PHENOLIC COMPOUNDS, PHYSICO-CHEMICAL PROPERTIES AND ANTIOXIDANTS ACTIVITY OF DIFFERENT RESIDUES FROM VINIFICATION (*VITIS VINIFERA* L.) PRODUCED IN BRAZIL

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ABSTRACT

The present study aimed to determine the physical and chemical characteristics, color, anthocyanin content, total phenolics, total flavonoids and antioxidant activity in vinification residue of red grapes (*Vitis vinifera* L.) varieties: Merlot, Shiraz, Pinot Noir and Cabernet Sauvignon. The residue had an optimum pH for its conservation as biomass, high fiber content, a protein influenced by its nitrogen content probably from the fertilization of grape cultivation and interesting energy content. The Merlot residue presented higher content of anthocyanins (118,92 mg cyanidin-3-gl/100g). The total phenolic compounds varied significantly between the varieties, and Merlot was the residue that had the highest content at 3902,54 (mg gallic acid/100g dry weight). The Shiraz variety had the highest content of total flavonoids equivalent to 26,56 (mg quercetin/100g dry weight). It was observed that the Merlot residue differed significantly ($p<0,05$) from the others and showed greater antioxidant activity against DPPH, ABTS and reducing power.

KEYWORDS: GRAPE RESIDUE. PHYSICOCHEMICAL COMPOSITION. ANTIOXIDANT ACTIVITY. ANTHOCYANINS. PHENOLICS. FLAVONOIDS.

COMPOSTOS FENÓLICOS, PROPRIEDADES FÍSICO-QUÍMICAS E ATIVIDADE ANTIOXIDANTES DE DIFERENTES RESÍDUOS DA VINIFICAÇÃO (*VITIS VINIFERA* L.) PRODUZIDOS NO BRASIL

ABSTRACT

O presente estudo teve como objetivo determinar as características físicas e químicas, cor, teor de antocianinas, teor de fenóis totais, teor de flavonóides totais e atividade antioxidante no resíduo de vinificação das variedades de uvas vermelhas (*Vitis vinifera* L.): Merlot, Shiraz, Pinot Noir e Cabernet Sauvignon. O resíduo possuía um pH ótimo para sua conservação como biomassa, alto teor de fibras, proteína influenciada por seu teor de nitrogênio provavelmente proveniente da fertilização do cultivo da uva e de um interessante conteúdo energético. O resíduo Merlot apresentou maior teor de antocianinas (118,92 mg de cianidin-3-gl/100g). Os compostos fenólicos totais variaram significativamente entre as variedades, e Merlot foi o resíduo que teve o maior teor em 3902,54 (mg de ácido gálico/100g de massa seca). A variedade Shiraz apresentou o maior teor de flavonóides totais equivalente a 26,56 (mg de quercetina/100g de massa seca). Observou-se que o resíduo Merlot diferiu significativamente ($p <0,05$) dos demais e apresentou maior atividade antioxidante para DPPH, ABTS e poder redutor.

PALAVRAS-CHAVE: RESÍDUO DA UVA. COMPOSIÇÃO FÍSICO-QUÍMICA. ATIVIDADE ANTIOXIDANTE. ANTICOCIANAS. FENÓLICOS. FLAVONÓIDES.

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1. INTRODUCTION

Brazil is a country of high agricultural activity and is one of the largest producers of agro-industrial residue, where different research centers are looking for new alternatives to use this generated organic matter in a usable source with high added content. The Gaucha region of the state of Rio Grande do Sul is the main area wine production and is responsible by 97% of the national wine production, with 188.5 tons of grapes industrialized, 300 million liters of wine and must, about 134 thousand tons of residue is generated annually, formed by bark and seeds [16,20,26].

Technical information reports that from approximately 100 liters of wine produced, 31,7% residue is generated, the grape that is used in the food industry generates a lot of residue of low economic value, usually used as animal feed. Therefore, the use of this residue as a by-product of high added content could represent significant economic gains and prevent or reduce the environmental problems caused by the accumulation of vinification bagasse [4,10,14].

The wine-making by-products are also characterized by a high content of phenolic compounds due to inadequate extraction during vinification. The by-products obtained after vinification, both seeds and bagasse, constitute a cheap source for the extraction of antioxidant flavonoids and can be used in the production of phytochemicals. In addition, anthocyanins are considered as potential substitutes for synthetic dyes because of their brightness, color and attractive water solubility, which allow their incorporation into a variety of food matrices [6,17].

