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RESEARCH ARTICLE

DETERMINATION OF THE ANTIOXIDANT ACTIVITY OF RED AND WHITE WINES PRODUCED IN RIO GRANDE DO SUL, BRAZIL

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ABSTRACT

The polyphenolic components of wines can be correlated to their antioxidant activity, which can provide human's health with several benefits. Samples of eight different red and white wines, produced in the region were characterized in terms of their antioxidant capacities, resveratrol concentration, color index, tartaric acid content, and total polyphenols concentration. The total polyphenols content found in red wines was higher than in white wines. Among the varieties tested, the red wine Tannat accounted for the highest polyphenols content (19.1 µg/L) compared to the white variety, Chardonnay with 10.96 µg/L. Burgundy showed the highest resveratrol content (43.29 µg/mL). The highest concentration of tartaric acid was found in the red wines Burgundy (0.60%) and Cabernet Sauvignon (0.58%) while the dry whitewine accounted for the lower concentration (0.47%).

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INTRODUCTION

The state of Rio Grande do Sul, located in the south region of Brazil, is one of the most important producers of grapes, and this culture has a significant importance to the state's economy. The state represents 95% of the national production, accounting for 330 million of liters of wine produced annually. The climate has a notable influence on the vines's characteristics (Gruppelli *et al*., 2008). In this context, the region of Alto Uruguai can be considered new to the cultivation of vines. Although it has peculiar characteristics, such as an early harvest period compared to other regions of the same state, specially the famous region known as Serra Gaúcha.

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Consequently, contributing with the competitiveness on the wine sectornacionally. The main substances that compose wines are sugars, organic acids, salts of mineral and organic acids, phenolic compounds, nitrogeneous substances, pectins, volatile and aromatic compounds (ethers, aldehydes, ketones), vitamins e sulfur dioxide (Hashizume *et al*., 2001). In addition, wine is one of the main sources of polyphenols, substances that exhibit high antioxidant properties (Alen-Ruiz *et al*., 2008). The polyphenols are present in both red and white wines, but in a higher concentration in red wines where they contribute to its astringency, bitterness, to the wine's color and its level of oxidation. The different phenolic compounds found in grapes are phenolic acids (gallic acid), flavonoids that are present in the skins and seeds (red anthocyanins, cathechins), and polymeric condensed tannins (Waterhouse; Wrolstad, 2001). Several studies demostrate that the consumption of food rich in antioxidants can contribute for the prevention of a number of

diseases, such as cardiovascular or neurological diseases, and cancer. In wines this antioxidant property is attributed to components like polyphenols and resveratrol. (Pretti, 2016). The concentration and properties of the phenols in wines might be influenced by the grape variety, soil type, growing conditions, the harvesting system and the vinification process (maceration, fermentation, clarification, aging, etc.). In the production process of red wines, the whole grapes are utilized, including the seeds and skins, which contain significant amounts of phenols. On the other hand, the white wines are processed by pressing off the skins and seeds right after collecting the fruits. Therefore, yielding a lower amount of phenols (Adamski, 2015).

Resveratrol (trans-3,5,4-trihydroxystilbene) is a phytochemical that exists as *trans* and *cis* isomer. It belongs to the stilbene family, and is the major active compound at this family. The interest in resveratrol increased when epidemiological studies proved that the consumption of red wine decreases the incidence of cardiovascular diseases (French paradox). Resveratrol also has anticancer, antifungal and antimicrobial activities (Kolouchova-Hanzlikova, 2004). The presence of organic acids in wines influence significantly on organoleptic properties such as flavor, color and aroma; and in the stability microbiologic control. These acids result from the fermentation process or come directly from the fruit (Matos, 2005). Tartaric acid is considered the most important and strongest acid present in wines. It influences on the color, taste, and chemical and biological stability of the product (Oliveira, 2009). However, studies regarding the quality of the wines produced in this region of the state of Rio Grande do Sul – Brazil.

MATERIALS AND METHODS

Eight samples of wines: red (Cabernet Sauvignon, Burgundy, Tannat and Merlot), and white wines (White Niágara, Chardonnay, Dry white wine and Muscat Embrapa); were provided by eight different wineries located in the north region of the state of Rio Grande do Sul known as Médio Alto Uruguai Region.

