Ohmic heating for polyphenol extraction from grape berries: an innovative prefermentary process

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ABSTRACT
High energy consumption is often required to increase the extraction of phenolic compounds from grapes during alcoholic fermentation. Processes such as thermovinification require significant temperature changes over a long period of time to ensure the diffusion of phenolic compounds from the grape skin layer to the must. In this study, the capability of the ohmic heating (OH) process (E = 55 V/cm, t = 60–90 s, T = 72 °C) to improve the extractability of valuable intracellular compounds from grape skins of Aglianico and Barbera grape matrices before the alcoholic fermentation step was investigated and compared with both untreated and conventional thermally (CH) treated (T = 72 °C, t = 90 s) samples. Total phenolics and antioxidant capacity were monitored during fermentation (10 days). In comparison to the conventional thermal treatment, the results showed that the phenolic compound content of musts was twice as high immediately after OH treatment. This process could drastically improve the classic prefermentary maceration (thermovinifications, cold macerations, etc.) time. In finished wines produced from the treated musts, the total polyphenolic content of OH wines was up to 17 % higher than that of CH wines, and 30 % higher than that of untreated wines. No differences in concentrations of total tannins and anthocyanins were observed between conventional and ohmic heated musts. However, an increase of 30 to 200 % for some aromatic esters was observed in wines from ohmic heated musts. Overall, the outcomes of this work proved that, in addition to the thermal effect, the moderate electric field (MEF) applied during ohmic heating has the potential to induce an instantaneous release of polyphenolic compounds due to the electroporation phenomenon of cell membranes, thus saving energy and reducing processing time.

KEYWORDS
Ohmic heating, wine, extraction, polyphenol, electroporation.
INTRODUCTION

From an oenological point of view, extracting phenolic compounds (e.g., anthocyanins, tannins and their polymers) from grapes during the maceration/fermentation phase shows great interest. The presence of these compounds determines the quality of red wines in terms of their structure, colour and mouthfeel, as well as their beneficial health properties (El Khawand et al., 2018). The phenolic content and composition of wine depends on the initial content in the grapes, which is a function of both the variety and cultivation factors, as well as the winemaking techniques (Donsì et al., 2013; Icier and Ilicali, 2005). Different winemaking techniques will also influence the final phenolic concentration of wines, as well as their chemical composition (in terms of, for example, sulfites, alcohol content and acidity) (Nioi et al., 2020; Yammine et al., 2020). Over the past 30 years, several modifications have been noticed in grape composition; for example, there is more sugar and less organic acids, resulting in a higher pH (van Leeuwen and Destrac-Irvine, 2017). However, only a fraction of the large amount of different phenolic compounds can be extracted using traditional winemaking techniques, with about 40% of anthocyanins and 20% of tannins from grape skins being transferred to the wine. This is because polyphenols and anthocyanins are mainly contained within the vacuoles of the hypodermal cells; therefore, their extraction encounters two main resistances to mass transfer in the form of the vacuole membrane and the cell membrane respectively (Donsì et al., 2010).

Traditional techniques for obtaining wines of high phenolic content consist in extending the maceration time beyond the time required for fermentation by up to 3 or 4 weeks (Bautista-Ortin et al., 2005). Additionally, they can involve physical treatments (e.g., thermovinification, must freezing and flash détente) or the use of maceration enzymes (pectolytic enzymes) to induce damage to the cell and vacuole membranes, and thus improve the extraction of phenolic compounds (Maza et al., 2019; El Darra et al., 2013; Donsì et al., 2010). These methods typically involve long production times and high energy costs, which are significant drawbacks that create a need for gentler and more efficient processing technologies for improving the extraction of phenolic compounds from the grape skin cells during the maceration step.

Therefore, improving the extraction processes is of great interest for liquid-phase alcoholic fermentation.

