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Effect of Pre-Fermentation Cold Maceration on the Quality of Tannat Wine

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ABSTRACT

Background: The wines produced with grapes from this region present intense coloration, high tannin concentration, balanced acidity and high alcohol content. However, despite being produced in the system of simple espalier with good handling of the canopy, leaf removal and productivity control per plant, the wine still presents pronounced astringency due to the incomplete phenolic ripeness. Based on this context, the purpose of this work was to evaluate the effect of PFCM on the winemaking process of Tannat grapes, particularly highlighting the effects on coloration, tannins and trans-resveratrol. This objective is based on the hypothesis that PFCM favors better extraction of polymerized tannins *trans*-resveratrol and anthocyanins from the skin, contributing to a higher color intensity and lower astringency of Tannat wine. By analyzing the basic quality-control variables of wine we found that density (0.9929 to 0.9964), alcohol content (12.38 to 12.95% v/v), total acidity (84 to 88 meq.L^{-1}), volatile acidity (6.3 to 8.7 mEq L^{-1}) and pH (3.51 to 3.58) were consistent with Tannat wines. Those values indicate that the fermentation process occurred as expected, considering that grapes were harvested with an average of 22.7° Brix and that the wines had an alcohol content between 12.95% and 12.38% and residual reducing sugars between 2.34 g L^{-1} and 3.35 g L^{-1}, which characterize them as dry wines. PFCM of Tannat grapes from the region of Campanha, RS, Brazil, increases the extraction of anthocyanins, *trans*-resveratrol, total polyphenols and tannins with higher polymerization, thus contributing to the production of a wine with greater color intensity and tasting quality.

INTRODUCTION

As it did in Uruguay, the Tannat grape variety adapted relatively well to the region of Campanha, RS, Brazil. The wines produced with grapes from this region present intense coloration, high tannin concentration, balanced acidity and high alcohol content (Garmendia and Vero, 2016). However, despite being produced in the system of simple espalier with good handling of the canopy, leaf removal and productivity control per plant, the wine still presents pronounced astringency due to the incomplete phenolic ripeness. This characteristic is the result of the presence of highly soluble monomeric or oligomeric tannins, which is generally more characteristic of seeds than epidermis (Jackson, 2008).

To obtain the maximum expression of Tannat grapes with greater phenolic structure, the main oenological practices employed are based on the use of pectolite enzymes, temperature variations during maceration and/or

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prolonged maceration, a solid/liquid ratio increase, *délestages*, *piegeages* and/or reassembly (Bautista-Ortín *et al*., 2004; Gómez-Plaza *et al*., 2002, López-Roca and Gómez-Plaza, 2004, Villaño *et al*., 2006, Zurbano *et al*., 1999). However, by intensifying the color extraction, some of these practices also improve the extraction of hydrolysable tannins, enhancing the astringency (Gómez-Plaza *et al*., 2002). Pre-fermentation cold maceration (PFCM), a procedure that is performed before alcoholic fermentation, is proposed as a way to optimize the color and polymeric tannins extraction, thus contributing to the production of a more balanced wine. PFCM consists of maintaining destemmed, crushed grapes at low temperatures (≤4˚C), followed by alcoholic fermentation at temperatures of 20-25°C (Stafussa *et al*., 2016).

One of the variables usually affected by PFCM is the content of phenolic compounds, an important factor because it is responsible for the color (anthocyanins), structure (anthocyanins, condensed tannins) and astringency (flavanols) of the wine, the latter being attributed to tannins with low-polymerization, also called soluble tannins (Gonzalez *et al*., 2004). 3-Flavanols are present in grapes as monomers and low-polymerized forms, constituting catechin tannins that are mainly located in the seeds and skin, even though monomers and dimers have been found in the pulp. The main 3-flavonol monomers of grapes are (+) catechin and its isomer, (-) epicatechin. Epicatechin can also be found as epicatechin 3-gallate.Gallocatechin, catechin 3-gallate and gallocatechin 3-gallateare also detected in grapes but seem to only be present in some varieties (Souquet *et al*., 1996, Granato *et al*., 2016). In grapes, catechin polymerization produces polymers called procyanidins (condensed tannins), but proanthocyanidins or prodelphinidins (Gonzalez-Manzano *et al*., 2004, Jackson, 2008) may also be produced.