Total polyphenols

The total polyphenols content was determined by the FolinCiocalteau method, which is based in the reduction of the phosphomolybdic and phosphotungstic acids in the presence of phenolic forms, resulting in the formation of blue colored complexes that strongly absorb light between 620 e 700 nm. The wine samples were diluted indistillated water (1:200). Subsequently, 1 mL of each diluted sample was transferred to a test tube, and was added 1 mL of the reagent Follin Ciocalteauand 1 mL of Na_2CO_3 (10% m/V).

The mixture was left reacting for 2 h protected from radiation. Following, the absorbance of the samples was measured in spectrophotometer UV/VIS at 765 nm wavelength. The quantification was made based on angallicacid standard curve at the following concentrations: 50, 40, 30, and 10 μg/L. The reaction occurred in the same conditions as the wine samples, and the absorbance was measured at 765 nm.

Determination of resveratrol content

The resveratrol content was determined by high performance liquid chromatography method (HPLC). The chromatograph used was a Perkin Elmer … equipped with a C18 column and a 20 μL injector. The elution was made in the isocratic mode (flux of 1,5 mL/min), the mobile phase used was acetonitrile (80%) and deionized water (20%). The absorbance was measured at 306 nm. The retention time at the column was in average 1.79 minutes. Samples of 100 mL of wine were extracted by liquid- liquid extraction by using 3 portions of 50 mL of ethyl acetylate. The organic phases were washed and taken for drying in a rota evaporator, and after filtration in a cellulose nitrate membrane 0.45 μm the samples were analyzed by chromatography. For quantification, was used a resveratrol standard curve in the following concentrations: 40, 30, 20, 10 and 1 μg/mL.

Determination of tartaric acid

The tartaric acid concentration was determined by neutralization titration. 75 mL of deionized water were heated. Right after the boiling started, 5 mL of wine were added, so the CO_2 and SO_2 present could be eliminated, and avoid their interference. Next were added 2 drops of indicator Bromothymol blue to the solution, and the titration was performed utilizing as titrant a solution of NaOH 0.1 mol/L standardized with Potassium Biphthalate. When the equivalence point was achieved, the volume of NaOH consumed to neutralize the organic acids was noted, and then were made the calculations in order to quantify the content of tartaric acid in each sample analyzed.

Color intensity and tonality

The samples were diluted in distilled water (10 times diluted), filtered in a cellulose nitrate membrane (0.45μm), then the absorbance of each sample was determined in spectrophotometer UV/VIS in three wavelengths: 420, 520 and 620 nm. From the spectral data obtained, the color intensity (equation 1) and color tonality (equation 2) were calculated according to the methodology described by Glories and cited by Castillo-Sánchez (2006).

$$CI = Ab._{420\ nm} + Ab._{520\ nm} + Ab._{620\ nm} \quad (1)$$

$$CT = Ab._{420\ nm} / Ab._{520\ nm} \quad (2)$$

Determination of the antioxidant activity

The antioxidant capacity was evaluated by the commonly used DPPH assay. The method consists of a photometric test where the radical 2, 2 difenil-1-picrilhidrazil (DPPH), which has intense purple color in alcoholic solution, is reduced in the presence of antioxidant molecules, forming a colorless compound. The samples were diluted in ethanol (1:1dilution). Subsequently, 0.1 mL of each solution was placed in a test tube covered in aluminum paper, and 4 mL of ethanolicsolution of DPPH 0.1 mMol/Lwas added. After 30 minutes the absorbance was measured. The following equation was used to determine the antioxidant activity:

$$\%\ \text{Antioxidant activity} = [(Ab._{DPPH} - Ab._{Sample}) \times 100] / Ab._{DPPH}$$

RESULTS AND DISCUSSION

Antioxidant activity

According to the results obtained, the variety Cabernet Sauvignon (81.64%) showed the highest antioxidant activity while the dry white wine had the lowest activity (37.47%). The results can be observed on the table 1 that follows. The red wines showed a higher capacity on inhibiting the free radical DPPH, so they have higher antioxidant activity. This behavior can be explained by the fact that the red wines have greater quantity of phenolic compounds comparing to the white wines. Most of the red wines are produced utilizing the seeds, skins and pulp, resulting in a higher amount of phenolic compounds (Frankel; Waterhouse; Teissedre, 1995).