In the by-products of the wine industry, recent studies have identified and quantified the presence of hydroxycinnamic acid derivatives, such as caffeic and coumaric acids; flavonols (such anthocyanin) and proanthocyanidines [1,2,25].

In the phytochemical composition of the residue, peels and seeds are the main areas of accumulation of phenolic compounds and antioxidant activity are concentrated in the vacuoles of the peel cells. They have important antioxidant and anti-inflammatory functions, which work in the prevention of cardiovascular diseases and cancers. The pulp of the grape, in turn, is rich in tannic compounds, which have a high nutraceutical and pharmacological potential [23,35].

The more intense the color of grape, the more interesting it becomes from the functional point of view, reports indicate that the dark colored grapes have a high content of anthocyanins, flavonoids, phenolic compounds, as well as high antioxidant

activity [23]. Therefore, the aim of this study was to determine the physicochemical characterization, anthocyanin content, total phenolics, total flavonoids and antioxidant activity in vinification residue of red grapes (*Vitis vinifera* L.) of Cabernet Sauvignon, Merlot, Shiraz and Pinot Noir varieties.

2. MATERIALS AND METHODS

2.1. Materials

The following reagents: DPPH (2,2'-diphenyl-1-picryl-hydrazyl), ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) , Trolox (6-hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylic acid), potassium persulfate, sodium carbonate, Folin-Ciocalteu, quercetin, and gallic acid, were purchased from Sigma-Adrich. All chemicals used in the experiments were analytical grade and distilled deionized water was used throughout the process. Samples of grape residues of *Vitis vinifera* L. species of winemaking in its varieties: Merlot, Shiraz, Pinot Noir and Cabernet Sauvignon were obtained from the wine industry "Luiz Argenta" in Flores da Cunha in Caxias do Sul, RS, Brazil, in March of 2014. The samples were packed in insulated boxes and where they were frozen at -18 °C until use, in the Food Technology Laboratory-FURG.

2.2. Sample preparation

The grape residue samples in each of its varieties were thawed, mixed manually, ground, homogenized by mechanical deformation using a food processor Ultra-Turrax homogenizer (Kinematica Polytron-GnbH, Kriens-Luzern, Suíça) for 3 min or until a mass of equal parts was obtained, in the absence of light.

2.3 Physicochemical analyses of residues

The total soluble solids content, pH and titratable acidity, of the prepared samples were determined, following the methods recommended by the Association of Official Analytical Chemists [3].

2.4. Analysis of the proximal chemical composition

Moisture content, proteins, lipids, crude fiber and ash were determined in the samples, prepared in triplicate, following the methods recommended by the Association of Official Analytical Chemists. The carbohydrate was calculated by difference [3].

2.5. Determination of total anthocyanins

Total anthocyanin content (TAC) in the grape vinification residues was quantified using a pH differential method, described by GIUSTI and WROSLTAD [15]. Where it is dissolved in two buffer systems: potassium chloride pH 1.0 (0.025M) and sodium acetate pH 4.5 (0.4M), As a result, the difference in absorbance is proportional to the anthocyanin content and was measured at 520 nm and 700 nm with a UV spectrophotometer (Kasuaki model 10UV), The anthocyanin content was calculated using the molar absorptivity (ϵ), Dilution factor (FD), Cuvette thickness (l) and molecular weights (MW) of cyanidin 3-glucoside (ϵ= 26900; l= 1cm; MW = 449.2). A corrected absorbance value was calculated as $[(A_{520nm}-A_{700nm})_{pH1,0}- (A_{520nm}-A_{700nm})_{pH4,5}]$. Results are expressed as mg of cyanidin 3-glucoside equivalents (Cy3-GE)/g of grape residues tissue, described by GIUSTI and WROSLTAD [15]). They were calculated using the equation:

$$\text{Anthocyanin content } \left(\frac{mg}{g}\right) = AxMWxFDx10^3)/(\varepsilon xl) \quad (1)$$

2.6. Determination of total phenolics

The total phenolics contents were determined according to the Folin-Ciocalteau method, described by BUCIC-KOJIC et al. [9]. Which consisted of using a 5 mL extract from centrifugation of 5000 rpm for 15 min 0.5 ml of this extract was then separated from the supernatant in a test tube, to which 8 mL of distilled water and 0.5 mL solution of Folin-Ciocalteau (0.25M) were added and was allowed to stand for 3 min. Afterward, 1.0 mL of 1M sodium carbonate (Na_2CO_3) solution was added and kept for 10 min. The tube was centrifuged (9000 rpm for 30 min) and kept in the dark for 2 h, in a spectrophotometer (Kasuaki model 10UV) absorbance was read at 750nm over glass cuvettes, methanol and all reagents without the aliquot of the sample centrifuged were used as the white. The total phenol contents were expressed as mg EAG (equivalent of gallic acid)/100 g of dry residue, all analyzes were performed in triplicate. The construction of the calibration curve was prepared with 0, 2, 4, 8, 15, and 20 µL stock solution, was $Y = 4.8144X + 0.00003$. $R^2= 0.998$.