There are structural differences between the procyanidins from the skin, pulp and seeds, and these differences are due to the grape variety. In addition, tannins from the seeds suffer less polymerization than those from the skin. Tannins from the seeds contain on average 28 monomeric flavanol units, while the ones from the skin contain more than 74 monomeric flavanol units, which generally indicates higher evolution of phenolic ripening of the skin than the seeds (Jackson, 2008, Farhadi *et al*., 2016).

Gonzalez-Manzano *et al*. (2004) and Kennedy (2008) consider maceration and fermentation to have significant effects on the extraction of anthocyanins and polymeric tannins because these are mainly located in grape skins. It is believed that low-temperature maceration might result in a proportionally superior extraction of color components and the tannin fraction from the skin in relation to the seeds (Tomazetti *et al*., 2015). The subsequent alcoholic fermentation at higher temperatures between 20 and 28°C complements extraction, following the conventional process. Moreover, the *trans*-resveratrol content is strongly affected by edaphoclimatic conditions, culture, vineyard handling, plant-microorganism interaction and the winemaking process (Abril *et al*., 2005, Dourtoglou *et al*., 2005, Gambuti *et al*., 2004, Ribeirode Lima *et al*., 1999, Souto *et al*., 2001, Stervbo *et al*., 2007), contributing to the functional properties of wine (Aggarwal *et al*., 2004, Ito *et al*., 2003). Therefore, it is assumed that PFCM can increase the extraction of *trans*-resveratrol, which is mainly concentrated in the grape skin (Sautter *et al*., 2005).

Based on this context, the purpose of this work was to evaluate the effect of PFCM on the winemaking process of Tannat grapes, particularly highlighting the effects on coloration, tannins and *trans*-resveratrol. This objective is based on the hypothesis that PFCM favors better extraction of polymerized tannins *trans*-resveratrol and anthocyanins from the skin, contributing to a higher color intensity and lower astringency of Tannat wine.

MATERIALS AND METHODS

Tannat grapes used from 7-year-old vineyards in the region of Campanha, RS, Brazil, in the municipality of Bagé, from the agricultural year of 2008. The vineyard was formed with grafted seedlings in Paulsen rootstocks, with 1.2 meters between plants and 3 meters between rows, in the conduction system of a simple espalier and pruned using the double Guy to method. The canopy height was approximately 1.2 meters. The average productivity was 3.5 kg per plant. Grape ripeness was monitored by determining the total content of soluble solids, total acidity and empirical tasting of berries and seeds. Harvest was on March 13, 2008 and was conducted manually. The grapes were transported in plastic boxes with a 17-kg capacity. When harvested, the grapes presented an average of 22.7°Brix, 112 $mEqL^{-1}$ of total acidity and must density of 1.0947. The average period between harvesting and winemaking was 24 h, 5 h of which consisted of transportation and the rest incubation in a cold chamber.

For treatment, three repetitions (20-L bottles) were performed. For the control treatment without PFCM, 17 kg of grapes were destemmed and crushed, with the immediate addition of SO_2 (50 mg/L) and yeast (20 mg/L of *Saccharomyces cerevisiae*, Maurivin® brand), followed by maceration for 6 days at a temperature of 20–25˚C, with daily reassembly and devatting on the 7^{th} day. Once the alcoholic fermentation was concluded, racking was performed and malolactic fermentation was monitored. Fermentation took 4 months, after which the tartaric stabilization was conducted in a cold chamber at 4°C for 10 days. After tartaric stabilization, the wines were bottled. In the case of microvinifications for PFCM treatment, destemmed and crushed grapes were cooled and kept between 0°C and 4°C for 4 days. After they were removed from the cold chamber, SO_2 and commercial

yeast were addedin the same amounts as in the control treatment, and alcoholic fermentation occurred at the same conditions as well. The physico-chemical tests were conducted after the bottling and sensory analysis, 6 months after bottling.

