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High Voltage Electrical Discharges and Ultrasound-Assisted Extraction of Phenolics from Indigenous Fungus-Resistant Grape By-Product

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The possible use of high voltage electrical discharges (HVED) at different frequencies (20, 50, and 100 Hz) and ultrasound-assisted extraction (UAE) at different temperatures (20, 40, and 80°C) for the recovery of phenolics from indigenous fungus-resistant grape by-product was evaluated. All extractions were performed over the period of 5, 10, and 15 min and with methanol- and ethanol-based solvents. Grape pomace (the grape by-product) was collected during the preparation of grape jams. The main phenolics identified in grape pomace were anthocyanins, including malvidin, delphinidin, peonidin-3-*O*-glucoside, and cyanidin-3-*O*-glucoside. Beside anthocyanins, phenolic acids, flavan-3-ols, and flavonols were identified. The HVED-assisted extraction showed to be a superior extraction method for obtaining high yields of all analysed compounds. The highest amount of total phenolics (3023.57 mg GAE/100 g DM) was extracted from grape pomace, using 50% (*v/v*) ethanol and 60 kV/cm HVED at 100 Hz for 15 min. The antioxidant activity of the HVED extract was 2.17 mmol Trolox/g DM. The highest yield of the identified phenolics from grape pomace was obtained with electric field intensity of 60 kV/cm and total energy input of 22.27 kJ/kg, during the extraction.

INTRODUCTION

Plant phenolics are organic compounds found abundantly in plants with potential health benefits. According to the Transparency Market Research, global demand for phenolics in 2018 was estimated at USD 873.7 million [Ameer *et al.,* 2017]. One way of meeting the high market requirements is exploitation of fruit by-products to extract and isolate phenolic compounds. During the production of wine, grape jams and juices, huge amounts of the grape pomace (GP) are obtained as the by-product. In Europe, one way of exploiting grape pomace is for obtaining the enocyanins, *i.e.* food colorants E-163, which are anthocyanins isolated from red wine pomace. Current data on phenolics profile of GP vary greatly between different studies [Teixeira *et al.,* 2014]. It is found that GP contains flavan-3-ols, flavonols, anthocyanins, and phenolic acids [Yang *et al.,* 2009]. Grape pomace consists mainly of grape skin which in red grape varieties is rich with anthocyanins, including malvidin, cyanidin, delphinidin, peonidin, petunidin, and their glucosides. So far, most of the researches on the utilization of grape pomace have been made with the pomace produced from processing of conventional grape varieties. However, the data on the phenolics profile of varieties of fungus-resistant grapes (FRG) showed that FRG are rich in phenolic acids, dihydrochalcones, stilbenes, flavonols, flavan-3-ols, and anthocyanins [Kontić *et al.,* 2016; Ehrhardt *et al.,* 2014]. The FGR were bred by interspecific cross-breeding between the Mediterranean variate *Vitis vinifera* and American varieties with the aim to develop varieties resistant to fungal diseases, including powdery and downy mildews, and grey rot. FRG were introduced in Europe in the 19th century to counter the invasion of the vermin *Phylloxera* (*Viteus vitifoliae*) [Noah, 2016]. Although production of wine from some FRG including the *Noah, Othello, Isabelle, Jacquez, Clinton*, and *Herbemont* varieties is forbidden due to Regulation EU No. 1308/2013, FRG are still grown in several European countries. Fungus-resistant grapes are consumed mainly as fresh fruit; however, due to their high resistance and high yields, they represent a good raw material for the production of different products such as juices, jams, jellies, *etc*. The GP obtained after production of such products from FRG has a high content of phenolic compounds due to incomplete extraction during the processing. These phenolic compounds are of interest because they have a positive effect on human health through their antioxidant, antibacterial, and anticarcinogenic potential, and the capability to prevent cardiovascular diseases, *etc*. [Rasouli *et al.,* 2017].

Nowadays, there is a trend in exploring non-conventional, green methods for the extraction of phenolic compounds. High voltage electrical discharges (HVED) is one of them. The HVED enhance the rate of extracted phenolic compounds per initial plant material at low energy input during processing [Li *et al.,* 2019; Boussetta *et al.,* 2012]. Applying high voltage

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between two electrodes submersed in water, or water solution, accelerates electrons that electrify water molecules creating the barrage of electrons called a streamer, and if the applied electric field is intense enough, the streamer propagates from the positive to the negative electrode. Consequently, chemical reactions and physical processes such as shock waves, cavitation and production of active species, will occur as well [Touya *et al.,* 2006]. These processes result in a rupture of the cell plant tissue which greatly improves extraction of intracellular compounds from plant material [Liu *et al.,* 2011]. The other method is ultrasound-assisted extraction (UAE) which is one of the most common extraction methods [Trojanowska *et al.,* 2019; Bakht *et al.,* 2018] used for recovering phenolics from plant material. UAE enhances a possibility to improve extraction yields while reducing the use of solvents, providing the opportunity to use greener alternative solvents and enhancing extraction of heat-sensitive components. The mass transfer during UAE is intensified by the implosion that creates high temperature and pressure spots between the solution and the solid matrix [Da Porto *et al.,* 2013]. Ultrasound-assisted extraction proved to be more efficient at different temperatures and with different solvents used for extraction, than shaking water bath extraction method.

Considering solvents used for the extraction of phenolics, the most used ones are water, methanol, and ethanol. In food systems, ethanol and water are preferred extraction solvents because of the hygiene, low cost, and abundance in addition to being compatible with health [Moure *et al.,* 2001].

The objective of this study was to compare the extraction methods for the extraction of phenolics from indigenous fungus-resistant grape by-product and to evaluate the extracts obtained. This by-product has not been studied in depth before (to the best of our knowledge) and could be a good source of bioactive phenolic compounds. The reason for comparison of extraction methods is to enable the recognition of new green methods for the extraction of valuable bioactive compounds from grape by-products and to obtain extracts rich with phenolic compounds.

