

Exploratory analysis of bioactive compounds and antioxidant potential of grape (*Vitis vinifera*) pomace

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ABSTRACT. The aim of this study was to explore the profile of bioactive compounds of Merlot grape pomace by different methodologies including phenolic composition by spectrophotometry, chromatography and spectroscopy and its antibacterial activity. Additionally, the correlation between phenolic compounds and antioxidant activity by principal component analyses (PCA) was evaluated. Merlot grape pomace showed different classes of bioactive compounds such as phenolic acids, flavonoids and stilbene with high antioxidant activity according to the DPPH ($2.58 \pm 0.07 \text{ mg mL}^{-1}$), autooxidation of system β -carotene/linoleic acid ($70.60 \pm 0.91\%$), ABTS ($1.22 \pm 0.01 \text{ mg mL}^{-1}$) and FRAP ($23.83 \pm 0.64 \mu\text{M FeSO}_4 \text{ g}^{-1}$) assays. The extracts showed minimum inhibitory concentration for gram-positive and gram-negative bacteria with high values for *Bacillus cereus* (MIC = 12.5 mg mL^{-1}) and *Staphylococcus aureus* (MIC = 25 mg mL^{-1}), respectively. In addition, fingerprint regions of the phenolic compounds were found by mid-infrared, supporting the results of the chromatographic analyses. Through the PCA, the antioxidant potential assays had high correlation with phenolic composition. Therefore, considering the significant concentrations of the different bioactive compounds found in Merlot grape pomace, it can be inferred that this cheap by-product can be reused by the food industry in the development of new products.

Keywords: Phenolic compounds; antimicrobial activity; winemaking; multivariate analyses; spectroscopy analyses.

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Introduction

Bioactive compounds are essential and non-essential compounds that are naturally present as constituents of food and can be defined as substances with biological activity capable of modulating metabolic processes resulting in the promotion of human health (Biesalski et al., 2009; Shirahigue & Antonini, 2020). These chemical compounds are secondary metabolites and are classified according to their routes and biosynthetic structure, in addition, they are divided into three main groups: (1) polyphenolic compounds; (2) terpenoids and (3) nitrogen-containing alkaloids and sulfur-containing compounds according to Vuolo, Lima, and Maróstica Junior (2019), and it is known that the polyphenolic compounds are the most abundant secondary metabolites reported in plants (Shirahigue & Antonini, 2020).

The polyphenolic compounds have great structural diversity and complexity, including phenolic acids, flavonoids, tannins, lignans, proanthocyanidins, among others. However, some factors widely influence the content of these compounds, such as genetic factors, environmental conditions and the degree of maturation of the plant as well as the variety of fruit, especially in grapes (Martelli & Giacomini, 2018; Peixoto et al., 2018).

Grapes are one of the world's most popular fruit, with approximately 79 million tons of production in 2018, and China, Italy and the United States are the leading grape producers (Food and Agriculture Organization of the United Nations [FAO], 2020). And about 75% of the total production is used to produce wine. In this process, the grape pomace generated corresponds from 20% to 30% of the original weight of the grape, being constituted mainly by seeds (38%–52%) and skins (5%–10%), and sequentially by remnants of pulp, stem, small pieces of stalks and yeast cells from the wine fermentation process (Venkitasamy, Zhao, Zhang, & Pan, 2019).

Typically, wine pomace has been used in the production of different types of "wine alcohol", as a compound to increase the organic matter, nitrogen and mineral content of the vineyard soils, as a fertilizer

or it can also be used in animal feed. However, the application of pomace as a fertilizer or in animal feed has disadvantages due to the presence of phenolic compounds that decrease the pH of the pomace and increase the resistance to biological degradation. In addition, inappropriate pomace disposal can lead to pollution of surface and groundwater, can attract pests that can spread diseases and oxygen depletion in the soil and groundwater by tannins and other compounds (Beres et al., 2017; Venkitasamy et al., 2019).

In general, phenolic compounds found in grape pomace are phenolic acids (hydroxybenzoic and hydroxycinnamic acids), flavonoids (catechins, flavonols and anthocyanins) and proanthocyanidins (Peixoto et al., 2018). These compounds exhibit numerous benefits including antioxidant activity, inhibition or induction of enzymes, inhibition of receptor activities, and induction and inhibition of gene expression, and antimutagenic, anticarcinogenic, antimicrobial, and other biological properties (Shirahigue & Antonini, 2020).

However, the antioxidant potential of these bioactive compounds depends on the number and arrangement of hydroxyl groups that can interact with free radicals by different mechanisms whether by hydrogen atom transfer (HAT) and/or single electron transfer (SET), but which allow chelation of metals, inhibition generating enzymes free radicals or participation in the process of repairing oxidative damage that may occur (Shahidi & Ambigaipalan, 2015; Shirahigue & Antonini, 2020).

For these reasons, the bioactive compounds of the by-products of winemaking have been explored in recent years in several scientific studies, for example: Beres et al. (2017) did a review about utilization of grape pomace from winemaking process; Lavelli, Kerr, and González-SanJosé (2017) indicated the use of bioactive compounds derived from grape pomace for the development of innovative products; Peixoto et al. (2018) reported in their studies as the grape pomace can be a source of phenolic compounds and with diverse bioactive properties; Li, Li, Yang, Yin, and Ming (2019) investigated the composition of eleven grape cultivars and compared the phenolic profiles and antioxidant activities in the skins and pulps; Chen et al. (2020) assessed the bioactivity of grape seed and its application in the food industry and Monteiro et al. (2021) studied the bioactive compounds and the antioxidant capacity of grape pomace flours.