2.7. Determination of total flavonoids

The total flavonoids content was determined by colorimetric test using aluminum chloride as chromophoric agent, according to the methodology described by

YANG, MARTINSON and LIU [13]. Aliquots of 250 µL of each sample were mixed with 1.25 mL of deionized water. Then, 75 µL of 5% aqueous sodium nitrite solution ($NaNO_2$) was added and vortexed. After 5 min, 150 µL of 10% aqueous aluminum chloride solution ($AlCl_3$) were added and homogenized. After 6 min, 0.5 mL aqueous NaOH of 1.0M solution were added, and the volume was completed to 3.0 mL with deionized water. The solutions were mixed thoroughly and the absorbances were measured at 510 nm using a spectrophotometer (Kasuaki 10UV model) having methanol as white solution and all reagents excluding the extracts. For the construction of the calibration curve stock solution and dilutions of 0, 20, 40, 80, 100, and 175 µL were prepared. The equation obtained was Y = 9.3799X + 0.0062, where X corresponds to the total flavonoid concentration, Y is the absorbance and R^2 = 0.9998. The results were expressed in mg QE (quercetin equivalent)/100g of dry sample.

2.8. Determination of antioxidant activity - DPPH Method

This analysis was performed according to the DPPH radical method described by NENADIS et al. [24]. This method consisted of weighing 5 g of extract, after which it was centrifugation at 5000 rpm for 25 min. The supernatant was then separated, taking 150 µL of this extract in a test tube to which 2.85 mL of dilute DPPH solution was added and stirred for 15 min in the absence of light. The absorbance was measured using an electro-spectrometer (Kasuaki 10UV model) with a wavelength of 515 nm, using glass cuvettes. The antioxidant activity was determined by interpolation of the absorbance of the samples with respect to the standard curve plotted with Trolox (6-hydroxy-2,5,7,8-tetramethylchrom-2-carboxylic acid) and the results were expressed in mg TEAC (Equivalent antioxidant capacity of Trolox)/100g of dry sample). All the analyses were done three times. For the construction of calibration curve, 2000 µM/mL of stock solution Trolox and the following dilutions were made; 80, 160, 240, 320, 405 and 525 µM. The linear equation obtained was: Y=0.0012X + 0.0001, where X corresponds to the concentration in µM (micromolar) of Trolox. Y is the absorbance measured at 515 nm and the correlation coefficient was R^2=0.999.

2.9. Determination of antioxidant activity - ABTS Method

This analysis was carried out using the SAMAVARDHANA et al. method [28]. The $ABTS^{\bullet+}$ radical was formed by reacting 5 mL of potassium persulfate solution with 88 µL of a stock solution of ABTS (192 mg of ABTS reagent in 50 mL of distilled water). Once the $ABTS^{\bullet+}$ radical was formed, it was diluted in ethanol until an absorbance measurement reached 0.70 (±0.02) at a wavelength of 734 nm and a temperature of

30°C. The absorbance was measured using a spectrophotometer model (Kasuaki model 10UV) at periods of 1, 4 and 7 min after the addition of the extract. The content was determined by interpolating the standard curve constructed with Trolox (6-hydroxy-2,5,7,8-tetramethylchromo-2-carboxylic acid), a synthetic antioxidant similar to the vitamin E. The results were expressed in mg TEAC (Equivalent antioxidant capacity of Trolox)/100g of dry sample). For the construction of calibration curve, a solution of 2 mM/mL was made the following dilutions were made: 0, 100, 500, 1000 and 2000 µM. The linear equation Y= 0.0003X + 0.0053 was obtained where X corresponds to the concentration (micromolar) of Trolox, Y is the absorbance measured at 734 nm with R^2= 0.997.