The density, alcohol content, total acidity, volatile acidity, pH and reducing sugars in wine were determined using the methods described by Amerine and Ough (1976). Total polyphenolic content, anthocyanin content and color index were determined according to Ribéreau-Gayon and Stonestreet (1965). The *trans*-resveratrol content was quantified by high-performance liquid chromatography (HPLC), following the method optimized by Souto *et al*. (2011) and adapted by Sautter *et al*. (2005) who modified the column temperature to 50°C.

Ca, Mg, Mn, Fe, Cu and Zn contents were measured by atomic absorption analysis, while the K, Na, Li and Rb were obtained by flame emission (Perkin-Elmer Corporation, 1976). The P content was determined by colorimetry using ammonium molybdate. The volatile components ethyl acetate, methanol, propan-1-ol, 2-methylpropan-1-ol, 2-methylbutan-1-ol, 3-methylbutan-1-ol and the sum of higher alcohols were quantified by gas chromatography. For gas chromatography, a system with flame ionization detectorand a Carbomax 600 (3.2 m long, internal diameter 1/8") stainless-steel column was used. The carrier gas was nitrogen with flow rate of 30 mL/min. The vaporizer temperature was 140°C, the oven was 98°C, and the detector was 160°C. The wine sample (3 µL) was directly injected after receiving 10% of volume from a1 g/L 4-methylpentan-2-ol solution as the internal standard (Bertrand, 1975).

Flavanols were separated using a preparatory C18 Sep-Pak (Waters) column in three major fractions (monomeric, oligomeric and polymeric), following the method by Sun, Spranger, Roque-do-Vale, Leandro, and Belchior (2001) and Labarbe *et al*., 1999), which has also been employed by Cosme *et al*. (2009). The fractions obtained by elution with methanol/chloroform varying from 25:75 (v/v) to 100:0 (v/v) were analyzed by HPLC to confirm the polymerization degree and quantification. The flavan-3-ol content of each fraction was determined by using the test with vanillin according to the method also described by Sun *et al*. (2001).

The antioxidant capacity of wine was determined based on the method offree radical scavenging from 2,2-diphenyl-1-picrylhydrazyl (DPPH).For each test, 990 µL of a 20 mgL^{-1}methanolic DDPH solution was used with the addition of 10 µL of wine. The absorbance reading at 517 nm was performed at 45 minutes of reaction in absence of light. At the same test conditions, reactions with Trolox were carried out to express the results in µmol Trolox equivalentmL^{-1} of wine.

The sensorial analysis was conducted by a panel of 11 trained tasters with the ability to quantify aromatic, tasting and visual descriptors in red wine. The evaluation was performed on three consecutive days, each one of them analyzing a repetition of the treatments, assigning grades from 0 to 9 according to the intensity perceived. The descriptors were selected to test the hypothesis of this work: color intensity, violaceous-red intensity, aromatic intensity, red fruits, dry fruits, tobacco/chocolate/tea, vegetable/herbaceous aroma, mouth volume, tannic sweetness, acidity, astringency, persistency and global evaluation. The experiment was organized into a completely randomized experimental delineation with two treatments, the first one conducted with the conventional winemaking process and the second one with PFCM, both with three repetitions. The results were analyzed statistically using STATISTICA 5.0 software. Means of all physico-chemical variables were compared between treatments using Tukey's test with 5% confidence intervals.

RESULTS AND DISCUSSION

By analyzing the basic quality-control variables of wine (Table 1), we found that density (0.9929 to 0.9964), alcohol content (12.38 to 12.95% v/v), total acidity (84 to 88 meq.L^{-1}), volatile acidity (6.3 to 8.7 mEq L^{-1}) and pH (3.51 to 3.58) were consistent with Tannat wines, as previously observed by other authors, although with grapes from other regions (Rizzon and Miele, 2004).Those values indicate that the fermentation process occurred as expected, considering that grapes were harvested with an average of 22.7° Brix and that the wines had an alcohol content between 12.95% and 12.38% and residual reducing sugars between 2.34 g L^{-1} and 3.35 g L^{-1}, which characterize them as dry wines.