These and other studies indicate the possibility of reusing grape pomace for various purposes in the food industries. Moreover, exploitation of these by-products may promote a set of benefits by reducing industrial waste discharge and increasing the economic gains by reutilization. In this context, the aim of our work was to explore bioactive compounds by investigating the profile of phenolic compounds and antibacterial activity of grape (*Vitis vinifera*) pomace and to evaluate the correlation between phenolic compounds and antioxidant potential by using multivariate statistical analysis (PCA).

Material and methods

Preparation of the samples

Merlot (*Vitis vinifera*) grape pomace sample was obtained in a company located in the state of Paraná, Brazil. The sample was a byproduct of the winemaking process, obtained after the grapes had been pressed. Once in laboratory, the grape pomace sample was dried in an air circulation oven (Marconi MA 035, Brazil) at 80°C for 36 hours. The dried residue was milled (grape pomace), stored in polyethylene film bags under vacuum packing and stored at -20°C. The extracts of grape pomace sample were obtained in the ratio 1:50 (m v⁻¹) using hydroalcoholic solutions varying the concentrations of ethanol and water (100, 80, 60, 40, 20 and 0%) (Haminiuk, Plata-Oviedo, Mattos, Carpes, & Branco, 2014). The mixtures (solute and solvent) were shaken for 24 hours on a shaker (TECNAL/TEC-420, Brazil) at 25°C. The tubes containing the solutions were centrifuged (EXCELSA II 206 BL, FANEM, Brazil) at 3493 x g (5000 rpm) for 25 minutes and the supernatants were separated and filtered in qualitative filter paper for further analyses.

Evaluation of the bioactive properties

The bioactive properties of Merlot grape pomace extracts were evaluated by means of spectrophotometric, chromatographic, spectroscopic and microbiological analyses. In Figure 1, there is a summary of these characterization analyses.

Bioactive compounds

The bioactive compounds were determined by spectrophotometric analyses using a spectrophotometer (UV-vis 1600, PróAnálise, Brazil). The total phenolic compounds content in the grape pomace extracts were estimated by a colorimetric assay (*Folin-Ciocalteu reagent*) at 765 nm and the results were expressed as

milligrams of gallic acid equivalents (GAE) per 100 g dry weight of grape pomace (Singleton & Rossi, 1965). The total flavonoids contents were determined at 510 nm with values expressed as milligrams of catechin equivalents (CAE) per 100 g dry weight of grape pomace (Meyers, Watkins, Pritts, & Liu, 2003). The total anthocyanins content was determined using the pH differential method at 510 and 700 nm and the results were expressed as total milligrams of cyanidin-3- glucoside per 100 g dry weight of grape pomace (Giusti & Wrolstad, 2001).

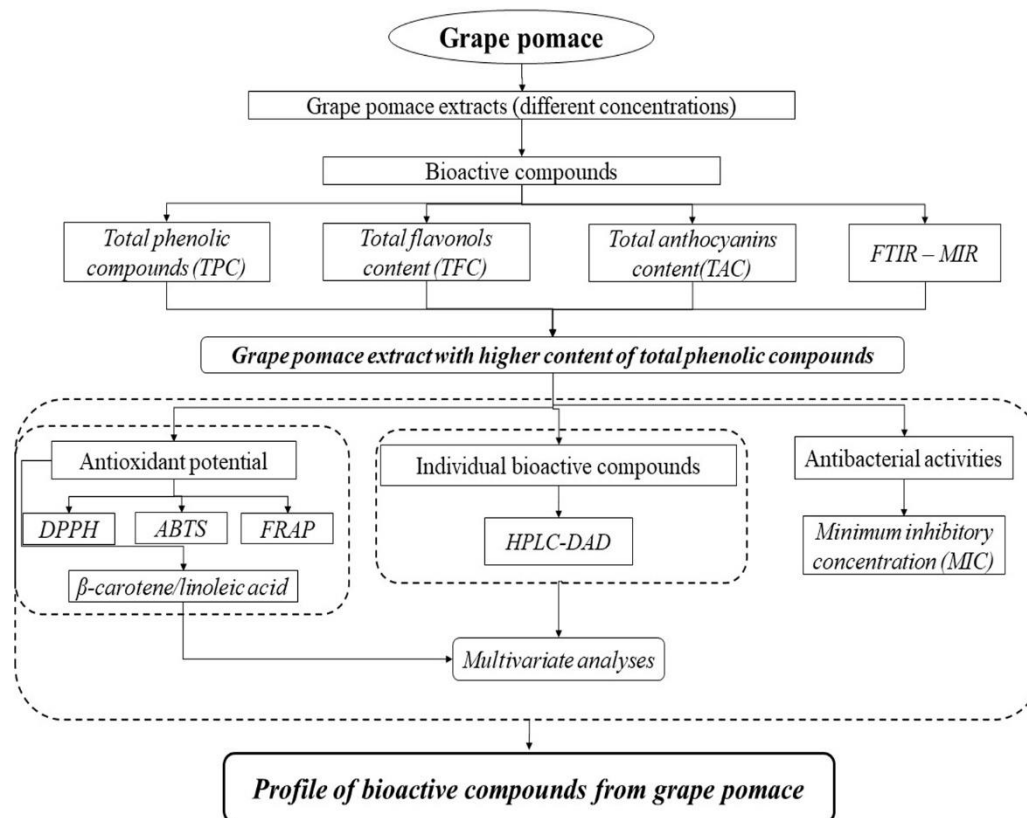


Figure 1. Summary diagram of the characterization analyses of the profile of bioactive compounds in grape pomace.

