Bioactive compounds recovery optimization from vine pruning residues using conventional heating and microwave-assisted extraction methods

Meirielly S. Jesus, Zlatina Genisheva, Aloia Romaní, Ricardo N. Pereira, José A. Teixeira*, Lucília Domingues

CEB – Centre of Biological Engineering, University of Minho, Campus Gualtar, Braga, Portugal

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ABSTRACT

Polyphenol compounds from vine pruning residue (VPR) were extracted by conventional heating and microwave-assisted treatments. For each treatment, total phenolic compounds and their antioxidant activity were optimized by experimental design. Maximal extraction of polyphenolic compounds (2.17 g/100 g VPR) was obtained at 80 °C, 120 min and 45% of ethanol by conventional heating, and 2.37 g/100 g of VPR were extracted by microwave-assisted process at 120 °C, 5 min and 60% of ethanol. Ellagic acid and apigenin were the predominant polyphenolic compounds in the extracts, achieving concentration of 68.65 and 208.23 mg/100 g VPR, respectively for conventional heating and 185.15 and 118.84 mg/100 g of VPR for microwave-assisted treatment. The results showed reduction of extraction time and energy consumption for microwave-assisted treatment leading to cost-effective technology for the extraction of polyphenol compounds. Furthermore, the results hereby compiled allow for the tailor-made extraction of specific high-value compounds from a renewable biomass as vine pruning residue.

1. Introduction

High volumes of agricultural wastes and byproducts are produced from agro-food industry every year. In Mediterranean regions, the wine industry generates a significant amount of these residues (namely stems, peels, seeds, vine leaves, vine pruning or shoots) with no economic value, which are directly discarded causing environmentally problems (Delgado-Torre et al., 2012; Teixeira et al., 2014). Recycling and reusing these wastes to obtain added-value compounds is mandatory to promote the circular Economy, and thus achieve a sustainable growth of our society. In this context, these residues are recognized sources of polyphenolic compounds with interesting biological activities which help for the prevention of inflammatory processes, cardiovascular problems and prevention of oxidative reactions (Teixeira et al., 2014). Therefore, the research on the extraction of these natural bioactive compounds from winemaking byproducts has recently increased (Domínguez-Perles et al., 2014; Lachman et al., 2016; Piñeiro et al., 2017; Pintać et al., 2018).

Nomenclature

Abbreviations

ABTS	2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid
CCD	Central composite design
CHE	Conventional heating extraction
DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
FPU	Filter Paper Units
GAE	Gallic Acid Equivalent
FE (II)	ferrous equivalents
mha	Million Hectares
mhl	Million hectoliters
MHz	Megahertz
MAE	Microwave-assisted extraction
RSM	Response surface methodology
UHPLC	Ultra-high-performance liquid chromatography

VPR	Vine Pruning Residue

Parameters and constants

A_0	Absorbance of control (DPPH or ABTS)
A_1	Absorbance of extracts
$\beta_{0i}, \beta_{1i}, \beta_{2i}, \beta_{3i}, \beta_{11i}, \beta_{22}, \beta_{33i}, \beta_{12i}, \beta_{13i}$ and β_{23i}	Are regression coefficients calculated from experimental
Ia	Inhibition activity (%)
IC_{50}	Concentration of sample or standard that can inhibit 50% of DPPH or ABTS (mg / mL)
R^2	Coefficient determination
x_1	Extraction time (min)
x_2	Extraction Temperature (˚C)
x_3	Concentration of ethanol (%)
y_i	Concentration total phenolics extracted, antioxidant capacity or phenolic compounds (Apigenin and Ellagic acid)

Among these residues, vine pruning residue is a lignocellulosic material composed by non-structural components (extractives, ashes, proteins) and structural components (cellulose, hemicellulose and lignin) which can be processed for the production of oligosaccharides and cellulosic ethanol (Jesus et al., 2017). In addition, extractives can be also considered as target fraction for an integral valorization of these lignocellulosic materials (Gullón et al., 2017). The content of the extractives in vine pruning residue can vary from 3.1 to 16.6 %16.6% (Dávila et al., 2016; Buratti et al., 2015; Jesus et al., 2017), which could depend on the cultivar, different growth regions (Delgado-Torre et al., 2012) and/or conditions of post-pruning and time of storage (Cebrián et al., 2017). In spite of representing a low percentage fraction, the recovery and separation from extractives of valuable products with high market price (such as terpenes, esters, stilbenes, flavonoids or other antioxidant phenols) could improve the economic profitability of the process (Cebrián et al., 2017).

Traditional methods used for the phenolic compounds extraction employ toxic solvents such as methanol-water mixtures (Delgado-Torre et al., 2012) and long times of heating, which results in high energy requirements and also in the degradation of thermal-labile molecules (Galanakis et al., 2013). The application of the extracted bioactive compounds for a human use makes mandatory the optimization of processes based on nontoxic solvents such as water or ethanol-water mixtures. Alternative techniques of extraction have been used for the recovery of phenolic compounds from vine pruning residues, including hydrothermal treatment at high temperatures (Jesus et al., 2017; Gullón et al., 2017), ultrasonic-assisted extraction (Jing et al., 2015; Kazibwe et al., 2017), alkaline hydrolysis treatments (Max et al., 2010), pulsed electric field extraction (Rajha et al., 2015) and microwave-assisted extraction (MAE) (Calinescu et al., 2017; Ranic et al., 2014; Spigno and De Faveri, 2009). Among these alternatives, the microwave-assisted treatment is considered an efficient method of extraction since, it requires low energy and short times of extraction (Delgado-Torre et al., 2012; Luque-Rodríguez et al., 2006; Piñeiro et al., 2017; Proestos and Komaitis, 2008). The electromagnetic dipole rotation between the molecules and the heating generated in the MAE solvent are responsible for the higher recovery of extracts with high content of the target compound. Polar molecules, such as water inside the plant cell, are heated instantaneously by microwave, where evaporation generates high pressure on the walls of cells due to the swelling of the plant cell, pushing and stretching the cell wall causing them to be destroyed. This process facilitates the release of the bioactive compounds from the cells, increasing the extraction yield. This efficiency can vary accordingly to the parameters adopted for the extraction such as time, temperature and solid-liquid ratio, concentration and type of solvent used (Mandal et al., 2007; Proestos and Komaitis, 2008).

Recently, vine pruning residue was subjected to two sequential stages of hydrothermal treatment to produce cellulosic ethanol and hemicellulosic liquors enriched in xylooligosaccharides and phenolic compounds with antioxidant activity (Jesus et al., 2017). On the basis of the previous work, a study of phenolic compounds extraction, as first stage for the biorefinery of vine pruning residue, is proposed. Thus, the aim of this work was the optimization of phenolic compound extraction by conventional heating and microwave assisted methods through an experimental design in which temperature, time and percentage of ethanol-water were evaluated. The antioxidant activity (based on FAP, DPPH and ABTS methods) were determined and phenolic compounds were identified and quantified.

2. Materials and methods

2.1. Raw material and analysis of chemical composition

Vine pruning residue (VPR) from V. vinifera variety Loureiro were collected and gently given away by a producer from the Minho region (North of Portugal), in January of 2015. The material was dried at room temperature, milled (Retsch SM100) in to pass an 8 mm mesh and homogenized in a single batch. The moisture content was determined using a Moisture Analyser (Radwag MAC 50/1/NH) and was 17.6 ± 0.12 and stored at room temperature in a dark and dry place until its use. The chemical composition of the VPR was previously analyzed by Jesus et al. (2017). VPR composition was as follows (expressed in g per 100 g VPR on oven-dry basis ± standard deviation based in three replicate determinations): 32.9 ± 0.66 of cellulose (as glucan); 14.87 ± 0.17 of xylan; 0.40 ± 0.06 of arabinan; 3.95 ± 0.52 of acetyl groups; 29.5 ± 1.21 of Klason lignin; 13.7 ± 1.02 of extractives in water; 2.94 ± 0.89 of extractives in ethanol and 3.32 ± 0.56 of ashes.

2.2. Extraction of antioxidant compounds

2.2.1. Conventional heating extraction (CHE)

Conventional heating extraction (CHE) treatment was carried out in silicone oil bath with circulating heater and temperature control using 160 mL cylindrical reactors fabricated from 316 stainless steel. Previous study was performed to evaluate the range of variables to be evaluated (Table 1). For CHE assays, 0.5 g of VPR was mixed with 20 mL of ethanol-water (according to Moreira et al., 2018) ranging from 30 to 60%, varying the temperature between room temperature (25 ˚C) and 100 ˚C and time between 60 and 180 min. Then, the extracted solution was recovered by filtration and the VPR extracts were stored at 4 ˚C until further use.

Table 1
Experimental runs of the preliminary analyzes using different time (min) temperature (°C) and ethanol (%) and total polyphenolic compound responses obtained.

Runs	Ethanol (% v/v)	Temperature (°C)	Time (min)	TPC (g GAE/100 g VPR)
1	60	80	120	1.9 ± 0.05[cd]
2	30	80	120	2.0 ± 0.09[cd]
3	45	80	60	1.7 ± 0.01[bc]
4	45	80	180	2.1 ± 0.03[d]
5	45	60	120	1.5 ± 0.01[a]
6	45	100	120	2.0 ± 0.01[cd]
7	45	25	120	1.2 ± 0.02[a]

* The averages followed by the same letters do not differ by the *Tukey* test ($p < 0.05$). TPC: total phenolic compounds; GAE: gallic acid equivalents.

After evaluation of preliminary results, an experimental design (2^3) was carried out for the optimization of phenolic compounds extraction, where the variables extraction time (19–221 min), ethanol concentration (20–70% v/v) and extraction temperature (46–114 °C) were evaluated. A liquid to solid ratio of 40:1 mL/g of VPR was selected for CHE experiments. Table 2 shows the conditions of experiments carried out. After conventional extraction, VPR extracts were recovered by filtration, as described above. All the experiments were conducted in triplicate.

