beverages

Article

Pilot Scale Continuous Pulsed Electric Fields Treatments for Vinification and Stabilization of Arinto and Moscatel Graúdo (*Vitis vinifera* L.) White Grape Varieties: Effects on Sensory and Physico-Chemical Quality of Wines

Filipa Vinagre Marques Silva [1,*], Riccardo Borgo [1], Andrea Guanziroli [1], Jorge M. Ricardo-da-Silva [1], Mafalda Aguiar-Macedo [2] and Luis M. Redondo [3,*]

[1] LEAF—Linking Landscape, Environment, Agriculture and Food, Associate Laboratory TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisbon, Portugal
[2] EnergyPulse Systems, Lda, Pólo Tecnológico de Lisboa, 1600-546 Lisbon, Portugal
[3] Pulsed Power Advanced Applications Group, Instituto Superior de Engenharia de Lisboa, GIAAPP/ISEL, Rua Conselheiro Emídio Navarro 1, 1959-007 Lisbon, Portugal
* Correspondence: fvsilva@isa.ulisboa.pt (F.V.M.S.); lmredondo@deea.isel.ipl.pt (L.M.R.)

Abstract: Pulsed electric field (PEF) processing of white grapes (Arinto, Moscatel Graúdo also known as Moscatel de Setúbal) before pressing for must extraction (1.2 and 1.6 kV/cm) and before bottling for finished wine physical stabilization (10 kV/cm) was implemented in a pilot-scale winery to produce about 540 L of wine for each variety. PEF was applied at these two different stages of wine production, and its effects on the sensory and physico-chemical quality of the wines were investigated. The sensory triangle tests revealed no significant change in both wine varieties' colour, odour, and taste with PEF extraction and PEF stabilization treatments. However, for colour coordinates assessed with a spectrophotometer, a significant increase in CIE b* colour coordinate was registered for PEF-extracted Arinto and Moscatel wine samples, showing a development of a more intense yellow colour. Concerning physico-chemical quality parameters, the PEF extraction increased both wine varieties' turbidity and pH, although total acidity was not affected. The total phenols also increased in Arinto with extraction. The second PEF treatment applied for wine stabilization did not affect any of the quality parameters, except total phenols, which decreased in Moscatel wine. The results encourage the application of PEF in the wineries at different stages of vinification of white wine grape varieties.

Keywords: PEF; continuous; pilot scale; vinification; decontamination; non-thermal pasteurisation; Moscatel de Setúbal; muscat; white wine; table wine; colour; flavour

Citation: Silva, F.V.M.; Borgo, R.; Guanziroli, A.; Ricardo-da-Silva, J.M.; Aguiar-Macedo, M.; Redondo, L.M. Pilot Scale Continuous Pulsed Electric Fields Treatments for Vinification and Stabilization of Arinto and Moscatel Graúdo (*Vitis vinifera* L.) White Grape Varieties: Effects on Sensory and Physico-Chemical Quality of Wines. *Beverages* **2024**, *10*, 6. https://doi.org/10.3390/beverages10010006

Academic Editor: Elena Bartkiene

Received: 30 October 2023
Revised: 28 December 2023
Accepted: 3 January 2024
Published: 8 January 2024

1. Introduction

During the last two decades, non-thermal preservation methods have developed and gained interest in the food industry in response to consumer demand for products with higher quality and safety. The main advantages of these processes are the increase in food quality, safety, and shelf life by both avoiding the negative effects of high temperatures and reducing the necessity of food additives [1–3]. With technological progress, the introduction of new techniques can also increase the food and beverage industries' competitiveness, sustainability, and productivity [4]. Studies applying high-pressure processing, ultrasound, and pulsed electric fields (PEF) to preserve/stabilise alcoholic beverages, such as beer and wine, have been carried out [5–10]. Among these technologies, PEF is one of the most appropriate for beverages' preservation, as it can be set in continuous operation for industrial-scale production [5,11]. PEF can be used at various stages of vinification for must or wine decontamination through the electroporation of wine spoilage microorganisms, thus reducing SO_2 additions [12,13] (Figure 1).

