

Effect of Cold Pre-Fermentative Maceration on 'Touriga Nacional' Wine Varieties in the Campanha Gaúcha Region, Brazil.

ABSTRACT

Aims: The objective of this work was to evaluate the use of cold pre-fermentative maceration in wines elaborated with the *Touriga Nacional* cultivar in the region of Campanha Gaúcha. 60 kg of grapes of the *Touriga Nacional* cultivar, with 19° Brix, were obtained from the municipality of Bagé - Rio Grande do Sul, Brazil.

Study design: The microvinification was divided in two treatments with three replicates each, with the first treatment (T1) corresponding to traditional maceration carried out for 8 days at temperatures of 22 °C, and the second treatment (T2) corresponded to the cold pre-fermentative maceration performed for three days, with temperatures of 5 to 8 °C.

Place and Duration of Study: The experiment was carried out at the experimental winery and the physicochemical analyses were carried out at the TPOAV laboratory of the Federal University of Pampa (UNIPAMPA), Dom Pedrito campus, during the cycle 2016/2017.

Methodology: The first treatment (T1) corresponds to traditional maceration, where the maceration occurred along with the alcoholic fermentation, in which the skins remained in contact with the wine for eight days under a temperature of 22°C. The second treatment (T2) corresponds to the cold pre-fermentative maceration, where the must remained in a cold room (5 to 8°C) for three days, and afterwards, the alcoholic fermentation was carried out in the presence of the grape skin for eight days, at the same temperature as the previous treatment. The variables analyzed were pH, total acidity (meq.L⁻¹), volatile acidity (g.L⁻¹), alcoholic content (% v/v), reducing sugars (g.L⁻¹), gluconic acid (g.L⁻¹), phenolic compounds, color indices (420, 520 and 620 nm), color intensity (420 + 520 + 620 nm), and color tone (420/520 nm). The total polyphenols index (TPI), ethanol index (%), HCL index (%), total anthocyanins (mg.L⁻¹), total tannins (g.L⁻¹) and gelatine index (%) were also analyzed. And the sensorial analysis as well.

Results: The alcoholic content of wines corresponding to the traditional maceration (T1) was higher than that observed in wines submitted to cold pre-fermentative maceration (T2). In relation to pH, T2 obtained a lower value than T1, conferring a more acidic character to the wine. The color tone of T1 was higher than T2. There was no significant difference in the other indices analyzed.

Conclusion: The results indicate that other techniques should be evaluated to increase the extraction of the phenolic compounds. Further studies on the *Touriga Nacional* cultivar and the vinification methods are necessary to improve the quality of the wines from this cultivar.

Keywords: Vitiviniculture, *Vitis vinifera*, phenolic compounds, color indexes.

1. INTRODUCTION

The Campanha Gaúcha Region, in Rio Grande do Sul, Brazil, in the Pampa Biome, is located in Parallel 31° South, consisting of 19 municipalities, in a total area of 62,681,157 km². This region has undergrowth and slightly undulating soils. Traditionally, it has always been linked to the exploitation of livestock and rice. However, in recent decades, favored by factors such as climate and soil, it has stood out as one of the grapes and fine wine producing centers.

One of the most challenging aspects of modern oenology is the use and improvement of effective technologies to explore the characteristics of grapes and their preservation in final wines [1]. The quality of a wine is related to factors such as the sanitary conditions of the grape and the vinification technology used [2]. In addition, soil, climatic conditions and vine handling also influence the final product [3]. These factors are responsible for determining the chemical characteristics and influence on the sensorial attributes of wine.

The *Touriga Nacional* cultivar is a *Vitis vinifera* variety, characterized by moderate productivity, due to the low weight of the bunch, although its fertility is high. The musts of this variety show high levels of probable alcohol and acidity, yet, very balanced. Due to the aromatic complexity, structure and quality of the phenolic compounds, it is a cultivar of excellence used to produce varietal wines or to improve a batch with other varieties [4].

Among fruits, grapes are considered one of the major sources of phenolic compounds. However, the great diversity among the varieties results in grapes with different characteristics, in flavor as well as coloring, a factor associated to the phenolic compounds content. There are several health benefits related to phenolic compounds, such as antioxidant, anti-inflammatory, antimicrobial and anticarcinogenic activity [5].