In the light of this, the use of electro-technologies such as Pulsed Electric Fields (PEF) and Ohmic Heating (OH), also known as Moderate Electric Fields (MEF), are becoming more popular. They intensify the extractability of the target intracellular compounds, due to the applied electric field (1-5 kV/cm for PEF, and < 1 kV/cm for OH) causing electroporation of the plant cell membranes (Lebovka et al., 2007; Pataro et al., 2017; Praporscic et al., 2006; Sensoy and Sastry, 2004; Vorobiev and Lebovka, 2013). In OH processing, the food product (liquid, solid or particulate-liquid mixture) is placed in direct contact with the electrodes of an ohmic heater across which an electric field is applied in the form of sinusoidal or bipolar pulsed square wave with frequencies ranging from 50 Hz to 100 kHz (Sarkis et al., 2013). The main advantage of OH is its ability to heat materials rapidly and uniformly, including particulate foods, while ensuring a highly efficient energy transfer (Pereira et al., 2016). This reduces the processing time and results in improved flavour (no temperature gradient and no hot wall effects) and nutrient retention, leading to a higher quality product from both a nutritional and organoleptic point of view. The potential of the combined electro-thermal effect of OH technology to significantly improve the extraction yields of target intracellular compounds (e.g., polyphenols and pectin) from different foods and food wastes during the extraction process has already been demonstrated (El Darra et al., 2013; Loypimai et al., 2015; Pereira et al., 2016). For instance, Pereira et al. (2016) found that the application of OH treatment (15 V/cm, 90 °C, 10 min) on potatoes enhanced the extractability of phenolic compounds by 30% compared to a conventional heating process (90 °C, 10 min). The authors attributed the greater capacity of OH in promoting mass transfer to the electroporation phenomena which occurs along with sample heating. Similarly, when Loypimai et al. (2015) investigated the influence of OH treatments on the recovery of anthocyanins from rice bran, they found that the concentrations of cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, delphinidin and malvidin in extracts were 60, 12, 17 and 7% higher respectively in comparison with conventional solvent-assisted steam extraction. Pereira et al. (2020) studied the possibility of obtaining aqueous extracts of anthocyanin from winemaking residues (grape skins) through Ohmic Heating (OH) treatment.
Two different electric pretreatments (40 °C for 20 min) and flash heating from 40 to 100 °C in less than 20 s were evaluated. In both cases, OH increased the extraction of total phenolic compounds and soluble solids, and increased the intensity of the red colour. When applied at high temperature and for a short time, OH induces anthocyanins to increase by 756 to 1349 g/g (+78%). These results show that OH have potential as an efficient and environmentally friendly technology which can contribute to a shift towards more sustainable processes in the food industry.

The use of OH in winemaking could thus potentially improve the extractability of polyphenols located in the vacuoles of crushed grape cells, thereby improving wine quality while reducing energy consumption. To date, no publication has dealt with the application of ohmic heating to must and its impact on the finished wine. The present study aimed to investigate the use of ohmic heating with MEF for improving the extraction of useful compounds during winemaking and its effects compared with those of conventional thermal treatment of crushed grapes. The chemical, physical and organoleptic properties of musts and wines obtained from untreated (control), OH or conventionally thermal treated samples were evaluated.

MATERIALS AND METHODS

1. Raw material

In this study, a blend of the two grape varieties Barbera and Aglianico was used. The grapes were harvested at their optimum ripening degree in 2016 from vineyards located in the province of Avellino (Italy).

During winemaking, both grape varieties were manually destemmed for all the steps (control, conventional heating and ohmic heating), weighed, equally mixed (50/50 w/w) and crushed. The resulting batches of must were subsequently subjected to either conventional or OH treatment prior to the alcoholic fermentation phase. Some batches did not undergo any treatment; these were the control samples.

2. Ohmic and conventional heating treatments

Ohmic heating treatments were performed in a laboratory batch heating system previously described in detail by Guida et al. (2013) (Figure 1). Briefly, the system consisted of a static ohmic heater comprising an open cylindrical polycarbonate tube (8.5 cm in diameter, 20 cm in length) with a disk-shaped stainless steel (type AISI 316 L) electrode with Teflon pressure caps on each side.