The independent variables: extraction time (x_1, min), extraction temperature (x_2, °C), and concentration of ethanol (x_3, %) were correlated with the dependent variables (phenolic compounds and antioxidant activity) by the follow equation:

$$y_i = \beta_{0i} + \beta_{1i} x_1 + \beta_{2i} x_2 + \beta_{3i} x_3 + \beta_{11i} x_1^2 + \beta_{22i} x_2^2$$
$$+ \beta_{33i} x_3^2 + \beta_{12i} x_1 x_2 + \beta_{13i} x_1 x_3 + \beta_{23i} x_2 x_3 \quad (1)$$

Where, y_i (i = 1 to 6) are the dependent variables corresponding to concentration of total phenolics extracted, antioxidant activity (g GAE/100 g VPR, g FE (II)/100 g VPR and g TE/100 g VPR) or phenolic compounds (apigenin mg/100 g VPR and ellagic acid mg/100 g VPR);

x_1, x_2 and x_3 value of independent variables; β_{0i}, β_{1i}, β_{2i}, β_{3i}, β_{11i}, β_{22i}, β_{33i}, β_{12i}, β_{13i} and β_{23i} are regression coefficients calculated from experimental data by multiple regression using the least-squares method. The experimental data were fitted to the proposed model using commercial software (Statistica). The goodness of model fitting was evaluated by the coefficient determination R^2 and the statistical significance by the *Fisher's F-test* for analysis of variable with a 95% confidence level. Significant differences among the results were evaluated by the one-way analysis of variance (*ANOVA*) followed by the *Tukey's* HSD.

2.2.2. Microwave-assisted extraction (MAE)

Alternative microwave-assisted extraction of phenolic compounds from VPR, were carried out in a Speedwave 4 microwave digester. Conditions of microwave extraction were selected based on conventional heating extraction treatment. For the extraction of phenolic compounds, the liquid to solid ratio was 40 mL of ethanol-water per 1 g of VPR. The percentage of ethanol-water varied from 0 to 60% (v/v). Microwave extraction assays were carried out at temperature ranging from 60 to 120 °C for 5 or 40 min (Table 3). Extracts were recovered by filtration. The experiments were conducted in triplicate.

2.3. Total phenolic compounds (TPC) analysis

Total phenol compounds in VPR extracts were analyzed based on the spectrophotometric method described by Meneses et al. (2013). Briefly, 5 μL of the extract, 60 μL of 7.5% (w/v) aqueous sodium carbonate solution, 15 μL of Folin-Ciocalteu reconstitution agent and 200 μL of distilled water were mixed in 96-well microplate. The mixture was incubated for 6 min at 60 °C and the absorbance was measured at 700 nm against a blank sample using a Microplate reader for UV–vis absorbance (Synergy HT-BIOTEK). A calibration curve was prepared using standard solution of gallic acid (125, 250, 500, 750, 1000, 2000, and 3000 mg/L). The results were expressed as g of gallic acid equivalents per 100 g dry material (g GAE/100 g VPR).

Table 2
Experimental runs using coded levels of time (min. x_1), temperature (°C x_2) and ethanol (% x_3) according to the 2^3 full factorial central composite design and extraction of polyphenolic compounds and antioxidant activity obtained under those conditions.

Independent variables			Symbol	Range and levels				
				−1.68	−1	0	1	1.68
Extraction time (min)			x1	19	60	120	180	221
Extraction Temperature (°C)			x2	46	60	80	100	114
Concentration of ethanol (%)			x3	20	30	45	60	70

Runs	Coded variables levels							
	x_1 (min)	x_2 (°C)	x_3 (% v/v)	TPC (g GAE/100 g VPR)	FRAP (g FE/100 g VPR)	DPPH (g TE/100 g VPR)	IC$_{50}$ (g/L)	ABTS (g TE/100 g VPR)	IC$_{50}$ (g/L)
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1	60	60	30	1.5 ± 0.04[ab]	1.8 ± 0.06[a]	3.4 ± 0.22 [abc]	12.3 ± 0.02	1.0 ± 0.01[a]	8.1 ± 0.41
2	60	60	60	1.6 ± 0.06[bcd]	1.7 ± 0.03[a]	3.1 ± 0.05[ab]	12.8 ± 0.05	1.3 ± 0.02[b]	6.7 ± 0.15
3	180	60	30	1.2 ± 0.013[a]	1.9 ± 0.05[a]	4.2 ± 0.14[cde]	6.2 ± 0.11	1.8 ± 0.02[c]	3.7 ± 0.10
4	180	60	60	1.5 ± 0.05[abc]	3.0 ± 0.16[c]	3.9 ± 0.12[1abcd]	7.6 ± 0.26	2.0 ± 0.02[cd]	3.3 ± 0.04
5	60	100	30	1.5 ± 0.03[abc]	2.3 ± 0.08[ab]	4.0 ± 0.12[bcd]	12.8 ± 0.22	2.1 ± 0.02[def]	5.2 ± 0.06
6	60	100	60	1.8 ± 0.06[def]	1.9 ± 0.05[a]	2.9 ± 0.18[ab]	12.6 ± 0.01	2.2 ± 0.05[fghi]	2.9 ± 0.11
7	180	100	30	1.7 ± 0.08[cdf]	2.9 ± 0.07[bc]	3.8 ± 0.14[abcd]	7.7 ± 0.07	2.3 ± 0.03 [fghi]	2.9 ± 0.14
8	180	100	60	2.1 ± 0.05[fg]	3.0 ± 0.04[c]	4.4 ± 0.12[bcd]	6.6 ± 0.02	2.4 ± 0.01[hi]	2.6 ± 0.03
9	19	80	45	2.0 ± 0.15[efg]	2.0 ± 0.13[ab]	3.7 ± 0.07[abcd]	8.4 ± 0.05	2.2 ± 0.01 [efgh]	3.0 ± 0.09
10	221	80	45	2.2 ± 0.05[g]	1.9 ± 0.05[a]	5.3 ± 0.02[ef]	5.6 ± 0.08	2.5 ± 0.06[a]	2.5 ± 0.15
11	120	46	45	1.5 ± 0.03 [bcd]	2.3 ± 0.07[ab]	3.1 ± 0.03[ab]	13.0 ± 0.01	2.4 ± 0.06[ghi]	3.2 ± 0.05
12	120	114	45	2.2 ± 0.02[g]	2.3 ± 0.17[a]	5.7 ± 0.04[f]	4.9 ± 0.12	2.3 ± 0.01 [efgh]	2.3 ± 0.09
13	120	80	20	1.3 ± 0.03[ab]	2.2 ± 0.2[a]	2.8 ± 0.08[a]	7.3 ± 0.15	2.0 ± 0.02[de]	3.1 ± 0.02
14	120	80	70	1.5 ± 0.16[2bcd]	2.1 ± 0.13[a]	4.5 ± 0.06[de]	6.9 ± 0.31	2.0 ± 0.02[cd]	2.2 ± 0.01
15	120	80	45	2.2 ± 0.08[g]	4.2 ± 0.04[d]	4.7 ± 0.18[def]	6.5 ± 0.10	2.2 ± 0.09 [efgh]	2.3 ± 0.21
16	120	80	45	2.2 ± 0.06[g]	4.3 ± 0.18[d]	4.3 ± 0.11[cde]	6.5 ± 0.08	2.2 ± 0.01[efgh]	2.1 ± 0.19
17	120	80	45	2.1 ± 0.06[g]	4.5 ± 0.17[d]	4.7 ± 0.18[cde]	6.6 ± 0.04	2.3 ± 0.01[fgh]	2.3 ± 0.14
18	120	80	45	2.2 ± 0.03[g]	4.7 ± 0.03[d]	4.7 ± 0.23[cde]	6.2 ± 0.17	2.3 ± 0.02[fgh]	2.7 ± 0.02

* The averages followed by the same letters do not differ by the *Tukey* test ($p < 0.05$). TPC: total phenolic compounds; GAE: gallic acid equivalents; FE ferrous equivalents; TE: trolox equivalents. Antiradical activity is expressed as a mean (n = 3) of IC$_{50}$ values (g of extract/L of solution).

Table 3
Total phenolic content and antioxidant activity from extracts obtained by microwave-assisted extraction.

