Influence of Different Solvents on the Phenolic Compounds Content Grape Bark Extracts (*Vitis vinifera*) from Syrah Variety

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Grape bark from the wine industry still contains significant amounts of phenolic compounds. Specific characteristics of the applied solvents influence the extraction of phenolic compounds directly. This study aimed to evaluate the influence of different solvents obtaining phenolic compounds in Syrah grape bark extracts by High-Performance Liquid Chromatography (HPLC). Grape peel extracts were prepared with three different solvents (water, 50%, and 80% grain alcohol), and evaluated using standard curve with 15 phenolic compounds. It was not possible to quantify the phenolic compounds, but the chromatograms suggest the presence of some of them. The solvent that provided the best interaction with grape peel was 50% grain alcohol.

Keywords: Antioxidants. Waste. Grape Peel. Chromatography.

Numerous researches over time have shown interest in studying the beneficial health effects attributed to phenolic compounds present in most foods. Studies highlight the multiple biological and clinical effects related to these diet compounds such as antioxidant, cardioprotective, anticancer, antibacterial, antiviral, vasodilatory, antidiabetic, and anti-inflammatory actions [1].

Phenolic compounds constitute one of the largest groups of secondary metabolites in plants with over 8000 already known. They are important compounds of foods. Grapes contain significant amounts of these substances compared to other fruits and vegetables [2]. The total phenolic content of grape skins changes through cultivar, such as topography, geology, drainage, climate, microclimate, grape variety, human intervention [3].

Researches show outstanding effectiveness in the reuse of grape marc because it contains significant amounts of phenols and antioxidants. The bark has components such as flavonols (kaempferol, quercetin and myricetin) [4], anthocyanins (cyanidin, delphinidin, peonidin, petunidin, malvidin), stilbene (resveratrol), phenolic acids (caftaric acid and p-coumaroyl tartaric acid), as well as a wide variety of tannins [5-7].

We used different solvent systems for the extraction of phenolic compounds from different matrices [8]. The solvent extraction is the most common method for separating natural antioxidants. These, when serially in foods with diversified solvents, may be more efficient in extracting bioactive compounds, since it takes into account the diversity of each sample [9]. The solvent system used in the extraction of grape marc directly influences the contents of total phenolic, anthocyanin, and antioxidant activity of the extracts [6].

The type and polarity of the solvent are characteristics that affect the transfer of electrons and hydrogen atoms, a vital aspect in the extraction of polyphenols, and, consequently, in the antioxidant capacity [10]. Beyond the yield, the choice of solvent system to be used also influences the composition of the extract [11].

The present study aimed to evaluate the influence of different solvents on the phenolic compounds obtained in Syrah grape bark extracts by High-

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Performance Liquid Chromatography, considering the importance of phenolic compounds for human health.

Methods

We used methanol, DMSO (dimethyl sulfoxide) (Sigma-Aldrich Chemical Co.- St. Louis, MO, USA) and acetic acid for HPLC grade, and grain alcohol (92.8% - Anidrol); a 0.20 μm regenerated cellulose membrane filter (Agilent Captiva, California, USA); caffeic acid (CAS number 331-39-5), gallic acid (CAS number 149-91-7), trans-cinnamic acid (CAS number 205-398-1), crystalline p-coumaric acid (CAS number 501-98-4), biochanin A (CAS number 91-80-5), catechin (CAS number 7295-85-4), epicatechin (CAS number 490-46-0), formononetin (CAS number 485-72-3), isoliquiritigenin (CAS number 961-29-5), myricetin (CAS number 529-44-2), narigenin (CAS number 67604-48- 2), quercetin (CAS number 117-39-5), resveratrol (CAS number 501-36-0), and hydrated rutin (CAS number 207671-50-9) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) and trans-ferulic acid (CAS number 537-98-4) from Fluka.

Obtaining Extracts

The samples of Syrah grape marc (*Vitis vinifera L.*) were obtained from wine industries in the San Francisco Valley region in August/2018. The barks were separated from seed and stalk and stored in an ultra-freezer (-20 °C) until the time of use. Three Syrah grape peel extracts with solvent variation were obtained: aqueous extract (EA), 50% grain alcohol extract (EAC50), and 80% cereal alcohol extract (EAC80). The peel was crushed with the solvent in proportion 1:5 m/v. Then, the mixture was placed in an ultrasonic bath (Elma Sonic, S40H, Germany) at 30 min/60°C. Subsequently, it was homogenized in a shaker incubator for 120 min/180 rpm, filtered and concentrated in a sample concentrator (Genevac, MiVac Concentrator, United Kingdom) at 50°C. The extracts were stored at freezing temperatures (-27 °C).