FIGURE 1. Schematic representation of the ohmic heating system.
The electrodes were connected to a 15 kW high frequency (25 kHz) pulsed power generator (model GR1520, Micropi Elettronica, Saviano, Italy) able to provide an electric current in the form of bipolar square waves at 25 kHz with adjustable amplitude (from 0 to 1500 V_{RMS}) and duty cycle (0 – 100 %), and limited only by a maximum current value of 20A. An integrated data-logger was employed to continuously and simultaneously record current intensity, voltage and product temperature.

For each OH treatment, 1 kg of must with an electrical conductivity of 1.2 mS/cm at 25 °C was loaded into the ohmic heater. Starting from an initial temperature of 22 ± 2 °C, the must was heated by applying a constant gradient voltage of 55 V/cm (duty cycle of 100 %) to reach a final temperature of 72 ± 2 °C for two different heating durations of 60 s (OH1) and 90 s (OH2). Once the set point temperature of 72 °C was reached, the samples were maintained at this temperature for a holding time of 15 s by reducing the applied voltage to a value well below the one used during the heating phase. Next, stabilisation at an ambient temperature (30 °C) was ensured by using an ice water bath for all the steps (for less than 25 seconds). A long cold plate was used on which the liquid circulated on its way to to a large refrigerated tank to ensure the cooling process. The temperature of the sample was measured by using a type-T teflon-coated thermocouple (1 mm in diameter) placed at the geometric centre of the ohmic cell.

For the conventional heating (CH) process, the must was put into flexible pouches made of multilayer (polyethylene-aluminum-polypropylene) film and immediately placed into a water bath set at 95 °C in order to obtain same thermal profiles to the ohmic heating experiments. Once the target temperature of 72 °C was reached, samples were placed in a second water bath set at 72 °C for 15 s. The temperature of the samples was monitored using an ice-water bath prior to fermentation.

At the end of both the OH and CH treatments, the samples were immediately cooled down to 25 °C in an ice-water bath prior to fermentation.

3. System performance calculations

The energy delivered to the samples during the OH treatments ($Q_{oh}$) was calculated according to Eq.1 using the actual values of current ($I$) and voltage ($U$) recorded by the data logger during each experimental run.

$$Q_{OH} = \int_{0}^{t} U(t) \cdot I(t) \, dt$$  \hspace{1cm} \text{(Equation 1)}$$

This energy was compared to the theoretical energy required to conventionally heat ($Q_{th}$) the samples by temperature gradient (Eq. 2):

$$Q_{th} = mC_p\Delta T$$  \hspace{1cm} \text{(Equation 2)}$$

where $m$ is the treated mass (kg), $\Delta T$ is the difference between final and initial temperature of samples (°C), and $C_p$ is the specific heat of must. The latter had been estimated by Colombié et al. (2007) as 3.6 kJ.kg⁻¹.K⁻¹ for must containing 200 g/L of sugar.

The ratio between the theoretical and OH energy (r) needed to heat the samples was calculated according to Eq. 3.

$$r = Q_{th}/Q_{OH}$$  \hspace{1cm} \text{(Equation 3)}$$

The energy cost of ohmic heating in this experiment was estimated as < 250 kJ/kg, which is 40 to 50 % lower than a classic thermovinification treatment (420 kJ/kg) as reported by Darra et al. (2013).

TABLE 1. System performance calculations.

|       | kJ/kg | kWh/kg | R   |
|-------|-------|--------|-----|
| $Q_{oh}$ | 192   | 0.053  | 1   |
| $Q_{OH1}$ | 228   | 0.063  | 0.84|
| $Q_{OH2}$ | 241   | 0.067  | 0.8 |