MATERIAL AND METHODS

Materials

Gallic acid, catechin, epicatechin, *p*-coumaric acid, chlorogenic acid, caffeic acid, quercetin, cyanidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, malvidin, delphinidin, Folin–Ciocalteu reagent (FC), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical were purchased from Sigma Aldrich (Darmstad, Germany). Grape pomace of *Isabell* variety, belonging to FRG varieties, was obtained from local producers from Slatina, Croatia. The grape pomace was obtained after pressing the grapes and removing the juice, which was used for jam production. Grape pomace was freeze-dried using a freeze-dryer (Christ Freeze Dryer, Gamma 2–20, Osterode, Germany) at -55°C under 0.220 mbar.

Determination of physicochemical properties of grape by-product

Total acidity were measured by titration with 0.1 M NaOH with phenolphthalein as an indicator. Results were expressed as g of tartaric acid equivalents per 100 g of GP (g/100 g). pH was measured using a table top pH-meter (FiveEasy FE20, Mettler Toledo, Giessen, Germany). GP color was determined with a colorimeter (Minolta CR-300, Kioto, Japan) using the L*a*b* system.

Phenolic compounds extraction

Extractions of phenolics from GP powder were performed using two different solvents (50% (*v/v*) of ethanol and 1% (*v/v*) HCl or 50% (*v/v*) of methanol and 1% (*v/v*) HCl) and two techniques supporting extraction. All extractions were carried out in triplicate with a solvent to solid ratio of 50:1 (*v/w*).

High voltage electrical discharges treatment of GP powder suspensions was performed on an experimental apparatus (Inganiare CPTS1, Croatia) consisting of a high voltage power source which enabled the 30 kV discharges in a 600-mL chamber with electrodes of a needle-plate geometry. A sharp stainless steel needle 2.5 mm in diameter was used. A positive pulse voltage was applied to the stainless steel cylindrical needle (2.5 mm in diameter) and a stainless disc (45 mm in diameter) was used as a ground electrode with the distance between the electrodes of 5 mm. The treatment was conducted at room temperature (25°C) and on the magnetic stirrer at different HVED frequencies (20, 50, 100 Hz) and time (5, 10, 15 min).

Ultrasound-assisted extraction was performed at 35 KHz at different temperatures (20, 40, 80°C) and time (5, 10, 15 min) (Bandelin Sonorex Digitech, Berlin, Germany). Additionally, GP powder extraction without the assisted technique (as control) was carried out on a magnetic stirrer at 40°C for 30 min using 50% (*v/v*) ethanol acidified with 1% (*v/v*) HCl. After a suitable extraction time, for all types of extractions, suspensions were centrifuged (Multifuge 3 L-R Centrifuge, Heareus, Hanau, Germany) at 25°C for 15 min (6596.2 × *g*) and supernatants were filtered through 0.45 μm PTFE syringe-tip filter (Chromafil Xtra, Macherey-Nagel, Germany).

Determination of total phenolics content and antioxidant activity

The total phenolics content (TPC) was determined by employing an FC reagent according to a procedure described by Loncaric *et al.* [2014]. Briefly, 0.2 mL of the extract was mixed with 1.8 mL of deionized water, 10 mL of FC (1:10, *v/v*), and 8 mL of 7.5% sodium carbonate in test tubes. The development of blue color was monitored at 765 nm after 120 min (Jenway 6300 spectrophotometer, Bibby Scientific, Stone, UK). The values obtained were interpolated on a gallic acid calibration curve and expressed as mg of gallic acid equivalents per 100 g of dry matter (DM) of GP (mg GAE/100 g DM).

The antioxidant activity (AOA) was measured using a DPPH radical according to the methodology described by Brand-Williams *et al.* [1995]. The reaction mixture consisted of 0.2 mL of the extract and 3 mL of 0.5 mM DPPH radical solution in ethanol. The changes in the color of radical from deep violet to light yellow were measured at 517 nm using a UV-Vis spectrophotometer (Jenway 6300) and the results were expressed as mmol of Trolox equivalents per g of GP DM (mmol Trolox/g DM).

Determination of phenolics by HPLC

High performance liquid chromatography was performed with the Varian LC system (Palo Alto, CA, USA) equipped with a ProStar 230 solvent delivery module, and ProStar 330 photodiode array detector (PDA). Reverse phase chromatography analyses were carried out with the OmniSpher C18 column (4.6 × 250 mm) packed with 5 μm diameter particles (Varian, USA). Mobile phase consisted of 0.5% (*v/v*) water solution of phosphoric acid (solvent A) and 100% HPLC grade acetonitrile (solvent B); elution was conducted according to Loncaric *et al.* [2014] and injection volumes were 20 μL. The UV-Vis absorption spectra of the standards as well as the compounds present in samples were recorded in the range of 190 to 600 nm. Quantification has been performed by external standard calibration. The content of identified phenolics was expressed as mg per 100 g of GP DM (mg/100 g DM).

Statistical analysis

All measurements were done on three extracts and data were expressed as mean ± standard deviation. Normal distribution and homogeneity of variances was tested using the Shapiro-Wilkov and Levenovu test, respectively, after which the experimental data were analyzed statistically by one-way analysis of variance (ANOVA). Fisher's LSD was calculated to detect significant difference ($p \leq 0.05$) between the mean values. MS Excel (StatPlus, AnalystSoft Inc., Walnut, CA, USA) statistical program was used for statistical analysis.