The density of wine from PFCM (T2) was greater than the wine from the control treatment (T1). The higher density of the wine obtained from PFCM was most likely caused by the increased solid/liquid contact period in this treatment, leading to a greater extraction of compounds such as anthocyanins and phenolic compounds with the increase of the solids in the wine. In addition, the concentration of reducing sugars in PFCM wine was higher, in accord with the higher density of this wine.

The at did not differ between treatments, remaining between 84 and 88 mEq L^{-1}, which is considered adequate for red Tannat wine conservation (Rizzon and Miele, 2004). The decrease in total titratable acidity from 107 to 84 mEq $L^{-1}$of must in T1 and from 112 to 88 mEq $L^{-1}$of must in T2 in the final wine is normal, especially because of potassium bitartrate precipitation during the fermentation process and tartaric stabilization, and also due to malolactic fermentation. The volatile acidity of the wines was low, remaining between 8.3 and 6.7 in T1 and T2, respectively, indicating good control of the fermentation process. Although dynamic

microbiology was not monitored during winemaking, we believe that volatile acidity was lower in the T2 wine because of the lower temperatures used at the beginning of the process, which helped prevent volatile organic acid formation.

The pH values between 3.51 and 3.58 are adequate for stability of red Tannat wines, even if they are slightly lower than the values found by Rizzon and Miele (2004), in wines from another region. This information is important because for other cultivars, such as Cabernet Sauvignon, Cabernet Franc and Merlot produced in the same region with the same rootstock and handling system, a significant reduction of total acidity has been observed, as has an increase of the wine pH, which makes adequate ripening more difficult, unless previous corrections are made (Rizzon *et al*., 1998). There was no evidence of this problem with the Tannat cultivar.

Table 1: Physico-chemical characteristics of wine produced from Tannat grapes harvested in the region of Campanha, RS, Brazil, with and without pre-fermentation cold maceration.

Analysis	Control	Pre-Fermentation Cold Maceration	CV(%)
Density (20/20˚C)	0.9829b*	0.9964a	0.75
Alcohol (%v/v)	12.95a	12.38a	4.45
Total acidity (mEq L^{-1})	84a	88a	10.61
Volatile acidity (mEq L^{-1})	8.3b	6.7a	13.98
pH	3.58a	3.51a	2.15
Reducing sugars (g L^{-1})	2.34b	3.35a	20.15
K (mg L^{-1})	1321.20a	1324.07a	3.1
Na (mg L^{-1})	8.9a	8.6a	8.2
Ca (mg L^{-1})	73.9b	79.6a	4.7
Mg (mg L^{-1})	112.7a	109.2a	6.5
Mn (mg L^{-1})	2.5a	2.4a	3.4
Cu (mg L^{-1})	0.2b	0.3a	12.4
Fe (mg L^{-1})	1.1a	1.0a	10
Zn (mg L^{-1})	0.4a	0.2b	49.8
Li (mg L^{-1})	4.9a	4.8a	9.1
Rb (mg L^{-1})	6.4a	6.1a	7.9
P (mg L^{-1})	136.1a	119.1a	14.4
Acetaldehyde (mg L^{-1})	42.49a	31.63a	66.64
Ethyl acetate (mg L^{-1})	69.28a	74.37a	8.95
Methanol (mg L^{-1})	194.42a	197.21a	6.14
Propan-1-OL (mg L^{-1})	45.92a	43.55a	6.66
2-Methylpropan-1-OL (mg L^{-1})	50.25a	44.43a	9.28
2-Methylbutan-1-OL (mg L^{-1})	82.58a	68.87b	11.98
3-Methylbutan-1-OL (mg L^{-1})	277.09a	227.89b	12.85
Sum of higher alcohols (mg L^{-1})	455.85a	384.76b	10.93

*Distinct letters indicate significant difference between means according to Tukey's test with 5% probability

Treatment had no effect on the contents of the main wine minerals. However, attention is drawn to the major participation of K in mineral composition, with values between 1321.20 mg L^{-1} and 1324.07 mg L^{-1}. These values are low considering the data of Rizzon and Miele (2004) from Tannat wines of eight harvests, with contents varying between 1644 mg L^{-1} and 2221 mg L^{-1}. The lower K contents in our wines were most likely one of the reasons for their better pH preservation, contrasting with observations of wine with higher K content, whose pH increased to close to 4.0(Rizzon and Miele, 2004). The association of low K content and pH between 3.51 and 3.58 allows us to predict an adequate stability of wines with or without PFCM.