Phenolic compounds by spectroscopy analyses

All grape pomace extracts were analyzed in the mid infrared region (MIR) in the range of 4,000 – 400 cm^{-1} using a Tensor 37 FTIR spectrometer system (Bruker Optics, Ettlingen, Germany) equipped with Fourier transform, integrating sphere. OPUS software (v. 6.0 Bruker Optics, Ettlingen, Germany) was used for spectral acquisition and instrumental control. Transmittance data were recorded at a nominal resolution of 4 cm^{-1} and accumulating 128 scans.

Phenolic compounds by chromatographic analyses

HPLC-DAD analyses were done using an Agilent HPLC series 1200 (Agilent Technologies, Germany), diode array detector (DAD) and Quad pump. Column was Zorbax Eclipse C18 (5 μm , 4.6 $\times$ 150 mm) filled with 1.8 μm stationary phase of double endcapped C18 (Agilent, Germany). Simultaneous monitoring was performed at 280, 320 and 370 nm. The mobile phase consisted of 2% (v v⁻¹) acetic acid in water (eluent A) and 0.5% acetic acid in water and acetonitrile (50:50, v v⁻¹; eluent B) using a gradient program as follows: from 10 to 24% B (20 min.), from 24 to 30% B (20 min.), from 30 to 55% B (20 min.), from 55 to 100% B (15 min.), 100% B isocratic (8 min.), from 100 to 10% B (2 min.). The injection volume was 10 μL at flow rate of 0.7 mL min^{-1} .

Antioxidant potential

The antioxidant potential was assessed based on the DPPH, ABTS, FRAP and β -carotene and linoleic acid assays. The absorbance values were measured at 518 nm by DPPH• method and the results were expressed as EC₅₀ values (mg 100 mL^{-1}) (Mensor et al., 2001) as well as by the ABTS assay, however performed at 734 nm (Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, & Byrne, 2006). The antioxidant capacity of FRAP reagent

was monitored at 595 nm with results expressed as $\mu\text{M FeSO}_4 \text{ g}^{-1}$ dry weight of grape pomace (Benzie & Strain, 1996). And finally, the autoxidation of system β -carotene and linoleic acid was monitored at 470 nm over a 60-minutes period with results in percentage of oxidation (Emmons, Peterson, & Paul, 1999).

Antibacterial analyses

For antibacterial analyses, the extract of Merlot grape pomace was obtained in the ratio 1:10 (m v⁻¹) using hydroethanolic or hydromethanolic solution 40% (v v⁻¹), shaken for 4 hours on a shaker at 25°C, centrifuged at 3493 x g for 10 minutes and the supernatants were separated using rotary evaporator. The antimicrobial activities of the extracts were evaluated on the basis of the Gram-positive bacteria, *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 6538. In addition to being evaluated also using the Gram-negative bacteria, *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 and *Salmonella enteritidis* ATCC 13076.

The microdilution method was used to determine the minimum inhibitory concentration (MIC) (Wiegand, Hilpert, & Hancock, 2008). Bacterial suspensions were prepared with about 10⁸ colony forming units (CFU) mL⁻¹, the initial suspension being adjusted by comparison with the McFarland standard of 0.5. Subsequently, dilutions were prepared in 0.9 % saline solution, 1:100 from the initial bacterial suspension. For the broth microdilution test 50 μL of Mueller-Hinton nutrient broth (MH) was added to the wells of a sterile 96-well microplates, then 50 μL of grape pomace extract was added to the first row of the microplate and dilutions of extracts in broth MH (1:1) were added. The concentration range obtained for the extracts was 100 to 0.19 mg mL⁻¹. The microplates were incubated at 37°C for 16 to 20 hours. The 2,3,5-triphenyl tetrazolium chloride was used as a control to indicate the presence of microbial growth. The antibiotics used as reference were amoxicillin and cephalixin (initial concentration of 128 mg L⁻¹).

Statistical and multivariate analyses

The results were expressed as the mean values $\pm$ standard deviation (SD). The MIR spectral data were organized using the Origin Pro 8.0 software (OriginLab., Northampton, MA, USA). The multivariate analyses were carried out using the STATISTICA 7.0 (Stat-Soft Inc., Tulsa, OK, USA).

Results and discussion

Profile of the bioactive properties

The efficiency of the extraction process for bioactive compounds depends on several factors, among them, the type of solvent and its concentration. Thus, it is interesting to evaluate how the mixture of ethanol and water can influence the extraction of these compounds present in the solid residues of the winemaking processes. Based on these considerations, a mixture of solvents, ethanol and water was used varying the concentration from 0 to 100% (v v⁻¹), respectively. Additionally, it was evaluated how this variation influences the bioactive compounds such as total phenolic compounds (TPC), total flavonoids content (TFC) and total anthocyanins content (TAC), and the results of the extraction processes are shown in Table 1.

Solvents with different polarities allowed the extraction of both polar (aqueous solvent) and nonpolar (ethanolic solvent) compounds, meaning an advantage in extraction (Cheng, Bekhit, McConnell, Mros, & Zhao, 2012). The majority presence of water, due to variations in concentration in the extracts, increases the permeability of the cellular tissue, and thus assists in the mass transfer by molecular diffusion, as well as in the recovery of the bioactive compounds soluble in water.