4. Winemaking procedure

Immediately after the CH and OH treatments, 3 g/L of SO2 was added to triplicate batches of 3 kg of untreated and treated samples to inhibit indigenous yeast. After a day long delay (ensuring a decrease in SO₂), musts were inoculated with the dry active yeasts *S.cerevisiae* FX10 (20 g/hL) (Laffort®, Bordeaux, France). Alcoholic fermentations were then conducted at 28 °C (± 1 °C) in 5L glass containers with plastic stoppers filled with water, and cap-punched daily and monitored by taking densitometric measurements (Densito 30 PX, Mettler Toledo, Columbus, OH). The end of the alcoholic fermentation was set as the time at which must volumetric mass decreased to 995 g/L. Afterwards, each batch was lightly pressed (< 1 bar) in a manually operated basket press (EnologicaMeola, Italy) to obtain fresh wines, which were subsequently conditioned into glass bottles.
In order to perform malolactic fermentations, the wines were inoculated with lactic bacteria *O. oeni* SB3 (Laffort®, Bordeaux, France) and stored at 20 °C. Malolactic fermentations were monitored with Randox RX (Monza, Italy) and considered complete when malic acid concentrations were inferior to 0.2 g/L.

5. Analyses of must and wines

5.1. Polyphenol Index

The polyphenol index was evaluated using the classical Folin-Ciocalteu Assay (FC). Briefly, in a 100 mL graduated flask, 1 mL of diluted wine (1/10 in distilled water, v/v) was mixed with 20 mL of 20 % Na$_2$CO$_3$ and 5 mL of Folin Ciocalteu reagent and completed with distilled water. After 30 min, the optical density at 760 nm in a quartz cuvette (1 cm optical path) was measured with a UV/Vis spectrophotometer (Jasco Inc., Easton, USA). The results were expressed as mg of gallic acid equivalent (GAE) per L of must. The $I_{fc}$ was measured during alcoholic fermentation.

5.2. Total Polyphenols Index (TPI)

The wine was diluted to 1/100 (v/v) in water and the optical density was measured at 280 nm in a 1 cm quartz cuvette. The result was expressed as TPI = OD x dilution.

5.3. Evaluation of ferric reducing antioxidant power (FRAP)

The antioxidant power of the musts was evaluated via a FRAP assay, following the procedure reported by Benzie and Strain (1996) with some modifications. For the analysis, 2.5 mL of freshly prepared FRAP working solution and 0.5 mL of 1/10 (v/v) diluted must were mixed and incubated for 10 min at ambient temperature. The observed change in absorbance was due to the reduction of ferric tripyridyl triazine (Fe III-TPTZ) complex by the antioxidants present in the samples, which was spectrophotometrically monitored at 593 nm against FRAP solution (blank). Ascorbic acid (Acros Organics, Geel, Belgium) was used as the standard for the calibration curve, and the FRAP values were expressed as mmol of ascorbic acid equivalents (mmol AAE) per L of must.

5.4. Quantification of total tannins and anthocyanins

The analysis of total tannin concentration was based on the transformation of proanthocyanidins into anthocyanidins by heating in acidic conditions. In two glass tubes, 4 mL of diluted wine (1/50 in distilled water, v/v), 2 mL of distilled water and 6 mL of HCl 12 N were mixed. One tube was placed in ice and the other in a hot water bath (100 °C) for 30 min. Afterwards, 1 mL of ethanol was added to the reactive mixtures. The optical density was measured at 550 nm with a 1 cm optical path length. The difference in optical density ($\Delta d_{550nm}$) between the two tubes provided the concentration in tannins, expressed in g/L as reported in Eq. (4) (Ribéreau-Gayon and Stonestreet, 1965):

$$C_{TAN} = 19.33 \times \Delta d_{550nm}$$

(Equation 4)

The total anthocyanin concentration analysis was based on sodium bisulfite bleaching (Ribéreau-Gayon and Stonestreet, 1965). A solution comprising 1 mL of wine, 1 mL of ethanol (0.1 % HCL) and 20 mL of HCl at 2 % was prepared. In one glass tube, 10 mL of this solution was mixed with 4 mL of distilled water. In another tube, the solution was mixed with 4 mL of sodium bisulfite 15 % (w/w). Optical densities at 520 nm (1 cm optical path length) were spectrophotometrically measured after 20 min. The difference in optical density ($\Delta d_{520nm}$) between the two tubes provided the concentration in mg/L as reported in Eq. (5):

$$C_{ANTH} = 875 \times \Delta d_{520nm}$$

(Equation 5)

5.5. Colour measurement of wines

Changes in the $L^*a^*b^*$ colour space were measured with a Konica Minolta CM-5 spectrophotometer (1 cm optical path length in a quartz cuvette). The Lab colour space mathematically describes all perceivable colours in the three dimensions using $L^*$ for lightness, the numbers 0 (black) to 100 (transparent) and $a^*$ and $b^*$ for the colour components green–red and blue–yellow. Differences in colour can be determined by numerically comparing the colour of a sample to that of the standard. This difference indicates the variation absolute colour coordinates and is referred to as Delta ($\Delta$). The general differences between the colour of the control and that of the treated samples are reported as $\Delta E_{ab}$.