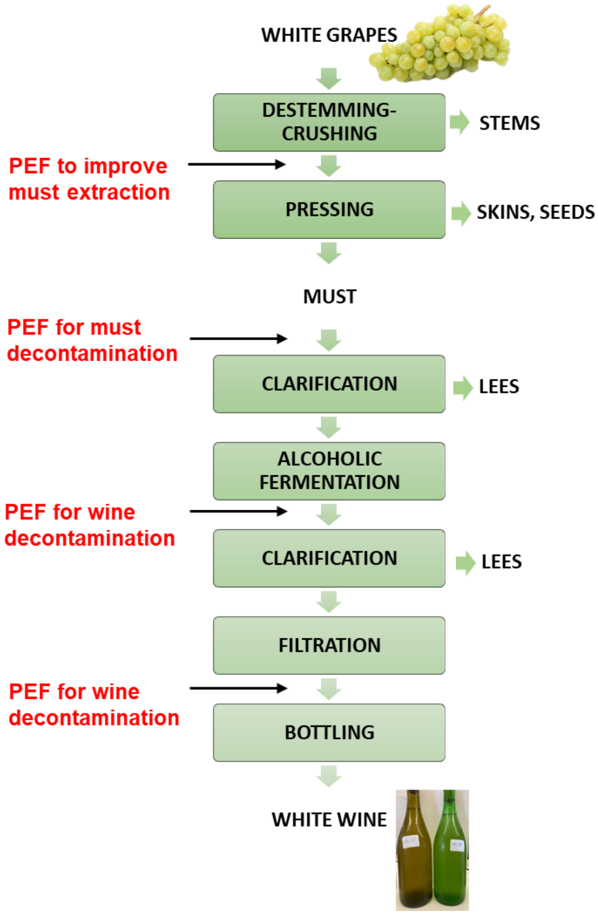


Figure 1. White wine vinification and potential applications of pulsed electric field technology (PEF) at different stages of production.

The other application of PEF is at the grape level to promote juice extraction at the beginning of vinification. High-voltage electrical impulses are also capable of forming micropores on the grape membrane cells, electroporating them. Electroporation of grape cells results in an increase in the permeability of the cytoplasmic membrane, which promotes the transport of intracellular components to the outside of the cell and vice versa [14,15]. At early stages of production, electroporation of skin and pulp of grapes' cells by PEF can promote mass transfer, which in this case translates into optimization of the grape juice extraction contained inside the grape cells (Figure 1). With the resolution OIV-OENO 634-2020, the International Organization of Vine and Wine (OIV) approved the use of this technology at the grape level, with the objective of facilitating and increasing the extraction of valuable substances, such as polyphenols, available nitrogen for yeasts, aroma compounds (including precursors and other substances located inside the grape cells), and, in the case of red varieties, also reducing maceration time [16,17].

A very important part of white wine vinification is the addition of sulphur dioxide (SO_2), which has multiple effects, the most considerable being its antimicrobial properties, consisting of the prevention of the proliferation of unwanted bacteria and yeasts, and antioxidant properties, contributing to the protection of the main components of the wine such as phenolic compounds and aromatic substances [18,19]. SO_2 also inhibits the activity of oxidoreductases such as polyphenoloxidase which prevents enzymatic browning reactions in must and wine [19]. Sulphur dioxide is usually added at various stages in the production of a white wine, in particular to the grapes, to the must, and lastly to the wine at each racking and pre-bottling [19]. It is important to mention that sulphur dioxide has negative effects on consumers if consumed in excessive quantities, and therefore a maximum of 200 mg/L or ppm has been set for white wines in the European

Union (Commission Delegated Regulation (EU) 2019/934 of 12 March 2019 supplementing Regulation (EU) No. 1308/2013 of the European Parliament and of the Council).

Arinto is a Portuguese vine variety widely cultivated in the entire country, with particular emphasis on DOC "Denominação de Origem Controlada" (designation of controlled origin) Bucelas, which generally shows a good affinity with traditional rootstocks. From an oenological point of view, Arinto wines generally have an open citrus colour and good acidity, with aromas of citrus fruits and sometimes some minerals, moderate intensity, and a high aging capacity. Wine made from Arinto grapes is light to medium in body, high in acid, and has flavours of lemon, apple, chalky minerality, and occasionally tropical undertones. Although it is frequently mixed with other grapes to provide acid to white wines, it can also be produced as a single varietal, particularly in the Bucelas region. A PEF study carried out with Arinto grapes revealed an increase of 5.47% in the *cuvée* wine (1 kV/cm) [20]. The Moscatel Graúdo, from the Setúbal region of Portugal, is a variety with medium-sized leaves, rounded, and three lobos. The bunch is large and conical, with wings and a long peduncle, while the berries are large, ovate, and yellowish green. Berries have medium-thick skin and firm flesh. From an oenological point of view, this variety is used to produce dessert and sweet wines, as well as table wines. It presents a high level of terpenoid compounds that confer high aromaticity to the final product. Wines can be aged, especially sweet ones, and can be generally described as strong, straw coloured, with an intense muscat aroma that is very persistent.

