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Grape juice by-products extracted by ultrasound and microwave-assisted with different solvents: a rich chemical composition

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Abstract By-products of the grape juice industry contain valuable compounds. The current work produced bioactive-enriched extracts from by-products of the grape juice, through three different extraction methods. Yields and chemical compositions varied, according to the extraction method (ultrasound, microwave, liquid–liquid). High-efficiency liquid chromatography with UV–Vis and high-resolution mass spectrometry characterised were used for chemical characterization, with glycosylated flavonoids evident. The crude extract was fractionated by open column, which has possibility carried-out fraction rich in resveratrol. The inhibition of DPPH radicals ranged from 14.2 to 74.2%, and the total phenolic content ranged from 0.1 to 107.0 mg gallic acid equivalents/100 g. Microwave-assisted extraction of grape juice by-products using polar solvents, such as ethanol and water, provided the best yield and chemical composition, obtaining extracts rich in flavonoids. In this way, this work has demonstrated the industrial grape by-products importances, which are a rich source of antioxidants if properly extracted.

Keywords Grape · Industrial by-products · Extraction · HPLC-UV · HRMS · Flavonoids

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Introduction

In the last century, the world population has increased five-fold. In 1900, it was about 1.5 billion people, while in 2000 there were almost 7 billion humans on the planet (United Nations Population Division, 2015). At this rate, the planet should reach 11.2 billion human beings in 2100, a growth of 53% compared to the present. This fact has required an industrial expansion, with a recurring increase in waste generated from processes, which need to be conveniently discarded.

The grape juice industrial process generates a large number of by-products. Grapes are cultivated all over the world but mainly in the temperate zone, with a total of 107.3 million tons, of which 35% was used for whole grape juice in 2014 (FAO, 2014). However, its residue has attracted researchers' attention because its chemical composition is based on phenolic compounds, which are natural antioxidants. These secondary metabolites are produced in plants, as a response to various forms of environmental stress (Naczk and Shahidi, 2004). These antioxidants act as free radical scavengers, electron or hydrogen donors and strong metal chelators, and thus, prevent lipid peroxidation, DNA damage and other adverse stress-induced phenomena (Afanasev et al., 1989; Blokhina et al., 2003).

Nevertheless, these compounds are not homogenously distributed in plant tissues, at the cellular and subcellular levels. The insoluble compounds are constituents of the cell walls, whereas the soluble ones are compartmentalised within the plant cell vacuoles. Also, the chemical nature of these compounds can range from low to highly

polymerised substances, including a variable proportion of phenolic acids, anthocyanins and tannins. Besides, the extraction efficiency of these bioactives is directly influenced by the extraction technique applies, the compounds thermal stability, the sample particle size, as well as the presence of interfering substances (in complex mixtures as plants extracts). In recent years, ultrasound (Yeo et al., 2015), and microwave (Zhang et al., 2011) have emerged as two of the most interesting techniques by which to intensify the extraction of active compounds from plants.

Therefore, this work aims to reduce the environmental problem caused by juice by-products, by generating value-added products as enriched phenolic extracts. Accordingly, three different extraction methods were tested, including microwave, ultrasound and a conventional liquid–liquid system, with various solvents. The chemical characterization was by High Performance Liquid Chromatography (HPLC–UV) and with High Resolution Mass Spectrometry (HRMS).

Materials and methods

Chemicals

Calibration curves for high-performance liquid chromatography (HPLC) were constructed for the following polyphenol standards: gallic acid, catechin, chlorogenic acid, epicatechin, rutin, ferulic acid, naringin, hesperidin, myricetin, resveratrol, quercetin and vitexin all obtained from Sigma–Aldrich (St. Louis, MO, USA). HPLC-grade acetonitrile purchased from Sigma–Aldrich (St. Louis, MO, USA) and ultra-pure water, prepared using a Milli-Q system, were used for the chromatographic analyses. The extraction solvents (hexane, chloroform, ethyl acetate, ethanol, water), all in P.A. grade, were purchased from Merck (Kenilworth, NJ, USA).