The concentrations of total higher alcohols in our wines, ranging between 455.85 mg L^{-1} in T1 wines and 384.76 mg L^{-1} in T2 wines, were similar to those found by Rizzon and Miele (2004) for Tannat wines. The difference between T1 and T2might be associated with the higher rate of fermentation in T1 wines. This behavior is corroborated by Glories and Maujean (2003), who state that practices that increase the fermentation velocity also increase higher alcohol formation.

The hypothesis of this work was that with PFCM before alcoholic fermentation, there would be a greater extraction of color and *trans*-resveratrol components, as well as the promotion of a proportionally superior extraction of more polymerized tannins. We clearly observed greater color extraction in wine elaborated with PFCM, where the highest values were found in yellow (DO 420), red (DO 520) and blue (DO 620) variables, resulting in higher color intensity (DO 420 nm + DO 520 + DO 620) (Figure 1). Moreover, the observed values, even in the T1 group, were higher than those normally cited for Brazilian red wines, including the wines produced from the Tannat variety (Rizzon and Miele, 2004). The most likely reason for this behavior is that the wine comes from espalier vineyards with good solar orientation (north-south), good handling of leaf removal, good leaf area/kg of grapes ratio (1 m^2/kg) and good productivity control per plant (3.5 kg/plant) in regions with climatic conditions allowing a relatively late harvest of this variety. In addition, PFCM contributed to a higher color extraction, supporting our hypothesis.

