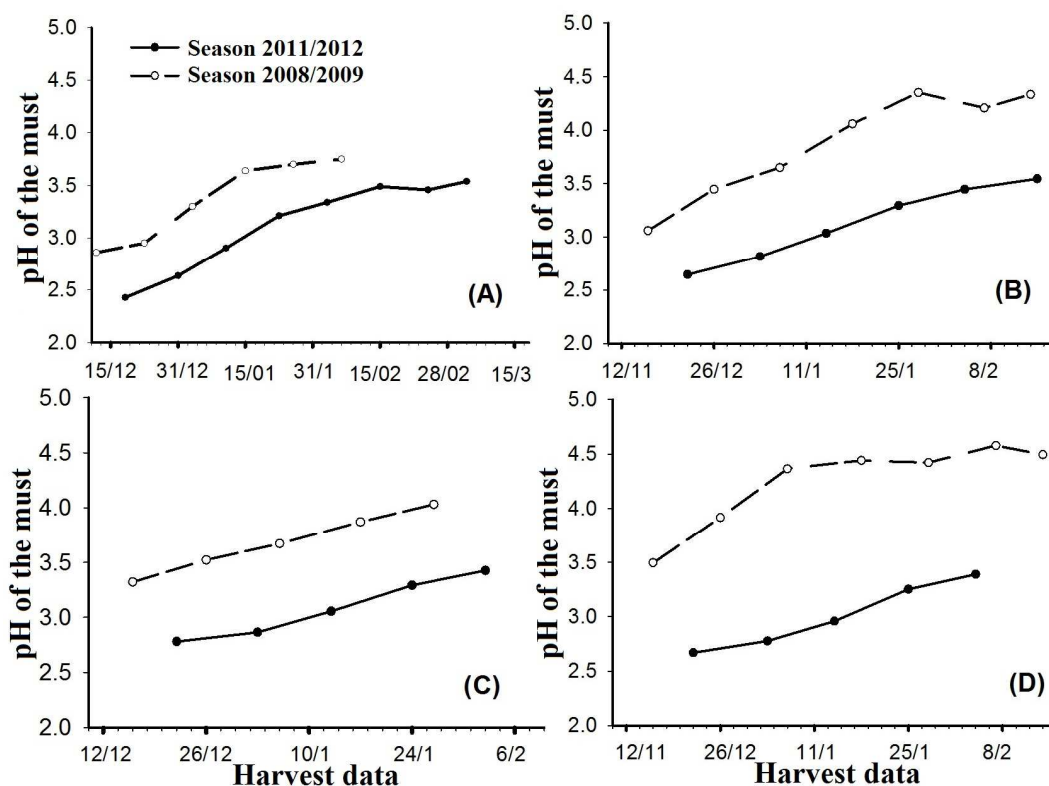
the nutrient translocation to the grape berries. This may have been responsible for the decrease in grape size during 2011/2012 growing seasons, except in Tempranillo, which was the least affected by these peaks in temperature, due to having a shorter cycle and being better adapted to regions with higher temperature ranges.

The pH progressively increased from *veraison* until harvest (Figure 4). However, the pH values obtained in the first growing season were greater than those values obtained in the second for all cultivars, and there were no significant differences between them (Table 1). All cultivars had a desirable pH in the

second growing season, since according to Rizzon and Miele (2002) the recommended pH for must is a maximum of 3.30 to guarantee microbiological and physico-chemical stability. Wines with a pH greater than 3.5 are not sufficiently preserved since they allow harmful microbial flora to develop. The pH is extremely important in several aspects of wine production, such as growth and development

of yeast and some fermentation mechanisms, and development of lactic bacteria, with pH being a requirement of malolactic fermentation. It is also a fundamental factor that influences the development of acetic bacteria as well as the activity of some pectolytic enzymes. Furthermore, pH determines the proportions of free and salified forms of each acid present in the wine.

Figure 4. Evolution of grape berries in pH during maturation of Cabernet Sauvignon (A), Merlot (B), Tempranillo (C), and Sangiovese (D) in Dois Vizinhos, PR during the 2008/2009 and 2011/2012 growing seasons. UTFPR Câmpus Pato Branco, 2012.



Source: Elaboration of the authors.