RESULTS AND DISCUSSION

Effect of control extraction on the physicochemical properties of grape pomace and its phenolic profile and antioxidant activity

The physicochemical properties of grapes and their by-products in general are highly influenced by grape variety, growing areas, cultural practices, and growing year [Stewart, 2013]. A dark red powder was obtained after freeze-drying and grinding grape pomace. The results of analyses of the physicochemical properties of FRG by-product powder are given in Table 1. Color parameters of the GP powder indicate dark red color of the powder (values of L* and a* parameters were positive) and the value of coordinate b* indicates a slightly purple color. The results obtained for total acidity and pH are in the range of those reported by other authors for FRG varieties from different growing areas [Slegers *et al.,* 2015; Vos, 2014; Nisbet *et al.,* 2014]. The low pH value (3.16) of the grape pomace is a consequence of phenolics present in it [Bustamante *et al.,* 2008]. As with the physicochemical properties, the content of individual phenolics can also vary significantly from one FRG variety to another [Pedneault & Provost, 2016; Ehrhardt *et al.,* 2014]. In our study, the GP powder had a high content of total phenolics (260.43 mg GAE/100 g DM) and a high antioxidant activity (6.92 mmol Trolox/g DM) compared to literature data [Pedneault & Provost, 2016; Yang *et al*., 2009]. The most abounded phenolics in the investigated by-product were anthocyanins (163.68 mg/100 g DM); malvidin, delphinidin, peonidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside, which is in accordance with the findings of Balík *et al*. [2013]. The second most abounded phenolic was quercetin (19.85 mg/100 g DM) from the flavonol family followed by flavan-3-ols (16.62 mg/100 g DM), including catechin and epicatechin. The content of phenolic acids was 15.02 mg/100 g DM with identified caffeic acid, *p*-coumaric acid, gallic acid, and chlorogenic acid.

Effect of high voltage electrical discharge and ultrasound-assisted extractions on total phenolics content and antioxidant activity of grape pomace

Figures 1 and 2 present the total phenolics content and the antioxidant activity of GP extracted with HVED and UAE. The HVED enabled a five-fold intensification of total phenolics extraction compared to UAE. This corresponds to the findings of Boussetta *et al.* [2012] who have noted in their study that HVED extraction intensity of total phenolics from grape pomace, seeds, skins, and stems was increased 7 times compared to the control extraction. The highest TPC (3023.57 mg GAE/100 g DM) and AOA (2.17 mmol Trolox/g DM) were obtained upon the extraction with the ethanol-based solvent, HVED at 100 Hz, treatment time of 15 min,

TABLE 1. Physicochemical properties, phenolic profile and antioxidant activity of grape pomace*.

Parameter	Value
Total acidity (g/100 g)	0.23±0.01
pH	3.16±0.01
Color	
L*	9.26±0.03
a*	11.68±0.12
b*	-0.59±0.09
Individual phenolic content (mg/100 g DM)	
Gallic acid	1.86±0.06
Catechin	8.63±0.03
Epicatechin	7.99±0.05
p-Coumaric acid	3.16±0.02
Chlorogenic acid	1.43±0.11
Caffeic acid	8.57±0.08
Quercetin	19.85±0.03
Cyanidin-3-*O*-glucoside	16.81±1.11
Peonidin-3-*O*-glucoside	28.64±0.98
Malvidin	74.56±1.26
Delphinidin	43.67±0.43
TPC (mg GAE/100 g DM)	260.43±0.14
AOA (mmol Trolox/g DM)	6.92±0.05

*grape pomace extracted using acidified 50% (*v/v*) ethanol without additional assistance (control extraction).
Results are expressed as mean ± SD (n=3). TPC – total phenolic content; AOA – antioxidant activity (DPPH assay), DM – dry matter.