The cold pre-fermentative maceration is a technique that consists of leaving the wort at low temperatures, which can range from 0 °C to 15 °C [6] [7]. This methodology allows a greater contact between the solid and liquid parts of the must. The objective of this practice is to increase the extraction and stabilization of phenolic compounds, besides reducing the extraction intensity during fermentation, thus avoiding the extraction of tannins and increasing the chemical and aromatic quality of wines [1].

Considering the importance of phenolic compounds for health, and the realization of new techniques to obtain wines with better quality, and consequently added value, the objective of this work was to evaluate the use of cold pre-fermentative maceration of wines made with the *Touriga Nacional* cultivar in the Campanha Gaucha region.

2. MATERIAL AND METHODS

For the vinification experiment, 60 kg of grapes of the *Touriga Nacional* cultivar, grafted on the Paulsen 1103 rootstock, were harvested from a commercial vineyard located in the municipality of Bagé, Rio Grande do Sul, planted in a soil referred to as Santa Tecla with 18% clay and pH 6.5. The altitude of the vineyard is 350m (the geographic coordinates are 31° 13' 49.16 "South and 53° 58' 58.72" West). This vineyard was conducted in the espalier system and short pruning was used. According to [8], the climate of this region is defined as Cfa, which means humid temperate climate with hot summer, with annual precipitations ranging from 1200 to 1500 mm, relatively well distributed.

The winemaking process was carried out at the experimental winery and the physicochemical analyses at the TPOAV laboratory of the Federal University of Pampa

(UNIPAMPA), Dom Pedrito campus. After harvesting, the grapes were weighed and stored in a cold room for about 12 hours, at a temperature of 8 °C. Soon after, the destemming and crushing of the grapes were carried out, with the solid and liquid parts obtained in the microvinification format being placed in 14L bottles, divided in two treatments with three replicates each. The first treatment (T1) corresponds to traditional maceration, where the maceration occurred along with the alcoholic fermentation, in which the skins remained in contact with the wine for eight days under a temperature of 22°C. The second treatment (T2) corresponds to the cold pre-fermentative maceration, where the must remained in a cold room (5 to 8°C) for three days, and afterwards, the alcoholic fermentation was carried out in the presence of the grape skin for eight days, at the same temperature as the previous treatment.

After the destemming and crushing of the grapes, sulfur dioxide (SO₂) was added in the form of potassium metabisulphite at a dose of 100 mg.L⁻¹ to the two treatments to prevent contamination and oxidation. About 40 minutes later, pectolytic enzyme was added at a dose of 5 g.hL⁻¹, aiming at greater extraction of the compounds present in the skin of the grapes. For the beginning of the alcoholic fermentation in both treatments, active dry yeast (*Saccharomyces cerevisiae*) at a dose of 20 g.hL⁻¹ and fermentation activator at a dose of 20 g.hL⁻¹ were used. The alcoholic fermentations occurred at a temperature of around 22°C, with two setups daily for each repetition.

The chaptalization of the musts was carried out, considering the difficulty found in the maturation of the grape in the 2016 crop, in order to correct the alcoholic strength of the wine in 1.5% v/v, using 25.5 gL⁻¹ of sucrose, respecting the quantities described by Brazilian law.

After the end of the alcoholic fermentation, the wines were separated and transferred to 4.6L bottles for the malolactic fermentation, which occurred spontaneously. At the end of the experiment, SO₂ was corrected with the addition of potassium metabisulfite to reach 1 mg.L⁻¹ of molecular SO₂. The bottling was carried out four months after the end of the malolactic fermentation, in 750ml bottles.

The physicochemical analyses were performed using the Foss Wine-Scan SO2 equipment, at the TPOAV laboratory of the Federal University of Pampa, in Dom Pedrito. The technology principle used by WineScan consists of Fourier transform infrared (FT-IR) spectroscopy, at 1060 wavelengths. The calibration performed by the manufacturer, from hundreds of samples and through multivariate analysis techniques of PLS (Partial Least Square), results in the simultaneous analysis of different parameters of the wine, which can also be validated or adjusted by the user. The variables analyzed were pH, total acidity (meq.L⁻¹), volatile acidity (g.L⁻¹), alcoholic content (% v/v), reducing sugars (g.L⁻¹), gluconic acid (g.L⁻¹), phenolic compounds, color indices (420, 520 and 620 nm), color intensity (420 + 520 + 620 nm), and color tone (420/520 nm).