Few studies combining the effect of PEF extraction and PEF stabilization on white wine quality have been carried out, in particular at a pilot scale and using continuous operation. Therefore, in this work, continuous PEF was employed in a pilot-scale winery for the vinification and stabilization of two white grape varieties from Portugal (Arinto and Moscatel Graúdo) for the production of table wines. The main goal was to assess the colour, aroma, taste, and physico-chemical quality properties of the finished wines. The specific objectives of the experiments were:

(i) To characterise the organoleptic properties of the two wine varieties;
(ii) to use PEF in continuous mode to extract must from grapes at a pilot-scale winery;
(iii) to compare the quality of wine produced from must extracted using PEF technology vs. wine produced from must conventionally extracted;
(iv) to treat finished wine with continuous PEF at a pilot-scale winery before bottling;
(v) to compare the quality of wine stabilised with PEF vs. the quality of conventional wine not submitted to a PEF treatment before bottling, aiming for a reduction in the amount of pre-bottling added with SO_2.

2. Materials and Methods

In this study, PEF technology was applied to the production and stabilization of two table white wines, Arinto (or Pedernã) and Moscatel Graúdo (or Moscatel de Setúbal).

2.1. Vinification

Approximately 1 tonne of high-quality Arinto grape clusters was purchased from Quinta do Cerrado da Porta, located in Sobral de Monte Agraço, Lisbon, in 2021. The timing of the harvest was chosen after considering the climate and the ripeness of the grapes (Brix, total acidity, pH). All grapes' clusters were hand-picked on the day of harvest, sorted into boxes of 15 kg each, and then brought immediately to the pilot-scale lab/winery located in Gouveia. The grape clusters were split into two portions of roughly equal weight (500 kg), being one of them subjected to pulsed electric fields for must extraction optimization (PEF1) while the other was left untreated (CE = conventional extraction). These two lots of grapes were allowed to dilute grape homogeneity problems as grapes from the same provenience (*terroir*) and grown in similar biological, environmental, and farming practices were used for the vinification. Both lots of grape clusters were crushed/destemmed, where berries were separated from the stems and crushed. Then, the juice and solid parts of the grape fell into the receiving hopper of the peristaltic pump. The heliflex hoses linked the peristaltic pump, which pumped the must through the PEF treatment chamber (see Section 2.3.1), leading it to its final destiny, the pneumatic press (Bucher Vaslin®XPro 5, Chalonnes-sur-Loire, France). For the control (conventionally extracted musts), the same system was used but without the application of PEF. During the process of pressing, only the first part of must—free-run must (*lágrima*), obtained by applying 1 bar for 20 min—was collected and used for vinification and further testing and analysis, as this will produce a higher-quality wine for quality testing. For Moscatel Graúdo, 270 L of control must and 265.7 L of PEF must were obtained from 518 and 503 kg of grape clusters. "Lágrima" must samples were collected for quality control of vinification progress. After pressing, the must was moved to a tank, SO_2 (5 g/hL) was added, and the temperature was lowered to 10 °C for 48 h to promote static cold settling, leading to the deposition of suspended particles at the bottom of the tank. No enzymes or additives were used for accelerating must or wine clarification. After a first rack, a yeast nutrient composed of ammonium sulphate, ammonium biphosphate, and thiamin-hydrochlorid (Enovit—AEB Group, Brescia, Italy) was added to the must (20 g/hL) to promote yeast growth, and the temperature was raised to 14–16 °C. Then, the must was inoculated with *Saccharomyces cerevisiae* (20 g/hL, Fermol Complete Killer Fru—AEB Group, Brescia, Italy) to allow better control of microbial species involved in the alcoholic fermentation (AF) process. During AF, which lasted about 15 days, each vat was monitored twice a day for density and temperature. When the density dropped below 1000 with glucose + fructose < 2 g/L, AF was deemed to be finished. The vats were closed once there were no visible gas bubbles. The alcoholic fermentation finished at the end of September 2021. After AF, and when necessary, until the quality analyses (May 2022), the level of free SO_2 in all tanks was corrected to 38 mg/L by adding SO_2. During this period, static settling took place, aided by the low winter temperatures of the region. The alcoholic content and volatile acidity of finished Arinto wine were 13.6% (v/v or alcohol by volume) and 0.41 g acetic acid/L. Moscatel Graúdo was characterised by 12.13% (v/v) alcohol and 0.17 g acetic acid/L of volatile acidity.