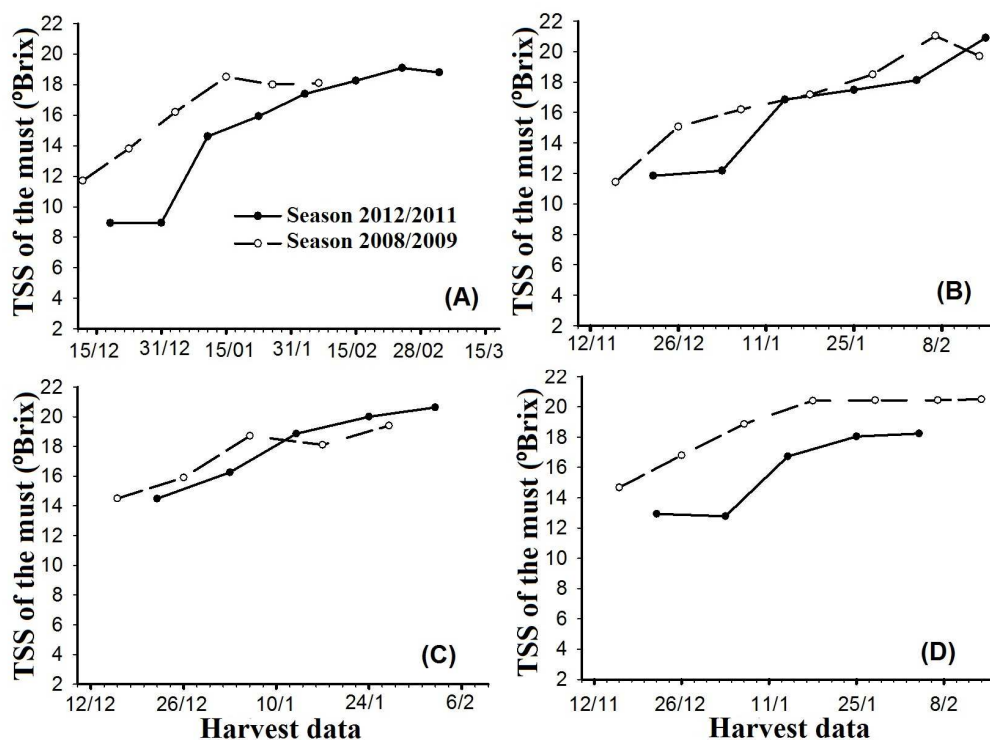
Where cultivars have a pH higher than the optimum, the pH levels can be reduced by defoliation around the flowering or bunches regions to create better light conditions. Studies carried out by Pötter et al. (2010) in several wine-growing areas showed that slightly defoliating the zone around the bunch of grapes in several phenological stages increased the total soluble solid content and decreased acidity, pH, and potassium (PÖTTER et al., 2010).

During the sampling dates, it was observed for all cultivars an increase in total soluble solid content (Figure 5). The greatest TSS content was found in Merlot and Sangiovese in the 2008/2009 growing season, whilst there was no difference between Merlot and Tempranillo in the 2011/12 growing season. Cabernet Sauvignon during the 2008/2009 growing season had the lowest TSS content (Table 1). When compared to results from other studies,

Merlot had a higher result than that obtained by Rizzon and Mielle (2003) who verified a content of 18.4 °Brix. Sangiovese experienced a decrease in TSS content in the second growing season compared

to the first; however, the results obtained were similar to those found by Intrieri et al. (2008) who obtained TSS values between 19.10 and 20.09 °Brix according to the different degrees of defoliation.

Figure 5. Evolution of Total Soluble Solids in grapes during the maturation of Cabernet Sauvignon (A), Merlot (B), Tempranillo (C), and Sangiovese (D) in Dois Vizinhas, PR during the 2008/2009 and 2011/2012 seasons. UTFPR Câmpus Pato Branco, 2012.



Source: Elaboration of the authors.

To obtain high sugar content, ideal temperatures during maturation are required, since high temperatures are capable of irreversibly altering the accumulation of sugar in the grapes (DIAS, 2006). As the second growing season received more rainfall than the first, there was consequently less sunlight and the maximum temperatures were high during this growing season, which caused results for Cabernet Sauvignon to differ from those found by other authors. Santos (2011) considers the best growing cycles to be those with summers that have little rainfall and days with ideal levels of sunlight to reach the desired total sugar levels of between 19.5 and 22 °Brix for this cultivar.