The total polyphenols index (TPI), ethanol index (%), HCL index (%), total anthocyanins (mg.L⁻¹), total tannins (g.L⁻¹) and gelatine index (%) were also analyzed, as described by [9], using the spectrophotometer.

The sensorial analysis was performed immediately after the wines were bottled, through a group of ten trained tasters, at the experimental winery of the Federal University of Pampa - Dom Pedrito Campus. A form prepared by the author (Annex A), with the parameters to be evaluated by the tasters was used. Samples were coded and served randomly for the analysis (Figure 1). The results of the analyses were submitted to analysis of variance (ANOVA) and the means were compared by the Tukey test at 5% of significance.

3. RESULTS AND DISCUSSION

Table 1 presents the physicochemical analyses performed in the proposed treatments.

Table 1 - Physicochemical analysis of Touriga Nacional wines. Dom Pedrito /RS, 2017.

Variables	*T1	T2	CV (%)
Alcohol (% v/v)	12.15 a	11.78 b	1.34
Acidez Total (meq. L ⁻¹)	79.06 a	81.33 a	1.36
Volatile Acidity (g.L ⁻¹)	0.40 a	0.36 a	10.65
pH	3.62 a	3.53 b	0.69
Reducing Sugars	1.80 a	1.86 a	4.45
Phenolic Compounds	32.43 a	29.23 a	6.11
Glycerol (g.L ⁻¹)	7.80 a	7.46 a	2.33
Total Anthocyanins (mg.L ⁻¹)	310.62 a	288.45 a	11.79
Total Tannins (g.L ⁻¹)	0.59 a	0.72 a	41.32
Total Polyphenols Index	47.00 a	43.76 a	5.93
**OD 420 nm	0.689 a	0.695 a	4.78
OD 520 nm	1.095 a	1.158 a	4.95
OD 620 nm	0.317 a	0.327 a	5.70
Color Intensity (420+520+620 nm)	2.101 a	2.180 a	4.94
Color Tone (420/520 nm)	0.629 a	0.600 b	1.39

* T1 - Traditional maceration; T2 - Pre-fermentative maceration. The means followed by the same letter do not differ statistically from each other. The Tukey test was applied at a 5% probability level. ** OD - Optical Density.

The alcohol content of wines made from traditional maceration (T1) presented higher alcohol content in relation to wines submitted to cold pre-fermentation maceration (T2). This result may be related to the degree of maturation of the grapes. Brazilian legislation establishes that table wine must have an alcohol content of 8.6% to 14% by volume [10]. Thus, both treatments fall within Brazilian legislation.

In relation to pH, the wine made from the pre-fermentative maceration obtained a lower value than T1, giving a more acidic character to the wine and, consequently, a higher total acidity (T2 superior to T1). Similar results were found by [11] who, when vinifying "Tannat" variety grapes, observed that the pre-fermentation maceration treatment obtained lower alcohol content and pH values. According to [12], Brazilian wines have a pH ranging from 3.0 to 3.6 depending on the type of wine (red or white), the cultivar, and the crop. For [13], pH control of musts and wines is one of the most important steps in winemaking to provide color intensity and maintain acidity, in addition to preserving microbiological stability.

For glycerol concentrations, the wines resulting from the two vinification methods did not show any difference from each other. Glycerol is one of the most abundant compounds in wine, containing about 5 g.L⁻¹ to 12 g.L⁻¹, serving as an indicator of the presence of *Botrytis cinerea* [14]. Thus, the higher the concentration of this compound in the wine, the greater the incidence of rot in the grapes. Thus, the values found are within the parameters, indicating a good grape quality.

Polyphenols are very important because they give color, flavor and aroma to the wines. In the evaluation of the total polyphenol content, no difference was found between the two evaluated treatments. According to [15], the use of cold pre-fermentative maceration induces

an increase in the polyphenol extraction, with positive effects on the final product quality, observed analytically and/or sensorially.

Color is one of the main characteristics of red wine, as it is the first one to be appreciated by the consumer, thus presenting great commercial importance [16].