Grape by-products

Grapes of varieties of *Vitis labrusca* L. Bordo and Isabel were cultivated in Rio Grande do Sul State (southern Brazil), in conventional cultivation systems used for grape juice preparation and using an industrial-scale technological process. The grapes were manually harvested at the stage of technical maturity, with soluble solids readings between 14 and 20 °Brix, according to the International Organisation of Wine and Vine (OIV, 2017). After harvesting, grapes were kept separately at room temperature (20°C) until juice processing within 2 days. The juice was produced through a continuous-flow process, with the grapes berries crushed using an industrial mechanical crusher (EDA, São Paulo, Brazil) and immediately pumped through a tubular heat exchanger (85 ± 1°C for 10 s) (Boff, Vacaria, RS, Brazil). The extracted grape juice was centrifuged at 5000×*g* for 10 min, using a 600 Series Pieralisi decanter centrifuge (Jesi, AN, Italy). The solid by-product obtained from the centrifugation process was used in this work. After this process, and before the extraction, it was maintained at 4 °C in the absence of light, for a maximum of 20 days.

Extraction methods and fractionation

Ultrasound

Ultrasound-assisted extraction was performed as described by Benelli et al. (2010) and Oliveira et al. (2013). Briefly, 5 g of grape by-product was weighed, and 100 mL of each solvent added (hexane, ethylic alcohol, chloroform, ethyl acetate and ultra-pure water), respectively. Extractions were conducted with an ultrasound probe (model Vibra-Cell, Sonics®, Newton, MA, USA), operating at 30% amplitude and 500 W power for 20 min. Next, the samples were filtered, and the respective solvents were vacuum-dried by rotary evaporation. The resulting samples were conditioned at − 20 °C for further evaluation.

Microwave

The microwave extractions were performed as described by Barba et al. (2016). Briefly, to 2 g of grape by-products, 10 mL of each solvent (hexane, ethylic alcohol, chloroform, ethyl acetate and ultra-pure water) was respectively added. The extractions were performed at 110 °C for 20 min, at 850 W power, using a microwave (Monowave 300, Anton Paar® Houston, TX, USA). After, the samples were filtered and vacuum-dried (rotatory evaporator). The resulting samples were conditioned at − 20 °C for further evaluation.

Liquid–liquid

The liquid–liquid process was adapted from Mendoza et al. (2013). Briefly, 10 g of by-products were extracted with solvents in increasing order of polarity (hexane, after chloroform, after ethyl acetate, and after ethylic alcohol (3 × 30 mL, for each solvent) using a separation funnel. After separation, the solvent was filtered and vacuum-dried (rotatory evaporator) individually. The resulting samples were conditioned at − 20 °C for further evaluation.

Fractionation

The fractionation was conducted using an open column (4 cm × 45 cm) with 147 g of silica gel and 22.4 g of

grape by-product. Initially, chloroform (100–60%) and ethyl acetate (0–40%) was used as the mobile phase. After, the mobile phase was changed to methanol (5–100%) and ethyl acetate (95–0%). In total, 255 fractions were collected and analysed by thin layer chromatography (TLC), using chloroform:ethyl acetate (7:3), as the mobile phase. The TLC plates were visualised under UV light at 254 and 365 nm and also stained with vanillin-sulphuric acid spray and further heated. The fractions with similar chromatographic profiles were pooled, and the solvent was removed by rotary evaporation. Altogether, 12 different fractions were obtained.

HPLC analysis

All HPLC analyses were performed on a Hewlett-Packard 1100 system, equipped with a quaternary pump, autosampler, degasser and UV-Vis detector. The column used was a Lichosphere C18 250 mm × 4 mm, particle size 5 μm (Agilent, Santa Clara, CA, USA) with a guard column of the same material., Chromatographic data were acquired and processed using the HP ChemStation software. The mobile phase consisted of solvent A (aqueous solution with 1% v/v phosphoric acid) and solvent B (acetonitrile). The injection volume was 5 μL, and the polyphenols were eluted using a gradient system with 90% A (5 min), 60% A (5–40 min) and then 90% A (45–50 min). The total run time was 50 min, and the flow rate was 0.5 mL/min. The analysis was monitored at 210 nm, according to the modified chromatographic method reported by Morelli (2010). The samples were filtered through a 0.45-μm nylon membrane before analysis. Peaks were identified by comparing their retention time and UV–Vis spectra with reference compounds, and the data were quantified using the corresponding curves of the reference compounds as standards (gallic acid, catechin, chlorogenic acid, epicatechin, rutin, ferulic acid, naringin, hesperidin, myricetin, resveratrol, quercetin and vitexin). All standards were dissolved in methanol, and the results were expressed in mg/kg of extract.