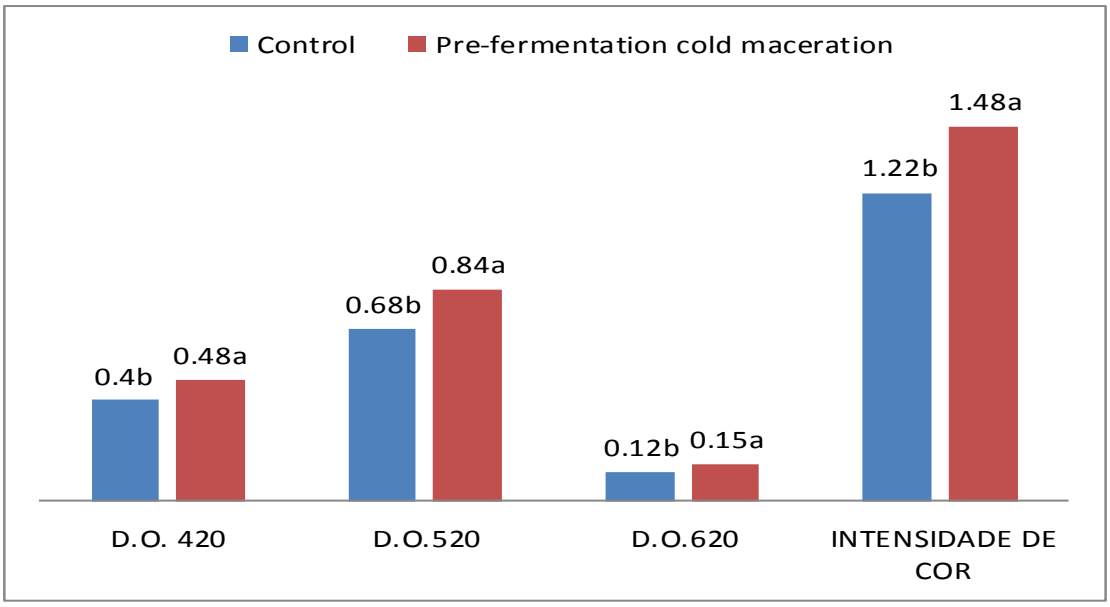
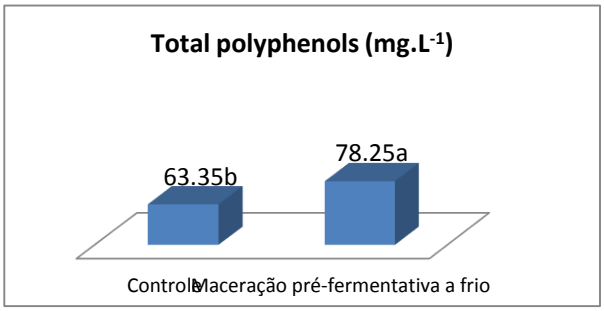
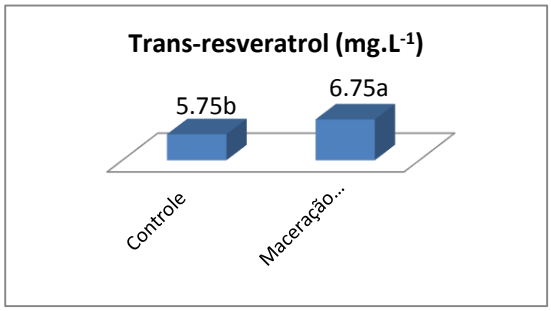
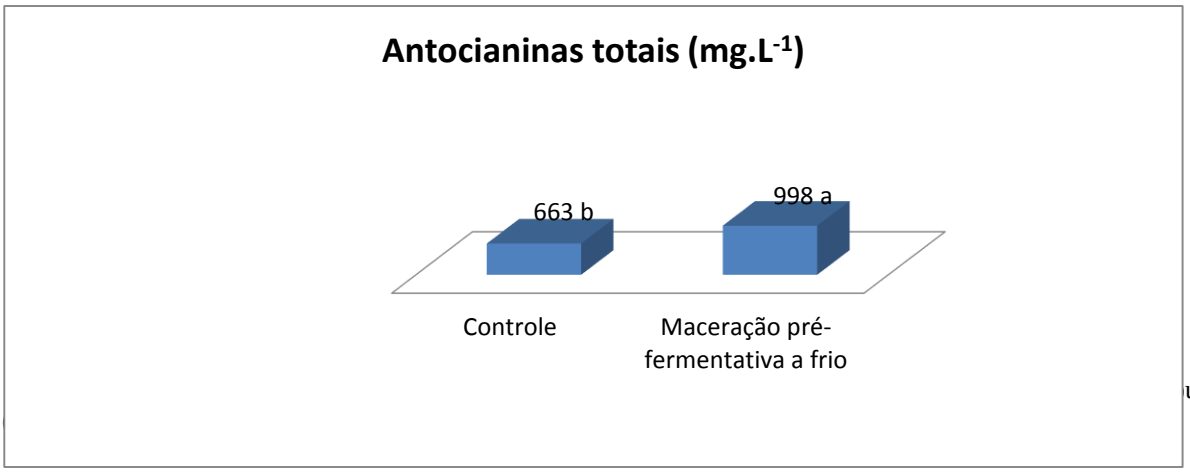


Fig. 1: Coloration of Tannat wine produced with and without pre-fermentation cold maceration.

Polyphenols, which have a direct impact on wine characteristics and quality, especially in red wine, also were significantly more abundant in PFCM wines (Figure 2). Jackson (2008) stated that polyphenols are important because they affect the appearance, taste, aroma and antimicrobial properties of wine.Concerning the anthocyanins, we found that control wine had a content of 663 mg L^{-1}, and wine produced via PFCM had 998 mg L^{-1}anthocyanins, which contributed to the higher coloration of PFCM wines (Figure 2). The polyphenol content also followed this trend, with values of 62.35 mg L^{-1}in control wines and 78.25 mg L^{-1}in PFCM wines. These results are relevant because anthocyanins and tannins are the major components of phenolic fractions in the wine (Ribéreau-Gayon *et al*., 2003). When polymerized with tannins, anthocyanins form more stable pigments, promote better color stability and contribute to the structure of red wine (Kennedy, 2008). Our physico-chemical evaluations showed that wines obtained by PFCM had higher concentrations of phenolic compounds (Figure 2) and polymeric fractions (Table 2).







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Although extraction of these components was not monitored during the fermentation process and no separate evaluation of seeds and skin was performed, at regional edaphoclimatic conditions, the phenolic ripeness of skin occurs faster and more completelythan that of the seeds. Therefore, PFCMmay have contributed to a proportionally higher extraction of these compounds from the skin in relation to that from the seeds, resulting in wine with higher concentrations of color components and tannin complexes.