Table 1. Spectrophotometric quantification of bioactive compounds in different hydroalcoholic concentrations

Hydroalcoholic extraction	Total Phenolic Compounds (mg GAE 100 g ⁻¹ dw)	Total Flavonols Content (mg CTE 100 g ⁻¹ dw)	Total Anthocyanins Content (mg cya-3-glu 100 g ⁻¹ dw)
E1	1249.59 $\pm$ 0.11 ^d	514.88 $\pm$ 0.25 ^d	31.28 $\pm$ 0.29 ^c
E2	1803.79 $\pm$ 0.69 ^c	1376.14 $\pm$ 1.11 ^b	13.14 $\pm$ 0.02 ^d
E3	2360.99 $\pm$ 0.77 ^b	1793.38 $\pm$ 0.53 ^a	8.96 $\pm$ 0.02 ^{de}
E4	2915.20 $\pm$ 1.69 ^a	1696.48 $\pm$ 0.40 ^a	66.24 $\pm$ 0.02 ^a
E5	1323.18 $\pm$ 0.86 ^d	813.55 $\pm$ 0.19 ^c	41.19 $\pm$ 0.02 ^b
E6	648.82 $\pm$ 0.59 ^e	254.39 $\pm$ 0.03 ^e	7.24 $\pm$ 0.01 ^e

Abbreviations: E1 – ethanol:water (100:0 – v v⁻¹); E2 – ethanol:water (80:20 – v v⁻¹); E3 – ethanol:water (60:40 – v v⁻¹); E4 – ethanol:water (40:60 – v v⁻¹); E5 – ethanol:water (20:80 – v v⁻¹); E6 – ethanol:water (0:100 – v v⁻¹). All results are expressed as mean $\pm$ standard deviation (n = 3). Means followed by different letters in the columns represent significant difference by Tukey's test (p $\leq$ 0.05).

Among the obtained hydroalcoholic extracts, E4 extraction (ethanol:water – 40: 60 – v v⁻¹) presented the most significant content of total phenolic compounds, 2915.20 ± 1.69 mg GAE 100 g⁻¹ dw, according to *Tukey's* test ($p \leq 0.05$). In addition, as there are flavonoids in the skins, seeds and stems, the complex formed between the flavonoids of the analysis matrix and aluminum chloride (AlCl₃) a considerable quantity of total flavonoids was obtained, 1696.48 ± 0.40 mg CTE 100 g⁻¹ dw, obtained in the same extraction (E4), as well as for total anthocyanins, 66.24 ± 0.02 mg cya-3-glu 100 g⁻¹ dw, since they are also a class of flavonoids and are the major compounds in the skins of dark grapes.

Grape pomace is mainly composed of skins and seeds. High concentrations of total phenolic compounds were found (Table 1). These compounds represent the largest group of bioactive compounds, which include other compounds classified in subgroups such as flavonoids (anthocyanins, flavonols, flavanols, among others) and non-flavonoids (phenolic acids, stilbenes and tannins, for example). The content and composition of phenolics compounds remaining can be affected by variety, climate, growing conditions, ripening stage of the grapes and inefficient processing (Topalović et al., 2020).

All extracts (E1 - E6) were also evaluated using infrared spectroscopy in the mid infrared region (MIR) by transmittance because the use of this method can assist in the qualitative identification of phenolic compounds present. In general, the application of the FT-MIR in the routine analyses of grapes or wines is of special analytical interest due to the presence of sharp and specific absorption bands for specific constituents such as phenolic compounds groups (Cozzolino, 2015).

The MIR region spectrum between 4000 and 400 cm⁻¹ was segmented into two broad regions: one with well-defined peaks with maximum and minimum from 750 to 1750 cm⁻¹ and other with an enlarged region from 3000 to 3750 cm⁻¹. The first derivative was applied to highlight the most important analytical signals (Figure 2).

In Figure 2, all extracts showed the same behavior, regardless of the proportion of water and ethanol used. In Figure 2a there was a stretch region O – H, with characteristic water vibration peaks between 3200 and 3600 cm⁻¹, with minimum peaks at 3400 cm⁻¹. It was also observed peaks close to 3000 cm⁻¹ with maximum and minimum points characteristic of aldehyde C – H related to phenolic groups (Cozzolino, 2015; Pavia, Lampman, Kriz, & Vyvyan, 2001).