High-resolution mass spectrometry (HRMS) analysis

The ethyl acetate total extract and the fractions were diluted in a solution of 50% (v/v) acetonitrile (Tedia, Fairfield, OH, USA), 50% (v/v) deionised water and 0.1% formic acid. The solutions were individually infused directly or with HPLC (Shimadzu, Kyoto, KA, Japan) assistance into the ESI source using a syringe pump (Harvard Apparatus, Holliston, MA, USA), at a flow rate of 10 μL min^{-1}. ESI (+)-MS were acquired using a hybrid high-resolution and high accuracy (5 μL) microTOF-QII mass spectrometer (Bruker Daltonics, Inc., Billerica, MA, USA), with the capillary and cone voltages set at + 3500 and + 40 V, respectively, and a de-solvation temperature of 100 °C. Diagnostic ions were identified by the comparison of exact *m/z* with compounds determined in previous studies. For data acquisition and processing, Hystar software (Bruker Daltonics, Inc. Billerica, MA, USA) was used. The data were collected in the *m/z* range of 70–800 at the speed of two scans per second, providing a resolution of 50,000 (FWHM) at *m/z* 200. No important ions were observed below *m/z* 100 or above *m/z* 800.

Total phenolic content

Ethyl acetate total extract and the fractions (No. 04–11) were individually diluted in a hydrochloric solution (70%) at 0.5 mg/mL. Each sample (0.5 mL) was diluted in a 0.2 N Folin-Ciocalteau solution (2.5 mL) for 5 min. Afterwards, 2 mL of 7.5% sodium carbonate solution was added, and the samples were incubated at 50 ± 1.0 °C for 5 min. The spectrophotometric absorbance of the reactions was measured at 760 nm (Roesler et al.,, 2007). Gallic acid (20–220 μg/mL) was used to construct a standard curve.

Antioxidant activity

The 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radical scavenging activity of the extracts was measured using a modified method of Yamaguchi et al. (1998). The extracts obtained in the ultrasonic, microwave and liquid–liquid extractions were added, respectively, to the Tris-HCl buffer (100 nM, pH 7.0) containing 250 μm of $DPPH^{\bullet}$ dissolved in ethanol. Tubes were stored in the dark for 20 min, and then the absorbance was read at 517 nm (UV-1700 spectrophotometer, Shimadzu, Kyoto, KA, Japan). Results were expressed as the percentage of inhibition of the $DPPH^{\bullet}$ radical.,

Statistical analysis

Phenolic quantification and antioxidant activity were expressed as mean ± standard deviation obtained from three independent experiments. Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, version 21.0) for Windows (Chicago, IL, USA). The Kolmogorov–Smirnov test was used to assess the parametric distribution of the data. Statistical significance was evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test. Results were deemed significant at $P < 0.05$.

Results and discussion

As a result of the accelerated increase in the population density, there is a greater demand for industrial products, with a consequent increase in waste generation (Fontana et al., 2013). Some of these residues, such as those from the grape juice processing industry and their derivatives, have a high incidence of active antioxidant compounds. The state of Rio Grande do Sul is the main producer of wines and derivatives in Brazil (Burin et al., 2014).

Anastasiadia et al. (2012) and Melo et al. (2015) determined the existence of phenolic compounds in wine by-products, which can be reused in the pharmaceutical, cosmetic and food additive sectors. However, there is a need to develop efficient and selective extraction methods to obtain extracts enriched in phenolic compounds. In this context, we tested three different approaches, including using solvents with different polarities and two different energy forms (microwave and ultrasound). Table 1 shows the yield results (m/m) for the tested processes.

Liquid–liquid extraction, which is related to the solubility difference between chemical compounds, is a traditional technique to isolate metabolites from plants and plant derivatives (Barba et al., 2016). The advantage of this procedure is the possible adjustment of the parameters, such as pH, temperature, time, the particle size of the initial solid, among others. In this work, a higher relative yield in the extracts with apolar (hexane) and polar (ethanol and water) solvents were observed. In a previous study, the bioactive yields ranged from 0.1 to 2.0% with water, ethanol and hexane, respectively (Mendoza et al., 2013).