2.2. Experimental Design

For each wine variety, four samples of finished wines were submitted to the following treatments and stored in different vats: Conventional extraction of must (CE), pulsed electric field-assisted extraction of must (PEF1), and the same wine samples submitted to a pulsed electric field stabilization treatment (PEF2) before bottling (CE + PEF2, PEF1 + PEF2) (Figure 2). The CE was the control sample used to study the effect of PEF1 and PEF2 on wine quality. All wine was bottled directly into antique green Burgundy bottles (0.75 L), closed with 4.0 × 2.5 cm cork stoppers (Neutrocork®, Amorim, Portugal) with the aid of a manual wine corker. The wines were stored at 20 °C until analysis. Sensory tests and physico-chemical analyses were performed with Arinto and Moscatel Graúdo wine samples submitted to different treatments.

Sensory triangle tests (colour, flavour) with the finished Arinto and Moscatel Graúdo wines were carried out to compare: (i) wines vinified from must conventionally extracted (CE) and PEF-assisted extracted must (PEF1); (ii) wines stabilised with PEF treatment (CE + PEF2, PEF1 + PEF2) with control wines (CE, PEF1).

With respect to physico-chemical parameters, the following analyses were carried out with Arinto and Moscatel wines in two different bottles of the same wine: Electrical conductivity (in CE and PEF1 wine samples), analytical L*a*b* CIE colour coordinates, total phenols, flavonoids and non-flavonoids, turbidity, pH, and total acidity. No microbiology analyses were performed, as the experiment's main focus was to assess the quality parameters.

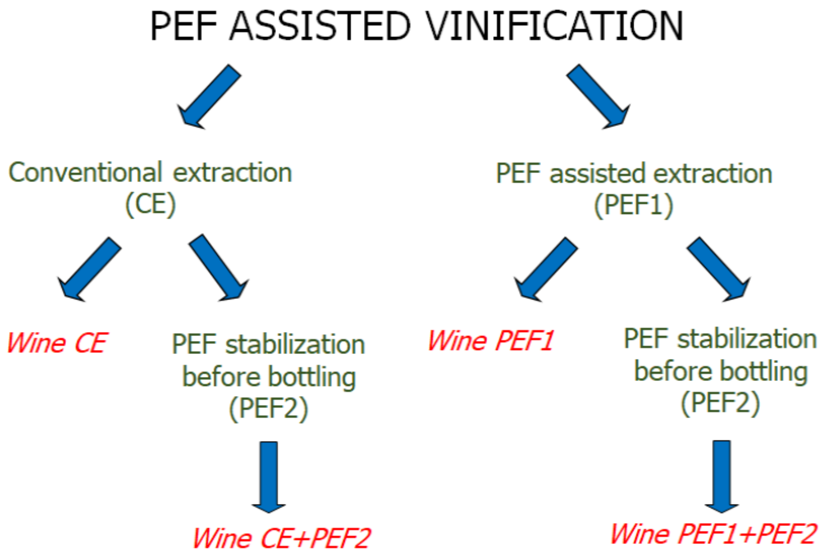
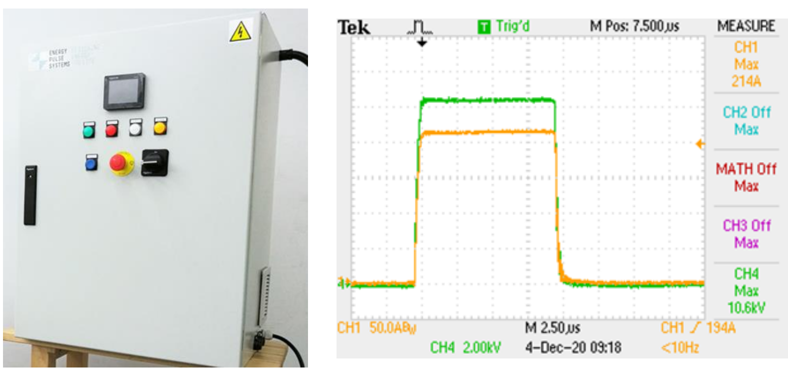


Figure 2. Pulsed electric field (PEF)-assisted vinification: four wine samples produced.