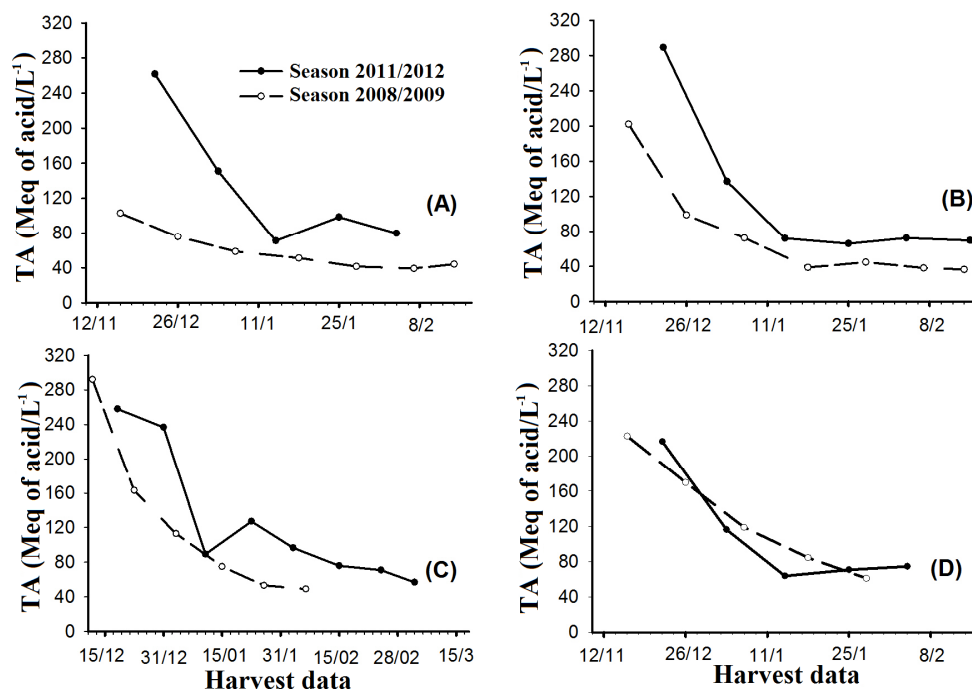
These TSS contents make all cultivars potentially capable of producing alcohol, up to the minimum requirement of 8.6% set by the legislation for fine table wines, without needing chaptalization of the must (BRASIL, 1998).

The TTA of all cultivars decreased during maturation (Figure 6). This decrease is due to the process of respiration of organic acids, and their dilution as the size of the grape berries increases, as well as salification (RIZZON; MIELE, 2003). For Dias (2006), during the crop growth phase the sugar produced gave rise to malic acid, which is stored in the cells of the grape berries and is reduced due to

degradation during *verasion*. This enables the grape to obtain energy, since the consumption of sugar is

inhibited, thereby reducing the acid content in the grape berries until maturation is complete.

Figure 6. Changes in the total acidity of grape must during maturation of grape cultivars Cabernet Sauvignon (A), Merlot (B), Tempranillo (C), and Sangiovese (D), in Dois Vizinhos, PR during the 2008/2009 and 2011/2012 growing seasons. UTFPR Câmpus Pato Branco, 2012.



Source: Elaboration of the authors.

The highest TTA was found in the Sangiovese grapes at 79.51 meq L⁻¹ of tartaric acid during the 2011/12 growing season, and the lowest was obtained by Merlot during the 2008/2009 growing season followed by Sangiovese and Cabernet Sauvignon. The highest TTA values occurred during the 2011/12 growing season (Table 1). The variation in TTA values from one growing season to another may be explained by greater rainfall during 2011/12 (2008/2009: 110 mm; 2011/2012: 434.2 mm, which was the total rainfall during sampling dates – Figure 1), since higher water availability increases the volume of the grape, thereby increasing the pH and TTA (DIAS, 2006).

Bevilaqua (1995) found that higher rainfall interfered with the acidity and sugar content of

the must, causing a decrease in must quality, and consequently, in the wine. This, according to Dias (2006), together with higher maximum temperatures accelerates the degradation, primarily of malic acid, thereby decreasing the TTA. These factors are responsible for the large variations observed during the study seasons.

In the 2008/2009 growing seasons only Tempranillo met the total minimum acidity required by legislation for fine wine, which states a minimum acidity of 55.00 meq L⁻¹. In the second growing season (2011/2012), all cultivars met the minimum acidity content required by law (Table 1). The total acidity in must and wine is hugely important in enology, as it directly influences the organoleptic properties (taste, color, and smell), and

the microbiological and physicochemical stability of wines (JACKSON, 2008).