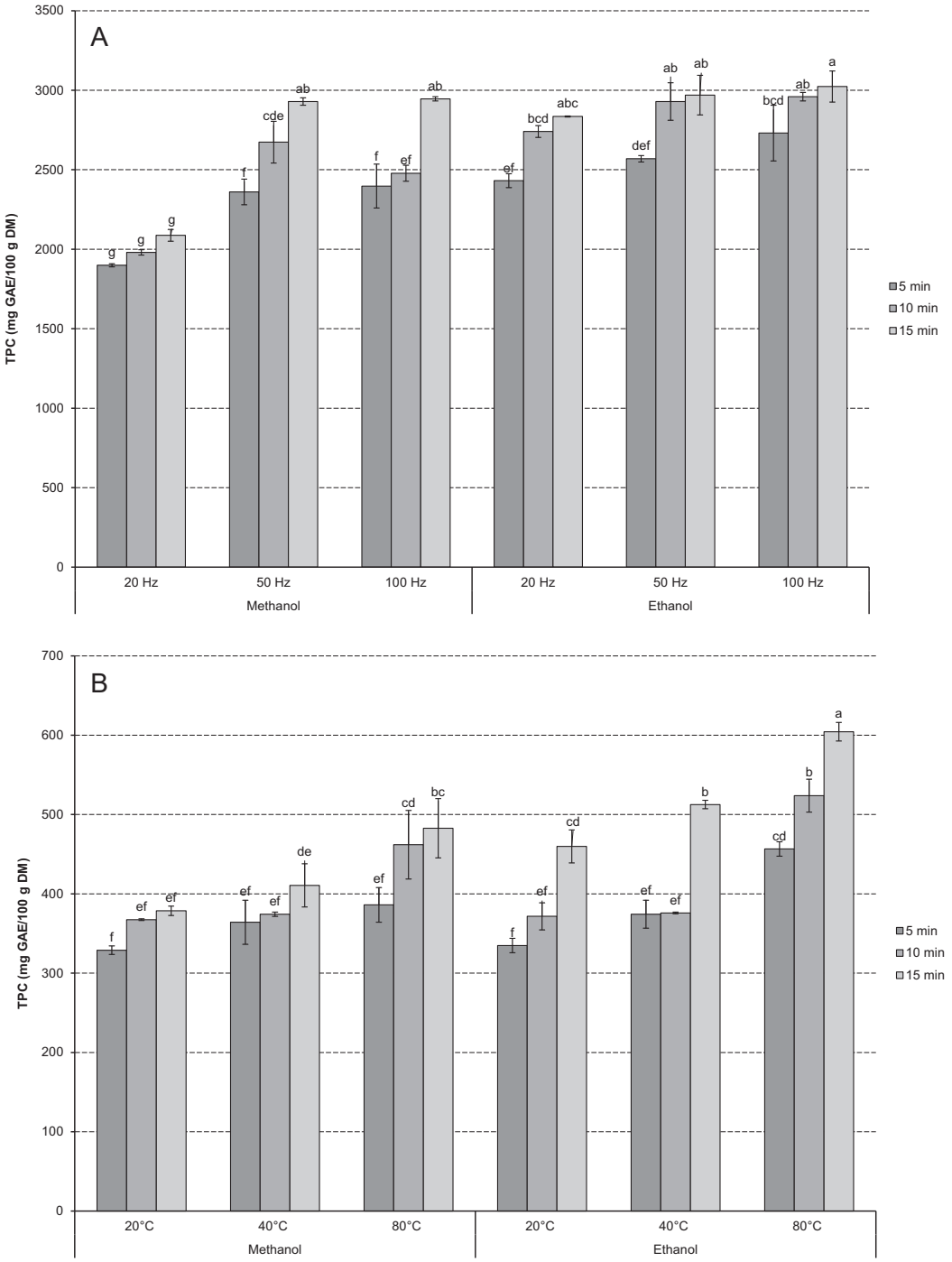

FIGURE 1. Total phenolic content (TPC) of grape pomace extracted with high voltage electrical discharges (HVED) treatment (A) and with ultrasound assistance (UAE) (B) for 5, 10 and 15 min. Results are expressed as means and SD (n=3) and calculated on the dry matter (DM) basis. Different letters above bars indicate significant differences between means at 95% confidence level as obtained by the LSD test.

and electric field intensity of 60 kV/cm. However, these values did not differ significantly ($p>0.05$) from those obtained when shorter extraction times at 100 Hz and also lower HVED frequency (50 Hz) were used. Considering UAE, the highest TPC (604.42 mg GAE/100 g DM) and AOA (1.52 mmol Trolox/g DM) were achieved at 80°C, and treatment time of 15 min. For total phenolics content and antioxidant activity, increasing the time and temperature during UAE produced higher recoveries with significant differences ($p\leq0.05$). The extraction of phenolic compounds from the plant material is influenced by their chemical nature, the sample size, extraction method, and extraction time as well as the structural and compositional features of the plant matrix and the strength with which they are bound to the plant matrix. However, when it comes to the solubility, differences in the structure of phenolic compounds determine their solubility in solvents [Shi *et al*., 2003b]. A mixture of some organic solvent (ethanol or methanol) and water proved to be more efficient in extracting phenolic compounds than the mono-component solvent system, which was reported by other authors for different products

