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# Using Ohmic Heating effect on grape skins as a pretreatment for anthocyanins extraction

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## ABSTRACT

This study aims to obtain aqueous extracts of valuable phytochemicals, in particular anthocyanin's, from winemaking residues by using grape skins as natural electrical conductors allowing internal heat dissipation through Ohmic Heating (OH) effect. Two different electric pretreatments were evaluated: i) using mild temperatures at 40 °C during 20 min; ii) flash heating from 40 to 100 °C in less than 20 s (no holding time). These pretreatments were followed by aqueous extraction in water at room temperature. Independently of the temperature applied, OH allowed to boost extraction levels increasing concentration of total phenolic compounds, as well as conductivity, soluble solids and red color intensity of the obtained extracts, as shown through principal component analysis (PCA). OH pretreatments at high-temperature short-time (HTST) due to the fast internal heating of grape skin structure allowed increase total concentration of anthocyanins from 756 to 1349 µg/g, with malvidin-3-O-glucoside being the main compound identified and quantified in the aqueous extracts through HPLC analysis (corresponding to about 60% of the total). These results showed that OH bring potential to be an efficient and environmentally friendly technology towards sustainable food processes.

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## 1. Introduction

Grape is one of the world's largest fruit crop, with worldwide production of about 74 million tons in 2017 (FAOSTAT, 2019). In European countries such as France, Italy, Spain and Portugal, wine industry constitutes an important part of the economy and culture. Europe is responsible for about 34.9% of worldwide grapes production. In grape producing countries such as Portugal, which according to FAO (FAOSTAT, 2019) produced 868,635 t of grapes during 2017, grape waste management resultant from wine-making remains one of the main problems. Grape pomace is the most abundant solid waste of the wine industry, resulting from the pressing of grapes and representing about 20% (w/w) of total grapes used for wine production. This by-product is left after juice extraction and can be used for the production of distilled drinks or ulti-

mately incinerated, dumped, or used for animal feed or as a fertilizer. This by-product if not treated effectively can initiate a number of environmental issues. Grape skins constitute an average of 65% of grape pomace, and are an interesting source of organic compounds being used as natural food additives for development of functional ingredients. A wide range of compounds can be recovered depending on the grape variety, methodology of winemaking and extraction technique, such as ethanol, tartrates, citric acid, dietary fibers, cellulose, hemicellulose, proteins and tannins (Mendes et al., 2013). Also, it has been reported that grape skins have valuable phenolic compounds, which are attractive from a nutritional standpoint due to their potential application as food antioxidants (Mendes et al., 2013; Mourtzinou and Goula, 2019). In fact, the quality and organoleptic properties of wine products are intrinsically related to the presence of phenolic compounds that were extracted from the grape during the winemaking process. The most important phenolic constituents of red grapes pomace include anthocyanins, catechins, epicatechin, rutin and quercetin derivatives (Peixoto et al., 2018; Rockenbach et al., 2011). The standard extrac-

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tion methodologies to obtain these phytochemicals relies on the use of organic solvents (i.e., ethanol, acetone, ethyl acetate, diethyl ether, methanol, etc.) and chemical agents (e.g. sulphur dioxide) allied to thermal or physical treatments (Pinelo et al., 2005; Boussetta et al., 2009). These methodologies are time and solvent consuming, presenting elevated energetic costs and environmental issues. For these reasons, there has been a growth in the development and optimization of non-conventional extraction technologies. Some of these technologies include: ultrasounds (USN), microwave assisted extraction (MAE), subcritical fluid extraction (SbFE), supercritical fluid extraction (SFE), high hydrostatic pressure (HHP), accelerated solvent extraction (ASE), extraction assisted by hydrotropic solvents, among others. These technologies are recognized by their high extraction yields (Duba et al., 2015; Barba et al., 2016; Caldas et al., 2018). Emerging technologies based on application of electric fields – e.g. pulse electric fields (PEF), high voltage electrical discharges (HVED) - have been applied for extraction of bioactive compounds. They have shown potential to provoke electrical permeabilization (i.e. electroporation) on cell membranes in smaller periods of time (Boussetta et al., 2013; El Darra et al., 2013a; Medina-Meza and Barbosa-Cánovas, 2015; Maza et al., 2019). However, the industrial application of these referred methodologies can be limited due to inherent investments costs, lack of regulatory approval and poor efficiency regarding microbiological or enzyme inactivation. Ohmic heating (OH) technology is also based on application of electric fields but contrary to PEF or HVED can be distinguished by its thermal nature allowing a controllable heating rate (with no upper temperature limit), unrestricted treatment time scale (for seconds to hours), unrestricted type of waveform and frequency (typically sinusoidal with frequencies ranging from Hz to kHz), and presence of alternating moderate to low electric fields (that can go below 0.1 kV/cm). OH is also recognized by its high energy conversion and easier scalability at pilot or even industrial level when compared with microwave heating for example (Silva et al., 2017). This technology also brings an important environmental and competitive benefit, once through OH principle a given food product can behave as an electric conductor and be a source of internal heat. This processing principle brings the opportunity to reduce gas emissions and water consumption during processing (Rocha et al., 2018). OH has been successfully used for thermal processing of foods (i.e. blanching, pasteurization, sterilization) (Jaeger et al., 2016; Pereira et al., 2016; Coelho et al., 2019). This energy efficiency can be achieved because OH is capable of a precise control of the temperature allowing volumetric HTST process avoiding contact with hot surfaces. For these reasons, this technology offers the potential to overcome the limitations of slower heat transfer (no thermal conduction) and overheating, while allowing to reach the desired holding temperature of the process in a short time, usually few seconds. These features are very important for pigment extraction (i.e. tannins and anthocyanins), since heating treatments will influence the yield of extraction depending on time/temperature binomial applied; it was described that HTST is one the best condition for anthocyanin release (Lambri et al., 2015). OH has now recognized potential to process fruits derivatives maintaining phytochemical concentration and fresh-like characteristics (Martín-Belloso and Morales-de la Peña, 2018). The objective of this work is to evaluate, to our knowledge for the first time, the effects of using OH as HTST pretreatment on the aqueous extraction of total soluble matter and phenolic compounds from red grape skins by-product resultant from winemaking process. Non-thermal effects related with electric field are also addressed.