Except for hexane and ethanol, the extract yields acquired by ultrasound were quite similar to those obtained with liquid–liquid extraction (Table 1). Ultrasonication involves the cavitation phenomenon, which improves the transference of heat through the plant cell wall, facilitating the extraction of bioactive compounds. Benelli et al. (2010) determined, through mathematical modeling, the best ratio between orange pomace, solvent volume and ultrasound power for extraction of carotenoids and derivatives, which was used here ($\approx$ 5 g $\times$ 100 mL). Palma and Barroso (2002) assessed the efficacy of ultrasound to extract tartaric and malic acids from wine-making by-products and reported yield ranges of 130–199 and 33–41 ppm, respectively. The authors used methanol and water as solvents while varying the volume, temperature and time, as well as ultrasound amplitude.

The microwave-assisted extraction showed the highest relative yields. This procedure has already been used to obtain bioactives from plants and plant derivatives (Barba et al., 2016). In this process, the electromagnetic energy, between 300 MHz and 300 GHz, is transferred in the form of heat by ionic conduction that is sufficient to break the cells and release the active compounds. In the same way, the process involves diffusion of the solvent through the sample matrix, consequently releasing the metabolites (Barba et al., 2016). In addition, it is possible to create a temperature and energy gradient, facilitating the extraction without degradation of the active principles, as evidenced from previous works that evaluated the effects of microwave-assisted extraction with polar solvents (water and ethanol) on wineries by-products, which were dried in various ways (Drosou et al., 2015; Torre et al., 2013). The yields obtained by the authors ranged from 2 to 16%, with air drying providing the best results.

The chemical composition of the extracts and fractions is directly related to the solvents and the extraction methods used. For the qualification and quantification of the chemical compounds present in the extracts, we used an HPLC/UV-Vis method adapted from Morelli (2010). The method have demonstrated the selectivity for 12 compounds (gallic acid, catechin, chlorogenic acid, epicatechin, rutin, ferulic acid, naringin, hesperidin, myricetin, resveratrol, quercetin and vitexin), with R^2 ranging from 0.9926 to 0.9999, LOD from 0.02 ($\mu g\ mL^{-1}$) to 0.15 and LOQ from 0.06 to 0.50 ($\mu g\ mL^{-1}$). In our extracts, six of these compounds detected previously were identified (Anastasiadia et al., 2012), as presented in Table 2. Gallic acid and naringin were the most prevalent, with ranges of 1.8–33.9 and 0.3–5.6 mg kg^{-1}, respectively, in the microwave-assisted extraction. Regarding the solvents, the most polar (ethanol and water) presented the highest amounts between the compounds identified.

Chromatographic techniques with different detectors have been widely used for the qualification and quantification of phenolic compounds in extracts of plants and industrial residues. In the characterisation of grape by-products by HPLC-UV, Brazinha et al. (2014) and Anastasiadi et al. (2012), demonstrated selective analysis of four (gallic acid, catechin, epicatechin and ferulic acid) and

Table 1 Yields (%) related to the methods of extracting of winery by-products

Method/Solvents	Hexane (%)	Chloroform (%)	Ethyl acetate (%)	Ethanol (%)	Water (%)
Liquid–liquid	0.64 ± 0.02	0.15 ± 0.03	0.21 ± 0.01	0.47 ± 0.05	0.44 ± 0.02
Ultrasound	0.23 ± 0.05	0.15 ± 0.03	0.24 ± 0.02	1.03 ± 0.01	0.65 ± 0.05
Microwave	4.15 ± 0.04	4.68 ± 0.01	0.45 ± 0.02	2.06 ± 0.03	3.12 ± 0.04

Date are expressed as mean ± SD (n = 3)

Table 2 Polyphenolic composition of extracts studied (mg/Kg)