Color intensity and tonality

The variety Tannathad the more intense color (1.42). The color intensity of the white wines did not showed a significant variation among the samples analysed. The lower intensity was observed in the variety Muscat Embrapa (0.16). This behavior is related, aside from other factors, to the existence of the anthocyanins in red wines. These are phenolic compounds that have high antioxidant activity, therefore, absent in the white grapes (Hashisume *et al*., 2001). Therefore, it can be concluded that the higher the color intensity, the higher is the antioxidant activity. The absorbance of all the samples decreased as the wavelength was increased, except for the variety Tannat, which showed higher absorbance at 520 nm than at 420 nm. This fact might lead to believe that this wine has some substance different from the other samples that highly absorbs at 520 nm. In addition, this substance probably has some influence on the intense color of this wine, and can be related to its antioxidant activity. According to the results, the variety Cabernet Sauvignon (1.10) had the most intense tonality while the variety Tannat (0.97) had the less intense.Among the white wines, Chardonay (1.54) showed the highest tonality (Table 1).

Total polyphenols

Due to the great complexity of phenolic compounds existent in wines, the polyphenolic content was expressed in Equivalents of Gallic acid. From the data obtained, it can be noticed that the polyphenols content in red wines in greater than in white wines. The variety Tannathad the greater concentration of polyphenols (19.10µg/L) while the variety dry white wine (4.52 µg/L) had the lower content (Table 1). A correlation between the percentage of antioxidant activity and the polyphenols content was not observed since the wine with the higher activity, Cabernet Sauvignon (81.64%) did not show the higher polyphenol content. This can be explained by the fact that the different polyphenols present in wines have antioxidant activity in different levels. Although, the dry white wine showed the lowest concentration of polyphenols and the smaller antioxidant capacity (37.47%).

Resveratrol content

The resveratrol content was calculated based on standard resveratrol curve that is represented as follows. Analyzing the data shown, we can say that the red wines had the most significant resveratrol contents, pointing out the variety Burgundy (43.29 µg/mL). In the meanwhile, the Chardonay (10.96 µg/mL), had the lower quantity of resveratrol (Table 1). The main reason for this propensity, is that the red wines are fermented with the skins, allowing a better absorption of the resveratrol, which is produced in the skins. According to Frémont (2000), the concentration of resveratrol depends on the type of grape, its geographical origin, the type of wine, the winemaking practices and the plant's degree of injury by *Botrytis cinerea*, a fungus responsible for the rotting of grapes. In red wines the concentration is bigger than in white wines. High concentrations are found, mainly in wines that had a prolonged contact between the must and the skins (Rodriguez-Delgado *et al*., 2002).

Tartaric acid

The greater content of tartaric acid was found in the wines Burgundy (0.60%) and Cabernet Sauvignon (0.58%) while the the dry white wine had the lowest (0.47%) (Table 1). The low content of tartaric acid found in the variety Muscat Embrapa was probably due to the acidity correction which is applied to its must. Analyzing the data obtained, we can see that the tartaric acid content found in the samples tested is in agreement with the limit accepted in wines, which is from 0.4 to 0.65% (Rosa, *et al*., 2013).

Table 1. Response of different wine variety for the parameters: Antiox. activity (%), resveratrol (µg/mL), polyphenols (µg/L), color intensity, color tonality and tartaric acid (%)

Wine variety	Antiox. Activity (%)	Resveratrol (µg/mL)	Polyphenols (µg/L)	Color intensity	Color tonality	Tartaric acid (%)
Carbenet S.	76.10	43.29	16.19	0.69	1.03	60.00
Burgundy	81.64	30.57	13.77	1.08	1.10	58.00
Chardonnay	46.17	10.96	11.71	0.17	1.54	0.47
Dry white wine	37.47	13.86	4.52	0.17	1.40	0.56
Merlot	76.00	20.21	12.23	0.79	1.03	0.48
Muscat Embrapa	54.50	19.99	12.09	0.16	1.34	0.49
Tannat	76.70	29.68	19.10	1.42	0.97	0.51
White Niágara	38.39	18.98	12.36	0.17	1.52	50.00

Conclusion

As expected the red wines showed an overall superior antioxidant activity compared to the white ones. This is due, mainly, to the differences in the fabrication process. Considering the great complexity of chemical substances that compose wines, it becomes difficult to make a correlation between certain data, like antioxidant activity and polyphenols content.

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