2.10. Determination of antioxidant activity - reducing power Method

The power of the samples to reduce the Fe^{3+} to Fe^{2+} ion was determined by YOU-TUNG et al. method [34]. A volume of 2 mL of sample (5 mg/mL) was added in 2 mL of 0.2M phosphate buffer (pH 6.6) and 2 mL of potassium ferricyanide (1%). The mixture was then incubated at 50°C for 20 min, and then 2 ml of 10% trichloroacetic acid (TCA) was added to the reaction. A 2 mL aliquot was mixed with 2 mL of distilled water and 0.4 mL of 0.1% ferric chloride in test tubes. After 10 min of reaction, absorbance of the resulting solution was read at 700 nm in a spectrophotometer (Kasuaki model 10UV). The increase in the absorbance of the reaction indicated an increase in reducing power. All samples were prepared in triplicate and in the absence of light.

2.11. Color Determination

The determination of the color parameters of the samples was obtained in the CIELAB system using a Konica Minolta portable colorimeter which was calibrated with a white porcelain plate, according to method described by SIEDE and ZAPATA [31].

2.12. Statistical Analysis

Data analysis was performed by applying the ANOVA and Tukey test to identify significant differences between means using Statistica 5.0 software. The level of significance for the difference between the means was 5% ($P<0.05$). All analyses were performed in triplicate.

3. RESULTS AND DISCUSSION

3.1. Physicochemical analyses of the vinification residues of different grape varieties

Results from physicochemical characterization carried out for grape residues from winemaking are shown in Table 1.

TABLE 1. pH, soluble solids and total acidity values of the grape residues of different varieties.

Parameters	pH	Soluble solids (^{o}Bx)	Total titratable Acidity*
Merlot	3.72 ± 0.02 ^{a}	3.50 ± 0.10 ^{a}	4.59 ± 0.14 ^{a}
Cabernet Sauvignon	3.84 ± 0.03 ^{a}	2.83 ± 0.08 ^{b}	2.98 ± 0.03 ^{b}
Shiraz	3.92 ± 0.02 ^{a}	1.83 ± 0.15 ^{c}	4.48 ± 0.12 ^{a}
Pinot Noir	3.95 ± 0.01 ^{a}	3.07 ± 0.06 ^{a}	3.10 ± 0.13 ^{b}

* (mg tartaric acid mL^{-1}. Results are expressed as mean ± standard deviation (n = 3). Averages with different letters in the same row are significantly different ($p<0.05$) by Tukey test.

The pH values found in the grape residues showed that the Pinot Noir variety had the highest value compared to other varieties, presenting significant difference ($p<0.05$) compared to Cabernet Sauvignon and Merlot varieties, and the Merlot variety had the lowest pH. In addition to the low pH, these are parameters that influence the conservation of a food, thus, it was from this pH value that the grape residue was within this context. These values were in the same range as those reported by LAFKA, SINANOGLOU, and LAZOS [19]. Regarding the values of total soluble solids, it was determined that there was no significant difference statistically ($p<0.05$) between Pinot Noir, Cabernet Sauvignon and Merlot grape variety residues. However, the Shiraz variety was the one that had significant difference from the other varieties. Even so, the Merlot variety had the highest total soluble solids compared to the others, this difference can be attributed to the characteristic of the variety of grape residue since it already had the highest moisture content, allowing the solubility of the soluble solids in its medium. DENG, PENNER and ZHAO [12] obtained similar results when evaluating the physicochemical characteristics of by-products of vinification of Cabernet Sauvignon, Merlot, Pinot noir grape (*V. vinifera*). These residues showed great pH and titratable acidity results to prevent the proliferation of molds and yeasts, and facilitated

their physicochemical stability, as an important parameter to ensure their conservation and proving ideal for use in industrial processes. These results are due to the fact of the residues contain a low amount of water, since they were extracted during the vinification, remaining practically as a solid residue filled with insoluble and fibrous cells.

3.2. Proximal chemical composition in winemaking residues from different varieties

The results of the proximal composition of winemaking grape residues are shown in Table 2. In analyzing the results, it was determined that for moisture there was no significant difference ($p<0.05$) among all varieties of winemaking residues, with the Merlot residue presenting the highest moisture content (59.33%) while Pinot Noir the lowest (50.69%). The resulting humidity values obtained in this study were lower to the pomace of winery waste (grape skin and seeds) from red winemaking determined by SINANOGLOU, and LAZOS [19], which were of 73.6% respectively.

TABLE 2. Proximal composition and calorific value of grape residues of different varieties.