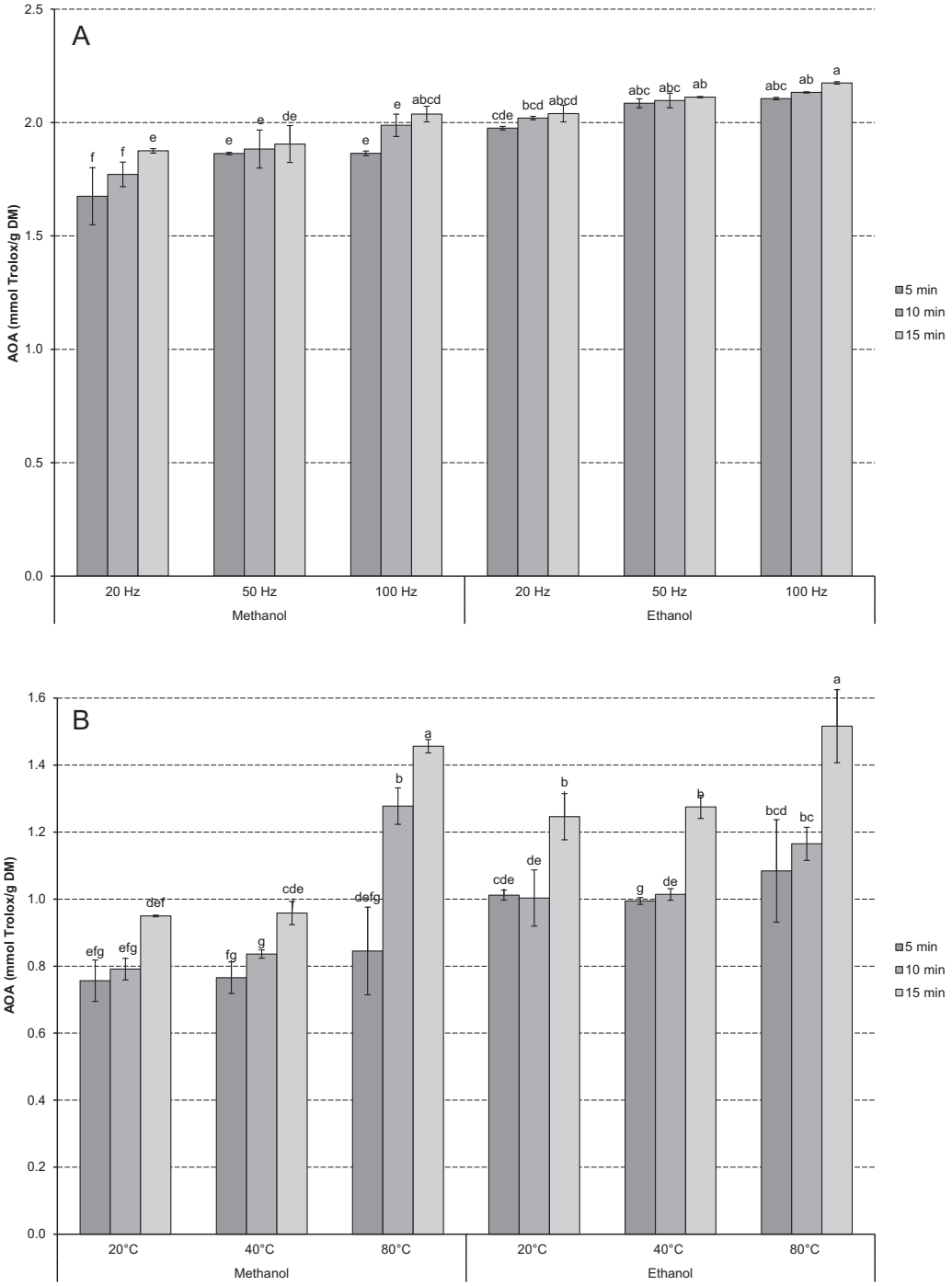

FIGURE 2. Antioxidant activity (AOA) of grape pomace extracted with high voltage electrical discharges (HVED) treatment (A) and with ultrasound assistance (UAE) (B) for 5, 10 and 15 min. Results are expressed as means and SD (n=3) and calculated on the dry matter (DM) basis. Different letters above bars indicate significant differences at 95% confidence level as obtained by the LSD test.

[Yan *et al*., 2018; Spigno *et al*., 2007; Pinelo *et al*., 2005]. When it comes to the extraction of phenolics from grape by-products, some authors [Do *et al*., 2014; Shi *et al*., 2003a] reported aqueous ethanol as a superior solvent while others [Pinelo *et al*., 2005] reported aqueous methanol as a superior solvent for the phenolics extraction. Therefore, the type of extraction solvent as well as the extraction method have a significant impact on the extraction yield of individual polyphenols from each individual plant material. There are some reports concerning optimization of extraction conditions of phenolic compound content and antioxidant activities of some plant foods but as some researches indicated the optimal procedure to be usually different for different plant matrices and different extraction methods [Rababah *et al*., 2010; Pellegrini *et al*., 2007].

Effect of high voltage electrical discharge-assisted extraction on phenolic profile of grape pomace

Table 2 shows contents of individual phenolics in grape pomace determined after HVED-assisted extraction. The effect of HVED extraction on the extraction of individual phe-

TABLE 2. Phenolic profile of grape pomace extracted with high voltage electrical discharges (HVED) treatment (mg/100 g DM).

Extraction conditions		Gallic acid	*p*-Coumaric acid	Caffeic acid	Catechin	Epicatechin	Quercetin	Cyanidin--3-*O*-glucoside	Peonidin--3-*O*-glucoside	Malvidin	Delphinidin
5 min											
MeOH	20 Hz	1.13±0.01^{o*}	3.66±0.08^{j}	28.25±0.02^{m}	8.66±0.04^{o}	7.95±0.09^{q}	16.24±0.02^{q}	17.62±0.03^{n}	28.97±0.01^{j}	79.61±0.08^{n}	42.19±0.09^{o}
	50 Hz	2.18±0.05^{j}	3.78±0.04^{i}	40.02±0.01^{h}	15.24±0.04^{j}	15.36±0.09^{l}	25.37±0.01^{l}	17.93±0.02^{m}	29.44±0.00^{i}	82.62±0.09^{j}	46.32±0.01^{i}
	100 Hz	2.28±0.01^{i}	4.78±0.01^{f}	39.05±0.03^{i}	22.93±0.01^{n}	19.69±0.03^{n}	25.13±0.01^{m}	19.42±0.04^{i}	31.64±0.09^{e}	86.51±0.13^{f}	49.15±0.11^{e}
EtOH	20 Hz	1.10±0.03^{op}	2.80±0.04^{m}	24.88±0.04^{o}	5.47±0.07^{q}	8.82±0.07^{p}	20.09±0.03^{o}	16.44±0.02^{p}	27.45±0.07^{l}	77.71±0.12^{p}	42.11±0.16^{o}
	50 Hz	1.41±0.04^{n}	2.92±0.01^{l}	33.81±0.07^{l}	23.34±0.06^{i}	14.10±0.04^{o}	28.98±0.07^{i}	17.12±0.02^{o}	28.19±0.05^{k}	79.15±0.09^{o}	44.08±0.13^{l}
	100 Hz	1.63±0.01^{l}	3.18±0.03^{k}	42.33±0.05^{f}	25.76±0.09^{h}	25.72±0.14^{h}	31.18±0.03^{g}	18.57±0.05^{k}	28.91±0.02^{j}	80.19±0.10^{m}	46.41±0.09^{i}
10 min											
MeOH	20 Hz	1.48±0.04^{m}	3.69±0.04^{ij}	39.08±0.10^{i}	19.61±0.04^{m}	18.46±0.10^{m}	16.73±0.09^{p}	19.55±0.14^{h}	29.84 ±0.08^{h}	82.41±0.13^{k}	44.82±0.04^{k}
	50 Hz	2.91±0.04^{f}	4.48±0.05^{g}	43.95±0.01^{e}	39.25±0.04^{f}	36.81±0.13^{f}	28.94±0.10^{i}	20.87±0.09^{e}	30.15 ±0.08^{g}	85.74±0.11^{g}	48.12±0.04^{fg}
	100 Hz	3.80±0.02^{c}	5.14±0.04^{e}	41.01±0.07^{g}	34.07±0.04^{g}	31.96±0.24^{g}	25.47±0.06^{k}	22.61±0.08^{b}	33.69 ±0.09^{b}	89.63±0.09^{b}	49.78±0.09^{c}
EtOH	20 Hz	1.06±0.05^{p}	3.67±0.04^{j}	26.83±0.01^{n}	7.27±0.08^{p}	20.07±0.30^{k}	21.47±0.09^{n}	18.11±0.04^{l}	28.88 ±0.13^{j}	79.61±0.16^{n}	43.10±0.07^{n}
	50 Hz	2.10±0.07^{k}	3.73±0.05^{ij}	38.57±0.04^{j}	46.73±0.05^{e}	36.58±0.18^{f}	30.45±0.11^{h}	18.96±0.06^{j}	29.73 ±0.08^{h}	81.19±0.11^{l}	46.09±0.03^{j}
	100 Hz	2.42±0.06^{h}	3.88±0.06^{h}	48.48±0.10^{b}	49.74±0.02^{c}	40.19±0.12^{e}	31.34±0.04^{f}	20.14±0.12^{f}	31.18 ±0.07^{f}	83.11±0.14^{j}	47.98±0.01^{g}
15 min											
MeOH	20 Hz	2.18±0.05^{j}	5.69±0.16^{d}	39.13±0.07^{i}	22.36±0.02^{l}	23.17±0.12^{j}	27.32±0.05^{j}	20.11±0.08^{f}	31.55±0.16^{e}	86.91±0.15^{e}	48.19±0.14^{f}
	50 Hz	2.62±0.03^{g}	5.84±0.06^{c}	44.11±0.07^{d}	48.22±0.05^{d}	41.24±0.21^{d}	33.11±0.02^{e}	22.64±0.01^{b}	32.39±0.01^{c}	89.33±0.13^{c}	50.08±0.09^{b}
	100 Hz	3.47±0.01^{d}	6.80±0.02^{a}	47.54±0.04^{c}	52.17±0.06^{b}	44.77±0.26^{c}	41.02±0.03^{b}	26.84±0.01^{a}	34.57±0.06^{a}	92.27±0.11^{a}	51.83±0.01^{a}
EtOH	20 Hz	3.19±0.05^{e}	5.69±0.03^{d}	35.16±0.02^{k}	22.66±0.03^{k}	24.65±0.49^{i}	33.37±0.02^{d}	19.89±0.01^{g}	30.12±0.11^{g}	81.24±0.09^{l}	43.64±0.08^{m}
	50 Hz	4.71±0.06^{a}	5.63±0.02^{d}	48.53±0.02^{b}	49.74±0.01^{c}	45.76±0.29^{b}	38.28±0.03^{c}	21.64±0.06^{d}	31.67±0.10^{e}	83.67±0.16^{h}	46.87±0.04^{h}
	100 Hz	4.21±0.05^{b}	5.97±0.06^{b}	54.09±0.01^{a}	53.68±0.07^{a}	53.19±0.29^{a}	45.31±0.06^{a}	22.44±0.04^{c}	32.08±0.09^{d}	88.09±0.15^{d}	49.56±0.11^{d}