5.6. Ester quantification by SPME GC-MS

For the ester analysis, the samples were prepared using solid-phase microextraction (SPME) following the protocol established by Antalick et al. (2010). The solution of sample and internal standards was homogenised and then loaded into a autosampling device. Gas chromatography analyses were carried out on an HP 5890 GC system coupled to an HP 5972 quadrupole mass spectrometer.
A BP21 capillary column (50 m, 0.32 mm, 0.25 µm film thickness; SGE, Courtaboeuf, France) was used and the carrier gas was helium N55 with a column-head pressure of 8 psi. The oven temperature was programmed at 40 °C for 5 min, then at a rate of 3 °C/min it was raised to 220 °C, at which it was held for 30 min. The mass spectrometer was operated in electron ionisation mode at 70 eV with selected-ion-monitoring (SIM) mode. For the quantification of the esters, 20 µl of a stock solution of internal standards comprising ethyl-d5 butyrate, ethyl-d5 hexanoate, ethyl-d5 octanoate and ethyl-d5 cinnamate at about 200 mg/L each in absolute ethanol was added to 25 ml of the samples.

6. Sensorial analyses

Sensorial analyses were conducted to evaluate any qualitative differences between wines obtained from untreated and treated musts. Triangle test was performed to determine whether there were any differences between each couple of samples of the finished wine. Three coded samples in black ISO glasses were presented to 44 panelists with moderate to expert experience in wine tasting from the ISVV (University of Bordeaux, France). The samples were evaluated in individual booths with a controlled room temperature of 20 °C (NF EN ISO 8589:2007). The panelists were presented with the samples in random order and asked to identify the different samples via olfactory examination.

In order to characterise the differences, 11 panelists were asked to provide a descriptive profile of each wine using seven distinctive parameters: aromatic intensity, fruity, fermentative, floral, empyreumatic, vegetal and global preference.

7. Statistical analysis

ANOVA with 5 % confidence interval was performed on the collected data (XLSTAT Software) followed by the Tukey HSD test. The results of the triangle test on the sensorial analyses were analysed by applying the binomial law corresponding to the distribution of answers in this type of test, with 5 % level of confidence.

RESULTS AND DISCUSSION

1. Temperature profiles

Figure 2 shows the heating curves from 20 to 72 °C obtained during both the CH and OH treatments. During the first 30 s, higher temperatures were achieved for the conventional thermal treatment. The profiles of both OH treatments were similar. Any differences in polyphenol extraction rate can be attributed to the electroporation effect due to the applied field strength during OH treatment.

![FIGURE 2. Heating curves of crushed grape berries during conventional heating (CH) and OH at different heating durations (60 s for OH1 and 90 s for OH2).](image-url)
2. System performance calculations

The electrical energy provided to the system and calculated using the current and voltage recorded during the OH experiments was compared to the theoretical energy required to heat the samples without any thermal loss. The r values obtained were 0.84 for OH1 and 0.80 for OH2 (Table 1). Icier and Ilicali (2005) reported values of 0.67 at 55 V/cm for apricot puree. The difference in r values between the two OH treatments may be due to the absence of thermal insulation of the ohmic cell. Coherently, shorter treatment times led to lower energy losses to the surrounding environment, as previously reported in the work of Darvishi et al. (2013) and Icier and Ilicali (2005). However, attention should be paid to the experimental conditions in the present study (i.e., non insulated walls), which could be drastically improved in an industrial design, leading to better yields.