## 2. Materials and methods

### 2.1. Sample material

The red grape skins were kindly provided by Adega Cooperativa de Ponte da Barca, after their use in maceration process for the production of local red Vinho Verde. The grape skins were a mixture of different varieties of red grapes authorized for the production of red Vinho Verde. As pre-processing step,

the grape skins were oven-dried at 45 °C (for 4 days) to avoid oxidation reactions and microbial spoilage that may occur during storage time due to its high moisture content (60%). Dehydration is considered an adequate alternative to prevent deterioration providing simultaneously mass and volume reduction (Pedroza et al., 2012), and when preformed at low temperatures allow the preservation of phytochemicals nearly as well as a freeze-drying process (Larrauri et al., 1997).

### 2.2. Thermal-pretreatment

Approximately 1.5 g of dry grape skins were rinsed briefly in NaCl solution (0.1 mol L<sup>-1</sup>) allowing to standardize electrical conductivity for OH effect and helping to remove unwanted superficial adhered material. Three different type of pretreatments were made independently before the aqueous extraction take place at room temperature, as follows: 1) low-temperature and long-time treatment – i.e. 40 °C for 20 min using OH (labelled as OH40); 2) low-temperature and long-time treatment – i.e. 40 °C for 20 min using conventional indirect heating (COV40); and 3) HTST using OH – i.e. ~100 °C for 1 s (labelled as OH100). This set of experiments allowed to disclose the putative occurrence of non-thermal electrical effects of OH (comparison between OH40 and COV40) and synergy between electrical and thermal effects though joule effect (comparison between OH40, COV40 and OH100).

#### 2.2.1. Conventional heating (COV)

Conventional heating was performed in a double-walled water-jacketed glass reactor vessel connected circulating thermo-stabilized water-bath. Temperature was measured with a type-K thermocouple (temperature precision of ±1 °C, Omega Engineering, Inc., Stamford, CT, USA), placed at the geometric center of the sample volume and connected to a data logger (USB-9161, National Instruments Corporation, Austin, TX, USA).

#### 2.2.2. Ohmic heating (OH)

Treatments were performed in a double-walled water-jacketed cylindrical glass tube vessel of 30 cm total length and an inner diameter of 2.3 cm. Stainless steel electrodes were placed at each edge of the glass tube and isolated with polytetrafluoroethylene (PTFE) caps. A gap between the electrodes of 5 cm was used for the experiments and heating was controlled through the supplied voltage using a function generator (1 Hz–25 MHz and 1–10 V, Agilent 33220A, Penang, Malaysia) connected to an amplifier system (Peavey CS3000, Meridian, MS, USA). During heating phase and holding treatment, the electric field applied was of 80 and 16 V/cm respectively. Electrical frequency was set to a value of 25 kHz avoiding electrochemical reactions at the electrode interface and thus potential leakage of electrodes material to the medium (Patara et al., 2014). Temperature was measured as described previously for conventional thermal extraction.

### 2.3. Aqueous extraction

Pre-treated grape skins (1.5 g of treated material) were placed together with 20 mL of distilled water at room temperature (c.a. 22 °C) under gentle orbital shaking at 120 rpm (KL-2 Edmund Buhler, Germany) for 1 h. The extraction mechanism was characterized by fast washing step and diffusion of compounds within the first 30 min of extraction (results not shown). Aqueous extracts were then were clarified by cen-

trifugation at 10.000 g for 5 min (MIKRO 120, Hettich, Germany) before further analysis.

#### 2.4. Chemical and physical characterization of the grape skin extracts

Aliquots of aqueous extracts previously prepared were used for physical and chemical determination. Unless otherwise stated all experiments were run at least in triplicate and performed at room temperature (ca. 20 °C).

##### 2.4.1. Total phenolic compounds (TPC)

The total content of polyphenol compounds in grape skins extracts was determined using the Folin–Ciocalteu method (Singleton and Rossi, 1965), adapted to a 96-well microplate. To a 5 µl of filtered and diluted extract were added 60 µl of sodium carbonate solution at 7.5% (w/v) and 15 µl of Folin–Ciocalteu reagent. After that, 200 µl of distilled water was added, and the solutions were mixed. The samples were heated at 60 °C for 5 min and were allowed to cool at room temperature. The absorbance was then measured by means of a spectrophotometric microplate reader (Sunrise Tecan, Grödig, Austria) set at 700 nm. Gallic acid was used for the calibration curve. As a control sample, distilled water was used. The content of total polyphenols was expressed as milligram gallic acid equivalent per g of dry weight (DW) sample (mg GAE/g<sub>DW</sub>).