Results are expressed as mean ± SD (n=3). Extracts were obtained after 5, 10 and 15 min of extraction in methanol (MeOH) and ethanol (EtOH) based solutions. Different letters in superscripts in each column indicate significant differences between means at 95% confidence level as obtained by the LSD test, DM – dry matter.

nolics varied. For all identified phenolic acids (gallic acid, *p*-coumaric and caffeic acids), all investigated parameters had a significant influence ($p \leq 0.05$) on the extraction yields. The highest extraction yield of gallic acid (4.71 mg/100 g DM) was achieved in the ethanol-based solvent after 15 min of extraction and HVED frequency of 50 Hz. The yields of *p*-coumaric and caffeic acids were increased with increasing the HVED frequency and with the duration of the extraction. The highest contents of *p*-coumaric acid (6.80 mg/100 g DM) and caffeic acid (54.09 mg/100 g DM) were achieved in GP after 15 min and HVED frequency extraction of 100 Hz in methanol-based and ethanol-based solvents, respectively. Both flavan-3-ols (catechin and epicatechin) were extracted with similar yields under each individual HVED extraction condition. Both, extraction time and HVED frequency significantly ($p \leq 0.05$) influenced the extraction yield of flavan-3-ols with their highest content in GP obtained after 15 min at 100 Hz in the ethanol-based solution. The highest content of quercetin in GP (45.31 mg/100 g DM) was obtained after extraction at HVED frequency of 100 Hz and treatment time of 15 min in the ethanol-based solvent. Other authors also reported better solubility of quercetin in ethanol-water mixtures compared with methanol-water mixtures [Razmara *et al.,* 2010]. All four identified anthocyanins were better extracted in the methanol-based solvent. The highest yields of those anthocyanins were obtained at HVED frequency of 100 Hz. However, extraction yields of the other two identified anthocyanins: peonidin-3-*O*-glucoside and malvidin, were significantly ($p \leq 0.05$) influenced by all process parameters. The highest contents of cyanidin-3-*O*-glucoside (26.84 mg/100 g DM), peonidin-3-*O*-glucoside (34.57 mg/100 g DM), malvidin (92.27 mg/100 g DM), and delphinidin (51.83 mg/100 g DM) were obtained in GP extracted by the methanol-based solvent after 15 min and frequency of 100 Hz. When choosing a solvent for the HVED extraction, water is a good choice since it is capable of dissolving most of the organic matter. However, some phenolics are poorly soluble in water and to increase their solubility it is useful to add organic solvents such as ethanol or methanol [Yan *et al.,* 2018], but if the organic solvent concentration is too high it will decrease solvent conductivity. Solvents used in this study had similar conductivity, *i.e.* 112.4 mS/cm in the case of the ethanol-based solvent and 111.1 mS/cm in the case of the methanol-based one. It is interesting that so far most of the HVED extractions were conducted in a relatively low liquid to solid ratio. However, this study showed that a higher liquid to solid (50:1, *v/w*) ratio could provide good phenolic yields, which is also supported by the findings of Barba *et al.* [2015]. In their study, they achieved a better yield of phenolic extraction from raw rapeseed (1.00 mg GAE/100 g) and rapeseed press cake (559.17 mg GAE/100 g) with a higher liquid to solid ratio (20:1, *v/w*) compared to the lower ratio (5:1, *v/w*) [Barba *et al.,* 2015].