Table 2: Concentrations of monomeric, oligomeric and polymeric flavanols (mg.L[1]) in Tannat wines from control and pre-fermentation cold maceration protocols.

Winemaking	Monomeric	Oligomeric (2 to 20)	Polymeric (over 20)	Total
Control	9.74±2.39	69.38±9.68	258.14±19.68	337.26
Pre-fermentation cold maceration	5.24±1.68	76.23±10.27	299.25±9.65	380.72

Like the color intensity and total phenolic compounds, the *trans*-resveratrol content was also higher in PFCM wines, reaching an average of 6.75 mg L^{-1}, while the control treatment resulted in values of 5.85 mg L^{-1} (Figure 2). This result indicates that these wines had high concentrations of *trans*-resveratrol compared to wines from other regions, which typically have 0.125-5.75 mg L^{-1} *trans*-resveratrol, except for one that was close to 14.3 mg L^{-1} (Abril *et al*., 2005, Dourtoglou *et al*., 2005, Gambuti *et al*., 2004, Ribeiro de Lima *et al*., 1999, Souto *et al*., 2001, Stervbo *et al*., 2007).This fact is relevant because resveratrol has functional properties (Aggarwal *et al*., 2004, Ito *et al*., 2003).

The cause of the elevated content for *trans*-resveratrol in Tannat wine produced with grapes from the region of Campanha, RS, was not evaluated by this study, but it is known that synthesis of this component is the result of abiotic (hydric, thermal and UV radiation variations, among others) and biotic stresses, such as attack by microorganisms (Stervbo *et al*., 2007), which are frequent factors in the region of this experiment. Stervbo *et al*. (2007) state that no region can claim the production of grapes with the highest resveratrol content, as this parameter has a multivariate and complex origin. The interaction between genotype, soil, climate, vineyard handling (Abril *et al*., 2005, Dourtoglou *et al*., 2005, Gambuti *et al*., 2004, Ribeiro de Lima *et al*., 1999, Stervbo *et al*., 2007) and winemaking process (Gambuti *et al*., 2004) affects resveratrol content. In this work, the use of pre-fermentation cold maceration contributed to a higher extraction of *trans*-resveratrol. However, the extraction kinetics were not monitored, and we only determined its total content in stabilized wine after 6 months of bottling. Although our data do not provide a conclusive evaluation, the presence of high *trans*-resveratrol content and the positive contribution of pre-fermentation cold maceration represent relevant information for future classification of grapes from this region and for the establishment of oenological procedures for winemaking.

The higher antioxidant capacity values obtained with PFCM (25.5% higher) in winemaking are consistent with the behavior of other variables that contribute favorably to this property, such as the higher phenol (25.5% higher), anthocyanin (50% higher) and resveratrol contents (17% higher). Their absolute values were relatively high compared to normally detected values in Brazilian red wines (between 10.3 and 22.0 µmol mL^{-1}) (Abe *et al*., 2007, Cataneo *et al*., 2008), and also in wines from other countries (between 12.8 and 25.2 µmol mL^{-1}) (Beer *et al*., 2003, Burns *et al*., 2000, Landrault *et al*., 2001). It is probable that regional climate conditions, characterized by low levels of rainfall during the ripening period, high solar exposure by the conduction and handling system and a good day-night thermal gradient favor the strong expression of antioxidants in Tannat grapes from this region, similar to what occurs in Uruguay (Echeverry *et al*., 2005). In addition, PFCM significantly contributed to an increased extraction of these components, resulting in an antioxidant activity 27.37% higher than that in wine elaborated without PFCM.