##### 2.4.2. Electrical conductivity

Electrical conductivity were measured at room temperature (25 °C) using a conductivity meter (LF5838, WTW, Germany) by immersion of the electrode (WTW TetraCon 323) on untreated and treated aqueous extracts. Results were expressed in mS/cm.

##### 2.4.3. Soluble solids

Total soluble solids were measured through a refractometer (HI96081, Hanna, Singapore) by dropping 100 µl of untreated and treated aqueous extracts in the prism surface. Results were expressed in Brix ° scale.

##### 2.4.4. Spectrophotometric analysis

Turbidity and red color of aqueous extracts were evaluated through absorbance measurement using microplate reader (Sunrise Tecan, Grödig, Austria) set at wavelengths of 600 and 530 nm, respectively.

##### 2.4.5. Total anthocyanins

Total anthocyanin content was measured in accordance with using the pH differential method described elsewhere (Pereira et al., 2016; Giusti and Wrolstad, 2001) through Eq. 1:

$$\text{Anthocyanin} = \frac{\text{Abs} \cdot \text{Mw} \cdot d}{\varepsilon \cdot l} \quad (1)$$

where  $\varepsilon$  is the cyanidin-3-O-glucoside molar absorbance, Abs is absorbance, Mw is the molecular weight, d is the dilution factor, and l is the cell path length. Results were expressed as µg of cyanidin-3-O-glucoside equivalents per g of sample.

##### 2.4.6. Anthocyanins analysis by HPLC

Anthocyanin profile (quantitative and qualitative) was carried out on HPLC-DAD (Waters Series 600 Mildford MA. USA), using a UV-vis photodiode array detector. The absorption spectra were recorded between 212 and 720 nanometers

**Table 1 – Limits of detection (LOD) and quantification (LOQ) and coefficient of determination ( $R^2$ ) for each anthocyanin.**

Concentration/µg/g <sub>DW</sub>	LOD	LOQ	$R^2$
Dp3gl	66.1	200.3	0.99978
Cy3gl	80.9	245.3	0.99991
Pt3gl	28.0	84.8	0.99995
Po3gl	281.6	853.4	0.99626
Mv3gl	28.4	86.1	0.99995

(nm), according to (Coelho et al., 2019). A Symmetry® C18 column (250 × 4.6 mm, 5 µm, Agilent, USA) was used as the stationary phase. Two mobile phases were employed, the solvent A: Acetonitrile (100%) with 0.2% TFA; Solvent B: acetonitrile/water (5:95 v/v) (Merck pure grade and pure water) with 0.2% TFA (Sigma-Aldrich, Germany); A linear gradient was used with a flow rate of 0.75 mL/min, under the following conditions: 0–30 min (0–100% B); 30–60 min (60% B); 10 min (100% B) with an injection volume of 40 µl. Samples were measured in triplicate. Calibration curves were obtained at a detection wavelength at 510 nm, using standards solutions of delphinidin-3-O-glucoside (Dp3gl), cyanidin-3-O-glucoside (Cy3gl), petunidin-3-O-glucoside (Pt3gl), peonidin-3-O-glucoside (Po3gl), malvidin-3-O-glucoside (Mv3gl) (Extrasynthese, Lyon, France). Calibration curves were linear in the interval between 0.002 and 50 mg/L with correlations coefficients above 0.99 (see Table 1).

##### 2.4.7. Energy consumption

Energy consumption of OH ( $P_{OH}$ ) and COV ( $P_{COV}$ ) treatments was expressed in kJ/kg and calculated as described elsewhere (El Darra et al., 2013a; Pereira et al., 2016; El Darra et al., 2013b) through in Eqs. (2) and (3), respectively:

$$P_{OH} = \frac{\int_0^t U \cdot I \cdot dt_{OH}}{m} \quad (2)$$

$$P_{COV} = C_p \cdot \Delta T \quad (3)$$

where U is the voltage applied (V), I is the current intensity (A), t is time of OH treatment (s),  $C_p$  is specific heat capacity of water (4.18 kJ/kgK),  $\Delta T$  is the increase of temperature and m is the mass of treated product (kg).

#### 2.5. Statistical analysis

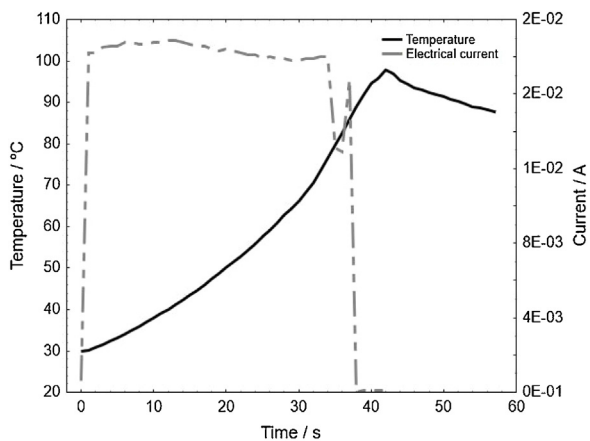
Statistica package software version 10.0.228.8 (StatSoft Inc.) was used for principal component analysis (PCA) and one-way analysis of variance (ANOVA) at a confidence level of 95%.