The highest TSS/TTA ratio was observed for Merlot at 72.28 during the 2008/2009 growing season. In 2011/2012 growing season the ratio TSS/TTA was lower than 2008/2009 growing season. The low TSS/TTA values obtained in that growing season may be due to the increase in total acidity of the grape berries in the vineyard studied, probably due to changes in rainfall during grape maturation. However, they were greater than the minimum values recommended, with approximately TSS/TTA = 20 (CHOUDHURY, 2000).

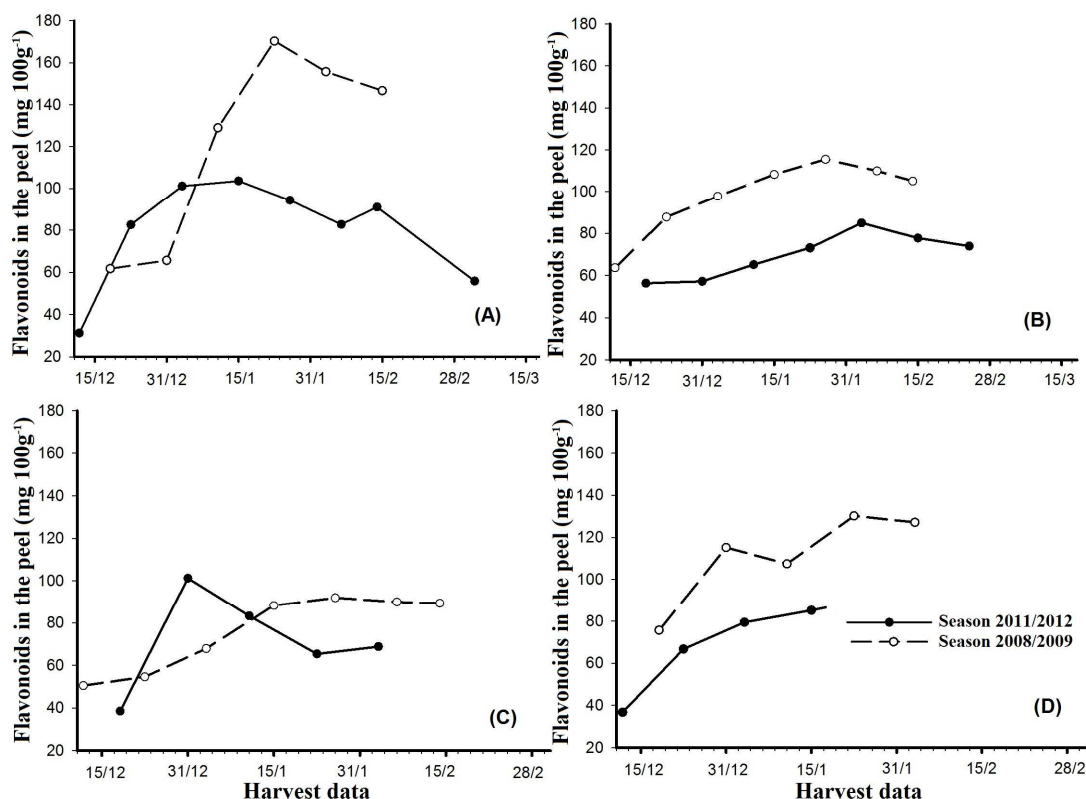
Besides technological maturation, which is defined by soluble solid content and total acidity, phenolic maturation is an important tool when defining the maturation of red wines, as it helps determine the best time to harvest and the potential

quality of the harvest or crop region (GUERRA; ZANUS, 2003).

The flavonols concentration in Cabernet Sauvignon, Merlot, and Tempranillo stabilized close to 31 January indicating degradation of these compounds and the start of over-ripening around this date (Figure 7). The best result was obtained by Cabernet Sauvignon, which had a mean flavonols concentration of $146.61 \text{ g} \times 100 \text{ g}^{-1}$ of peel (Table 1). For all cultivars, the higher contents of flavonols were obtained in the 2008/2009 growing season.