Runs	Ethanol concentration (% v/v)	Temperature (°C)	Time (min)	TPC (g GAE/100 g VPR)	FRAP (g FE (II)/100 g VPR)	DPPH (g TE/100 g VPR)	IC$_{50}$ (g/L)	ABTS (g TE/100 g VPR)	IC$_{50}$ (g/L)
1	60	120	40	2.3 ± 0.10[c]	9.5 ± 0.45[e]	3.2 ± 0.02 [ab]	4.9 ± 0.40	3.3 ± 0.03[b]	1.3 ± 0.17
2	60	120	25	2.2 ± 0.10[bc]	8.5 ± 0.02[ef]	3.2 ± 0.02 [ab]	5.4 ± 0.43	3.2 ± 0.10 [ab]	1.4 ± 0.27
3	60	120	10	2.3 ± 0.02[c]	6.5 ± 0.03 [ab]	3.1 ± 0.05 [ab]	5.3 ± 0.21	3.3 ± 0.04[b]	2.4 ± 0.25
4	60	120	5	2.4 ± 0.01[c]	7.5 ± 0.07[bcd]	4.3 ± 0.06[c]	6.4 ± 0.12	3.4 ± 0.20[b]	3.0 ± 0.38
5	60	90	10	2.15 ± 0.02[bc]	7.2 ± 0.01[abc]	2.3 ± 0.06[a]	6.5 ± 0.50	3.0 ± 0.10[ab]	2.0 ± 0.04
6	60	90	5	2.1 ± 0.02[bc]	6.2 ± 0.03 [ab]	3.8 ± 0.01[bc]	6.8 ± 0.64	2.7 ± 0.04 [ab]	4.0 ± 0.26
7	60	60	10	1.6 ± 0.02[a]	6.3 ± 0.03 [ab]	3.2 ± 0.01 [ab]	–	2.9 ± 0.04 [ab]	–
8	60	60	5	1.8 ± 0.05[ab]	5.8 ± 0.09[a]	3.2 ± 0.02 [ab]	–	2.8 ± 0.02 [ab]	–
9	30	120	10	2.3 ± 0.01[c]	8.4 ± 0.03[cde]	4.0 ± 0.10[bc]	5.5 ± 0.13	2.7 ± 0.03 [ab]	2.2 ± 0.19
10	30	120	5	2.4 ± 0.01[c]	7.5 ± 0.10[bcd]	4.0 ± 0.01[bc]	3.6 ± 0.14	3.3 ± 0.10[b]	1.1 ± 0.13
11	30	90	10	2.3 ± 0.02[c]	6.8 ± 0.03[abc]	3.9 ± 0.01[bc]	3.7 ± 0.39	2.8 ± 0.03 [ab]	2.6 ± 0.67
12	30	90	5	2.3 ± 0.02[c]	4.1 ± 0.02 [ab]	4.0 ± 0.04[c]	6.7 ± 0.07	2.8 ± 0.07 [ab]	2.4 ± 0.05
13	30	60	10	1.6 ± 0.01[a]	6.2 ± 0.09 [ab]	3.3 ± 0.03[bc]	–	2.5 ± 0.04 [ab]	–
14	30	60	5	1.8 ± 0.03[ab]	5.9 ± 0.06 [ab]	3.4 ± 0.06[ab]	–	2.2 ± 0.02[c]	–
15	0	120	10	1.5 ± 0.01[a]	5.6 ± 0.06[a]	3.5 ± 0.02[bc]	–	2.7 ± 0.01 [ab]	–
16	0	120	40	2.4 ± 0.01[c]	6.3 ± 0.05[c]	3.7 ± 0.20 [ab]	6.9 ± 0.67	3.0 ± 0.03 [ab]	2.6 ± 0.25

*The averages followed by the same letters do not differ by the *Tukey* test ($p < 0.05$). TPC: total phenolic compounds; GAE: gallic acid equivalents; FE (II) ferrous equivalents; TE: trolox equivalents. Antiradical activity is expressed as a mean (n = 3) of IC$_{50}$ values (g of extract/L of solution).

2.3.1. UHPLC analysis

Phenolic compounds present in the VPR extracts from conventional heating and microwave treatments were analyzed and quantified by Shimatzu Nexpera X2 UHPLC chromatograph equipped with Diode Array Detector (Shimadzu, SPD-M20 A). Separation was performed on a reversed-phase Aquity UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μm particle size; from Waters) and a precolumn of the same material at 40 °C. The flow rate was 0.4 mL/min. The HPLC grade solvents used were water/formic acid (0.1%) as solvent A and acetonitrile as solvent B. The elution gradient for solvent B was as follows: from 0.0 to 5.5 min eluent B at 5%, from 5.5 to 17 min a linear increase to 60%, from 17.0 to 18.5 min a linear increase to 100%, then column equilibration from 18.5 to 30.0 min at 5%. Phenolic compounds were identified by comparing their UV/Vis spectra and retention times with that of corresponding standards. Compounds were quantified and identified at different wave-lengths: ellagic acid at 250 nm; vanillic acid at 254 nm; catechin, narigenin, *p*-cumaric acid, epicatechin, gallic acid, syringic acid, *o*-cumaric acid and cinnamic acid at 280 nm, *trans*-resveratrol at 308 nm; apigenin and rosmarinic acid at 329 nm; rutin at 350 nm and quercetin at 370 nm. All calibration curves had R^2 between 0,997 and 0,999. Concentration of the individual compounds in the calibration curves were between 250 mg/L and 1.25 mg/L.

2.4. Determination of antioxidant capacity

2.4.1. Ferric reducing antioxidant power (FRAP)

The antioxidant activity by the ferric reducing antioxidant power (FRAP) was made according to the methodology previously described (Ballesteros et al., 2015; Meneses et al., 2013). FRAP reagent was freshly prepared by mixing a 10 mM 2,4,6-tris (1-pyridyl)-5-triazine (TPTZ) solution in 40 mM HCl with a 20 mM, FeCl$_3$ solution and 0.3 M acetate buffer (pH 3.6) in a proportion 1:1:10 (v/v/v). A calibration curve was prepared with aqueous solution of FeSO$_4$ (10, 30, 50, 60, 70, 80, 110, 140, 200, and 275 mg/L). In a 96-well microplate was added 10 μL of the filtered and duly diluted extract with 290 μL of FRAP reagent. Then, the reaction mixture was incubated at 37 °C for 15 min. After that, the absorbance was determined at 593 nm against a blank prepared with distilled water. FRAP values were expressed as grams of ferrous equivalent per 100 g of VPR extract (g FE/100 g VPR).

2.4.2. DPPH radical scavenging activity

The activity DPPH was determined by measuring free radical scavenging the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (Meneses et al.,

2013; Sánchez-Gómez et al., 2017a). The method consists in the reduction of the DPPH radical in the presence of hydrogen-donating antioxidant and in the formation of the non-radical DPPH-H form at the end of the reaction. The percentage of remaining DPPH was inversely proportional to the antioxidant concentration. The reaction was carried out in a 96-well microplate, samples (10 μL) with extract were mixed with 290 μL of DPPH in methanol (6×10^{-5} M dissolved in methanol absolute to an absorbance value of 0.700 at 515 nm), incubated for 1 h at room temperature and the absorbance was measured at 515 nm. The negative and positive controls were made with methanol and the Trolox standard, respectively. The inhibition activity (%) of the DPPH radicals was calculated using the following equation:

$$\% \, inhibition \, activity = \frac{A_0 - A_1}{A_1} x \, 100 \qquad (2)$$

Where A_0 was an absorption of the negative control and A_1 for an absorbance of the extracts. The sample concentration for the 50% reduction of DPPH radicals (IC$_{50}$) was calculated by interpolation. The IC$_{50}$ values were expressed as g of Trolox equivalent per 100 g of dry weight material (g TE/100 g VPR).

2.4.3. Radical ABTS elimination capability

The ABTS (2,2-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) cation decolorization assay was according to the methodology described by Ahmad Mir et al. (2017) adapted for 96-well microplates to evaluate the ability of the sample to eliminate free ABTS + radicals. ABTS (7.4 mM) diluted in ethanol and potassium persulfate (2.6 mM) were mixed in 1:1 (v/v) ratio and incubated for 16 h at room temperature in the dark. To a 200 μL of ABTS radical solution were added 10 μL sample solution or ethanol and the mixture was allowed to stand for 1 h at 30 °C to obtain a stable absorbance. Absorbance was measured at 734 nm against a blank. The results were expressed as percent inhibition using the Eq. 2 as described in the item 3.4.2.

2.5. Statistical analysis

All experiments were performed in triplicate and the results were expressed as mean value ± standard deviation. The statistical analysis of model was carried out using StatSoft Statistica software (version 10).

3. Results and discussion

3.1. Conventional heating extraction (CHE)

3.1.1. Effect of temperature, time and ethanol percentage on phenolic compounds extraction: preliminary experiments

The temperature, solvent concentration and time of extraction are important factors that influence the efficiency of the extraction process of phenolic compounds. Preliminary experiments were carried out to evaluate the range of variables for the extraction of phenolic compounds. The conditions of these experiments and the results obtained are listed in Table 1.

As seen in Table 1, the lowest extraction of total phenolic compounds was achieved at the room temperature. Improvement in the extraction was observed, when the temperature was increased up to 80 and 100 °C. On the other hand, the percentage of ethanol, varying between 30–60%, showed similar results. For the time of extraction, the highest concentration of total phenolic compounds (TPC) was obtained at higher extraction periods. However, there were no significant differences between the experiments carried out at 120 and 180 min. The highest total phenolic compounds extraction (2.0, 2.1 and 1.9 g GAE/100 g VPR) was obtained under conditions of experiment 2 (30%, 80 °C and 120 min), experiment 4 (45%, 80 °C and 180 min) and experiment 6 (45%, 100 °C and 120 min), respectively. The lowest phenolic compounds extraction (1.2 and 1.5 g GAE/100 g VPR) was achieved in experiments 7 and 5 (Table 1), respectively. In this preliminary study, it is observed that the temperature is the most important factor for the extraction of polyphenols compounds from vine pruning residue. On the other hand, the time of extraction has a lower influence on the TPC extraction than the ethanol concentration and temperature.

3.1.2. Total phenolic compounds and antioxidant activity CHE method

Based on previous results (Table1), an experimental design (2^3) was proposed to optimize the phenolic compounds extraction and to evaluate their antioxidant capacity. The range of operational conditions (temperature, time and percentage of ethanol) and dependent variables studied in this work were listed in Table 2.