The absorbance indexes 420, 520 and 620 are related to the parameters of color intensity, color tonality, tannins, anthocyanins and total polyphenols [17]. There was no statistical difference between the two treatments for any of the evaluated wavelengths (420, 520 and 620nm). According to results shown by [17], the maximum absorption at 520nm (red), characteristic of new red wines, is due to anthocyanin composition, and decreases with wine aging, while the absorption of 420nm (yellow) increases.

Color intensity values are obtained by adding the wavelengths obtained at the indices 420 nm, 520 nm and 620 nm. It was observed that there was no statistical difference between the treatments. The color tone on the other hand is represented by the division of the values of the indices 420 nm and 520 nm. Thus, the color tone of T1 was higher than T2. This result is similar to that found by [15], who observed an increase in the color tone of wine in the treatment with cold pre-fermentative maceration. Despite the difference between the wines, the two treatments present a color tonality within the recommended values in the literature. Young wines have tonalities ranging from 0.5 to 0.7, which increases during aging and reaches a limit of 1.2 to 1.3 [18].

The analysis of the variables total acidity, volatile acidity, reducing sugars, phenolic compounds, total anthocyanins and total tannins did not present statistical differences between the two vinification methods. These results demonstrate the importance of the quality of the grape that is destined to the vinification. Traditional winemaking and alternative winemaking methods may change some parameters in the resulting wines, but in general the main characteristics are dependent on the source material.

Table 2 shows the results of the analysis of gelatine index, HCL index and ethanol index, as a percentage.

Table 2 - Tannin indices of the wines of the Touriga Nacional cultivar.

Variables	*T1	T2	CV (%)
Gelatin Index (%)	70.55 a	30.12 b	32.67
HCL Index (%)	29.07 a	22.93 a	36.68
Ethanol Index (%)	92.22 a	91.44 b	0.29

* T1 - Traditional maceration; T2 - Pre-fermentative maceration. The averages followed by the same letter do not differ statistically from each other. The Tukey test was applied at the 5% probability level.

The gelatin index indicates the percentage of tannins capable of reacting with proteins or astringents and is usually between 25% and 80%. Values above 60% indicate a very astringent wine with high levels of soluble tannins. Alternatively, values below 35% indicate that the wine needs body or that an accelerated complexity of the tannins occurred. While values between 40 and 60% are considered more convenient [9]. The vinification methods employed in this study resulted in significant differences. T1 obtained a Gelatin index of 70.55% and 30.12% for T2, thus characterizing the wine elaborated from the traditional maceration as astringent, a characteristic with an aging potential for wine. The wine made with a pre-fermentative maceration proved to be a light wine, which allows its consumption relatively fast.

The hydrochloric acid content in red wines is between 5% and 40%. In wines with values above 35% to 40%, there is a strong probability that precipitation occurs, since they indicate high concentrations of polymerized tannins. Values ranging from 10% to 25% are considered adequate for a wine to be aged [9]. There was no statistical difference between the treatments, however, according to the values found, the wine prepared from the pre-fermentative maceration presents potential for aging.

In relation to the ethanol index, which represents the percentage of tannins that are combined with polysaccharides, there was a statistical difference, where T1 presented 92.22% of tannins combined with polysaccharides and T2 presented 91.44%.

After stabilization, the wines were submitted to a sensorial analysis, and the results were submitted to statistical analysis. According to Table 3, the results of the sensorial analysis did not present significant differences, but some characteristics were perceived.

Table 3 - Results of the sensorial analysis of the treatments.