Entry/Solvent	Hexane	Chloroform	Ethyl acetate	Ethanol	Water
Liquid–Liquid					
Catechin	Nd	Nd	Nd	Nd	Nd
Resveratrol	Nd	Nd	Nd	0.94 ± 0.06^{Ab}	Nd
Hesperidin	Nd	Nd	Nd	0.59 ± 0.07^{Ab}	Nd
Gallic acid	2.03 ± 0.04^{Bb}	0.04 ± 0.001^{Cb}	0.04 ± 0.001^{Cb}	3.25 ± 0.13^{Ac}	1.94 ± 0.03^{Bc}
Epicatechin	Nd	Nd	Nd	Nd	Nd
Naringin	1.72 ± 0.06^{Ab}	0.01 ± 0.002^{Ca}	0.02 ± 0.001^{Cb}	0.28 ± 0.07^{Bb}	0.31 ± 0.04^{Bb}
Ultrasound					
Catechin	Nd	Nd	Nd	Nd	Nd
Resveratrol	Nd	Nd	Nd	Nd	Nd
Hesperidin	Nd	0.02 ± 0.002^{Ca}	0.16 ± 0.01^{Bb}	0.50 ± 0.06^{Ab}	Nd
Gallic acid	0.08 ± 0.003^{Dc}	0.08 ± 0.003^{Db}	0.23 ± 0.002^{Cb}	4.10 ± 0.03^{Bb}	5.01 ± 0.12^{Ab}
Epicatechin	Nd	Nd	0.06 ± 0.002^{Ab}	Nd	Nd
Naringin	0.04 ± 0.003^{Bc}	0.02 ± 0.003^{Ba}	0.04 ± 0.003^{Bb}	0.50 ± 0.08^{Ab}	0.45 ± 0.08^{Ab}
Microwave					
Catechin	Nd	Nd	1.40 ± 0.04^{Aa}	Nd	Nd
Resveratrol	Nd	Nd	1.17 ± 0.05^{Ba}	7.59 ± 0.49^{Aa}	Nd
Hesperidin	Nd	Nd	0.62 ± 0.09^{Ba}	3.16 ± 0.23^{Aa}	Nd
Gallic acid	15.41 ± 0.48^{Da}	16.44 ± 0.39^{Ca}	1.74 ± 0.04^{Ea}	33.87 ± 0.37^{Aa}	24.98 ± 0.29^{Ba}
Epicatechin	Nd	12.29 ± 0.16^{Aa}	1.14 ± 0.04^{Ca}	5.24 ± 0.25^{Ba}	Nd
Naringin	5.55 ± 0.64^{Aa}	Nd	0.27 ± 0.05^{Da}	1.37 ± 0.31^{Ca}	3.41 ± 0.55^{Ba}

Means followed by the same letter do not differ statistically by Tukey test ($P < 0.05$). Lowercase letters and uppercase letters correspond to lines and columns respectively between the same compounds

thirteen compounds (gallic acid, catechin, epicatechin, procyanidin B2, epicatechin gallate, *trans*-resveratrol, quercetin, kaempferol, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-coumaric acid and ferric acid) respectively.

In our extracts, six of these compounds detected previously (Anastasiadia et al., 2012) were identified, as presented in Table 2. Gallic acid and naringin were the most prevalent, with ranges of 1.8–33.9 and 0.3–5.6 mg kg^{-1}, respectively, in the microwave-assisted extraction. Regarding the solvents, the most polar (ethanol and water) presented the highest amounts of the evaluated compounds. Among the solvents investigated in this study, ethyl acetate and ethanol have been quoted for their efficiency in the extraction of phenolic compounds (Spigno et al., 2007). In this way, catechins were successfully isolated from grape berry using methanol and ethyl acetate as solvents, by the shaker extraction method (Jin et al. 2010). Likewise, Ribeiro et al. (2015) extracted phenolic acids, among them gallic acid, using the polar solvents, ethyl acetate and water. As one of the main features, resveratrol was identified in ethanol and ethyl acetate from liquid–liquid and microwave-assisted extraction, in concentrations ranging from 0.9 to 7.6 mg kg^{-1}. Careri et al. (2003) and Yilmaz and Romeot (2004) identified the presence of resveratrol in wine-making residues. *Trans*-resveratrol, a phytoalexin belonging to the class of stilbenes, is found in grape by-products, especially in red varieties.