However, it should be pointed out that for the extraction of phenolics with HVED, energy input (Eq. 1) is a most demanding factor combined with electric field intensity (Eq. 2) and treatment time [Rajha *et al.,* 2015].

$$W_{HVED} = \frac{E_p \times n}{m} \quad \text{Eq. 1}$$

$$E = \frac{V}{d} \quad \text{Eq. 2}$$

where: W_{HVED} is the energy input (kJ/kg), Ep is the energy of one pulse (kJ), m is the mass of suspension (kg); and E is the electric field intensity (kV/cm); V is the peak voltage (kV); d is the distance between electrodes (cm). Clearly, with the longer treatment time the energy input is higher, which leads to more complete destruction of plant cell structure and to extraction enhancement [Parniakov *et al.,* 2016]. Consequently, the yield would increase after a longer treatment time. However, higher energy inputs, over 100 kJ/kg, could lead to the degradation of target compounds or to the generation of H_2O_2 radicals [Rosello-Soto *et al.,* 2015]. In this study, the highest yields were obtained when treating the suspension for 15 min which gives an energy input of 22.27 kJ/kg, the electric field intensity was set on 60 kV/cm and energy of one impulse to 0.15 J. The energy input for treatment times of 5 and 10 min at 100 Hz was 7.43 and 14.85 kJ/kg, respectively.

Effect of ultrasound-assisted extraction on phenolic profile of grape pomace

The ultrasound power (35 kHz) used in this experiment is in the domain (18–40 kHz) for achieving much stronger ultrasound effects such as disruption of biological cell walls and cell membranes, as well as particle size reduction, as reported by Novak *et al.* [2008].

The effect of UAE on the contents of individual phenolics determined in GP is shown in Table 3. The phenolic acid profile was differently affected by the extraction parameters applied. The ethanol-based solvent proved better for the extraction of gallic acid and caffeic acid and the methanol-based one for the extraction of *p*-coumaric acid. Difference in polarity could influence the content of phenolic acids in the extracts, as the methanol polarity is 6.6 compared to 5.2 for ethanol [Betancourt *et al.,* 2008]. However, both extraction time and temperatures significantly ($p \leq 0.05$) influenced the extraction of phenolic acids. The highest extraction yields of gallic acid (2.32 mg/100 g DM) and caffeic acid (36.19 mg/100 g DM) were achieved in the ethanol-based solvent after 15 min at 80°C. The highest extraction yield of *p*-coumaric acid (5.87 mg/100 g DM) was obtained in the methanol-based solvent after 15 min at 80°C. The highest extraction yields of flavan-3-ols, including catechin (40.25 mg/100 g DM) and epicatechin (23.02 mg/100 g DM), were obtained in the ethanol-based solvent after 15 min at 80°C. Both epicatechin and catechin yields were significantly influenced by solvent type ($p \leq 0.05$), which was also noted during the HVED extraction. Other authors also reported that UAE of catechins was more efficient in the ethanol-water solvent and after longer extraction time [Alonso *et al.,* 1991]. The highest extraction yield of quercetin (24.97 mg/100 g DM) was obtained in the ethanol-based solvent after 15 min at 80°C. Anthocyanins were better extracted in the methanol-based solvent as in the HVED case. However, all identified anthocyanins were significantly ($p \leq 0.05$) influenced by all applied parameters equally. The higher extraction yields were obtained by increasing the temperature and extraction time. The high-

TABLE 3. Phenolic profile of grape pomace determined after ultrasound assisted extraction (UAE) (mg/100 g DM).