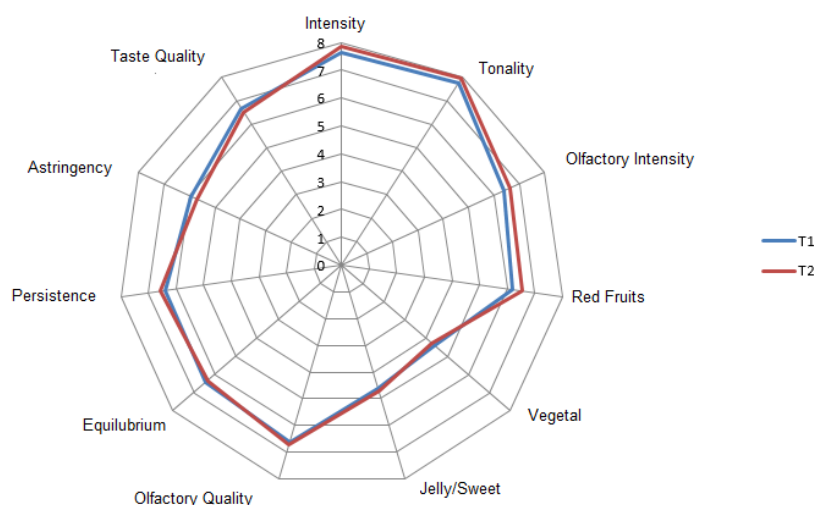
Variables	T1	T2	CV %
Intensity	7.61 a	7.85 a	3.46
Tonality	7.78 a	7.95 a	1.76
Olfactory Intensity	6.41 a	6.65 a	6.92
Red Fruits	6.20 a	6.56 a	4.04
Vegetal	4.40 a	4.30 a	6.3
Jelly/Sweet	4.60 a	4.70 a	8.6
Olfactory Quality	6.63 a	6.71 a	1.47
Equilibrium	6.46 a	6.36 a	4.76
Persistence	6.38 a	6.58 a	4.25
Astringency	5.93 a	5.70 a	7.23
Taste Quality	6.68 a	6.53 a	2.86
Overall Rating	81.70 a	79.76 b	0.85

**T1 - Traditional maceration; T2 - Pre-fermentative maceration. The averages followed by the same letter do not differ statistically from each other. The Tukey test was applied at a 5% probability level.*

In the visual analysis, the intensity and tone parameters did not present statistical differences between the treatments. However, as observed in Fig. 1, the wine elaborated with the pre-fermentative maceration has a higher color intensity and a more intense tonality. The intensity refers to the clarity of the color and, along with the tonality, provides clues about characteristics such as grape maturation, contact time in maceration, fermentation in barrels and the age of wine [19].

Regarding the olfactory characteristic, there were no statistical differences; however, the wine elaborated with the pre-fermentative maceration presented higher intensity and olfactory quality (Fig. 1). For the vegetal and jelly/sweet descriptors, there were no significant differences between the treatments. The fruity aroma, represented by the red fruits, characteristic of the cultivar, was more representative in T2. This result indicates that the pre-fermentative maceration indicates an increase in the fruity characteristics, associated with the *Touriga Nacional* cultivar, presenting a predominance of the varietal aroma.

228 Regarding the taste profile of the wines, it is observed that the wine elaborated by the
 229 traditional method presented the highest results for the descriptors of equilibrium,
 230 astringency and taste quality, while the other presented a higher value for the descriptor
 231 persistence.
 232



233 **Fig. 1 - Visual, aromatic and taste profile of Touriga Nacional wines.**
 234 * T1 - Traditional maceration; T2 - Pre-fermentative maceration. The Tukey test was applied at
 235 a 5% probability level.
 236
 237

238 In the overall evaluation, there was a statistical difference, where the wine elaborated by the
 239 traditional method obtained higher grades in relation to the wine elaborated with the pre-
 240 fermentative maceration, and the averages were 81.70 and 79.76 points, respectively.
 241 These values prove the good quality of the wines, so there is no need for corrections. When
 242 asked if they would buy the analyzed products, the tasters answered yes to both treatments,
 243 noting that the products were considered as pleasing to the palate of these consumers.
 244

245 According to results, one can observe the values that the tasters would be willing to pay for
 246 the product, and the values were close, with the average being R\$ 31.33 for the wine
 247 elaborated from the traditional methodology and R\$ 29.66 for the wine made with the pre-
 248 fermentative maceration.
 249

250 4. CONCLUSION

251
 252 The cold pre-fermentative maceration in *Touriga Nacional* wines obtained positive results,
 253 mainly observed in the sensorial analysis, where the parameters of intensity and color
 254 tonality were higher than in traditional maceration. Higher values for olfactory intensity, red
 255 fruit aromas and persistence were also found, showing that cold pre-fermentative maceration
 256 mainly emphasizes varietal aromas.
 257