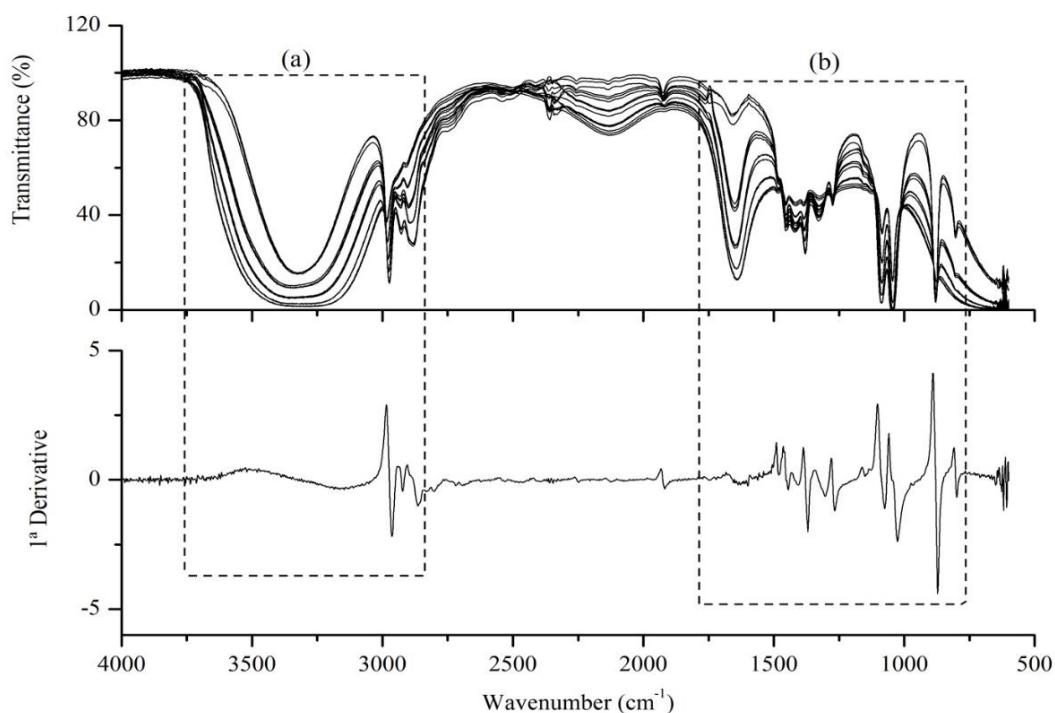


Figure 2. Mid-infrared transmittance spectra of all grape pomace extracts and first derivative transformed spectra. Region (a) corresponding to band from 2750 to 3750 cm⁻¹; region (b) from 750 to 1800 cm⁻¹.

Since region Figure 2b between 750 and 1300 cm⁻¹ contains a fingerprint region, it may be related to aromatic groups such as C – C and C – C – O functional groups associated with different phenolic compounds, ethanol, and organic acids. In the spectral region from 1168 to 1457 cm⁻¹ it was possible to verify gallic and

tannic acids and in the spectral regions (from 1133 to 1160 cm^{-1} , from 1238 to 1322 cm^{-1} , and from 1373 to 1457 cm^{-1}) phenolic compounds in samples of Merlot grape pomace. So, the spectroscopy technique in the MIR region can be used to qualitatively identify phenolic compounds in grape pomace (Fragoso, Aceña, Guasch, Mestres, & Busto, 2011).

Through the FTIR-MIR spectroscopy analyses there were signals with the same intensity for phenolic compounds from all grape pomace extracts (Figure 2). However, differences have been seen for the hydroalcoholic extract with 40% ethanol (E4), it had the highest content phenolic compounds, total flavonoids and total anthocyanins and, therefore, was the extract used for the other instrumental analyses because it indicates the potential for reuse of grape pomace.

Relationship between antioxidant potential and bioactive compounds

Eleven phenolic compounds were identified from Merlot grape pomace in the 40% ethanol (E4) extracts, such as flavan-3-ols (catechin = $19.13 \pm 0.81 \text{ mg } 100 \text{ g}^{-1}$), flavonols (quercetin = $31.15 \pm 0.14 \text{ mg } 100 \text{ g}^{-1}$, rutin = $4.20 \pm 0.50 \text{ mg } 100 \text{ g}^{-1}$ and kaempferol = $8.33 \pm 0.21 \text{ mg } 100 \text{ g}^{-1}$), hydroxybenzoic acid (gallic acid = $36.04 \pm 1.85 \text{ mg } 100 \text{ g}^{-1}$, vanillic acid = $35.41 \pm 2.08 \text{ mg } 100 \text{ g}^{-1}$ and syringic acid = $46.09 \pm 2.23 \text{ mg } 100 \text{ g}^{-1}$), hydroxycinnamic acid (*trans*-cinnamic acid = $4.42 \pm 0.28 \text{ mg } 100 \text{ g}^{-1}$, caffeic acid = $3.80 \pm 0.78 \text{ mg } 100 \text{ g}^{-1}$, and *p*-coumaric acid = $1.47 \pm 0.05 \text{ mg } 100 \text{ g}^{-1}$) and stilbene (resveratrol = $4.54 \pm 0.14 \text{ mg } 100 \text{ g}^{-1}$), as seen in Figure 3a, 3b and 3c.