Chromatographic Analysis of Extracts

High-Performance Liquid Chromatography (HPLC) performed the identification of the phenolic compounds (caffeic acid, gallic acid, p-coumaric acid, trans-cinnamic acid, trans-ferulic acid, biochanin a, catechin, epicatechin, formononetin, isoliquiritigenin, myricetin, naringenin, quercetin, resveratrol and rutin hydrate) in the Syrah grape bark extracts. Initially, solutions of 10 $mg.mL^{-1}$ were prepared and dissolved in methanol. Methanolic solutions of grape extracts were prepared (5500 ppm). The samples were filtered with a 0.20 μm nylon filter (Agilent Captiva) for subsequent injection into an HPLC system (Shimadzu, LC-20AT, Japan) equipped with an automatic injector and diode array detector (DAD) (Shimadzu, SPD-M20, Japan). The chromatographic separation was performed according to the methodology proposed by Salgueiro and Castro [12] and Cabral and colleagues [13]. A NUCLEODUR 100–5 C18 EC column (150 x 4 mm ID, 5-μm particle size) was used in conjunction with a ZORBAX Eclipse Plus C18 4.6 x 12.5 mm precolumn (Agilent, USA).

Analysis conditions were performed with an elution gradient with a mobile phase of 5% acetic acid and methanol in different proportions, and with a total analysis time of 42 minutes (from 0 to 35 min (0-92% B); 35 to 40 min (92-0% B); 40 to 42 min (0% B)). The injection volume was 20 μL, and the flow 1 $mL.min^{-1}$. The machine was operated at a temperature of 40±2°C. The DAD reading was adjusted in the range of 190 to 800 nm, and the chromatographic acquisition was defined between 260 and 370 nm. The compound identification was made by comparing the retention time, and the ultraviolet spectrum between samples and standards (Table 1), the working range of the compound was 0.5 to 30 $mg.L^{-1}$.

In order to ensure the reliability of the results obtained, the validation was performed according to the methodologies of the National Health Surveillance Agency (ANVISA) [14] and the National Institute of Metrology, Quality and Technology (INMETRO) [15]. This analysis was performed according to the parameters of

selectivity, linearity, precision, detection limits, and quantification limits.

Results and Discussion

The phenolic compound investigated in different grapes bark extracts are shown in Table 2.

It is noteworthy that, although no compounds are quantified, the extracts are shown in their chromatograms peaks that suggest the presence of some compounds in their phytochemical composition, which are flagged with <LD (below detection limit) in Table 2.

According to the results presented, it is suggested that the solvent that provided the best interaction with the grape peel was the 50% grain alcohol. Below, is the chromatogram (Figure 1) for rutin at a wavelength of 260 nm.

The myricetin is an isoflavonoid with antioxidant activity, and it is commonly distributed in fruits, vegetables, nuts, berries, tea, and red wine [16]. It is believed that mirecetine has multiple therapeutic effects, the potential for health as antioxidant properties [17,18], anti-carcinogenic, anti-inflammatory, anti-atherosclerotic, anti-thrombotic, anti-diabetic and antiviral [19], these have anti and pro-oxidants effects, as well as exhibiting mutagenic effects and antimutagenic potential [18,20-22].

The rutin, a natural flavone, has several properties, including antiallergic, anti-inflammatory, antitumor, antioxidant, antidiarrheal, antihypertensive, antimutagenic and protection against nitrosative stress and hepatocellular injury [18,23,24].

Wang and colleagues [25] analyzed grape extract obtained from solutions of HCl and methanol (1:1) and acetone (70%), and analyzed by the same chromatographic method, and found values of 34.8±3.3 μg.mg and 9.0±0.8 μg.mg to myricetin and rutin.

Table 1. Limit of detection and quantification of active compounds by HPLC DAD.