### 3. Results and discussion

#### 3.1. Non-thermal effects of ohmic heating method

OH pretreatments were performed using grape skins biomass as an electric conductor thus allowing passage of an electrical current and internal heat generation (Joule heating). Fig. 1 shows an example of thermal and electrical profile of OH pre-treatments at target temperature of approximately 100 °C (OH100).

It has been reported that indirect heating treatments in water at temperature of 50 °C during 20 min can increase extraction kinetics of phenolic compounds to a value of



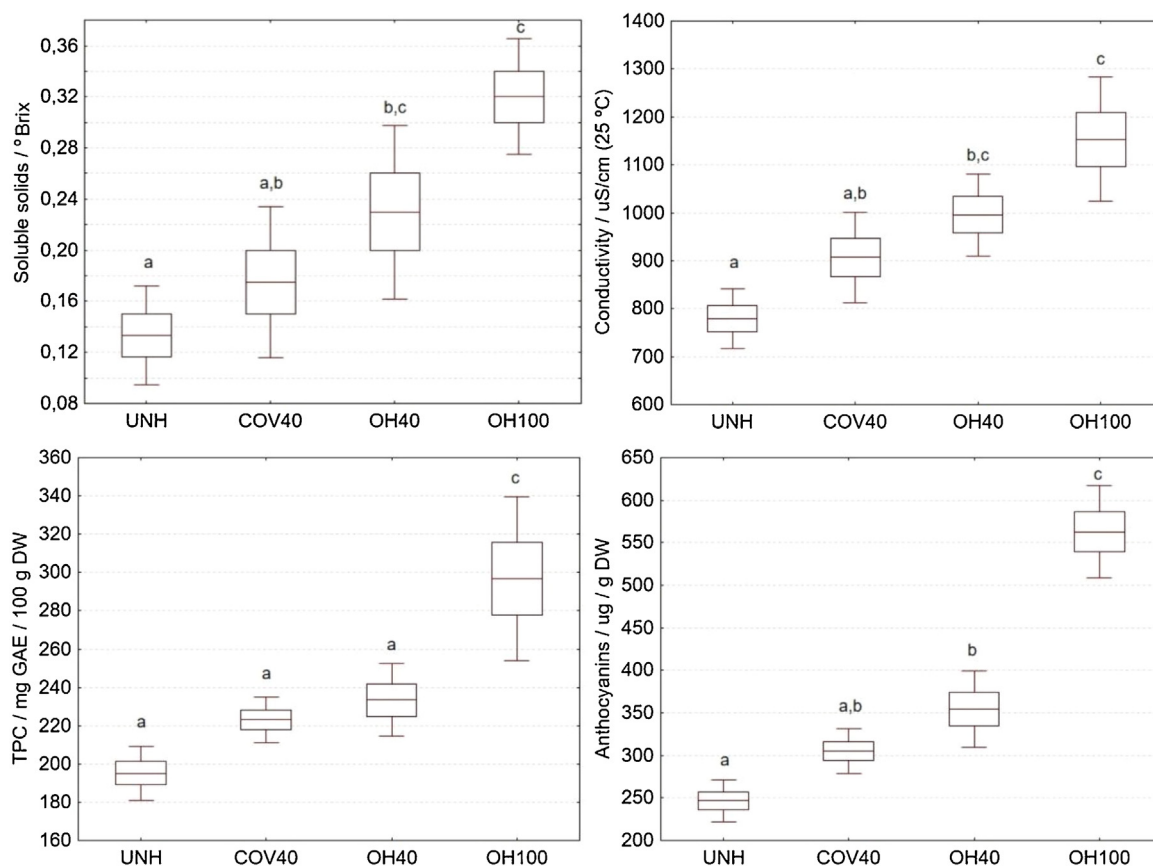
**Fig. 1 – Thermal profile of OH pretreatments at target temperature of approximately 100 °C (OH100).**

approximately 150 mg GAE/100 g of dry matter (El Darra et al., 2013b). But when PEF is combined with thermal effects at electric field of 400 V/cm (maximum temperature of ~45 °C) and 800 V/cm (maximum temperature of 82 °C), extraction of phenolic compounds increased to values of 200 and 300 mg GAE/100 g DW, respectively.

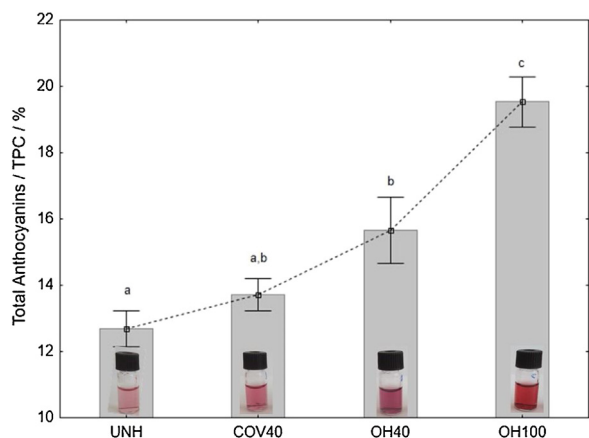
In this work, onset thermal extraction conditions - i.e. temperature between 35–40 °C for a treatment time of 20 min – were first evaluated in order to reduce thermal permeabilization and evaluate the occurrence of electrical effects of low intensity (<100 V/cm) otherwise hampered by high extraction temperatures (thermal permeabilization) and use of organic solvents, such as ethanol (chemical permeabilization). As shown through Fig. 2, effectiveness of aqueous

extraction of anthocyanins increases by increasing the intensity of the treatment conditions as follows: unheated < mild thermal effects (COV40) < mild thermal + electrical effects at 16 V/cm (OH40) < high thermal + electrical effects at 80 V/cm (OH100).