Oliveira et al. (2013) extracted wine-making by-products using ethanol, water and ethyl acetate with ultrasound and identified gallic acid, *p*-OH-benzoic acid, vanillic acid and epicatechin by HPLC/UV, in concentrations ranging from 0.5 to 5.1 mg kg^{-1}. Likewise, Melo et al. (2015) extracted grape pomace and rachis using solvents of various polarity, including water and ethanol. The authors identified 11 compounds, among them gallic acid, catechin and epicatechin, in amounts ranging from 0.1 to 7.4 mg kg^{-1}.

In order to obtain fractions enriched in phenolic compounds, we tested the open column fractionation using chloroform, ethyl acetate and methanol as the mobile phase and silica as the stationary phase. Thus, 255 fractions were separated, which after preliminary analysis (TLC), were pooled to form 12 groups with a similar composition. Table 3 describes the compounds (gallic acid, catechin and resveratrol) identified by HPLC/UV. Resveratrol was extracted in highest concentrations in the fractions with chloroform:ethyl acetate (7:3 to 3:4 v/v). All fractions collected by the fractionation method showed the presence of gallic acid. Nugroho et al. (2016) also performed open

Table 3 Polyphenolic composition of fractions studied (μg/mL)

Entry/Fraction	F1	F2	F3	F4	F5	F6
Catechin	Nd	Nd	Nd	Nd	Nd	Nd
Resveratrol	Nd	Nd	Nd	Nd	1.38 ± 0.06^{c}	1.50 ± 0.09^{c}
Galic acid	1.35 ± 0.07^{h}	1.46 ± 0.06^{h}	1.76 ± 0.07^{g}	2.65 ± 0.03^{e}	2.95 ± 0.02^{d}	3.04 ± 0.05^{b}
Entry/Fraction	F7	F8	F9	F10	F11	F12
Catechin	Nd	Nd	1.69 ± 0.07^{a}	0.48 ± 0.02^{b}	Nd	Nd
Resveratrol	5.89 ± 0.14^{a}	1.68 ± 0.03^{b}	1.18 ± 0.07^{d}	Nd	Nd	Nd
Galic acid	3.24 ± 0.04^{bc}	3.78 ± 0.06^{a}	2.54 ± 0.05^{e}	2.60 ± 0.05^{e}	2.18 ± 0.11^{f}	3.07 ± 0.06^{cd}

Means followed by the same letter do not differ statistically by Tukey test ($P < 0.05$)

column fractionation for the isolation of phenolics from leaves of *Carica papaya*. The authors used chloroform:methanol:water (70:30:10 v/v) as a mobile phase and retrieved 186 fractions, where myricetin-3-rhamnoside, kaempferol-3-rutinoside, quercetin, kaempferol, as well as other phenolics, were isolated.

HRMS is a powerful tool for identification of natural metabolites, for instance, phenolics (Rufatto et al., 2013) and alkaloids (Nicola et al., 1985), in complex mixtures (extracts). By HRMS, information, such as exact mass and the isotopic ratio can be used in the chemical identification (Bristow and Webb, 2003; Knolhoff et al., 2014). Furthermore, for unequivocal identification and differentiation of isobaric interferences, the fragmentation pathway is also a powerful tool. In the extracts and fractions, it was possible to identify 14 compounds by ESI (+) and ESI (−) modes, Table 4.

The technique used in this work allowed the identification of two glycosides, malvidin-*O*-glycoside and quercetin-3-*O*-glycoside. Casas et al. (2016) extracted grapes using methanol and water under pressure and determined the polyphenolic compounds by HRMS, among which gallic acid, catechin and epicatechin were identified. Also, the authors documented the presence of resveratrol, stilbenes and derivatives, such as *O*-glucosides, found in the grape stems. The flavonoids, such as quercetin, malvidin and resveratrol, beyond the glycosylated, such as quercetin-3-*O*-glucoside and malvidin-3-

Table 4 Chemical compounds identified in *V. labrusca* extracts by high resolution mass spectrometry, in positive and negative mode