Components	Merlot	Cabernet Sauvignon	Shiraz	Pinot Noir
Moisture (%)	59.33±0.59^{a}	57.61±0.62^{b}	51.02±0.16^{c}	50.69±0.21^{c}
Ashes (%)	5.62±0.53^{b}	7.45±0.62^{a}	8.09±0.16^{a}	7.81±0.21^{a}
Lipids (%)	3.31±0.14^{a}	2.03±0.20^{c}	2.59±0.08^{b}	1.92±0.27^{c}
Protein (%)	13.16±0.51^{b}	15.83±0.41^{a}	14.19±0.09^{b}	15.51±0.48^{a}
Crude fiber (%)	46.99±0.12^{a}	40.06±0.71^{c}	41.52±0.62^{c}	43.24±0.21^{b}
Carbohydrate(%)	30.92±0.20^{c}	35.64±0.97^{a}	33.61±0.74^{b}	31.52±0.73^{c}
Kcal/100g	206.07±1.82^{b}	220.17±2.68^{a}	214.53±2.38^{a}	205.38±1.63^{b}

Results are expressed as mean ± standard deviation (n = 3); Averages with different letters in the same row are significantly different ($p<0.05$) by Tukey test.

These values indicate that the Merlot sample was the moistest residue, which did not happen with the other residues, this characteristic was probably due to the type of pressing that was used during the winemaking process. The ash content was not significantly different ($p<0.05$) between Cabernet Sauvignon and Pinot Noir residues, but showed a significant difference with the Shiraz and Merlot varieties. The Shiraz residue was higher to that obtained by PENNER and ZHAO [12]. The ash content

corresponded to the presence of minerals in the grape residues such as potassium, phosphorus, calcium and magnesium.

The lipid content showed no significant difference ($p<0.05$) between the Cabernet Sauvignon and Pinot Noir residues and even so these had significant differences with the Merlot and Shiraz residues. Pinot Noir residue showed a relatively lower content than the other residues (1.92%), with the Merlot residue presenting the highest value of 3.31%. These results were lower than those obtained by LAFKA, SINANOGLOU and LAZOS [19] in winery waste (6.3%) and seeds of Cabernet Sauvignon (6.6%). Other authors have reported much higher levels with respect to residues in the present study, getting up to 13.5% in red grape pomace and 9.5% for white grape pomace [22]. The lipid content is mostly associated with grape seeds, and at the moment our vinification residues contain low amounts of lipids and indicating that this content is due to the fact that the residue is constituted by peel and seed fractions, and that the difference in lipid content is in the types of grape varieties that contain less amounts of seeds than others. The protein content showed a significant difference ($p<0.05$) among all Merlot and Shiraz residues, and the Cabernet Sauvignon and Pinot Noir residues did not differ from each other having higher values than the other two grape residues, these results ranged from the lowest values, 13.16% for Merlot, to the highest value corresponding to 15.83% for the Cabernet Sauvignon residue. The results herein were superior to those reported by Sanchez-Alonzo, Sola and Borderias [29] for red grapes pomace with a 8.1% protein and 7.3% for white grapes pomace. The protein content of the grape residues in all its varieties is probably due to the variety, soil type, farming practices and the pressing technology that are subjected to for the wine production. It is believed that these characteristics were responsible for generating a variation in all components of the grape vinification residues [29,32].

The crude fiber content showed significant difference ($p<0.05$) among all residues, ranging from 40.06% to 46.9%. However, our results were within the range of those reported by BRENES et al. [8] on the grape pomace (32.5% - 56.3%). The carbohydrate content showed significant difference ($p<0.05$) among all residues, with Merlot having the lowest (30.92%) and Cabernet Sauvignon residue showing the highest with 35.64%. These carbohydrate values were within the range for grape pomace (12.2-48.3%) reported by BRENES et al. [8]. This difference probably occurred due to the high cellular metabolism during the development of the cultivar subjected to high production systems, organic system, with the chemical composition of grape being defined by the maturity stage, genetic potential, climate and handling, and is linked to the composition conditions for factors involved in the development of the fruit [32].

Regarding calories, the grape residues in this study showed significant differences ($p<0,05$) among all residues, but were very close to those reported by BRENES et al. [8] who found values of 288.4 – 210.1 kcal, demonstrating that these residues have a low caloric content and can be used as an ingredient in the food industry in order to add value and reduce calories.

3.4. Total phenolics, total flavonoids and anthocyanins

The anthocyanin levels found in the grape vinification residue were statistically different ($p<0.05$) among samples studied as can be seen in Table 3.