258 However, longer cold pre-fermentative maceration times should be assessed for a better
 259 evaluation of the characteristics provided by this method. In addition, other techniques
 260 should be evaluated in order to increase the extraction of phenolic compounds. Further
 261 studies on the *Touriga Nacional* cultivar and vinification methods are necessary to improve
 262 the quality of the wines made with this cultivar.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Côte-Real, DCC. Effects of pre-fermentative cold maceration and application of oenological tannins in red winemaking. Dissertation (Master in Viticulture and Oenology) - Higher Institute of Agronomy, Technical University of Lisbon, 2009. Portuguese.
2. Castilhos, MBM, Del Bianchi, VL. Red wines of the Northwest Paulista: Relationship Between the Physical-Chemical and Sensorial Profiles. HOLOS, 2012; 28 (4) Portuguese.
3. Chavarria, G, Santos, HP. Management of vines under protected cultivation. Rural Science, 2009; 39 (6): 1917-1924. Portuguese.
4. Palma, AFJ. Effect of deficit irrigation on physiology, vegetative growth, grape production and quality, Touriga Nacional grape variety (*Vitis vinifera* L). Dissertation (Master in Viticulture and Enology) - Higher Institute of Agronomy, Technical University of Lisbon, 2014. Portuguese.
5. Abe, LT, Da Mota, RV, Lajolo, FM, Genovese, MI. Compostos OL. and *Vitis vinifera* L. Ciênc. Techn. Aliment., 2007; 27 (2): 394-400. portuguese.
6. Albanese, D, Attanasio, G, Cinquanta, L, Di Matteo, M. Volatile compounds in red wines processed on an industrial scale by short pre-fermentative cold maceration. Food and Bioprocess Technology, 2013; 6(11): 3266-3272.
7. Cai, J, Zhu, BQ, Wang, YH, Lu, L, Reeves, MJ, Duan, CQ. Influence of pre-fermentation cold maceration treatment on aroma compounds of Cabernet Sauvignon wines fermented in different industrial scale fermenters. Food chemistry. 2014; 154: 217-229.
8. Köppen, William.1931. Climatology. Mexico, Fund for Economic Culture.
9. Zamora, F. Elaboration and Breeding of Red Wine: Scientific and practical aspects. 1.ed. Madrid: Ediciones Mundi-Prensa, 2003. Spanish.
10. Ministry of Agriculture. Law No. 7678, of November 08, 1988, as amended by Law No. 10970 of November 12, 2004. Accessed 26 september 2016. Available:: <http://www.ibravin.org.br/Legislacao-Brasileira>.
11. Zocche, RGS. Oenological potential of Tannat, Cabernet Sauvignon and Merlot grapes produced in the municipality of Bagé-RS. 2009, Thesis, UFPel, Pelotas, RS. Portuguese.
12. Tecchio, FM, Miele, A, Rizzon, L.A. Sensorial characteristics of Bordô wine. Pesq. Agropec. Bras. 2007; 42 (6): 897-899. Portuguese.
13. Lasanta, C, Caro, I, Perez, L. The influence of cation exchange treatment on the final characteristics of red wines. Food Chemistry. 2013; 138: 1072-1078.
14. Hipólito-Reis, C. Wine, Gastronomy and Health. University of Porto. 2008. Portuguese

- 316 .
317
318 15. Dal'Osto, MC. Use of cold maceration in the extraction and stabilization of phenolic
319 compounds in Syrah wines cultivated in the October-winter cycle. Dissertation (Master in
320 Food Science and Technology) - Luiz Queiroz College of Agriculture, University of São
321 Paulo, Piracicaba, 2012. Portuguese.
322
323 16. Mateus, N, Freitas, V. Latest scientific progress on wine pigments. Internet Magazine of
324 Viticulture and Enology, 5 p. 2006. Portuguese.
325
326 17. Costa, VB, Guedes, LC, Gabbardo, M. Physico-chemical and sensorial analyzes of table
327 wines made from Isabel and Jacquez varieties. Spaces. 2016; 37 (26): 28. Portuguese.
328
329 18. Ribéreau-Gayon, P, Dubourdieu, D, Donèche, B, Lonvaud, A. Treaty of winemaking:
330 microbiology of wine, winemaking. Buenos Aires: Hemisfério Sur, 2003. v.1. Spanish.
331
332 19. Jackson, R. Sensory analysis of wines, manual for professionals. Zaragoza - Spain
333 Editora Acribia, 2009. Spanish.
334
335