Extraction conditions		Gallic acid	*p*-Coumaric acid	Caffeic acid	Catechin	Epicatechin	Quercetin	Cyanidin--3-*O*-glucoside	Peonidin--3-*O*-glucoside	Malvidin	Delphinidin
5 min											
MeOH	20°C	0.27±0.01^{m}	0.58±0.01^{k}	19.02±0.05^{m}	10.65±0.02^{n}	5.16±0.03^{m}	3.93±0.03^{lm}	13.15±0.01^{o}	26.51±0.09^{n}	77.36±0.07^{l}	40.33±0.06^{n}
	40°C	0.50±0.03^{l}	0.85±0.00^{j}	19.87±0.05^{l}	11.66±0.08^{m}	5.63±0.00^{l}	7.57±0.01^{g}	16.11±0.00^{l}	27.33±0.07^{k}	77.98±0.06^{k}	41.21±0.04^{l}
	80°C	1.03±0.01^{ijk}	1.09±0.01^{h}	20.52±0.04^{j}	13.81±0.18^{k}	8.37±0.00^{j}	9.03±0.39^{f}	18.46±0.04^{f}	29.61±0.07^{e}	81.61±0.08^{f}	45.63±0.05^{f}
EtOH	20°C	0.89±0.01^{k}	0.26±0.01^{n}	18.08±0.07^{o}	6.66±0.04^{q}	8.46±0.03^{j}	3.69±0.16^{m}	12.53±0.06^{q}	24.19±0.08^{p}	73.31±0.08^{r}	38.62±0.04^{q}
	40°C	0.98±0.08^{jk}	0.34±0.02^{m}	18.56±0.06^{n}	8.66±0.04^{o}	10.99±0.01^{gh}	4.42±0.13^{k}	13.09±0.03^{p}	25.08±0.07^{o}	74.15±0.05^{q}	38.99±0.06^{p}
	80°C	1.46±0.03^{ef}	0.35±0.02^{m}	20.69±0.04^{j}	14.87±0.05^{i}	13.37±0.25^{d}	14.75±0.25^{c}	17.59±0.03^{j}	26.87±0.08^{l}	78.46±0.08^{j}	41.32±0.08^{k}
10 min											
MeOH	20°C	0.98±0.03^{jk}	1.95±0.05^{f}	21.69±0.02^{i}	13.52±0.05^{l}	7.87±0.04^{k}	5.33±0.35^{j}	16.11±0.01^{l}	28.61±0.07^{h}	79.31±0.04^{i}	41.36±0.04^{k}
	40°C	1.03±0.03^{ijk}	2.31±0.04^{e}	22.78±0.04^{h}	14.48±0.04^{j}	7.94±0.04^{k}	8.76±0.07^{f}	18.52±0.00^{e}	28.99±0.08^{g}	80.39±0.05^{h}	44.67±0.02^{g}
	80°C	1.10±0.05^{hij}	2.57±0.02^{d}	25.16±0.26^{f}	26.14±0.01^{c}	11.06±0.03^{g}	12.26±0.18^{e}	20.82±0.02^{b}	32.67±0.07^{b}	86.11±0.05^{b}	48.31±0.02^{b}
EtOH	20°C	1.29±0.08^{efg}	0.38±0.01^{m}	20.26±0.03^{k}	7.18±0.08^{p}	9.33±0.12^{i}	4.04±0.34^{l}	15.55±0.04^{n}	26.67±0.07^{m}	75.87±0.08^{p}	39.26±0.04^{o}
	40°C	1.28±0.07^{fgh}	0.44±0.06^{l}	25.03±0.26^{f}	11.66±0.02^{m}	10.70±0.00^{h}	8.69±0.28^{f}	15.93±0.03^{m}	27.94±0.04^{j}	76.49±0.05^{n}	40.56±0.03^{m}
	80°C	1.47±0.03^{de}	0.85±0.08^{j}	27.57±0.02^{e}	17.69±0.02^{f}	16.70±0.01^{c}	16.81±0.20^{b}	17.94±0.01^{h}	29.37±0.05^{f}	80.56±0.09^{g}	45.73±0.03^{e}
15 min											
MeOH	20°C	1.20±0.20^{ghi}	2.00±0.01^{f}	27.73±0.07^{e}	15.48±0.07^{h}	8.49±0.02^{j}	5.88±0.18^{i}	17.88±0.02^{i}	30.54±0.04^{d}	82.64±0.04^{d}	44.39±0.03^{h}
	40°C	1.65±0.03^{cd}	2.76±0.03^{c}	31.21±0.04^{d}	15.99±0.00^{g}	9.17±0.56^{i}	7.48±0.09^{g}	19.36±0.01^{d}	32.62±0.07b	84.82±0.04^{c}	47.61±0.07^{c}
	80°C	1.69±0.37^{c}	5.87±0.01^{a}	34.53±0.17^{c}	25.46±0.04^{d}	11.99±0.08^{f}	14.90±0.25^{c}	24.43±0.03^{a}	33.97±0.09^{a}	89.91±0.06^{a}	49.82±0.09^{a}
EtOH	20°C	1.81±0.06^{bc}	1.79±0.00^{g}	24.01±0.05^{g}	27.12±0.05^{b}	12.43±0.31^{e}	7.06±0.19^{h}	17.23±0.02^{k}	28.37±0.07^{i}	76.34±0.07^{o}	41.62±0.04^{j}
	40°C	1.95±0.04^{b}	1.03±0.03^{i}	35.32±0.09^{b}	22.82±0.04^{e}	19.44±0.23^{b}	14.05±0.03^{d}	18.06±0.04^{g}	29.61±0.08^{e}	76.99±0.04^{m}	43.55±0.05^{i}
	80°C	2.32±0.08^{a}	3.27±0.05^{b}	36.19±0.03^{a}	40.25±0.03^{a}	23.02±0.14^{a}	24.97±0.05^{a}	20.37±0.07^{c}	31.12±0.05^{c}	82.49±0.07^{n}	47.08±0.04^{d}

Results are expressed as mean ± SD (n=3). Extracts were obtained after 5, 10 and 15 min of extraction in methanol (MeOH) and ethanol (EtOH) based solutions. Different letters in superscripts in each column indicate significant differences between means at 95% confidence level as obtained by the LSD test, DM – dry matter.

est yields of cyanidin-3-*O*-glucoside (24.43 mg/100 g DM), peonidin-3-*O*-glucoside (33.97 mg/100 g DM), malvidin (89.91 mg/100 g DM), and delphinidin (49.82 mg/100 g DM) were obtained in the methanol-based solvent after 15 min of extraction at 80°C. Our findings are consistent with previous reports despite obvious differences in extraction conditions and plant materials. With UAE of dried chokeberries in 50% (*v/v*) ethanol, Ćujić *et al.* [2016] reported that the total anthocyanin contents were significantly greater after longer period of extraction. Wang *et al.* [2018] found the highest yields of TPC and TFC with extraction time of 15 min when extracting blueberry leaves using ultrasound-negative pressure cavitation extraction. The highest yields in all UAE experiments were obtained at 80°C (Table 3), this is because at higher temperatures diffusion coefficient and generally solubility of solute are increased [Palma & Taylor, 1999]. As during the HVED extractions, liquid to solid ratio is another important parameter since it facilitates the mass transfer driving force. The phenolic acid yields obtained in this experiment with the liquid to solid (50:1, *v/w*) ratio confirm this (Table 3). The results obtained are consistent with the findings of Bamba *et al.* [2018] who investigated the influence of extraction conditions on ultrasound-assisted recovery of bioactive phenolics from blueberry pomace and their antioxidant activity. Accordingly, the optimal extraction solvent was 50% (*v/v*) ethanol, and extraction was more efficient with a higher liquid to solid ratio and longer treatment time.

CONCLUSION

The FRG by-product was rich in phenolics, especially anthocyanins. The HVED-assisted extraction of grape pomace showed to be a superior extraction method for obtaining high yields of anthocyanins, phenolic acids, flavan-3-ols, and flavonols. The best parameters for extracting phenolics were: electric field intensity of 60 kV/cm, energy of one impulse of 0.15 J, treatment time of 15 min, and HVED frequency of 100 Hz, giving the total energy input of 22.27 kJ/kg during the extraction. Regarding UAE, it was shown that higher yields were achieved at higher temperature (80°C) and at longer treatment time (15 min), with ultrasound power set on 35 kHz. Since phenolics have different solubility, applying a solvent system with medium polarity seems to be more effective and appropriate to maximize contents of individual phenolics in the extract. Depending on the phenolic compound of interest, ethanol- or methanol-based solvents should be used.

CONFLICT OF INTEREST

Authors declare no conflict of interests.

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