



Experience of Cold Maceration on ‘Touriga Nacional’ Wine Varieties in the Campanha Gaúcha Region, Brazil

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MRL and VBC designed the study and wrote the first draft of the manuscript. Author MRL performed the experiments. Authors DPE and WMC participated in fieldwork and laboratory analysis. Authors MG and RLS managed the analyses of the study. Author SBA managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2018/40119

Editor(s):

(1) Moreira Martine Ramon Felipe, Associate Professor, Departamento de Enxéñaría Química, Universidade de Santiago de Compostela, Spain.

Reviewers:

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(2) Maria Carla Cravero, Italy.

Complete Peer review History: <http://www.sciencedomain.org/review-history/24084>

Short Research Article

Received 25th January 2018

Accepted 3rd April 2018

Published 10th April 2018

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 into two treatments, 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. Each treatment was replicated three times contented 4.6 liters of wine, finally.

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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 remain in a cold room (5 to 8°C) for three days, and afterward, 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. The wine sensory analysis was performed immediately after bottling.

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 cold pre-fermentative maceration in *Touriga Nacional* wines seems to give positive results, even if the statistical analysis evidenced significant results only for the wine sensory global pleasantness. 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 vinific

Keywords: *Brazilian wines; cold maceration; phenolic compounds; color indexes; touriga nacional; sensory analysis.*

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 are very balanced, presenting high levels of probable alcohol and acidity. 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 with 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 most important 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. MATERIALS AND METHODS

2.1 Materials

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 Köppen [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.

2.2 Treatment T1 and T2

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 contented 4 liters of wine, finally. 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.

2.3 Fermentation

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.

2.4 Physicochemical Analyses

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 107 acid (g.L⁻¹), phenolic compounds, color indices (420, 520 and 620 nm), color

intensity (420 + 108 520 + 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 Zamora [9], using the spectrophotometer.

2.5 Sensory Analysis

The sensory 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 with the parameters to be evaluated by the tasters was used. Samples were coded and served randomly for the analysis. In particular, the parameters were: intensity, tonality, olfactory intensity, red fruits, vegetal, jelly/sweet, olfactory quality, equilibrium, persistence, astringency, taste quality, global pleasantness.

2.6 Statistical Analysis

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.

A coefficient of variation (CV) is a statistical measure of the dispersion of data points in a data series around the mean. It is calculated as follows: (standard deviation) / (expected value). The coefficient of variation represents the ratio of the standard deviation to the mean, and it is a useful statistic for comparing the degree of variation from one data series to another, even if the means are drastically different from one another.

3. RESULTS AND DISCUSSION

Table 1 presents the physicochemical analyses performed in the proposed treatments. The alcohol content in T1 was higher than in T2. The alcohol high content can be explain through yeasts activities because the T1 increased fermentation activities of yeast strains. In fact, the temperature (22°C) improve the yeast growth than the low temperature (5-8°C). 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 T2 obtained a lower value than T1, giving a more acidic character to the wine and, consequently, a higher total acidity. Similar results were found by Zocche [11] who, when vinifying "Tannat" variety grapes, observed that the pre-fermentation maceration treatment obtained lower alcohol content and pH values. According to Tecchio et al. [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 Lasanta et al. [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.

The wines resulting from the two vinification methods showed no statistical difference for glycerol concentrations. 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. As the values found are within the parameters, the grapes used in the experiment had good quality in relation to the fungal contamination.

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 Dal'Osto [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 620 nm). According to results shown by Costa et al. [17], the maximum absorption at 520 nm (red), characteristic of new red wines, is due to anthocyanin composition, and decreases with wine aging, while the absorption of 420 nm (yellow) increases.

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. Means followed by the same letter are not different according to ANOVA and Tukey-test ($p < 5\%$)

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 Dal'Osto [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. 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%.

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. Means followed by the same letter are not different according to ANOVA and Tukey-test ($p < 5\%$).

Table 3. Results of the sensory 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
Global pleasantness	81.70 a	79.76 b	0.85

*T1 - Traditional maceration; T2 - Pre-fermentative maceration. Means followed by the same letter are not different according to ANOVA and Tukey-test ($p < 5\%$).

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

In the visual analysis, the intensity and tone parameters did not present statistical differences between the treatments. However, as observed in Fig. 1, T2 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, T2 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.

Regarding the taste profile of the wines, it is observed that T1 presented the highest results for the descriptors of equilibrium, astringency and taste quality, while the other presented a higher value for the descriptor persistence.

There was a statistical difference in the global pleasantness: the wine T1 obtained a higher rate than the wine T2 and the averages were 81.70 and 79.76 points, respectively. These values prove the good quality of the wines, so there is no need for corrections. The tasters expressed a preliminary opinion on the pleasantness of the two wines. They were asked if they would buy the analyzed products, and they did not expressed a preference for one product or the other. Both wines were considered equally pleasant.

According to results, one can observe the average values that the tasters would be willing to pay for both products were very



Fig. 1. Visual, olfactory and taste profile of the touriga nacional wines

(No significant statistical differences were found between the sensory profiles of the T1 and T2 wines, with the ANOVA and Tukey test, $p < 95\%$)

* T1 - Traditional maceration; T2 - Pre-fermentative maceration.

similar: R\$ 31.33 for the wine elaborated from the traditional methodology and r\$ 29.66 for the wine made with the pre-fermentative maceration.

4. CONCLUSION

The cold pre-fermentative maceration in *Touriga Nacional* wines seems to give positive results, even if the statistical analysis evidenced significant results only for the wine global pleasantness. However, the parameters of intensity and color tonality are slightly higher than in the traditional maceration as well as the values of the olfactory intensity, the red fruit aromas and the persistence, showing that cold pre-fermentative maceration mainly emphasizes varietal aromas.

However, longer cold pre-fermentative maceration times should be assessed for a better evaluation of the characteristics provided by this method. In addition, other techniques should be evaluated in order to increase the extraction of phenolic compounds. Further studies on the *Touriga Nacional* cultivar and vinification methods are necessary to improve the quality of the wines made with this cultivar. Larger scale studies are also needed, as in the present study wines were tested only on a laboratory scale.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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