The total phenolic compounds obtained by CHE ranged from 1.2 to 2.2 g GAE/100 g of VPR. Maximum concentration of total phenolic compounds (2.2 g GAE/100 g) was obtained under conditions of the central points (120 min, 80 °C and 45% ethanol concentration) and minimum concentration of total phenolic compounds (1.2 g GAE/100 g) was obtained in run 3 (180 min, 60 °C and 30% ethanol concentration). These results are in agreement with the values found by other authors (Çetin et al., 2011; Karacabey and Mazza, 2008; Luque-Rodríguez et al., 2006; Moreira et al., 2018) for CHE using different ethanol concentrations. Studies in the literature have shown that the total content of phenols in VPR can vary significantly according to the grape variety (Çetin et al., 2011; Delgado-Torre et al., 2012; Farhadi et al., 2016). Previous work confirmed the influence of solvent concentration on the extraction process of phenolic compounds (Alexandru et al., 2014; Moreira et al., 2018). Mixtures of alcohols/water were found to be more efficient in extraction of phenolic constituents than the corresponding mono-component solvent system due to the increase in permeability in the membrane of the lignocellulosic material (Alara et al., 2018; Bouras et al., 2015). However, despite increasing the extraction of total phenolic compounds, it does not have much influence on the antioxidant activity (Pinelo et al., 2006; Spigno et al., 2007; Yilmaz et al., 2006).

The antioxidant activities of the extracts varied, according to the analysis method, between 1.7 and 4.7 g FE/100 g for the FRAP, 2.9–5.7 and 1.0–2.4 g GAE/100 g and IC_{50} 0.6–1.3 and 0.2 to 0.8, for DPPH and ABTS, respectively. Maximum antioxidant activity for FRAP (4.7 g FE/100 g) were obtained under conditions of the central points (120 min, 80 °C and 45% ethanol concentration). For the ABTS and DPPH methods, the maximum antioxidant activity was achieved in one of the

axial points; runs 10 and 12, respectively, both made using ethanol 45%. The highest antioxidant activity measured by DPPH was 2.4 g TE/100 g VPR (run 12) and the highest antioxidant activity quantified by ABTS was of 5.7 g TE/100 g VPR (run 10). The lowest concentration of total phenolic compounds was obtained in the run 3 (180 min, 60 °C and 30%). The antioxidant activity of vine pruning extracts was superior to the results obtained by Moreira et al. (2018), for FRAP (0.72 g AAE/100 g VPR) and DPPH (0.83 g TE/100 g VPR).

The experimental variables were correlated following the polynomial Eq. 1, of the second order, presented in point 2.2.2. The proposed mathematical models describing the extraction time (x_1), temperature time (x_2) and ethanol concentration (x_3) as function and using normalized values are described by Eqs. 3–5 and 6 for TPC (y_1), FRAP (y_2), DPPH (y_3) and ABTS (y_4), respectively.

$$y_1 = 2.75 + 0.11x_1 + 0.09x_1^2 + 0.08x_2 - 0.25x_2^2 - 0.35x_3 - 0.48x_3^2 \\ + 0.09x_1x_2 + 0.04x_1x_3 + 0.03x_2x_3 \tag{3}$$

$$y_2 = 4.64 + 0.11x_1 - 0.97x_1^2 + 0.04x_2 - 0.91x_2^2 - 0.61x_3 - 0.84x_3^2 \\ + 0.03x_1x_2 - 0.16x_1x_3 + 0.20x_2x_3 \tag{4}$$

$$y_3 = 2.53 + 2.25x_1 + 0.12x_1^2 + 0.03x_2 - 0.13x_2^2 - 0.12x_3 - 0.24x_3^2 \\ - 0.13x_1x_2 - 0.03x_1x_3 + 0.01x_2x_3 \tag{5}$$

$$y_4 = 4.66 + 0.24x_1 + 0.53x_1^2 + 0.12x_2 - 0.14x_2^2 - 0.17x_3 + 0.43x_3^2 \\ - 0.06x_1x_2 + 0.01x_1x_3 + 0.20x_2x_3 \tag{6}$$

The R^2 of the models presented in Eqs. 3–6 were: 0.94, 0.90, 0.69 and 0.73, respectively. The calculated F-value ($F_{5,3}$) were: 38.35 (Eq. 3); 67.21 (Eq. 4); 16.95 (Eq. 5) and 78.16 (Eq. 6). The models were validated by ANOVA analysis and F-test at 95% of confidence level before building the response surface graphs presented in Fig. 1a–d. All models were statistically significant since the calculated F-values were higher than the listed F-value ($F_{5,3} = 9.01$) at 95% of confidence level.

The statistical significance of the model (based on *Fischer´s F* parameter) showed the good fitting of the evaluated variables total phenolics compounds and FRAP ($R^2 \geq 0.9$).

Table 4 shows the regression coefficients and variance analysis of linear, quadratic and interactions between variables. The total phenolic compounds (TPC) and antioxidant activity of VPR extracts presented significant effects ($p < 0.05$, $p < 0.01$ or $p < 0.0001$), whereas the interactions between the variables presented different meanings according to the applied analysis method ($p < 0.05$, $p < 0.01$ or $p < 0.0001$). Therefore, the linear and quadratic effects of independent variables were the main determinant terms for antioxidant activities. Fig. 1a–d shows the effect of temperature and extraction time, with a fixed concentration of 45% ethanol, on the extraction of TPC and antioxidant activity measured by FRAP, DPPH and ABTS. As shown in Fig. 1a, concentration of total phenolic compounds above 2 g GAE/100 g of VPR was obtained when the temperature was > 80 °C, regardless of the extraction time. The temperature had a notable positive effect on the concentration of total phenolic compounds. Fig. 1b shows the response surface and the Pareto plot of the effects of the two variables, namely extraction time, extraction temperature (ethanol concentration set at 45%), for FRAP assays. Quadratic and linear effects of the variables were all significant. Control of model parameters, R^2, F-exp, confirmed the model adequacies for total phenolics compounds and FRAP (Table 4).

The antioxidant activity measured by DPPH, showed significant effects for all variables and their interactions, except for the interaction between the extraction time and the ethanol concentration, but increasing the temperature and extraction time at low ethanol concentrations reduced the antioxidant capacity of the extracts (Fig. 1c). With the increase of the extraction temperature, DPPH values increased significantly. However, no significant differences were found in DPPH when ethanol concentration increased above 45%. The interaction of
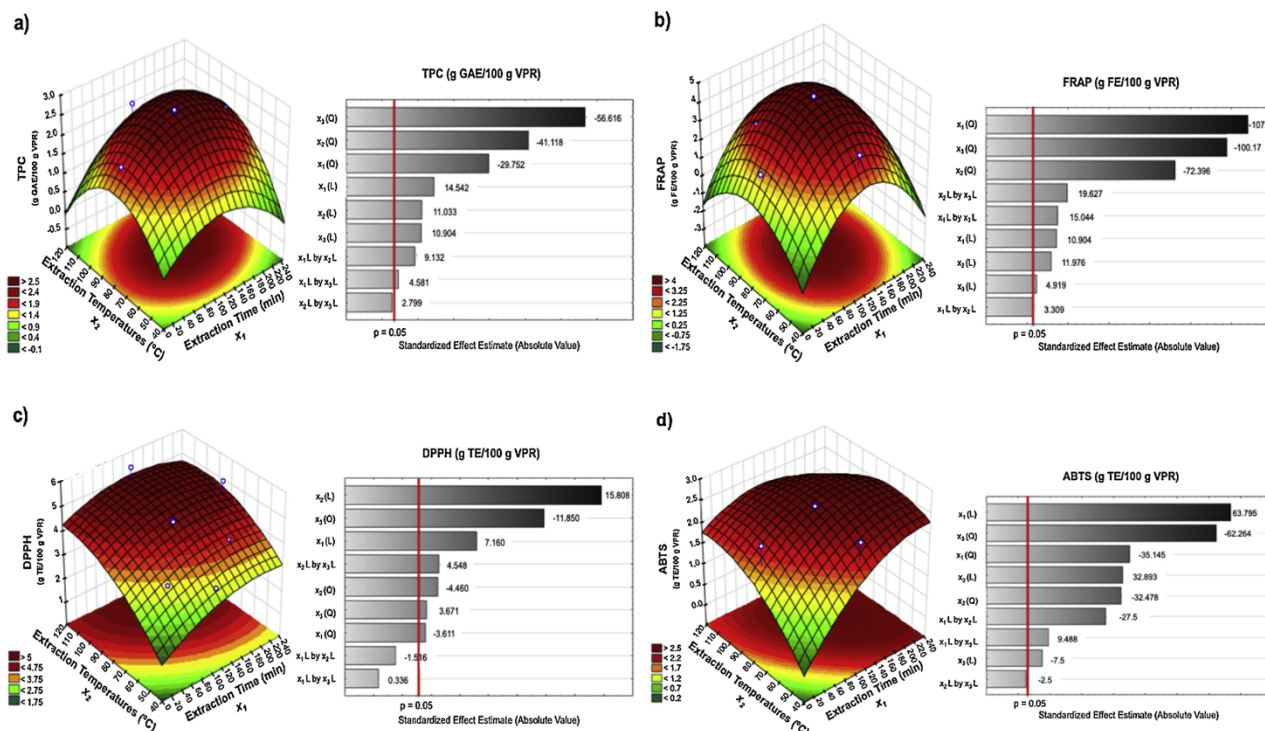
Fig. 1. Pareto diagram for standardized effects and response surface graphs as function of time of extraction and temperature of extraction (fixed enthanol con-centraction at 45% of: a) total phenolicas compounds in GAE g/100 g and antioxidant activity b) FRAP in g FE (II)/100 g. c) DPPH in g TE/100 g and d) ABTS in g TE/100 g ($p >$ 0.05).

Table 4
Analysis of variance of the regression parameters of the predicted second order polynomial models for total phenol compounds and antioxidant activities (FRAP, DPPH and ABTS).