TABLE 3. Contents of total anthocyanins, total phenolics and total flavonoids in winemaking residues from different varieties of grapes (*Vitis vinifera L*.).

Residues	TA (mg Cy3-GE/100g) (D.M.)	TP (mg GAE/100g) (D.M.)	TFV (mg QE/100g) (D.M.)
Merlot	118.92 ± 0.65 [a]	3902.54 ± 8.22 [a]	89.95±0.22 [a]
Cabernet Sauvignon	95.04 ± 0.47 [b]	2332.69 ± 7.06 [b]	21.01±1.74 [d]
Pinot Noir	47.54 ± 2.46 [c]	2047.32 ± 6.04 [c]	47.54±1.66 [c]
Shiraz	32.61 ± 0.78 [d]	1911.86 ± 3.68 [c]	64.64±1.01 [b]

Means with different letters in the same column are significantly different in Tukey test ($p<0.05$), Results are expressed in mean ± standard deviation (n = 3). TA = total anthocyanins; TP = Total phenolics; TFV = Total Flavonoids; D.M. = Dry Mass; Cy3-GE= cyanidin 3-glucoside equivalents

In Table 3, the Merlot grape variety residue had the highest amount of anthocyanins, equivalent to 118.92 mg cyanidin-3-glucoside/100g sample, followed by Cabernet Sauvignon variety with a content of 95.04 mg cianidin-3-glucoside/100g, these two grape varieties had the highest amounts of anthocyanins, which can be justified by the characteristic of being redder. These residues had an intense reddish hue compared to the other residues, which indicates the presence of anthocyanins pigments. Residues of Shiraz and Pinot Noir varieties showed lower anthocyanin content, with the Shiraz variety presenting a content of 32.61 mg cyanidin-3-glucoside/100g. These two last varieties initially showed low red hue parameter that was indicative of low content of anthocyanins, as can be seen from the results shown in Table 3. Compared to other research, the results of this study has shown that anthocyanins values of the residues were higher than those obtained by BOZAN, TOSUN and ÖZCAN [7], in their study of the Concord grape variety pomace, obtaining

a content of 560,38 mg cyanidin-3-glucoside/100g for Bordo grape pomace and also lower than the 1122 mg of anthocyanins/100g of dry bagasse value reported by ROCKENBACH et al. [26].

DWYER el al. [13] compared the amounts of anthocyanins found for grape berries of Cabernet sauvignon, Merlot and Pinot Noir variety in the order of 89, 142 and 29 mg/100g equivalents of cyanidin-3-glucoside respectively, and values of this work were within this range. Anthocyanins are compounds belonging to the class of flavonoids, which are presented as a group of natural pigments with different phenolic structures, they are responsible for blue, violet and all shades of red appearing in grapes, in addition to being present in juice they are also found in grape skins which form part of residues from grapes which are generated during processing [5]. The content of anthocyanins in the vinification residues was due to the fact these pigments were evenly dissolved in the vacuolar solution of epidermal cells and anto-cytoplasts of the residues.

For total phenolics in the winemaking grape residues, it was determined that there was a statistically significant difference ($p<0.05$) between the residues Merlot, Cabernet Sauvignon and Pinot Noir and Shiraz, which did not differ from each other, as can be seen in Table 3. The Merlot variety grape residue showed the highest amount of total phenolics equivalent to 3902.54 mg gallic acid/100g dry sample, varieties that followed in quantitative order were Cabernet Sauvignon and Pinot Noir with contents of 2332.69 and 2047.32 mg gallic acid/100g dry sample, respectively. The Merlot variety had the highest total phenolic content and the Shiraz variety had the lowest content. From these results, it can be argued that the total phenolics content was due to the high content of anthocyanins and probably to the presence of other phenolic acids and phenylacetic acids. ROCKENBACH et al. [26] reported higher values of total phenolic acid, obtaining 7474 mg gallic acid/100g dry mass in Cabernet Sauvignon, while LLOBERA and CAÑELLAS [22] reported higher values for Cabernet Sauvignon, Pinot Noir and Shiraz, lower than the residue from Merlot. They determined 2630 mg gallic acid/100g dry mass in the range of Manto Negro variety red grapes pomace. Compared with Chardonay grape variety peels with 9900 mg gallic acid/100g of peels on a dry basis reported by LUTHER et al. [21], the values in the present study were higher. Probably, the variability of these results is due to the fact that they were analyzed in whole berries, the type of soil where they were cultivated and the cultural practices. Total flavonoids presented significant difference ($p<0.05$) among all residues, with the residue of the Merlot variety showing the highest amount of total flavonoids equivalent to 89.9 (mg quercetin/100g) followed by Shiraz at 64.6 mg