3. Alcoholic fermentation

Following the thermal treatments, must loads of 3 kg were sulfited at 3 g/hL and inoculated after a 24 h delay with S. cerevisiae FX10 at 20 g/hL in glass vessels. The alcoholic fermentation was held at a constant temperature of 28 °C. Figure 3 shows that 11 days were necessary to ensure a complete fermentation process, regardless of the sample. As similarly reported by Donsi et al. (2010), tissue damage to grape skins occurring after the application of either conventional or ohmic heating was not found to influence the rate of fermentation.

Figures 4 and 5 show the kinetics of the polyphenol index and FRAP antioxidant activity respectively for untreated and thermally processed musts over time. The musts obtained following the ohmic heating process clearly show a significantly (p < 0.05) higher total polyphenol concentration throughout the duration of the fermentation in comparison to the untreated must. After one day, the total polyphenol concentration differed between the OH treatments and CH/control by 675 mg/L; after 6 days, it differed by 470 mg/L. At the beginning of fermentation, the FC of must subjected to conventional heating differed slightly to that of the control (+ 188 mg/L), which increased until the last observation day, when no statistical differences with OH treated samples were detected (p > 0.05), regardless of the processing duration. However, at the beginning of the fermentation step (days 1 - 2), the total polyphenol concentration of the OH treated samples was double that of the conventionally treated samples. The extraction of skin tannins and anthocyanins starts at the early stages of maceration, even in the absence of ethanol. A short maceration time results in wines with low proanthocyanidin concentration and low astringency, because the seeds and skins are in contact with the musts for a shorter period of time.
FIGURE 4. Kinetics of Folin Ciocalteu Assay (FC) of musts during alcoholic fermentation for untreated, conventionally heated (CH) and OH treated crushed grapes at different heating durations (OH1: 60 s; OH2: 90 s).

FIGURE 5. Kinetics of Ferric reducing antioxidant power (FRAP) of the musts during alcoholic fermentation for untreated, conventionally heated (CH) and OH crushed grapes at different heating durations (OH1: 60 s; OH2: 90 s).
A long maceration step, however, will lead to wines with higher proanthocyanidin concentration (Canals et al., 2005). Therefore, as regards the OH process, a short maceration time could be considered to limit the diffusion of tannins from the seeds.

To consolidate these results, the FRAP (Figure 5) of the musts were also measured during fermentation. Significant antioxidant activity related to phenolic content has been reported by Chira et al., 2008. The curves follow the same trends of those in Figure 2 and could be linked to the evolution in polyphenol concentration. In particular, Figure 5 shows that after one day of fermentation the FRAP of the OH treated samples was triple that of the untreated musts (6 mmol/L versus 2 mmol/L). Once again, no differences in FRAP were observed between the CH and OH treated samples at the end of the fermentation process (p > 0.05), which is in accordance with the similar concentrations of polyphenols found in the musts.

Further chemical and sensory analyses on the finished wines (i.e., obtained after malolactic fermentation) were also carried out in this study with the purpose of detecting any differences between the samples in terms of phenolic composition and aromas.

### 4. Wine analysis

Five months after alcoholic and malolactic fermentation, the wines underwent physicochemical and sensorial analysis. The results in terms of total tannins, anthocyanins and TPI are summarised in Table 2. Significant differences (p < 0.05) were found between the control, CH and OH musts: OH2 treated musts showed a 30 % increase compared to the control, and a 17 % increase compared to CH. These results indicate that the moderate electric field may have had a slight influence on polyphenol extraction during the OH treatment, despite the results obtained from the Folin Ciocalteau Assays during alcoholic fermentation. Regarding total tannin concentration, no significant differences were observed between the CH and OH treatments. Independently of the thermal effects, these results indicate that moderate electric fields do not have a significant impact on tannin extraction. Whatever the thermal treatment (CH or OH), the anthocyanin concentration was always greater than the evaluated one for untreated samples, despite no significant differences between samples being revealed (p > 0.05). It is commonly known that thermal treatment increases anthocyanin concentration (El Darra et al., 2013), but the extractability of phenolic compounds can

### Table 2. Tannin, anthocyanin and TPI measurements on finished wines. Different letters in the same column indicate significant differences between the mean values (p < 0.05).