Coeficientes	y_1	y_2	y_3	y_4	y_5	y_6
β_{0i}	2.75	4.64	2.53	4.66	0.071	0.218
β_{1i}	0.23[a]	0.11[a]	2.25[a]	0.24[a]	−0.002[a]	0.001[d]
β_{2i}	0.18[b]	0.97[a]	0.12[a]	0.53[b]	0.011[a]	0.031[a]
β_{3i}	0.17[b]	0.04[b]	0.03[a]	0.12[a]	0.006[a]	0.056[a]
β_{11i}	−0.50[a]	−0.91[a]	−0.13[a]	−0.14[a]	−0.007[a]	−0.025[a]
β_{22i}	−0.70[a]	−0.61[a]	−0.12[a]	−0.17[a]	−0.009[a]	−0.040[a]
β_{33i}	−0.96[a]	−0.84[a]	−0.24[a]	0.43[a]	−0.012[a]	−0.045[a]
β_{12i}	0.19[b]	0.03[c]	−0.13[a]	−0.06[a]	−0.005[a]	0.020[a]
β_{13i}	0.09[c]	−0.16[a]	0.03[d]	0.01[b]	0.006[a]	−0.021[a]
β_{23i}	0.06[d]	0.20[a]	0.01[a]	0.20[d]	0.001[a]	0.012[a]
R^2	0.94	0.90	0.69	0.73	0.74	0.90
F-exp	38.35	67.21	16.95	78.16	517.19	592.64
lack of fit	51.36[a]	434.36[a]	377.60[a]	803.74[a]	8217.06[a]	135.79[a]

[a] Significant coefficients at the $p < 0.001$ confidence level.
[b] Significant coefficients at the $p < 0.01$ confidence level.
[c] Significant coefficients at the $p < 0.05$ confidence level.
[d] not significant $p > 0.05$.

temperature and ethanol concentration was significant for DPPH method. Fig. 1d shows the effect of time and temperature on the anti-oxidant activity measured by ABTS. The extraction time presented a linear effect, while the extraction temperature and the ethanol con-centration had quadratic effects on the response. Under these condi-tions, the maximum antioxidant activity measured by ABTS can be obtained with an extraction temperature of 80 °C and 45% ethanol. However, the linear effects of the extraction time demonstrate that an increase on the extraction time, lead to higher antioxidant activity, according to the method ABTS. Previous studies with VPR showed a significant linear increase of the antioxidant activity (ABTS), with in-crease temperature and low ethanol concentrations (50%). However, the effect of temperature diminished in higher ethanol concentrations

(60%) (Karacabey and Mazza, 2010). The ethanol concentration, time and temperature on total phenolics compound and antioxidant activ-ities of VPR, has been also reported by Luque-Rodríguez et al. (2006), and Karacabey and Mazza (2010). The authors showed that the only significant variable was the temperature (16.4–240 °C); the percentage of ethanol (20–100%) and extraction time (20–90 min) had negative and positive effects, respectively. The lack of adjustment test at the 0.05 level does not indicate evidence of lack of fit for both the total phenolic compound models and antioxidant activity measured by FRAP, DPPH and ABTS.

3.1.3. Process optimization and validation

In the search to find the highest antioxidant activity (using FRAP, DPPH and ABTS methods), and the total polyphenolic content, main-taining the minimum concentration of ethanol, time and temperature, it was possible to optimize the extraction process. The optimal extraction conditions for the present study were different according to the analysis performed. Optimized parameters for antioxidant activities were in-cluded in Table 5. As observed, operational conditions to maximize the antioxidant activity for different methods varied in the range of 80–115 °C, 137–175 min and 45–53% of ethanol concentration. The antioxidant activities, measured by FRAP, ABTS and total phenolic compounds showed similar optimal conditions between them, tem-perature of 80 to 83 °C and ethanol concentration of 45 to 46%, how-ever the extraction time ranged from 124 to 175 min, where the po-tential values were 4.4 g FE/100 g VPR, 2.5 g TE/100 g VPR and 2.3 g GAE/ g, respectively. For DPPH the optimum conditions were: extrac-tion time of 147 min, temperature of 115 °C and ethanol concentration of 53%, where the antioxidant potential value was 5.1 g TE/100 g VPR. The validation experiments carried out under the predicted conditions derived from the experimental design analysis demonstrated that the experimental values were close to the predicted values, confirming the validity and adequacy of the predicted models. In addition, the ver-ification experiments also proved that the predicted values of total polyphenolic compounds and antioxidant activity (using FRAP method)

Table 5
Optimized conditions for antioxidant activity of vine pruning residue extract (including predicted and experimental values).

Responses	Process variables			Predicted value	Experimental value
	X_1 (min)	X_2 (°C)	X_3 (% v/v)		
TPC (g GAE/100 g)	137	83.45	46.68	2.26	2.27 ± 0.15
FRAP (g FeE/100 g)	124	81.72	45.42	4.38	4.45 ± 0.24
DPPH (g TE/100 g)	147	115.33	53.39	5.22	5.12 ± 0.24
ABTS (g TE/100 g)	175	80.01	45.06	2.37	2.54 ± 0.13

for the VPR model can be satisfactorily achieved within the 95% confidence interval. Therefore, the regression equations obtained in this study can be used to obtain extracts with optimal antioxidant activity and total polyphenols.

3.1.4. UHPLC analysis of phenolic compounds of CHE

Qualitative and quantitative analysis of phenolic compounds of the extract at optimized condition were analyzed by ultra high performance liquid chromatography (UHPLC). Table 6 lists the concentrations of the identified compounds (results expressed in mg/100 g of VPR). The highest concentration of phenolic compounds was obtained under conditions of the central point of experimental design (120 min, 80 °C and 45%). Fifteen phenolic compounds of different classes were detected: flavonoids, phenolic acids and stilbenes, the concentrations of the compounds varied according to the extraction conditions applied in each experiment. The flavonoids present in the extracts obtained were catechin (12.7–21 mg/100 g), narigenin (4.9–6.2 mg/100 g), apigenin (6.4–218.0 mg/100 g), quercetin (23.5–27.7 mg/100 g) and rutin (9–16.5 mg/100 g). The phenolics acids obtained were rosmarinic acid (13.5–15.3 mg/100 g), cinnamic acid (12.6–16.1 mg/100 g), syringic acid (13.5–20 mg/100 g), vanillic acid (19.7–23.1 mg/100 g), ferulic acid (5.8–6.9 mg/100 g), gallic acid (6.3–12.4 mg/100 g), *o*-cumaric acid (7.4–16 mg/100 g) and ellagic acid (5.0–75.5 mg/100 g). The retention time of the epicatechin and the *p*-coumaric acid were the same and it was not possible to separate these compounds, so the concentrations are presented together (5.3–6.7 mg/100 g). The stilbene resveratrol was found in concentrations between 13.3 mg and 14.9 mg/100 g VPR.

The most abundant flavonoids in VPR are (+) - catechin and (-) - epicatechin, however, in this work these compounds were not the most abundant which is not in accordance with previous studies (Çetin et al., 2011; Delgado-Torre et al., 2012; Luque-Rodríguez et al., 2006; Sánchez-Gómez et al., 2017b, 2014). The highest concentration of (+) - catechin was 21.5 mg/100 g of VPR (run 6, 60 min, 100 °C and 60% of ethanol). The highest content of *p*-coumaric acid + epicatechin 6.7 mg/100 g of VPR, was found at run 10 (221 min, 80 °C and 45% of ethanol). The highest concentration of resveratrol obtained in this study was 14.9 mg/100 g of VPR (221 min, 80 °C and 45%). These values were higher than those found by Sánchez-Gómez et al. (2014) in extracts of VPR (2.8 mg/100 g). But, the values of resveratrol concentration were lower than those reported for VPR hydroethanolic extracts (Cebrián et al., 2017; Soural et al., 2015; Vergara et al., 2012). However, in this work the most abundant flavonoid was apigenin with 207.9 mg/100 g of VPR (120 min, 80 °C and 45%), although it has not been reported in previous studies using VPR. However, apigenin was identified in extracts of grape marc (Pintać et al., 2018).

The concentrations of phenolic acids: gallic acid, vanillic acid, syringic acid and ferulic acid were within the range (0.5–2.1, 0.3–0.8, 0.6 and 0.2–14.3 mg/100 g, respectively) reported in literature (Cebrián et al., 2017; R. Sánchez-Gómez et al., 2017b, 2014). Among the identified phenolic acids, the most abundant in VPR extracts was ellagic acid, with concentration of 68.6 mg/100 g VPR at the central point (120 min, 80 °C and 45%), these values were higher than those found in the literature Cebrián et al. (2017) (1.36–2.1 mg/100 g VPR), Luque-Rodríguez et al. (2006) (17.9–57 mg/100 g VPR), Sánchez-Gómez et al.

(2017b) (0.8–5.4 mg / 100 g VPR), Sánchez-Gómez et al. (2014) (0.4–1.4 mg/100 g VPR). As a general trend, the total concentration of phenolic compounds in the extract, identified by UHPLC, increased with temperature. According to Luque-Rodríguez et al. (2006), the temperature was the most influential variable on the composition of the extracts of VPR, whereas the percentage of ethanol and extraction time had only an influence on the amount of each compound.

Ellagic acid is a polyphenol of great interest due to its high prebiotic, antioxidant and anti-inflammatory activity (García-Niño and Zazueta, 2015; Landete, 2011; Yu-Qing et al., 2017). In addition, ellagic acid was one of the most abundant compounds found in VPR extracts from this study. The ellagic acid content in extracts of VPR obtained using CHE ranged from 5.0 to 75.5 mg/100 g VPR. The results obtained in this work were superior to those described in previous works for the ellagic acid content in extracts of VPR obtained using the CHE technique (Cebrián et al., 2017; Luque-Rodríguez et al., 2006; Sánchez-Gómez et al., 2017b, 2014). According to our results, the highest ellagic acid content was achieved using 45% ethanol, temperature of 80 °C and extraction time of 120 min. On the other hand, the lowest content was obtained in the extract prepared using 60% ethanol, extraction temperature of 60 °C and 60 min of extraction time. Fig. 2a shows the response surface for the temperature-time relationship with the ethanol concentration set at 45% for the extraction of ellagic acid. All extraction parameters investigated showed a significant positive impact on the final ellagic acid content (Fig. 2b). The concentration of ethanol showed the greatest positive impact on ellagic acid content in VPR extracts. The levels of ethanol, extraction temperature and extraction time significantly affected the ellagic acid content ($p < 0.001$) (Table 4). The interactions between time/ethanol concentration, time/temperature and temperature/ethanol concentration, ($p < 0.001$) also significantly affected the ellagic acid content.