No significant differences ( $p > 0.05$ ) were observed between COV and OH40 extracts resulting from pretreatments at 40 °C, performed under similar thermal conditions. Results obtained with COV40 were similar to the ones observed for untreated sample ( $p > 0.05$ ) and mainly justified by natural diffusion of molecular compounds in water. This was already expected taking into account that pretreatments conditions were selected in order to avoid enhanced thermal extraction that would hinder additional extraction effects of OH. It is then important to highlight that the cumulative effects of electroheating at 40 °C (OH40) were sufficiently high to promote significant differences against unheated samples (UNH) regarding electrical conductivity, total phenolic compounds and anthocyanins concentration ( $p < 0.05$ ). For example, an increase of the electrical conductivity value of approximately 200  $\mu\text{S}/\text{cm}$  was observed after OH40 pretreatment when compared with UNH sample. This gives an indication that additional permeabilization of tissues has occurred allowing the leakage of components to the liquid medium; this was not observed for the pretreatment at COV40, where electrical conductivity of samples after extraction procedures was similar to the one observed for UNH sample ( $p > 0.05$ ). This additional permeabilization effect promoted by OH40 can be justified by additional permeabilization of tissues as result of internal electroheating of the material, and this is only possible given the nature of OH. OH40 allowed to enhance diffusion of phenolic compounds, including anthocyanins, to



**Fig. 2 – Quantitative analysis of aqueous extract resulting from OH and COV pretreatments. Whisker bars correspond to confidence interval of 95%. Different letters correspond to statistical significant differences ( $p < 0.05$ ).**



**Fig. 3 – Ratio of anthocyanin's to TPC expressed in percentage and corresponding extracts color evaluated at 530 nm. Different letters correspond to statistical significant differences ( $p < 0.05$ ).**

the liquid medium – i.e. about 56 and 26% more than observed in UNH and COV40 samples, respectively. The phytochemicals present in grape skins are mainly composed of polyphenols, which according with molecular structure can be divided in several classes, such as phenolic acids, flavonoids (in which anthocyanins are included), stilbenes and lignans (Zhu et al., 2012). This explains the higher amount of TPC present in the extracts with concentrations ranging from approximately 60–400 mg GAE/100 g DW, which are comparable with previous works (Castro-López, 2016; Ky et al., 2014). Pretreatments at mild conditions (at 40 °C) with low permeabilization effects, and without the use of any organic solvent, have only promoted diffusion of hydrophilic components of low molecular weight. This can explain the same levels of TPC observed between pretreated COV40 and UNH samples ( $p > 0.05$ ). Otherwise, knowing that flavonoids – in which anthocyanins are included – are in general compounds of low molecular weight, and thus easily extracted due to additional permeabilization induced by OH40.

The OH100 pretreatment at HTST conditions allowed to get best extraction performance with significant increases on TPC, particularly in anthocyanins concentration when compared with UNH, COV40 and OH40 samples ( $p < 0.05$ ). With OH100 soluble anthocyanins and TPC reached maximum values of approximately 600  $\mu\text{g/g}$  DW and 320 mg GAE/100 g DW increasing their initial values about 120 and 60%, respectively. The percentage of extracted anthocyanins of the total phenolic compounds greatly depends on the quality of source material (dried or fresh) and extraction methodology, particularly the type of the solvent used. For example it has been reported that the use of acetone (at 50%) can result in extraction of approximately 7% of total phenolic compounds, while the use of methanol (at 70%) often increases anthocyanin extraction up to 46% (Ramirez-Lopez and DeWitt, 2014). Fig. 3 shows the effects of different treatment strategies on the ratio between extracted anthocyanins to TPC (in percentage). OH100 pretreatment resulted in a markedly increase of the percentage of the extracted anthocyanins to the TPC corresponding approximately to a value of 20%; iratio between extracted anthocyanins and total phenolic compounds as a result of OH100 pretreatment increased from 5 to 8% when compared with OH40 and UNH, respectively. These results shows that within the established conditions thermal degradation of anthocyanins was somehow prevented. This

increase was also followed by a visible increase of red color of the obtained extracts (see picture inserts in Fig. 3): a linear relationship between extracted anthocyanins and red color (at 520 nm) was also established with high correlation coefficient ( $R^2 > 0.95$ ; results not shown).