Entry	Percursor ion *m/z*	Extract	Method	Identification	Elem. Comp.	Diff. ppm	References
Analysis in ESI(+): Hexane (A); Chloroform (B); Ethyl acetate (C); Ethanol (D); Water (E); Ultrasound (1); Microwave (2); Column (3); Liquid–liquid (4)							
1	129.0546	A–E	1–4	Furaneol	$C_6H_8O_3$	4.41	Sasaki et al. (2015)
2	169.0496	A–E	1;2;4	Vanillic acid	$C_8H_8O_4$	2.86	Ribeiro et al. (2015)
3	193.0725	E	4	Quinic acid	$C_7H_{12}O_6$	5.06	Silva et al. (2015)
4	181.0496	D–E	1;2;4	Caffeic acid	$C_9H_8O_4$	2.67	Ribeiro et al. (2015)
5	229.0885	B	1	Resveratrol	$C_{14}H_{12}O_3$	4.86	Koyama et al. (2017)
6	291.0855	D–E	1;4	Catechin/Epicatechin	$C_{15}H_{14}O_6$	4.68	Ribeiro et al. (2015)
7	303.0506	C–D	1;2	Quercetin	$C_{15}H_{10}O_7$	0.40	Ribeiro et al. (2015)
8	331.0810	E	1	Malvidin	$C_{17}H_{15}O_7$	2.35	Koyama et al. (2017)
9	465.1008	C–D	1	Quercetin-3-*O*-glucoside	$C_{21}H_{20}O_{12}$	5.38	Koyama et al. (2017)
10	493.1360	D–E	1;2	Malvidin-3-*O*-glucoside	$C_{23}H_{25}O_{12}$	2.84	Koyama et al. (2017)
Extracts analysis in negative mode ESI(−): Hexane (A); Chloroform (B); Ethyl acetate (C); Water (E); Ultrasound (1); Microwave (2); Column (3); Liquid–liquid (4)							
11	179.0558	A;D–E	1–4	Glucose	$C_6H_{12}O_6$	1.32	Kurt et al. (2017)
11	255.2364	A–E	1–3	Palmitic acid	$C_{16}H_{32}O_2$	4.41	Ribeiro et al. (2015)
13	279.2340	C–D	1–3	Linoleic acid	$C_{18}H_{32}O_2$	5.71	Ribeiro et al. (2015)
12	283.2698	A–C	1–4	Stearic acid	$C_{18}H_{36}O_2$	4.22	Ribeiro et al. (2015)

O-glucoside, are known for their *in vivo* antimicrobial, antifungal and antioxidant activities (Lomillo et al., 2014; Mendoza et al., 2013; Oliveira et al., 2013). These natural antioxidants act as free radical scavengers, promoting vasodilation and inhibiting enzymes, such as phospholipase, cyclooxygenase and lipoxygenase, in addition to reducing lipid peroxidation (Kabir et al., 2015).

Antioxidant capacity

Phenolic compounds are an important class of natural antioxidants. The extracts were evaluated by the DPPH method, Table 5.

With regards to the solvents, the extracts with the highest antioxidant capacity were those obtained with ethyl acetate and ethanol. Meanwhile, comparing the extraction modes, the ultrasound allowed us to achieve the highest percentage (74.22 ± 2.07%) of DPPH antioxidant activity with ethyl acetate solvent. In the liquid–liquid extraction, the highest percentage of this parameter corresponded to the ethyl acetate and ethanol extracts. In the microwave extraction, the highest percentage of DPPH antioxidant activity was 36.64 ± 2.44%, using ethanol as solvent. Similarly, Brazinha (2014) noted that hydroalcoholic extracts (60 wt.% ethanol and 3 g/L citric acid in water) displayed higher DPPH values (up to approximately 80%) than the single solvents

Table 5 The crude extracts antioxidant capacity determinated by DPPH· method

Solvents	Liquid–Liquid	Ultrasound	Microwave
Hexane	14.87 ± 7.01^{Ab}	16.59 ± 5.79^{Ab}	20.26 ± 1.22^{Aa}
Chloroform	19.61 ± 7.01^{Ab}	17.03 ± 0.30^{Ab}	17.89 ± 2.74^{Ab}
Ethyl acetate	33.62 ± 0.61^{Ba}	74.22 ± 2.07^{Aa}	31.03 ± 6.71^{Ba}
Ethanol	29.53 ± 3.96^{Aab}	31.47 ± 1.22^{Ab}	36.64 ± 2.44^{Aa}
Water	14.22 ± 3.05^{Bb}	28.66 ± 7.62^{Ab}	27.59 ± 1.22^{Aa}