The final predictive equation to describe the extraction efficiency to reach the maximum ellagic acid content (y_5) of VPR is as follows:

$$y_5 = 0.07 - 0.002\,x_1 + 0.011\,x_1^2 + 0.006\,x_2 - 0.007\,x_2^2 - 0.009\,x_3$$
$$- 0.012\,x_3^2 - 0.005\,x_1 x_2 - 0.006\,x_1 x_3 + 0.001\,x_2 x_3 \tag{7}$$

According to the p-value, it can be concluded that the model showed a good approximation for the investigated responses ($p < 0.001$). The R^2 for ellagic acid was 0.74, the non-significant value of lack of fit ($F = 135.79$) showed the model is fitted to the spatial influence of the variables to the response with good prediction, with a *F-value* of 517.19, higher than the *F-value* table ($F_{5.3} = 9.01$).

The content of apigenin in VPR extracts obtained using CHE ranged from 6.4 to 2.2 mg/100 g VPR. In our study, the extract with the highest apigenin content was obtained using the following parameters: 45% ethanol, temperature of 80 °C and extraction time of 120 min. However, it was not identified using 60 min of extraction time, extraction temperature of 60 °C and 30% of ethanol. The lowest content was obtained in the extract prepared using 180 min, 60 °C and 30% ethanol. The results show that the concentration of apigenin increased up to 80 °C and 120 min of extraction, at higher temperatures and times there was a decrease of the concentration, probably due to its degradation. The response surface shows the interaction of the extraction time and temperature with the ethanol concentration set at 45% (Fig. 2a). The

Table 6
Polyphenolic compounds identified in vine pruning residue extracts (expressed as mg per 100 g VPR).

Run	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Time (min)	60	60	180	180	60	60	180	180	19	221	120	120	120	120	120	120	120	120
Temperature (°C)	60	60	60	60	100	100	100	100	80	80	46	114	80	80	80	80	80	80
Ethanol concentration (%)	30	60	30	60	30	60	30	60	45	45	45	45	20	70	45	45	45	45
Phenolics Compounds (mg/100 g VPR)																		
catechin	13.2^{ab}	14.6	14.9^{bc}	14.9^{abc}	16.3^{cd}	21.0^{e}	15.8^{cd}	14.4^{abc}	16.4^{bc}	15.7^{bcd}	17.4^{d}	15.0^{bcd}	14.4^{bc}	12.7^{a}	17.6^{cd}	13.3^{abc}	15.1^{bc}	15.8^{de}
narigenin	ND	ND	ND	6.2^{d}	ND	ND	ND	5.5^{bc}	ND	5.9^{bc}	ND	5.4^{bc}	ND	ND	5.7^{bc}	4.9^{b}	5.3^{b}	5.4^{ab}
apigenin	ND	164.7^{d}	6.4^{a}	193.7^{ef}	7.8^{a}	60.4^{bc}	66.9^{c}	198.1^{ef}	160.4^{d}	187.4^{e}	76.1^{c}	191.5^{ef}	46.5^{b}	190.1^{fg}	215.1^{g}	194.9^{fg}	203.6^{g}	218.0^{g}
quercetin	ND	25.4^{cde}	23.5^{b}	24.3^{cde}	ND	25.3^{cde}	27.7^{e}	27.7^{e}	23.5^{b}	25.9^{def}	24.6^{bcd}	24.1^{bcd}	ND	23.9^{bc}	25.2^{cde}	23.8^{bcd}	24.3^{bcd}	26.1^{ef}
rutin	ND	11.9^{c}	13.5^{d}	14.9^{def}	ND	9.7^{b}	11.6^{c}	15.6^{efg}	14.2^{de}	15.3^{efg}	10.6^{bc}	15.0^{def}	9.0^{b}	14.9^{def}	16.2^{g}	14.7^{efg}	15.5^{efg}	16.5^{g}
p-cumaric acid + epicatechin	5.7^{ab}	6.1^{bcd}	5.3^{a}	5.8^{abc}	6.3^{b}	6.6^{cd}	6.4^{bcd}	6.6^{cd}	6.0^{bcd}	6.7^{d}	6.4^{bcd}	6.2^{bcd}	6.1^{bcd}	6.2^{bcd}	6.1^{bcd}	6.0^{bcd}	6.0^{bcd}	6.5^{bcd}
gallic acid	ND	11.1^{efg}	ND	10.2^{cde}	6.3^{b}	11.7^{gh}	9.4^{c}	11.3^{gh}	10.0^{cd}	11.8^{g}	9.8^{cd}	12.4^{h}	6.8	10.5^{def}	11.1^{fgh}	10.8^{fgh}	10.9^{fgh}	11.9^{gh}
syringic acid	20.0^{e}	16.1^{d}	16.0^{d}	15.3^{c}	15.1^{c}	ND	13.5^{b}	13.5^{b}	21.0^{abc}	ND	ND	ND	ND	ND	ND	ND	ND	ND
vanillic acid	19.7^{a}	21.1^{abc}	19.9^{ab}	21.4^{bcd}	21.1^{abc}	23.0^{fg}	22.6^{def}	23.0^{g}	21.0^{cde}	23.1^{g}	22.3^{def}	21.9^{cde}	21.2^{def}	21.4^{cde}	21.6^{cde}	20.6^{def}	20.8^{def}	22.7^{efg}
ferulic acid	6.1^{abcd}	6.5^{bcd}	6.0^{abc}	6.2^{bcd}	6.3^{bcd}	6.2^{abc}	6.4^{bcd}	6.6^{bcde}	6.0^{abc}	6.7^{bcd}	6.4^{bcd}	5.8^{ab}	6.5^{e}	5.8^{a}	6.5^{cde}	6.3^{bcd}	6.3^{bcd}	6.9^{ef}
o – cumaric acid	ND	14.6^{fgh}	16.0^{hi}	16.3^{i}	ND	7.4^{b}	9.0^{b}	14.4^{k}	11.4^{d}	14.1^{g}	12.5^{de}	13.5^{ef}	9.4^{c}	13.4^{ef}	15.5^{ghi}	13.6^{fg}	14.4^{fg}	15.4^{ghi}
ellagic acid	23.9^{b}	5.0^{a}	59.0^{g}	66.5^{ghi}	5.1^{a}	32.7^{cd}	37.2^{d}	50.3^{ef}	59.7^{g}	58.4^{fg}	49.6^{e}	59.8^{g}	27.4^{bc}	62.4^{gh}	75.5^{i}	60.9^{ghi}	67.6^{gh}	70.4^{hi}
cinnamic acid	13.2^{ab}	14.6^{def}	14.2^{bcd}	15.3^{cde}	12.6^{a}	14.0^{bc}	14.7^{def}	16.8^{g}	14.2^{bcd}	16.1^{efg}	13.3^{bcd}	15.3^{def}	14.0	14.9^{def}	15.8^{efg}	14.7^{def}	15.1^{def}	16.1^{fg}
rosmarinic acid	13.6^{b}	15.0^{cd}	13.9^{ab}	14.2^{bcd}	ND	15.2^{d}	14.7^{bcd}	15.2^{d}	13.9^{bc}	15.3^{d}	14.8^{bcd}	14.2^{bcd}	13.7^{bcd}	14.1^{bcd}	14.0^{bcd}	13.6^{bcd}	13.7^{bc}	15.0^{cd}
trans - resveratrol	ND	14.6^{bc}	13.5^{b}	13.8^{bc}	ND	14.8^{c}	14.3^{bc}	14.8^{c}	13.5^{b}	14.9^{c}	14.4^{bc}	13.9^{bc}	13.7^{bc}	13.8^{bc}	13.7^{bc}	13.3^{bc}	13.4^{b}	14.6^{bc}

* The averages followed by the same number do not differ by the Tukey test ($p < 0.05$).
ND: not detected.

Pareto graph (Fig. 2d) showed that the most dominant factor influencing the extraction of apigenin was the concentration of ethanol, and this factor positively affected its extraction ($p < 0.001$). The quadratic and linear levels of extraction temperature, quadratic extraction time and the interactions between time/temperature, time/ethanol concentration and temperature/ethanol concentration also significantly altered the apigenin content, while the linear parameter of extraction time was insignificant ($p > 0.5$) (Table 4). The predictive equation to describe the extraction efficiency to reach the maximum apigenin content (y_6) of VPR is as follows:

$$y_6 = 0.21 + 0.001x_1 + 0.0001\,x_1^2 + 0.031\,x_2 + 0.056\,x_2^2 - 0.025\,x_3$$
$$- 0.039\,x_3^2 - 0.045\,x_1x_2 - 0.020\,x_1x_3 + 0.021\,x_2x_3 \qquad (8)$$

The variance analysis (ANOVA) was able to confirm that the model was significant ($p < 0.001$), and with R^2 values of 0.90 and calculated F-value 592.64 higher than the table $F_{5,3} = 9.01$–95% of confidence level (Table 4). Negligible lack of fit ($p > 0.05$) was also evidence of good fit of the model.

3.2. Microwave-assisted extraction (MAE)

Based on the optimization of the conventional extraction method, study of the extraction of the polyphenolic compounds using microwave-assisted method was initiated in order to find the minimum conditions of extraction (concentration of ethanol (%), temperature (ºC) and time)) to obtain maximum extraction yield. The experiments were conducted at different concentrations of ethanol (0–60 %), temperatures (60–120 °C) and time (5–40 min).