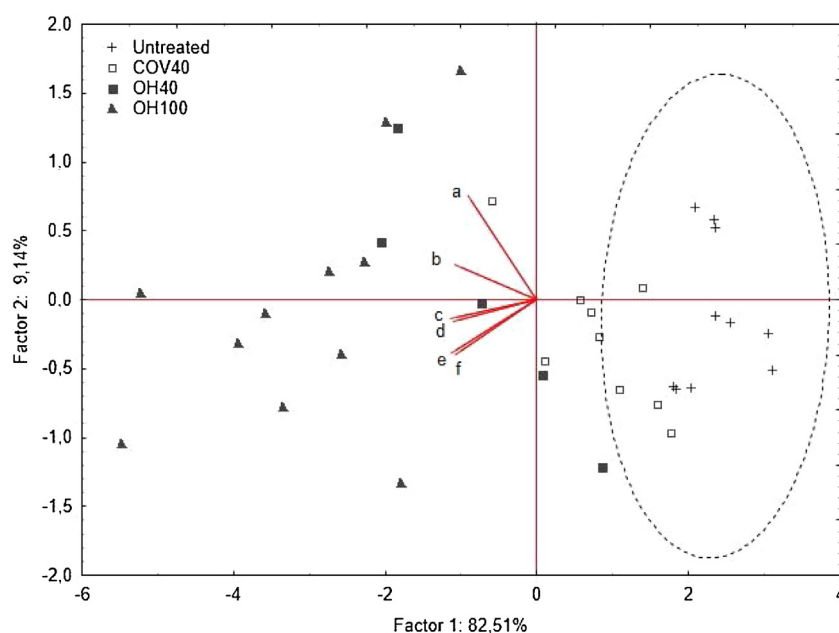
### 3.2. Multivariate analysis

For a comprehensive understanding of the effects of these pretreatments a principal component analysis (PCA) was performed in an attempt to find correlations between measured variables and obtained results. Fig. 4 shows the projection in two principal components - PC1 (horizontal axis) and PC2 (vertical axis). From this analysis, it is evident that thermal pretreatments are responsible for more than 90% of variability in the analyzed data. The effectiveness of extraction increases along the horizontal axis being UNH samples placed in positive axis of PC1, while the treated samples move from positive to negative axis of PC1 (extraction direction) depending on the treatment applied. Thus, all measured variables (anthocyanins and phenolic compounds, electrical conductivity, turbidity and red color intensity measurements) are correlated with each other (all move towards negative axis of PC1); anthocyanins and phenolic contents are well correlated and responsible for the case projection in negative axis of PC1 and PC2, while soluble solids and electrical conductivity are both correlated in negative axis of PC1 and positive axis of PC2. In a general way, PCA shows that OH pretreatments resulted in permeabilization of tissues favoring extraction of soluble compounds during aqueous extraction at room temperature, being this particularly evident for OH100 treatments. In addition, all OH pretreatments presented a tendency to increase extraction yield, being all the cases placed outside of the 95% confidence interval of control samples (UNH) contrary to COV40 samples. This underlies an enhanced efficiency of the thermal process on permeabilization/disruption on grape skin tissues. PCA allowed to show that properties such as electrical conductivity, soluble solids, turbidity and color (at 520 nm) are well correlated with total phenolic compounds, and therefore can be used as an expedite way of inferring about treatment efficiency regarding tissue permeabilization. Results from Section 3.1 and 3.2 evidences that the use of OH100 pre-treatments can avoid the use of organic solvents such as ethanol, and reduce drastically treatment times without compromising extraction yield and percentage of extracted anthocyanins.

### 3.3. Qualitative assessment of anthocyanins

OH pretreatments particularly favored the extraction of anthocyanins, whose stability is affected by physical and (bio)-chemical external factors such as light, pH and temperature (Burton-Freeman et al., 2016; Harbourne et al., 2013). Temperature can induce structural changes promoting degradation of anthocyanins into constituent phenolic acids. To understand the impact of pretreatments on qualitative profile of anthocyanins, HPLC analysis were performed on aqueous extracts resulting from UNH and treated (COV40, OH40 and OH100) grape skins. As shown in Fig. 5 and Table 2, HPLC analysis allowed the identification of five major anthocyanin's – i.e. delphinidin-3-O-glucoside (Dp3gl), cyanidin (Cy3gl), petunidin (Pt3gl), peonidin-3-O-glucoside (Po3gl) and malvidin-3-O-glucoside (Mv3gl).

These compounds are commonly identified in red grape skins (Corrales et al., 2009; Zhao et al., 2010; Kammerer



**Fig. 4 – Qualitative assessment of the effects of OH pretreatments on aqueous extraction through Principal Component Analysis (PCA) using measurement variables such as (a) electrical conductivity, (b) soluble solids, (c) total phenolic compounds, (d) anthocyanins concentration, (e) absorbance at 520 nm and (f) turbidity at 600 nm. Untreated samples are enclosed by an ellipse at 95% confidence level.**