|            | Total tannins (mg/L) | Total anthocyanins (mg/L) | TPI       |
|------------|----------------------|---------------------------|-----------|
| Control    | 3421 ± 164           | 903 ± 81                  | 64.4 ± 2.1|
| CH         | 4591 ± 342           | 1087 ± 41                 | 72.5 ± 0.7|
| OH1        | 4349 ± 369           | 1023 ± 99                 | 77.4 ± 1.2|
| OH2        | 4233 ± 102           | 1016 ± 50                 | 84.1 ± 4.0|

### Table 3. Concentration (in µg/L) of the main esters in finished wines from untreated and thermally (CH/OH) treated musts derived from GC-SPME. (a, b and c indicate significant differences between the control, CH, OH1 and OH2 at a 95 % confidence level).

| Perception threshold | Control | CH | OH1 | OH2 |
|----------------------|---------|----|-----|-----|
| Ethyl propanoate     | 2100    | 149.0 ± 18 | 192.2 ± 20 | 246.4 ± 20 | 245.1 ± 25 |
| Ethyl decanoate      | 200     | 6.0 ± 0.7  | 10.6 ± 1  | 12.1 ± 1,2 | 12.1 ± 1,5 |
| Ethyl dodecanoate    | NA      | 4.9 ± 0.6  | 7.2 ± 0.8 | 8.0 ± 0.9  | 6.3 ± 0.8  |
| Propyl acetate       | NA      | 5.9 ± 0.7  | 17.9 ± 2  | 19.4 ± 2   | 18.7 ± 2   |
| Isoamyl acetate      | 2       | 365.2 ± 40 | 310.5 ± 35 | 481.1 ± 50 | 491.7 ± 50 |
| Ethyl isobutyrate    | 0.1     | 88.3 ± 9   | 86.0 ± 9  | 140.2 ± 15 | 151.7 ± 18 |
| Phenylethyl acetate  | 250     | 41.9 ± 5   | 41.2 ± 5  | 65.7 ± 8   | 62.1 ± 8   |
| Ethyl dihydrocinnamate| 1.6    | 1.4 ± 0.2  | 1.6 ± 0.2 | 0.7 ± 0.1  | 0.9 ± 0.1  |
| Ethyl cinnamate      | 1.1     | 1.7 ± 0.2  | 1.9 ± 0.2 | 0.7 ± 0.1  | 0.8 ± 0.1  |
vary significantly depending on grape variety (Netzel et al., 2003; Ortega-Regules et al., 2008).

Similar to the results obtained by Pereira et al. (2020) on wine must residues, anthocyanins also increased in the present study: by 20% for conventional heating and by 12 - 14% for ohmic heating treatments. The grape skin was not in the same physiological state, but an impact on the extractability of these compounds should be noted. In terms of aromatic impact, the concentrations of only 9 out of 32 esters commonly present in red wine were found to significantly differ when comparing the OH treatments and control. Six of these 9 esters were significantly different in OH and CH (Table 3). When taking into account the perception threshold mentioned in the literature (Antalick et al., 2010), the significantly higher levels of isoamyl acetate (banana aroma) and ethyl isobutyrate (strawberry, fruity) in the wines from OH treated samples in comparison to the control and CH samples may have had a potential impact on the sensorial properties of the OH wines.

**TABLE 4.** L*a*b* colour analyses of thermally treated samples (Δ: difference between control and sample) (p < 0.05).

|       | ΔL*     | Δa*     | Δb*     | ΔE*ab   |
|-------|---------|---------|---------|---------|
| OH1   | -1.20 ± 0.18 a | -3.19 ± 0.40 a | -2.02 ± 0.28 a | 5.09 ± 0.52 a |
| OH2   | -1.45 ± 0.19 a | -3.75 ± 0.52 a | -2.42 ± 0.33 a | 4.36 ± 0.65 a |
| CH    | -0.46 ± 0.18 b | -1.28 ± 0.45 b | -0.73 ± 0.31 b | 3.97 ± 0.59 b |