3.2.1. Total phenolic compounds and antioxidant activity MAE method

According to Rombaut et al. (2014); Vinatoru et al. (2017) from an industrial point of view, the conventional extraction technique has some disadvantages, such as insufficient recovery of extracts, extensive extraction time and intensive heating and/or mixing, resulting in high energy consumption. In contrast MAE is a more advantageous methodology with its selective heating of the vegetal material versus solvent that improves the extraction mechanism by increasing the extraction yield and decreasing the extraction time. The predicted optimum conditions for TPC extraction were calculated as extraction time of 137 min, extraction temperature of 83.45 °C, ethanol concentration 46.7%, and the model predicted a maximum TPC concentration of 2.3 g GAE/100 g VPR. The experimental concentration achieved under these conditions was similar CHE 2.3 g GAE/100 g VPR. For MAE the total phenolic content concentrations ranged between 1.5 and 2.4 g GAE/100 g VPR. The total phenolic content (TPC) as function of the extraction conditions are shown on Table 3. The results showed that the highest concentration of TPC was reached using 30% ethanol, 120 °C and 5 min for MAE. According to these results, MAE can be considered a better method for polyphenols extraction (2.4 ± 0.01 g GAE/100 g VPR) than the conventional heating extraction (CHE) (2.2 ± 0.1 g GAE/100 g VPR). In addition, the antioxidant capacity of extracts obtained by MAE was significantly higher than the obtained by CHE. These values are in agreement with the results obtained by Çetin et al. (2011) (2.5 to 3.6 g GAE/100 g VPR) and were superior to those found by Cebrián et al., and Karacabey and Mazza, (2017; 2008) (0.1 to 0.2 and 0.3 to 0.8 g GAE/100 g VPR, respectively). The superheated extraction of ethanol and water from VPR polyphenols yielded 1.7 to 4.1 g of GAE/100 g, depending on the experimental parameters. Experiments 15 and 16 (0% ethanol, 120 °C, 10 min. and 0% ethanol, 120 °C, 40 min.) showed lower quantities of phenolic compounds in comparison to the other extractions using the same times and temperatures. This is due to the higher dielectric constant ($\varepsilon = 80$) that water has in comparison to other solvents. This feature causes significantly lower dissipation than other solvents ($\delta = 1500 \times 104$). This phenomenon causes overheating of the water leading to degradation of the bioactive
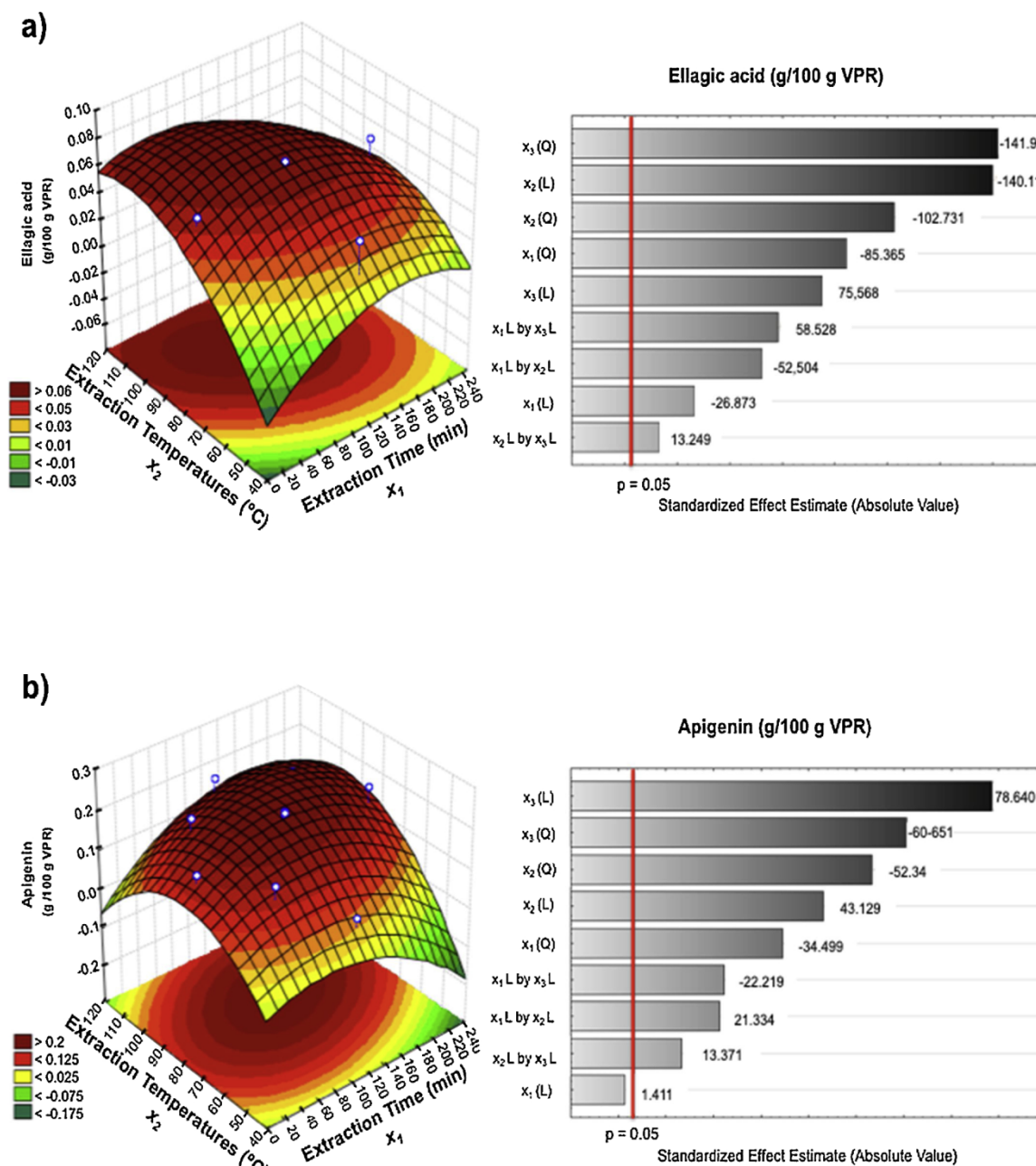
a)

b)

Fig. 2. Response surface and Pareto diagram for standardized effects showing the effect of temperature and time of extraction (fixed enthanol concentraction at 45% of: a) total phenolicas compounds in GAE g/100 g and antioxidant activity b) FRAP in g FE (II)/100 g c) DPPH in g TE/100 g and d) ABTS in g TE/100 g ($p > 0.05$).

compounds (Proestos and Komaitis, 2008).

The FRAP, DPPH and ABTS assays are often used to determine the total antioxidant activities of natural compounds (Oroian and Escriche, 2015). The antioxidant activities FRAP, DPPH and ABTS ranged from 4.1 to 9.5 g FE (II)/100 g VPR, 2.3 to 4.3 TE/100 g VPR and 2.2 to 3.4 g TE/100 g VPR, respectively (Table 3). The extracts that presented lower TPC concentrations were not submitted to IC_{50} inhibition analysis for DPPH and ABTS antioxidant activities. The highest antioxidant capacity measured by FRAP using MAE method was 9.5 g TE/100 g VPR using 60% ethanol at 120 °C for 40 min, these results were superior to those found in this work using the CHE method and that is in contrast to that described by (Gullón et al., 2017). These authors tested different severities in autohydrolysis for the extraction of VPR phenolic compounds. They described FRAP values between 0.7 and 2.7 g TE/100 g VPR. The results of ABTS radical censor sequestering activity were consistent with the DPPH tests, and showed moderate oxidant activity

relative to ABTS. The highest antioxidant activity was measured by DPPH and ABTS methods (4.3 TE/100 g of VPR and 3.4 g TE/100 g of VPR, respectively), using 60% ethanol, at 120 °C, during 5 min. The ABTS results were in accordance with those obtained by Karacabey and Mazza (2010) in conventional extractions using a temperature of 83.6 °C, solvent to solid ratio of 70 (mL/g) and 58% ethanol. The antioxidant activities (IC_{50}) of the VPR extracts obtained by MAE varied from (DPPH) $IC_{50} = 3.6$ to 6.9 g/L and (ABTS) $IC_{50} = 1.1$ to 4.0 g/L, respectively. These values show high efficiency as DPPH and ABTS reducers compared to those obtained by the CHE method ($IC_{50} = 4.9$ to 13 g/L, $IC_{50} = 2.1$ to 8.1 g/L, respectively). The greater ability to reduce DPPH and ABTS obtained in MAE was when 30% ethanol was used at a temperature of 120 °C for 5 min ($IC_{50} = 3.6$ g/L and 1.1 g/L, respectively). According to Ruiz-Moreno et al. (2015) the direct comparison of the antioxidant activities of wine derivatives with the literature is difficult due to the grape variety discrepancy and the variety

of extractive techniques and quantification methods.

3.2.2. UHPLC analysis of phenolic compounds of MAE

Among the 15 polyphenolics compounds identified in the extraction by the CHE method, only 12 compounds were identified in the extracts obtained by the MAE method. The major phenolic compounds identified by UHPLC for MAE were catechin, narigenin, apigenin, rutin, p-cumaric acid + epicatechin, ferulic acid, gallic acid, syringic acid, vanillic acid, o-coumaric acid, ellagic acid, cinnamic acid, rosmarinic acid, trans-resveratrol (Table 7). The presence of these compounds is in agreement with other authors (Cebrián et al., 2017; Çetin et al., 2011; Delgado-Torre et al., 2012; Jesus et al., 2017; Sánchez-Gómez et al., 2017b, 2014).