Means followed by the same letter do not differ statistically by Tukey test ($P < 0.05$). Lowercase letters and uppercase letters correspond to lines and columns respectively

Kabir et al. (2015) used enzymatic digestion to extract phenolic compounds from grape by-products. The obtained extracts exhibited around 60 and 70% DPPH radical scavenging activity. Rajha et al. (2013) studied ground grapes, using solid-liquid extraction with water as solvent, and varying the time and temperature parameters. According to the authors, the DPPH radical scavenging activity was 11 to 35%, which corroborates with the results of the present study.

Total phenolic compounds

The phenolic compounds found in grapes and grape derivatives can be classified into three main groups: (1) phenolic acids, such as benzoic and hydroxycinnamic derivatives; (2) flavonoids, like catechins, flavanols and anthocyanins, and (3) tannins and proanthocyanidins (Fontana et al. 2013). The total phenolic content was determined by the Folin-Ciocalteau method adapted from Roesler et al. (2007). Comparing the extractions methods, the samples with a higher content of polyphenols also had a higher antioxidant activity, Table 6.

In general, the ultrasound extractions were inferior to those obtained by microwave and liquid–liquid. However, the microwave crude extracts showed the best Folin-Ciocalteau results (0.04 ± 13.18–6.02 ± 107.03 mg gallic acid equivalents [GAE]/100 g). Kabir (2015), using enzymatic digestion for phenolic extraction, reported 33.19–41.05 mg chlorogenic acid equivalents/g dry bagasse. Yilmaz et al. (2015) observed higher total phenolic contents were found in the peel and seeds of the residues compared to the pulp. The polyphenols of grape differ significantly, depending mainly on the cultivar, vintage, degree of maturation and the technology applied during the vinification. Centeno et al. (2015) and Corrales et al. (2008) and extracted grape by-products with ultrasound, using water and ethanol as solvents and established total phenolic contents of 0.20 ± 6.17 and 0.56 ± 5.31 mg GAE/100 g, and DPPH antioxidant capacities of 1.22 ± 31.47 and 7.62 ± 28.66 mg GAE/100 g, respectively. Moreover, in a comparison of extraction methods

Table 6 Content (%) of total phenolic compounds mg GAE/100 g gallic acid

Solvents	Liquid–Liquid	Ultrasound	Microwave
Hexane	3.58 ± 0.25^{Bb}	0.05 ± 0.002^{Cb}	31.76 ± 0.66^{Ca}
Chloroform	0.09 ± 0.005^{Db}	0.07 ± 0.007^{Cb}	107.03 ± 6.02^{Aa}
Ethyl acetate	0.15 ± 0.003^{Cb}	0.92 ± 0.006^{Bb}	13.18 ± 0.04^{Ea}
Ethanol	12.75 ± 0.39^{Ab}	6.17 ± 0.20^{Ac}	73.33 ± 2.45^{Ba}
Water	10.23 ± 0.63^{Ab}	5.31 ± 0.56^{Ac}	26.15 ± 1.29^{Da}

Means followed by the same letter do not differ statistically by Tukey test ($P < 0.05$). Lowercase letters and uppercase letters correspond to lines and columns respectively

for grape by-products, the authors suggested that the application of ultrasound power improves the efficiency and yield of the extraction process, being faster and accelerating over time.

In summary, this work had demonstrated that *V. labrusca* varieties, widely cultivated in southern Brazil, and used for grape juice, also have a high antioxidant potential, which is associated with the presence of phenolic compounds. Among the extraction methods studied, the microwave technique with polar solvents offered the best results regarding the level of bioactive compounds. Also, we demonstrated that techniques, such as ultrasound and microwave, are capable of extracting glycosylated flavonoids, which are associated with various biological activities. These compounds are easily hydrolysed and, therefore, are not obtained by other methods of extraction. Furthermore, we have shown that the juice by-products, which are normally discarded, representing an environmental problem, can be reused.

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Compliance with ethical standards

Conflict of interest All authors declared that they have no conflict of interest.

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