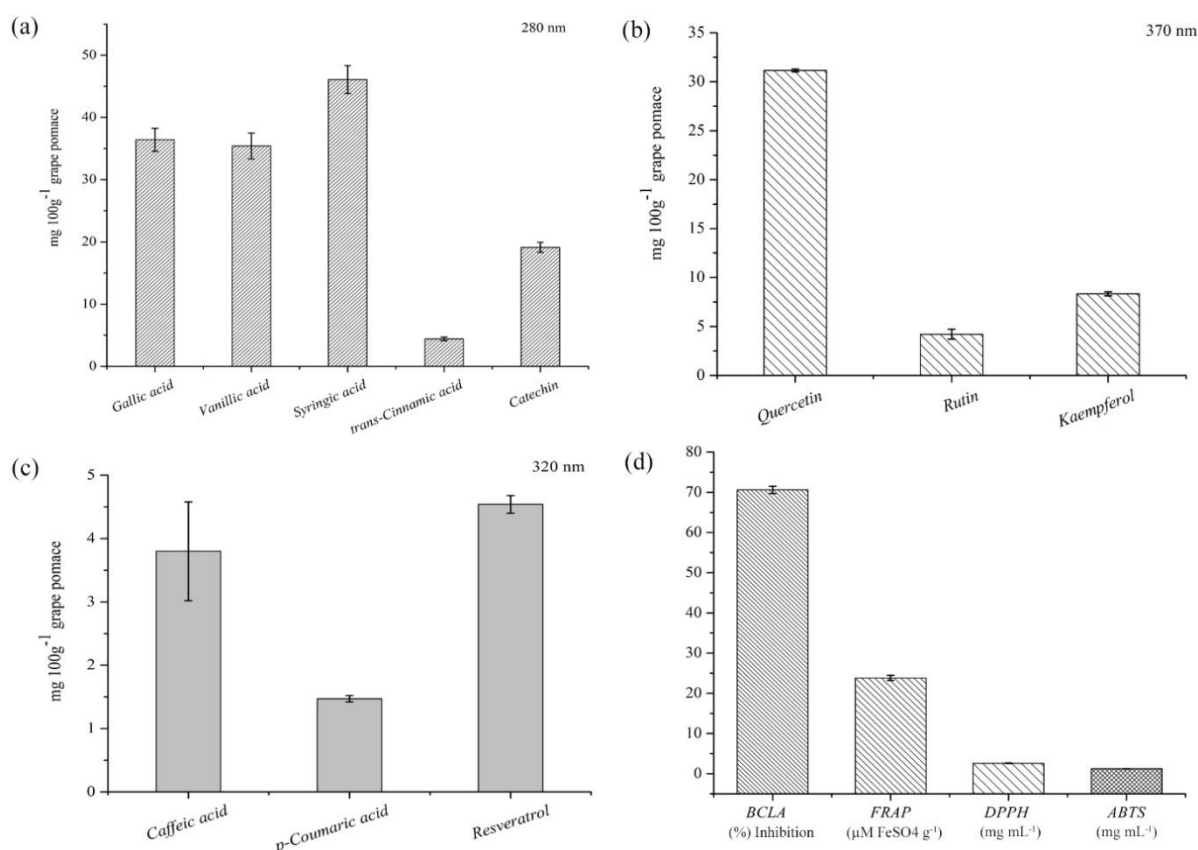


Figure 3. Results of the contents of individual bioactive compounds by high performance liquid chromatography (HPLC) at 280 nm (a), 320 nm (b) and 370 nm (c); antioxidant activity by BCLA, DPPH, ABTS and FRAP assays.

All of these phenolic compounds have been shown to have antioxidant potential, but as the antioxidant activity is highly complex, to use only one method is not enough to have an idea of the antioxidant capacity of a product, thus different methods are necessary for this purpose. The methods of evaluation of antioxidant capacity may differ with respect to the reaction mechanism, the target species, the conditions under which the reaction occurs and the way to express the results. Moreover, other factors may influence the determination of antioxidant capacity, including the type of solvent used in the extraction of compounds and their polarity, temperature and extraction time, the ratio of mass/volume, among others (Fragoso et al., 2011).

Thus, the results obtained for the antioxidant capacity assays based on the SET and HAT mechanisms were as follows: DPPH ($2.58 \pm 0.07 \text{ mg mL}^{-1}$), ABTS ($1.22 \pm 0.01 \text{ mg mL}^{-1}$), FRAP ($23.83 \pm 0.64 \mu\text{M FeSO}_4 \text{ g}^{-1}$) and

BCLA ($70.6 \pm 0.9\%$), which are shown in Figure 3d. The derivatives of hydroxycinnamic acids are better antioxidants for the DPPH assay than hydroxybenzoic acids because the double bond present in the cinnamic acid derivative molecule ($-\text{HC}=\text{CHCOOH}$) participates in the stabilization of the electron displacement resonance of unpaired radicals, whereas the benzoic acid derivatives do not exhibit this characteristic (Williams, Cuvelier, & Berset, 1995). So, the result of the DPPH assay for the Merlot grape pomace may be related to the presence of quantified hydroxycinnamic acids: *trans*-cinnamic acid, caffeic acid and *p*-coumaric acid.

While with respect to ABTS assay, the values of antioxidant activity may be related to phenolic acids levels, mainly, the gallic acid. As the Merlot sample has a high amount of gallic acid, the concentration of the insoluble bound fraction of this phenolic acid may influence the mechanism of reaction of this method because it had advantages such as reaction with hydrophilic and lipophilic compounds and can be applied in solutions both with aqueous and organic solvents and can react with compounds in a wide pH range (Haddouchi et al., 2014).

On the other hand, high levels of hydroxybenzoic acids may be more effective as antioxidants by the autoxidation of β -carotene/linoleic acid assay (BCLA), because the *o*-methoxyl groups present in the phenolic acids such as syringic acid and vanillic acid, for example, improve the stabilization of the phenoxyl radical (Von Gadow, Joubert, & Hansmann, 1997). The benzoic acids present in grape pomace have higher values than cinnamic acids. Thus, it can be inferred that the result for BCLA assay of $70.6 \pm 0.9\%$ of inhibition can be related to the significant content of hydroxybenzoic acids determined on the sample of Merlot grape pomace.

In relation to the FRAP assay, the efficiency of the antioxidant capacity for this assay depends on the redox potential of the analyzed compounds and is characterized by the complexity of their molecules. The FRAP components are highly related with their total phenolic and total flavonoid content, among polyphenols the greatest antioxidant efficacies in this assay can be associated for quercetin, kaempferol, rutin, tannic acid, caffeic acid and gallic acid (Pulido, Bravo, & Saura-Calixto, 2000). Thus, the value of FRAP ($23.83 \pm 0.64 \mu\text{M FeSO}_4 \text{ g}^{-1}$) obtained in this study can be associated with the different classes of flavonoids present in the Merlot grape pomace, such as flavonols: quercetin, kaempferol and rutin, flavan-3-ol (catechin) and stilbene, resveratrol.

As the antioxidant potential for all assays were strongly correlated with polyphenols, the results of the individual phenolic compounds (Figure 3), total phenolic compounds (TPC), total flavonoid content (TFC), total anthocyanin content (TAC) in Table 1 and of the antioxidants assays (DPPH, ABTS, FRAP and BCLA) of Figure 3d, were used to assess their correlation through the principal component analyses (PCA), according to Figure 4.