Among the quantified phenolic compounds, the most abundant flavonoids were apigenin (9.4–184 mg/100 g of VPR) and catechin (16.6–27.1 mg/100 g of VPR), their concentration varied according to the extraction conditions. The concentration of apigenin significantly exceeded the other flavonoids, with the highest concentration of 184 mg/100 g of VPR with 60% ethanol at 90 °C for 5 min. Meanwhile, this value was lower than the optimum obtained in CHE, 207.9 mg/100 g of VPR (120 min, 80 °C and 45%). Pintać et al. (2018) was able to extract between 0.30 and 9.1 mg/100 g of grape pomace apigenin, using CHE and 80% ethanol and acetone as extraction solvents, respectively. The highest concentration of catechin was 27.1 mg/100 g of VPR. The results obtained by MAE for catechin are in agreement with previous studies on extracts of grapevine shoots (Sánchez-Gómez et al., 2017b).

Stilbenes are the most studied group of compounds in VPR due to their important properties for the pharmaceutical industry (Guerrero et al., 2016; Piñeiro et al., 2017, 2016; Shen et al., 2009; Soural et al., 2015). Resveratrol is the most studied stilbene in VPR, in this study the resveratrol concentration ranged from 0.3 to 36.0 mg/100 g of VPR in the MAE method. The highest content of resveratrol was 36.0 mg/100 g of VPR when 60% ethanol was used at 120 °C for 5 min. These results are superior to those found by Çetin et al. (2011) and Sánchez-Gómez et al. (2014), in extracts of VPR, but still inferior to the results obtained by Delgado-Torre et al. (2012) using 1 g of solid and 20 mL of ethanol of 80% (v/v), Soural et al. (2015) 1.5 g of grape canes and 100 mL of methanol and Vergara et al. (2012) using 2 g of solid and 16 mL of ethanol of 80% (v/v), both used higher amounts of solid and organic solvent in a higher concentration than the one used in this work.

The most abundant phenolic acids were ellagic acids (6.67–185.1 mg/100 g of VPR), syringic (15.6–24.4 mg/100 g of VPR) and rosmarinic (15.87–36.25 mg/100 g of VPR). The highest concentration of syringic acid (24.4 mg/100 g of VPR) was obtained when using 60% ethanol at 60 °C for 10 min and 20.02 mg/100 g VPR, this concentration was higher than that obtained by CHE when submitted 30% ethanol at 60 °C for 60 min. These results are superior to those described in the literature (Cebrián et al., 2017; Sánchez-Gómez et al., 2014). Extracts submitted to 60% ethanol, at 120 °C for 5 min, achieved the highest ellagic acid extraction (185.1 mg/100 g of VPR) and rosmarinic acid (36.2 mg/100 g of VPR). These results were superior to those obtained by CHE (8.6 and 14.1 mg/100 g of VPR, respectively) using 45% ethanol at 80 °C for 120 min. The ellagic acid content varies remarkably according to the solvent and/or the extraction procedure used (Cebrián et al., 2017; Luque-Rodríguez et al., 2006; Sánchez-Gómez et al., 2014).

The experiments that presented the highest concentration of phenolic compounds were 3 and 4 (60% ethanol, 120 °C for 10 min and 60% ethanol, 120 °C for 5 min, respectively). The mixture of organic solvent and water is more efficient in the extraction of phenolic compounds than pure solvents Bouras et al. (2015). According to the results of Liazid et al. (2007), most phenolic compounds can be extracted without degradation at temperatures up to 125 °C for an extraction time of 20 min. The results obtained for the polyphenolic profile indicate that the concentration of each compound determined after the

Table 7
Phenolic compounds in the vine pruning residue samples extracted using the microwave-assisted extraction method (mg/100 g VPR).

Run	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Time (min)	40	25	10	5	10	5	10	5	10	5	10	5	10	5	10	40
Temperature (°C)	120	120	120	120	90	90	60	60	120	120	90	90	60	60	120	120
Ethanol concentration (%)	60	60	60	60	60	60	60	60	30	30	30	30	30	30	0	0
Phenolics Compounds (mg/100 g VPR)																
catechin	27.1^g	27.5^{gh}	26.6^{678}	25.5^{fgh}	23.4^{defg}	23.4^{defg}	24.4^{fgh}	19.2^{abc}	16.6^a	24.9^{fg}	23.3^{cdef}	22.6^{cdef}	20.8^{abc}	20.4^{bcd}	21.4^{cde}	17.3^{ab}
narigenin	3.1^a	1.5^a	1.1^a	0.3^a	0.7^a	0.2^a	0.6^a	0.2^a	0.5^a	ND	0.3^a	ND	ND	ND	0.4^a	0.2^a
apigenin	104.8^{def}	93.9^{de}	150.1^{fg}	118.8^{efg}	94.4^{def}	184.7^i	126.4^{hi}	174.0^{hi}	59.0^{bc}	16.0^a	70.0^{cd}	34.6^{ab}	30.8^{ab}	44.8^{bc}	12.4^a	9.4^a
quercetin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
rutin	13.3^d	13.2^d	10.6^{bcd}	11.2^{bcd}	12.3^{cd}	9.7^{bc}	10.9^{bcd}	9.6^{abc}	10.3^{bcd}	9.3^{abc}	9.1^{ab}	9.6^{bc}	9.7^{bcd}	11.1^{234}	4.3^a	9.4^{abc}
p-cumaric acid + epicatechin	6.9^{cd}	7.4^d	7.0^{cd}	7.3^d	6.9^{cd}	6.9^{bc}	7.2^d	6.8^{ab}	6.7^{bc}	6.8^{ab}	6.8^{bc}	6.6^{ab}	6.4^a	6.9^{34}	6.5^{ab}	6.8^{bc}
rosmarinic acid	32.8^e	29.7^{de}	35.5^{ef}	36.2^f	31.7^{ef}	32.5^e	34.0^{ef}	27.3^d	22.9^c	21.5^3	25.8^d	22.6^c	22.4^c	22.4^c	15.9^b	ND
cinnamic acid	9.3^b	10.0^{bc}	10.8^d	11.0^{cd}	9.2^b	11.1^{cd}	10.2	10.9^{cd}	9.9^{bc}	ND	9.7^{bc}	11.3^{cd}	10.9^d	10.2^{bc}	15.7^{ab}	ND
syringic acid	16.8^d	17.1^d	17.3^d	17.7^e	16.5^{bc}	16.6^{cd}	24.4^e	16.2^{abc}	16.6^{cd}	16.3^{abc}	16.3^{abc}	16.2^{abc}	15.6^a	16.6^{34}	15.7^{ab}	16.3^{bc}
vanillic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ferulic acid	6.9^{abcd}	7.1^{efg}	7.0^{bcd}	7.3^g	6.7^{ab}	7.1^{efg}	7.0^{bcd}	6.8^{abcd}	7.1^{efg}	7.2^{efg}	6.8^{abcd}	6.8^{abcd}	6.6^a	7.1^{def}	6.7^{abcd}	6.8^{abcd}
gallic acid	14.1^{de}	13.9^{de}	13.9^{de}	15.1^e	11.7^{cd}	10.8^{abcd}	10.9^{bcde}	10.3^{bcd}	10.5^{abcd}	14.4^{de}	9.7^{abc}	9.2^{abc}	6.8^a	7.1^{ab}	7.3^{abc}	11.8^{cd}
o-cumaric acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ellagic acid	75.3^c	64.8^d	172.5^h	185.1^i	73.5^{cd}	131.2^g	119.6^g	29.5^a	68.3^{cd}	88.5^{cde}	109.2^{ef}	97.7^{de}	74.4^c	86.0^{de}	ND	ND
Trans-resveratrol	29.5^{fg}	32.3^{gh}	34.9^{gh}	36.0^h	31.3^{gh}	32.3^g	33.6^{gh}	27.3^{cd}	22.9^{cd}	19.6^b	25.6^{bcd}	22.4^{cd}	22.4^{cd}	22.4^{34}	16.3^b	0.3^a

*The averages followed by the same number do not differ by the Tukey test ($p < 0.05$).
ND: not detected.
60

extraction with the different concentrations of ethanol, time and temperature increased significantly using microwaves in comparison with the conventional extraction, except for some compounds (apigenin, cinnamic acid, resveratrol, rutin, rosmarinic acid, ellagic acid, quercetin, vanillic acid and *o*-cumaric acid) and especially for the water extraction, possibly due to the higher dielectric constant. The obtained results are in accordance with Chemat et al. (2017), concluding that, compared to CHE, the MAE is a greener method, as it uses less energy, shorter extraction time and less percentage of ethanol.

Even though, MAE has been successfully used in the extraction of valuable components in laboratory scale, the number of industrial-scale applications remain very small. However, MAE is considered a key process for the application of the biorefinary concept to any industrial production, like is the extraction of natural products (Vinatoru et al., 2017).

4. Conclusions

The extraction of polyphenolic compounds (TPC) was optimized for conventional heating and microwave-assisted extraction using environmentally friendly solvents *i.e.* ethanol and water. The obtained TPC and antioxidant activity were according to the predicted by the linear and quadratic models developed from the optimization study. Extraction of TPC from VPR had greater efficiency when using MAE than CHE methodology. The use of MAE significantly reduced the extraction time and improved the extraction efficiency of TPC of high industrial interest such as ellagic acid and apigenin. The extraction of valuable TPC from VPR contributes to increase the integral valorization of this residue. For first time was reported the presence of the compound apigenin in the extracts of VPR. MAE generally increased extraction of ellagic acid, *trans*-resveratrol, rosmarinic acid, cinnamic acid and catechin when compared to CHE; similar values were obtained for *p*-coumaric acid + epicathechin, apigenin, rutin, syringic, ferulic and gallic acid. Lower amounts of naringin, quercetin, vanillic and *o*-cumaric acids were detected in MAE than in CHE.

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