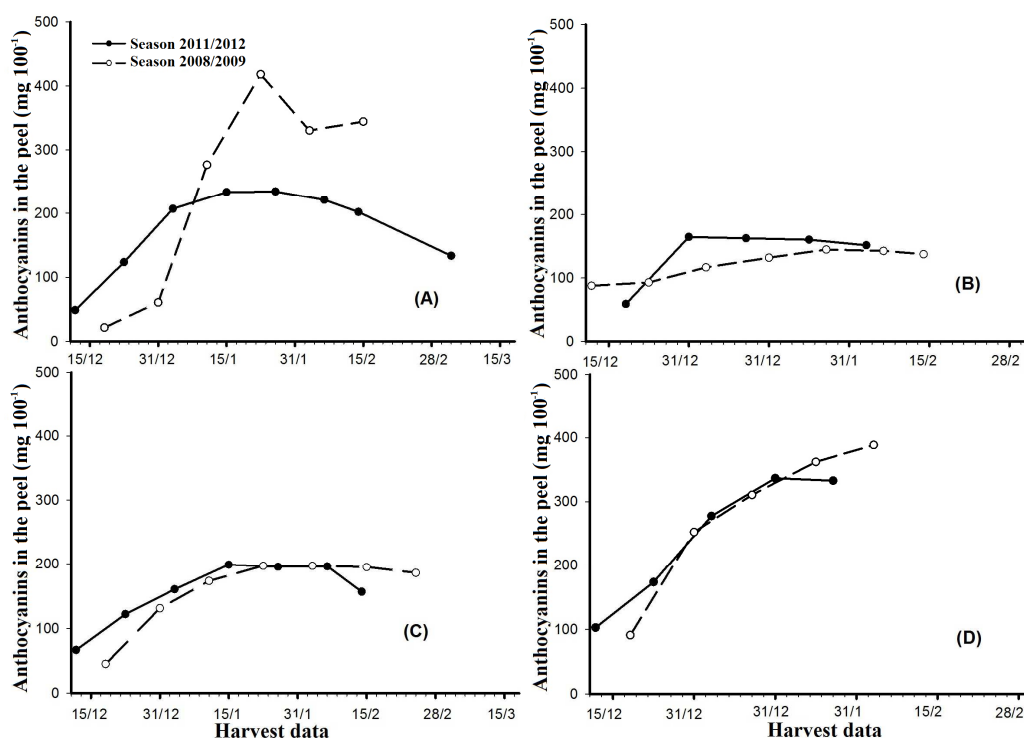
For the concentration of anthocyanins, Tempranillo and Cabernet Sauvignon showed the best results, 388.99 and $344.36 \text{ mg } 100 \text{ g}^{-1}$ of peel, respectively, both during the 2008/2009 season (Table 1). The growing season had no influence on this characteristic in Sangiovese (Figure 8).

Figure 7. Changes in the flavonoid concentration during maturation of Cabernet Sauvignon (A), Merlot (B), Tempranillo (C), and Sangiovese (D) grapes berries during the 2008/2009 and 2011/12 growing seasons. UTFPR Câmpus Pato Branco, 2012.



Source: Elaboration of the authors.

Figure 8. Changes in the anthocyanin concentration during maturation of Cabernet Sauvignon (A), Merlot (B), Tempranillo (C), and Sangiovese (D) grapes berries. UTFPR Câmpus Pato Branco, 2012.



Source: Elaboration of the authors.

In red wines, the anthocyanins represent the highest percentage of phenolic compounds, making it an important constituent in the production of red wines since it contributes to sensory characteristics, in particular the color of the wine (MUÑOZ-ESPADA et al., 2004).

According to Hernández-Hierro (2012), the biosynthesis and accumulation of anthocyanins in grape peel occur at the start of *veraison*, with maximum accumulation occurring close to harvest; however, there is a decrease in anthocyanin content during over-ripening. The decrease in anthocyanin content after this period occurs due to changes in environmental conditions and management techniques during maturation (RYAN; REVILLA, 2003). According to Dias (2006), higher temperatures influence the content of phenolic compounds, thereby decreasing, for example, the anthocyanin content.

The anthocyanin content is pH dependent. The pH change causes modifications in the structure of anthocyanins which are in equilibrium in the solution, and this changes the coloring of wine (BROUILLARD, 1982). This could explain, in part, the lower concentration of anthocyanins in the second growing season.

The wines must meet minimum quality standards required by legislation. In this sense, it is interesting to note that all cultivars met Brazilian's standards of identity and quality established by the Ministério da Agricultura e Abastecimento for fine dry red wines.

Conclusions

All cultivars did not have enough total acidity to give the wine the desired stability for long-term storage, making this ideal to be consumed as young wine. Only in the second year the acidity reach

adequate values, according to the standards set by the Ministério da Agricultura e do Abastecimento of Brazil (MAPA).

All the cultivars had good sugar content and did not require chaptalization of the must, and the alcohol content was within the limit prescribe in the established guidelines of MAPA.

Tempranillo grapes showed the best maturation with regard to physico-chemical attributes during the growing seasons when they were sampled, demonstrating that it was the cultivar best adapted to the environment studied.

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