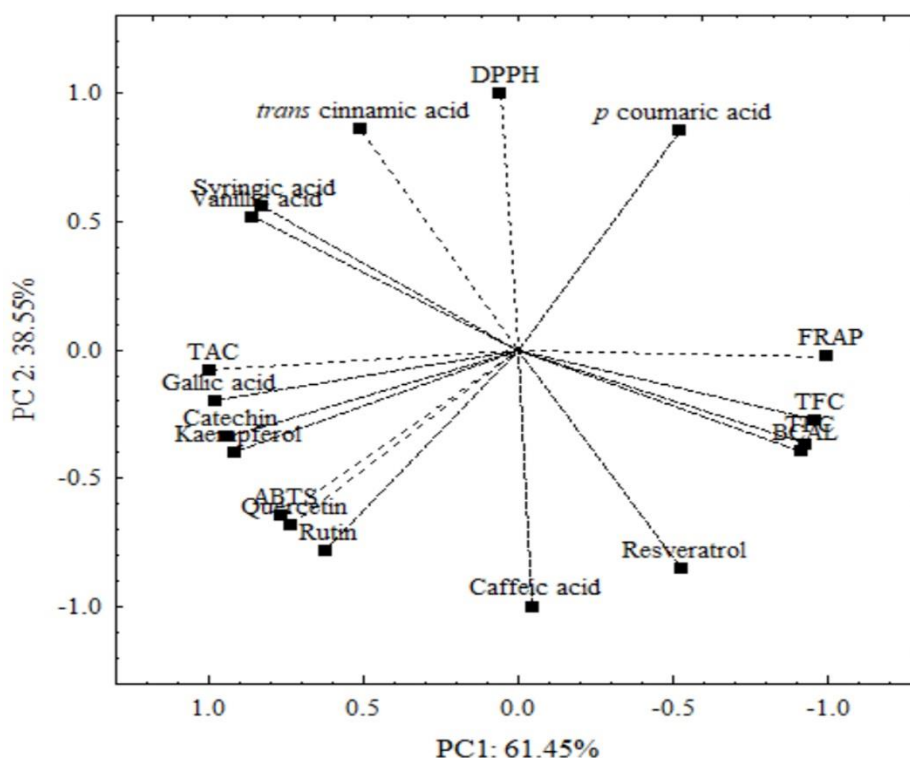


Figure 4. Principal components analyses between polyphenols (total phenolic compounds, individual phenolic compounds, total flavonoids content, total anthocyanins content) and antioxidant assays.

According to the PCA, there were positive similarities between the DPPH, BCLA and FRAP assays and differences for the ABTS method as shown by PC1 (61.45%). On the other hand, the PC 2 (38.55%) indicated differences between DPPH method and other assays. The possible similarities and differences between the antioxidant activity assays can be related to the contents of hydroxycinnamic acids, hydroxybenzoic acids and flavonoids that compose the profile of the phenolic compounds of Merlot grape pomace.

In this sense, it can be observed that the *trans*-cinnamic ($r = 0.8874$) and *p*-coumaric ($r = 0.8216$) acids had a positive contribution to the DPPH assay and a high negative correlation for caffeic ($r = -0.9999$). Both PC1 and PC2 grouped the results of the flavonoids to the data obtained for the ABTS assay, being possible to observe strong positive correlation (TAC: $r = 0.8122$, gallic acid: $r = 0.8770$, catechin: $r = 0.9371$, quercetin: $r = 0.9988$, rutin: $r = 0.9806$, and kaempferol: $r = 0.9577$) and strong negative correlation (*p*-coumaric acid: $r = -0.9496$). Such inferences are due to the mechanism of each antioxidant assay, because the ABTS assay is based on the generation of radical ABTS+ blue-green in color, which is applicable to both hydrophilic and lipophilic antioxidant systems; whereas DPPH assay uses a radical dissolved in organic media and is, therefore, applicable to hydrophobic systems (Floegel, Kim, Chung, Koo, & Chun, 2011).

BCAL assay based on the hydrogen atom transfer mechanism measures the ability of an antioxidant to eliminate free radicals by donating hydrogen, in this way, when relating the results of BCAL assay with polyphenols, it was noted that there was a high positive correlation (TPC: $r = 0.9995$, TFC: $r = 0.9915$, FRAP: $r = 0.9280$, and resveratrol: $r = 0.8196$) and high negative correlation (TAC: $r = -0.8844$, gallic acid: $r = -0.8212$, vanillic acid: $r = -0.9911$, and syringic acid: $r = -0.9830$) with phenolic compounds, flavonoid content and phenolic acids, as well as in the FRAP assay there was also a correlation with several polyphenols both positive (TPC: $r = 0.9394$, and TFC: $r = 0.9686$) and negative correlation (TAC: $r = -0.9946$, gallic acid: $r = -0.9747$ and catechin: $r = -0.9323$).

Similar results to those obtained in this study have been reported recently, for example, Peixoto et al. (2018) described that the antioxidant activity had strongly correlated with the presence of phenolic compounds, non-anthocyanins and anthocyanins, while Monteiro et al. (2021) indicated the correlation values between the antioxidant assay and the phenolic compound evaluated, so it can be seen that the total phenolic compounds showed a higher correlation with the antioxidant capacity (DPPH: $r = 0.81$; ABTS: $r = 0.95$, and FRAP: $r = 0.98$), than the total anthocyanins content (DPPH: $r = 0.59$; ABTS: $r = 0.69$ and FRAP: $r = 0.63$).