quercetin/100g) on a dry basis. Our results were superior to those reported by RUBERTO et al. [27] in Cabernet Sauvignon grape pomace (1.9 mg quercetin/100g dry pomace). The result of the flavonoids was mainly due to the value of anthocyanins and probably to the presence of functional groups such as chalcones, flavones, flavonols, flavonols and condensed tannins present in the residues. The residues of grapes studied showed high phenolic content in the present study, this fact is related to the fact that the residues showed an intense color of red-purple, attributed to the greater amount of anthocyanins, usually associated to the high value of total phenolics.

3.5. Analysis of antioxidant capacity - DPPH Method

The antioxidant activity measured by DPPH in grape residues showed a significant difference ($p<0.05$) among all the residue, as can be seen in Table 4, where the Merlot residue had the highest value, followed by Pinot Noir, Cabernet Sauvignon and Shiraz, with values of 3271.01; 2405.58; 1323.39 and 1032.91 (mg TEAC/100g dry sample), respectively. The values found in our study were lower than those reported by SAMAVARDHANA [28] on V*itis vinifera* grape variety pomace (4090.8 mg TEAC/100g dry sample).

TABLE 4. Antioxidant capacity by the of DPPH, ABTS and reducing power methods in residues from different winemaking grape varieties.

Residues	DPPH mg TEAC/100g D.M.	ABTS mg TEAC/100g D.M.	Reducing power (abs.)
Merlot	3271.01 ± 1.61 [a]	6070.71 ± 0.05 [a]	1.06 ± 0.05 [a]
Pinot Noir	2405.58 ± 1.77 [b]	4166.40 ± 0.10 [b]	0.74 ± 0.06 [b]
Cabernet Sauvignon	1323.39 ± 1.63 [c]	2943.83 ± 0.09 [c]	0.50 ± 0.04 [c]
Shiraz	1032.91 ± 1.35 [d]	1904.42 ± 0.08 [d]	0.37 ± 0.03 [d]

Results are expressed in mean ± standard deviation (n = 3), Means with different letters in the same column are significantly different ($p<0.05$) by Tukey test TEAC = Trolox antioxidant equivalent capacity; D.M.= Dry Mass; abs = absorbance.

The results in the present study were lower than those determined by ROCKENBACH et al. [26] on the Couderc grape variety pomace (4787.25 mg TEAC/100g dry sample), Pinot Noir grape pomace (9502.25 mg TEAC/100g dry sample on a dry basis) and Cabernet Sauvignon bagasse (5210.75 mg TEAC/100g dry sample on a dry basis). The highest value of antioxidant activity by DPPH was the Merlot residue, this was due to the significant effect of the high value in total anthocyanins, total phenolics and total flavonoids. Followed by the Pinot Noir residue,

where the effect of its antioxidant activity was due to the significant presence of total flavonoids.

3.6. Antioxidant capacity - ABTS Method

The antioxidant activity by the ABTS method in grape residues showed a significant difference ($p<0.05$) among all the residues in the following order: Merlot, Pinot Noir, Cabernet Sauvignon and Shiraz, as it can be seen in Table 4, where the Merlot residue presented the highest value, 6070.71 (mg TEAC/100g) or (242.83 µMol Trolox Eq/g) on a dry basis, after conversion of their units. The lowest content of 1904.42 mg Trolox Eq/100g or 76.17 µMol Trolox Eq/g sample on a dry basis belonged to the Shiraz grape variety, the Merlot residue had the highest antioxidant capacity in the present study. The value of the Cabernet Sauvignon grape residue was inferior to that obtained by RUBERTO et al. [27] on the grape peels of Cabernet Sauvignon (4075 mg trolox/100g dry sample), the authors considered that their samples had high antioxidant potential. The variability of values for both the DPPH and ABTS method is probably due to the characteristics of each sample, where the differences are due to the type of crop, variety, soil and climatic aspects. Both methods showed similar quantitative behavior when antioxidants sample react against the DPPH and ABTS radical transferring an electron or hydrogen and losing the characteristic color of the radical after reduction, thus evaluating the reducing power of an antioxidant, on donating an electron, it is oxidized, becoming a more stable compound. Therefore, its absorbance decreased, visually noted by discoloration of DPPH and ABTS solution by using a spectrophotometer. From the results of the antioxidant activity of the DPPH method were lower to the ABTS method, this was due to the fact that DPPH is a more selective method to react specifically with the hydroxyl groups of the antioxidants, whereas the ABTS is less selective and can react easily with any hydroxylated aromatic compound, even with flavonoids devoid of hydroxyl groups independent of their antioxidant capacity, which is why there existed a variation in the measurement of the antioxidant capacity of the vinification residues [18].