**Table 2 – Concentration of anthocyanin's ( $\mu\text{g/g DW}$ ) in aqueous extracts identified through HPLC.**

Concentration/ $\mu\text{g/g DW}$	Untreated	COV40	OH40	OH100
Dp3gl	<LOQ ( $163.8 \pm 7.3$ ) <sup>a</sup>	<LOQ ( $173.8 \pm 8.1$ ) <sup>a</sup>	<LOQ ( $192.5 \pm 23.9$ ) <sup>a</sup>	$269.0 \pm 36.1$ <sup>b</sup>
Cy3gl	<LOQ ( $184.1 \pm 17.3$ ) <sup>a</sup>	<LOQ ( $190.6 \pm 10.3$ ) <sup>a</sup>	<LOQ ( $206.9 \pm 11.5$ ) <sup>a</sup>	$293.7 \pm 69.0$ <sup>b</sup>
Pt3gl	$118.1 \pm 6.2$ <sup>a</sup>	$128.6 \pm 4.8$ <sup>a</sup>	$141.8 \pm 21.4$ <sup>a</sup>	$203.5 \pm 31.3$ <sup>b</sup>
Po3gl	<LOD ( $94.1 \pm 3.0$ ) <sup>a</sup>	<LOD ( $93.2 \pm 1.5$ ) <sup>a</sup>	<LOD ( $79.1 \pm 30.3$ ) <sup>a</sup>	<LOD $100 \pm 6.2$ <sup>b</sup>
Mv3gl	$444.4 \pm 7.9$ <sup>a</sup>	$477.1 \pm 41.3$ <sup>a</sup>	$549 \pm 115.7$ <sup>a</sup>	$876.4 \pm 142.7$ <sup>b</sup>
Total	$756.2 \pm 78.1$ <sup>a</sup>	$779.5 \pm 37.7$ <sup>a</sup>	$883.3 \pm 159.7$ <sup>a</sup>	$1348.8 \pm 208.1$ <sup>b</sup>

For each anthocyanin, means in the same row with different letters are significantly different ( $p < 0.05$ ).

et al., 2004). The percentage of phytochemicals extracted in dried grape pomace can be deeply influenced by the degradation imposed by the type of drying and way of storage. However, commercial dried grape pomace still retains large amounts of phenolic and anthocyanins in their composition. About six different types of anthocyanins are usually identified and quantified after different extraction procedures using as source material a commercial dried grape pomace, as follows: Cy3gl, Dp3gl, Mv3gl, pelargonidin-3-O-glucoside, Po3gl, and Pt3gl (Ramirez-Lopez and DeWitt, 2014). This profile is in agreement with anthocyanin's identified in this study. Moreover the total anthocyanin's concentration determined through HPLC - ranging from 750 to 1350  $\mu\text{g/g DW}$  - is well comparable with ones obtained through pH differential method, which ranged from approximately from 250 to 630  $\mu\text{g/g DW}$  (see Fig. 3). (Corrales et al., 2009) reported a total concentration of anthocyanin monoglucosides in grapes skin ranging from 5 to 7  $\text{mg/g DW}$ , using HHP as pre-treatment followed by extraction with ethanol, where Mv3gl corresponded to 60% of the extract. Results reported here maintain the same proportion of Mv3gl towards total concentration of anthocyanin monoglucosides (ranging from 58 to 60%). The maximum total concentration was obtained with OH100 treatment (i.e. 1.4  $\text{mg/g DW}$ ), which is slightly lower than the latter study but this can be justified by the use water instead of ethanol during extraction procedure.

Additionally, applied pretreatments did not change qualitative composition of identified anthocyanins on untreated grape skins. Previous works have shown that HTST processing can enhance mass transfer and thus higher solubilization and release kinetics of value added cell components such as anthocyanins (El Darra et al., 2013a; Lambri et al., 2015; Corrales et al., 2009). However, conventional thermal treatments applied in wine industry, such as thermovinification (at  $\sim 70^\circ\text{C}$  in less than 1 h) or flash heating (at temperatures ranging from 85 to  $95^\circ\text{C}$ , in less than 10 min), can also result in degradation of anthocyanins and also require high energy input - i.e. from 160 to 250  $\text{kJ/kg}$  (Maza et al., 2019).

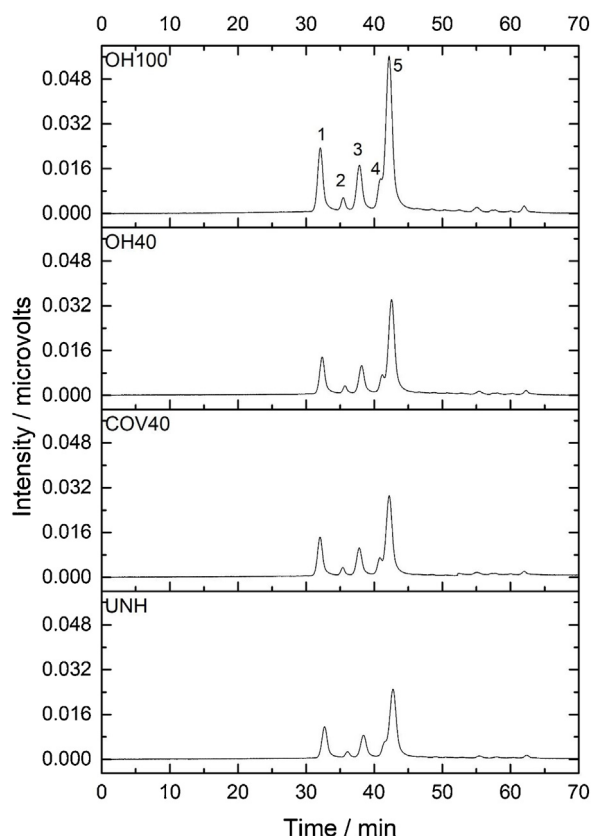
In this case, all thermal treatments provided an incremental increase of anthocyanins concentration in particular when OH was applied. This was evident for aqueous extracts that resulted from OH100 pretreatment, showing an increase of total anthocyanins content of about 70% when compared with extracts from untreated samples (UNH).