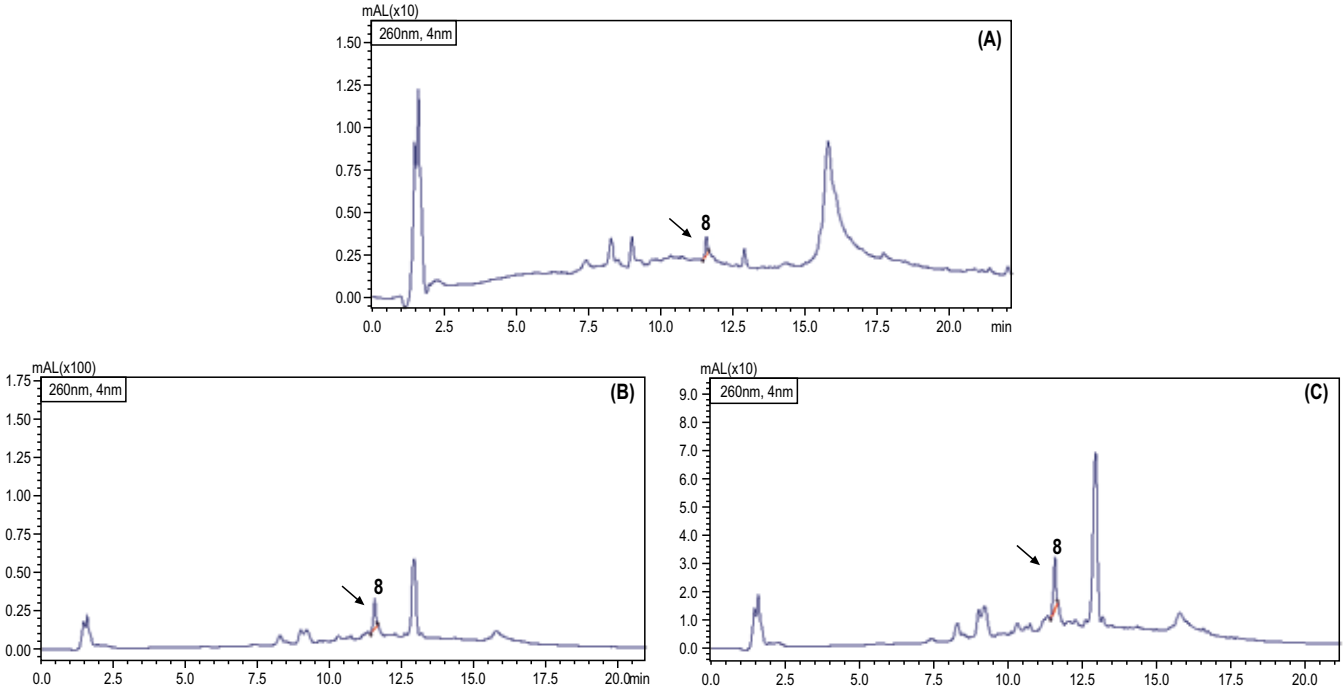
Phenolic Compound	tR (min)	ʎ (nm)	Detection Limit ($mg.g^{-1}$)	Limit of Quantification ($mg.g^{-1}$)
Coffee acid	8.20	320	0.29	0.96
Gallic acid	2.32	280	0.96	3.21
p-coumaric acid	10.35	300	1.04	3.48
Trans cinnamic acid	16.02	280	0.28	0.93
Trans-Ferulic Acid	11.33	320	1.99	6.64
Biochanin A	22.00	280	0.41	1.38
Catechin	6.59	280	1.07	3.58
Epicatechin	8.45	280	0.81	2.69
Formononetin	19.28	300	0.28	0.94
Isoliquiritigenin	18.70	370	0.34	1.14
Myricetin	13.06	370	0.72	2.39
Naringenin	16.54	280	0.24	0.79
Quercetin	15.30	370	0.77	2.57
Resveratrol	13.79	300	0.28	0.93
Rutin Hydrate	11.23	260	0.29	0.97

Table 2. Qualitative evaluation of the active compounds present in Shiraz grape extracts obtained with different solvents.

Phenolic Compound	EA	EAC50	EAC80
Coffee acid	<LD	<LQ	<LQ
Gallic acid	ND	<LD	<LD
p-coumaric acid	<LD	<LD	<LD
Trans cinnamic acid	<LD	<LQ	<LD
Trans-Ferulic Acid	ND	<LD	<LD
Biochanin A	ND	ND	<LD
Catechin	<LD	ND	<LD
Epicatechin	<LD	<LD	<LD
Formononetin	ND	ND	ND
Isoliquiritigenin	ND	ND	ND
Myricetin	<LD	<LQ	<LQ
Naringenin	<LD	<LD	<LD
Quercetin	ND	ND	ND
Resveratrol	ND	<LD	<LD
Rutin Hydrate	<LD	<LQ	<LQ

ND = Not detected; < LD = below the limit of detection; <LQ = below the limit of quantification.

Figure 1. (Chromatogram of grape extracts obtained with different solvents. EA (A), EAC50 (B) and EAC80 (C).



Conclusion

We observed that the extracts have phenolic compounds in their composition. However, the concentration analyzed of the samples was relatively low compared to the analytical curve used for the analysis method. Thus, it is inferred that the matrix since this study (grape peel) has phytochemical compounds, but a new analysis with a higher concentration is necessary to quantify these substances effectively.

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