**TABLE 5.** Results of the triangle tests for the coupled comparison of finished wines obtained from untreated musts (control), and conventional (CH) and ohmic (OH) treated musts (p < 0.05).

|                  | Correct answers | Wrong answers | Total | Significativity |
|------------------|-----------------|---------------|-------|----------------|
| OH1 vs control   | 24              | 20            | 44    | S              |
| OH2 vs control   | 24              | 20            | 44    | S              |
| CH vs control    | 19              | 25            | 44    | NS             |
| OH1 vs CH        | 29              | 15            | 44    | S              |

**FIGURE 6.** Results of the descriptive analysis carried out by the panel on the wines obtained from untreated (control), conventional (CH) and ohmic (OH) treated musts.
Other ester concentrations were below the perception threshold and no conclusions could be drawn other than that significant differences exist. These differences may be due to the improvement in the extraction of ester precursor compounds resulting from the combined effects of heating and electroporation during ohmic heating. Indeed, precursor compounds, such as amino acids in berries, are the basis for the production of higher alcohols and therefore esters (Quilter et al., 2003).

In terms of L*a*b* colour changes in the samples (Table 4), ∆a* and ∆b* decreased, corresponding to a very light browning of treated samples and more specifically to OH treated samples. A slight decrease in clarity (∆L*) is also observed in the treated samples with respect to the untreated ones. According to Martínez et al. (2001), an overall colour change (∆E*ab) higher than 3 can be detected by the human eye. These colour changes may be directly related to the higher concentrations of extractable compounds (Bakker et al., 1998).

Based on these results, 44 panelists carried out triangle tests (orthonasal olfaction) in order to determine whether there were any perceptible differences between the studied wines. The results of these tests are shown in Table 5. At a 95 % confidence interval, the OH treated wine samples were perceived as being different to both the control and the CH treated samples, which were both found to be similar. After this test, 11 panelists were asked to perform an olfactory descriptive analysis of the wines. The chosen descriptors were aromatic intensity, fruity, fermentative, floral, empyreumatic, vegetal and global preference (Figure 4).

Due to the low number of panelists, the statistical analyses did not show any significant differences between the investigated samples (p > 0.05). However, both OH wines were described as being less vegetal than the CH and the control; the CH and OH wines were perceived as being fruitier than the control; and the OH wines received the highest global preference score. These results may be linked to the ester concentrations (Table 3) may be linked to increases in ester concentrations leading to increases in fruity aromas.

The panelists did not perceive any clear differences in the floral, fermentative, and empyreumatic characteristics of the samples. These sensory characteristics mainly depend on the grape variety and the compounds it contains; additional experiments should therefore be carried out on other grape varieties at different stages of maturity.

CONCLUSIONS

In this study, the extraction potential of the ohmic heating treatment of grape must before alcoholic fermentation during the winemaking process was evaluated. The results show that the thermal-electric effect of OH processing causes the rupture of grape skin cell membranes, and thus a subsequent improvement in the release of phenolic compounds into the musts compared to conventionally treated samples.

In particular, the concentrations of phenolic compounds in the finished wines produced from ohmic heated musts are similar to those produced from musts subjected to traditional heat treatment (+10 to 30 % compared to wines produced from untreated musts), such as thermovinification. However, an immediate release of phenolic compounds was observed immediately after the ohmic treatments, highlighting the effect of electroporation. Therefore, this process could be a great improvement over traditional methods for wines produced from liquid-phase fermentation. In addition, a significant increase (from 30 to 200 %) in the concentrations of various esters was observed for musts subjected to the ohmic heating process compared to untreated musts or those subjected to conventional heating. Further studies would be required on the extraction of precursor compounds using moderate electric fields.

A direct comparison with hot and cold prefermentary macerations, as well as with thermovinification would be a logical follow-up to this study. Compared to these classic thermal treatments, similar extraction yields would be obtained from ohmic heating over a shorter period of time, leading to energy and time saving. Furthermore, different grape varieties should be studied in order to increase knowledge about grape MEF extraction potential. In all cases, ohmic heating shows great potential in terms of providing innovation and increasing employment in the winemaking sector.

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