Table 3: Antioxidant capacity (µmol Trolox equivalentmL^{-1}wine) of Tannat wines produced with and without pre-fermentation cold maceration

Wine	Antioxidant Capacity
Control	17.9 ± 0.9b
Pre-fermentation cold maceration	22.8 ± 0.5

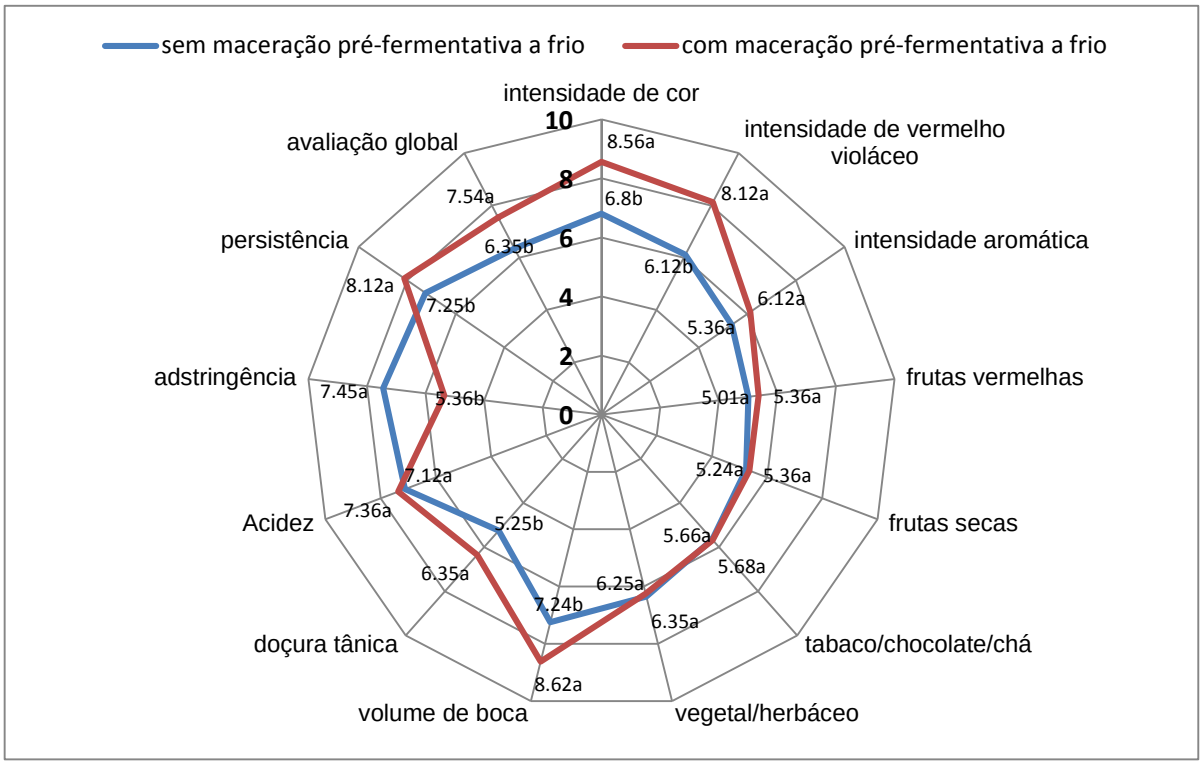

Fig. 3: Sensory analysis of Tannat wines with and without pre-fermentation cold maceration.

The sensorial analysis of wines (Figure 3) confirmed the physico-chemical data, meaning that the wine produced with PFCM was perceived by evaluators as having higher color intensity(especially the violaceous), greater mouth volume and tannin sweetness and lower astringency and persistency. This perception is consistent with the fact that PFCM favored a greater extraction of color components and more polymerized tannins (Figure 2, Table 3). The better global evaluation of the PFCM wine indicates the oenological viability of this practice for winemaking with Tannat grapes from the studied region of Brazil. This work presents a great contribution to the agricultural sciences mainly crop science, by revealing the performance of Tannat grape varieties used to produce wine in the campaign region in the state of Rio Grande do Sul, Brazil. Thus, possible to set the standard for quality and constituents of greater evidence and importance in the practice of enology, this information can be applied to further studies in research institutions, and winemakers with interest to better understand the wine characteristics produced in southern Brazil.

Conclusion:

PFCM of Tannat grapes from the region of Campanha, RS, Brazil, increases the extraction of anthocyanins, *trans*-resveratrol, total polyphenols and tannins with higher polymerization, thus contributing to the production of a wine with greater color intensity and tasting quality.

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