3.7. Antioxidant capacity - reducing power

The reducing power determined by the grape residues showed a statistically significant difference ($p<0.05$) among all residues, decreasingly from Merlot, Pinot Noir, Cabernet Sauvignon and Shiraz. The Merlot grape residue presented the highest absorbance, 1.06, and was the sample that had the best reducing power, Shiraz grape residue was the sample that had the lowest absorbance value equal to 0.37. From the

values found, the reducing power of the samples was a measure that provides an estimate of the ability of a compound to reduce Fe^{+3} in the form of ferricyanide to Fe^{+2} in the presence of an antioxidant compound through the solution color change from yellow to the blue green tone, forming the blue compound of Prussia [14].

3.8. Color Analysis

Table 5 presents the mean values and standard deviation of the CIELab color parameters, L*, a*, b*, Hue angle, and Chromaticity (C^o) determined from different winemaking grape varieties residues. It was observed that the values of the parameter (a*) did not present any statistically significant differences ($p<0.05$) between Merlot and Cabernet Sauvignon grape residues, equally Pinot Noir and Shiraz residues did not differ statistically. Due to the residues of the Cabernet Sauvignon and Merlot varieties presenting higher values of the parameter a*, these residues were redder. On the values of the parameter (b*) of the residues, Merlot variety presented a significant difference from the other samples which indicated a greater tendency to blue color than to yellow. On the brightness, Shiraz was the residue that had a darker color than the other residues, but this tone was not a proper expression of the red intensity.

TABLE 5. Color parameters in residues from different winemaking grape varieties.

	Color Parameters				
Residues	a*	b*	L*	C^*	H^o
Cabernet Sauvignon	15.8±1.2 [a]	4.6±1.7[a]	21.5±0.2 [a]	16.5±1.0 [a]	16.5±4.5 [b]
Merlot	15.1±0.4 [a]	0.6±0.2[b]	22.3±0.4 [a]	15.1±0.9 [a]	2.4±0.9 [c]
Pinot Noir	10.6±0.2 [b]	5.8±0.1[a]	19.9±0.2 [b]	11.9±0.6 [b]	27.3±3.4 [a]
Shiraz	11.2±0.5 [b]	5.3±0.1[a]	18.0±0.2 [b]	12.6±1.2 [b]	26.1±1.9 [a]

Means followed by the same letter in the column do not differ by Tukey test ($p<0.05$); L, a*, b* = color parameters; C * = Chromaticity; H^o = Tone (Hue angle)

For the chromaticity and tonality, Shiraz and Pinot Noir residues showed significant differences with other residues. Merlot and Cabernet Sauvignon residues had high chromaticity values, these two samples were darker and red. The characteristic tonality red, one can say it is due to the high content of anthocyanins, as the Merlot grape residue was the one that presented the highest content of anthocyanins. According to work by CLIFF [11], the highest concentration of

anthocyanins may have contributed to the higher chromaticity and color tone values of the grape products and by-products presented.

4. CONCLUSIONS

All winemaking grape residues evaluated showed pH values similar to grape berries, higher total titratable acidity and lower soluble solids of grape berries, due to the concentration of hydrogen atoms in this medium, facilitating its physicochemical stability as ideal substrate for its conservation and use in the industrial process. From the proximate chemical composition of all winemaking residues, the Merlot variety presented the lowest energy content, and that this was due to grape variety and residue production technology. There was an influence of the presence of anthocyanins on the content of total phenolics, where the Merlot grape variety residue showed the highest values in anthocyanins, total flavonoid and total phenolics. It was determined that anthocyanins, total flavonoid and total phenolics, had a direct relationship on ABTS and DPPH free radicals sequestration methods, where the Merlot grape variety residue showed the highest values of antioxidant activity and reducing power as an indicative of its high antioxidant activity. Greater red color residues showed higher levels of anthocyanins, phenolic compounds and antioxidant capacity. Merlot grape variety residue evaluated is a good source of anthocyanins, phenolic compounds and high antioxidant capacity, with values higher than many Brazilian fruits and can be used as a functional food.

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