So, the higher the phenolics content, the greater the antioxidant efficacy. It should also be considered that the antioxidant capacity of the compounds may depend on three factors: (a) the potential to chelate metals, because it is strongly related to the arrangement of hydroxyl and carbonyl groups around the molecule, (b) the presence of hydrogen or electrons substituents capable of reducing free radicals, and (c) the ability of flavonoid, for example, to localize the unpaired electron, leading to the formation of a stable phenoxyl radical (Musialik, Kuzmich, Pawlowski, & Litwinienko, 2009). Thus, it can be inferred that the antioxidant potential was correlated with the bioactive compounds present in Merlot grape pomace and can also have different properties, including anti-inflammatory, anticancer, antiproliferative and antibacterial activities.

Antibacterial analyses

The results for minimum inhibitory concentrations (MIC) are summarized in Table 2 for each antibiotic (amoxicillin and cephalexin) against *B. cereus*, *S. enteritidis*, *E. coli*, *S. aureus* and *P. aeruginosa* growth by microdilution broth method using ethanol and methanol as solvent.

The extracts were more effective (lowest MIC values) against Gram-positive bacteria, mainly *B. cereus* and *S. aureus*, comparing to Gram-negative ones (*P. aeruginosa*, *E. coli* and *S. enteritidis*). The reason for the greater or lesser resistance can be explained through the difference between the cell walls of Gram-positive and Gram-negative bacteria (Riazi, Zeinali, Hoseini, & Behmadi, 2015). Or also, some authors attribute the antimicrobial potential to the phenolic compounds (Côté et al., 2011), principally to the hydroxyl groups of the phenolic compounds such as gallic acid, *p*-coumaric acid, ferulic acid, caffeic acid, catechin, kaempferol, *trans*-resveratrol and others, because phenolic compounds are able to interact with the membrane proteins of bacteria by means of hydrogen bonding, which can result in changes in membrane permeability, causing cell destruction or coagulation of the cell content (Oliveira, Angonese, Gomes, & Ferreira, 2016; Riazi et al., 2015).

Table 2. Minimum inhibitory concentration (MIC) of Merlot (*Vitis vinifera*) grape pomace extracts with different solvents (ethanol and methanol).

Bacteria (extract)	MIC
<i>B. cereus</i> (amoxicillin)	8 mg L ⁻¹
<i>B. cereus</i> (cephalexin)	32 mg L ⁻¹
<i>B. cereus</i> (ME - ethanol)	12.5 mg mL ⁻¹
<i>B. cereus</i> (ME - methanol)	12.5 mg mL ⁻¹
<i>S. enteritidis</i> (amoxicillin)	1 mg L ⁻¹
<i>S. enteritidis</i> (cephalexin)	16 mg L ⁻¹
<i>S. enteritidis</i> (ME - ethanol)	>100 mg mL ⁻¹
<i>S. enteritidis</i> (ME - methanol)	>100 mg mL ⁻¹
<i>E. coli</i> (amoxicillin)	8 mg L ⁻¹
<i>E. coli</i> (cephalexin)	16 mg L ⁻¹
<i>E. coli</i> (ME - ethanol)	>100 mg mL ⁻¹
<i>E. coli</i> (ME - methanol)	>100 mg mL ⁻¹
<i>S. aureus</i> (amoxicillin)	0,5 mg L ⁻¹
<i>S. aureus</i> (cephalexin)	2 mg L ⁻¹
<i>S. aureus</i> (ME - ethanol)	25 mg mL ⁻¹
<i>S. aureus</i> (ME - methanol)	25 mg mL ⁻¹
<i>P. aeruginosa</i> (amoxicillin)	16 mg L ⁻¹
<i>P. aeruginosa</i> (cephalexin)	32 mg L ⁻¹
<i>P. aeruginosa</i> (ME - ethanol)	100 mg mL ⁻¹
<i>P. aeruginosa</i> (ME - methanol)	100 mg mL ⁻¹

Bacillus cereus ATCC 11778, *Salmonella enteritidis* ATCC 13076, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *P. aeruginosa* ATCC 27853.
Merlot initial sample concentration - [C]₀ME = 100 mg mL⁻¹. Initial concentration of antibiotics - [C]₀A = 128 mg L⁻¹.

As the Merlot grape pomace presented a profile of phenolic compounds containing phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), flavonoids (flavonols and flavan-3-ols) and stilbenes, it can be suggested that satisfactory MIC values may be related to the presence of these compounds. The results obtained in the present study were similar to the MIC values found in literature from grape seed extracts (Jayaprakasha, Singh, & Sakariah, 2001), grape pomace (Peixoto et al., 2018) and there are also some studies that indicate a slight tendency of red cultivars to be more effective against Gram-positive bacteria, while Gram-negative bacteria seems to be more affected by white cultivars (Katalinić et al., 2010).

Conclusion

Grape (*Vitis vinifera*) pomace is a residue with a complex composition. In our study different individual bioactive compounds were found, including phenolic acids, stilbenes and flavonoids. What is more, in accordance with the chromatographic analysis, the phenolic compounds were also identified by MIR spectroscopy. Furthermore, principal component analysis (PCA) demonstrated that the antioxidant potential can be associated with the phenolic composition of the winemaking by-product. Grape pomace extracts showed antibacterial activity for both gram-positive and gram-negative bacteria. Thus, considering the significant concentration of different compounds found in Merlot grape pomace, this residue can be reused by the food and pharmaceutical industries in the development of new products, such as functional foods, supplements or nutraceutical formulations.

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