### 3.4. Energy consumption

The use of physical extraction techniques are often limited to the threshold level of energy input to promote disruption of cellular materials and thus promote extraction. The levels of specific energy consumption of novel techniques such as HDEV, PEF and US on the extraction of bioactive compounds

**Table 3 – Energy consumption of performed OH pre-treatments and their comparison with other extraction techniques (thermal and non-thermal) using grapes or derived by products.**

Treatment	Electrical conditions	Time of treatment	Target temperature/ °C	Energy input consumption/kj/kg	Ref
OH40	10 V/cm/25 kHz	20 min	40	21.1 ± 3.3	Present study
OH100	70 V/cm/25 kHz	30 s	100	46.4 ± 14.7	
COV40	–	20 min	40	314	
PEF	0.8 kV/cm	100 ms	–	42	El Darra et al. (2013b)
PEF	5 kV/cm	1 ms	–	53	El Darra et al. (2013b)
PEF	13 kV/cm	10 μs	<30 °C	up to 564	Barba et al. (2015)
PEF	0.4 kV/cm	5 s	45 °C	38.1	El Darra et al. (2013a)
PEF	0.1 kV/cm	210 s	82 °C	178.8	El Darra et al. (2013a)
US	24 kHz	5 min	Na	121	El
US	24 kHz	10 min	Na	242	Darra
US	24 kHz	15 min	Na	363	et al. (2013b)

**Fig. 5 – Example of typical chromatograms of skin grape extract before and after pre-treatments (UNH, COV40, OH40 and OH100) where the following anthocyanin's were identified: 1) Dp3gl; 2) Cy3gl; 3) Pt3gl; 4) Po3gl; and 5) Mv3gl.**

from grape pomace increase drastically with increasing intensity of treatment conditions - such as electric field, number of pulses, treatment time (even in the range of ms) and temperature. Typical values for energy consumption of grape non-thermal pretreatments (prior to fermentation) using PEF and US range approximately between 40 to 50 kJ/kg and 120 to 360 kJ/kg, respectively (El Darra et al., 2013b). When PEF treatments are applied using typical conditions of OH technology (i.e. low electric field intensity, longer treatment times and high temperatures) energy consumption drastically increases to 178.8 kJ/kg, well above the one observed in OH.

Even when compared with treatments that combine PEF at mild temperature conditions (<50 °C), OH can attain equivalent energy consumption with the advantage of using high temperatures. Heating brings increased mass transfer (thus

reducing the use of organic solvents) and has an important microbiological benefit regarding extract quality. Depending on the level of extraction required, energy consumption can go up to 218, 564 and 2727 kJ/kg even at room temperature (<30 °C) for HDEV, PEF and US technologies, respectively (Barba et al., 2015). Table 3 shows energy consumption values for OH treatments performed in this work, as well as published data regarding the use of non-thermal technologies such as PEF and US to extract compounds from grape skins or pomace. Nonetheless, the inevitable differences of experimental protocols, nature of the technologies and material source, this work allows an approximate overview of the energy values involved in the different processes. OH immediately brings the advantage of a thermal process with high energetic efficiency using grape biomass as source of internal heat (no use of water as heat transfer medium). In these set of experiments energy consumption of OH100 was below 50 kJ/kg; more than 300 kJ/kg would be required taking into consideration an indirect heating process to achieve a temperature increase of water from 25 to 100 °C (with  $\Delta T = 75$  °C, and  $C_p = 4.18$  kJ/kg °C). Energy consumption of OH treatments (even at high temperatures) is well comparable with PEF treatments and even lower when compared with PEF treatments performed at high temperatures and moderate electric field (El Darra et al., 2013b), in which energy consumption can be higher than 100 kJ/kg.

#### 4. Conclusions

OH can be effectively applied to anthocyanin extraction from grape skins. OH and its joule effect (internal heat generation), avoids the use chemical solvents, reduces treatment times and the use of water, and lowers energy consumption when compared with conventional thermal processes used in wine industry. Given thermal nature of OH it was possible to establish a direct flash-heating extraction process thus allowing permeabilization of tissues without promoting thermal degradation of the molecules of interest. HPLC analysis confirm that total contents of extracted anthocyanins in water increased markedly (two times more) with OH100 pretreatment when compared with untreated samples. It was also evident that even at mild temperatures, OH can be potentially used to increase extraction of anthocyanins and this strategy should be further investigated for the selective recovery of labile molecules from fresh or unprocessed material. Another advantage of using HTST method relies on microbiological and enzymatic inactivation of the remaining grape lignocellulosic residues that have recognized potential to be used for the development of other value added products. From molecular

standpoint electrical and internal heat effects are intimately attached and both may have contributed to the enhanced permeabilization of grape skin tissues observed in these results. This research contributes to body of knowledge regarding the use of an alternative extraction strategies that reduce thermal load and chemical extraction (use of hazardous organic solvents). OH technology brings the opportunity to exploit the synergy between heat and electric fields effects through an efficient and environmentally friendly way towards food bioeconomy.

## Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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