Due to the large scale of the experiments conducted in a winery (500 kg of grapes were required for conventional extraction and 500 kg for PEF extraction), it was not possible to conduct replicates of the grape CE and PEF extraction treatments.

2.3. PEF Treatments

In this study, PEF operating in continuous mode was applied at a pilot-scale winery in two stages of wine production: (i) At the early stages for must extraction before pressing (PEF1), and after (ii) for stabilization/decontamination of finished wine before bottling (PEF2). The PEF equipment includes a high-voltage pulse generator with a control unit connected to a treatment chamber where the electric pulses are conveyed to the product. A solid-state Marx generator was used to generate electric pulses [21]. The high-voltage pulse generator was designed and set by the EnergyPulse System (Figure 3) and is able to produce both monopolar and bipolar pulses. The pulse generator control unit (model EPULSUS-PM1A-10) allows independent control of the applied voltage (0–10 kV), pulse width (1–200 µs), and electrical current intensity (up to 200 A), with a maximum power of 3 kW. It is the most expensive part of the PEF equipment and was used for the two PEF treatments applied at different stages of vinification. Only the treatment chambers were different for PEF1 and PEF2 treatments (see Sections 2.3.1 and 2.3.2).



(**a**) (**b**)

Figure 3. Model EPULSUS-PM1A-10 with a control unit, designed and set by EnergyPulse Systems: photos of the equipment (**a**); typical wave shape for 10 µs pulse width, 10 kV voltage and 200 A current intensity (**b**).

2.3.1. PEF Aided Juice Extraction Treatment before Pressing

The treatment applied to the Arinto grapes after crushing and destemming was 6 kV, equivalent to 1.2 kV/cm field strength (electrode gap distance of 50 mm), 30 A of current, with monopolar pulses of 50 µs width and 100 Hz frequency. Given that it is more difficult to press Moscatel Graúdo grapes due to their cellular structure, a higher value of field intensity was applied to Moscatel grapes. Therefore, for Moscatel Graúdo grapes, the same frequency and pulse width were used, with 8 kV, equivalent to 1.6 kV/cm, resulting in 50 A current intensity. Four tonnes/h of crushed and destemmed grapes flowed in a pipe with a 50 mm diameter (DN50) and a residence time of 176.7 ms. A co-linear treatment chamber composed of three (i.e., the middle one was connected to a high voltage generator output and the end ones to ground) stainless steel electrodes was used (Figure 4). The chamber was set vertically to avoid air entering the PEF treatment chamber and electrical discharges.

Figure 4. Co-linear pulsed electric fields treatment chamber with vertical configuration, used for must extraction before pressing (PEF1) (red arrow indicated the PEF treatment chamber designed byby EnergyPulse Systems).

2.3.2. PEF Stabilization Treatment before Bottling

PEF stabilization can also be referred to as PEF decontamination or PEF non-thermal pasteurisation of wine. The PEF treatment parameters were selected in accordance with the literature and the maximum capacity of the pilot-scale PEF system. Since a co-linear treatment chamber was used (Figure 5), the electric field strength is not homogeneously distributed. 200 L/h of wine flowed in a 10 mm pipe (DN10). The residence time was 28.3 ms. The processing parameters were 10 kV, monopolar pulses of 25 microseconds width, and a frequency of 150 Hz. As the distance between electrodes is 10 mm, the electric field intensity was 10 kV/cm. The real PEF treatment time was 106 microseconds. The measured current in the Arinto and Moscatel Graúdo wines was 85 A and 104 A, respectively, as the electrical conductivity of Moscatel was higher than that of Arinto. The specific energy delivered to Arinto wine with these processing conditions was estimated at 60 kJ/kg and caused a 14 °C increase in the wine temperature. For Moscatel, the specific energy delivered was 70 kJ/kg with a 16 °C increase in temperature, and this treatment was efficient for total yeast reduction from 1.2×10^3 cfu/mL of CE wine to no colonies detected in 1 mL of PEF stabilised wines, CE + PEF2 and PEF1 + PEF2.

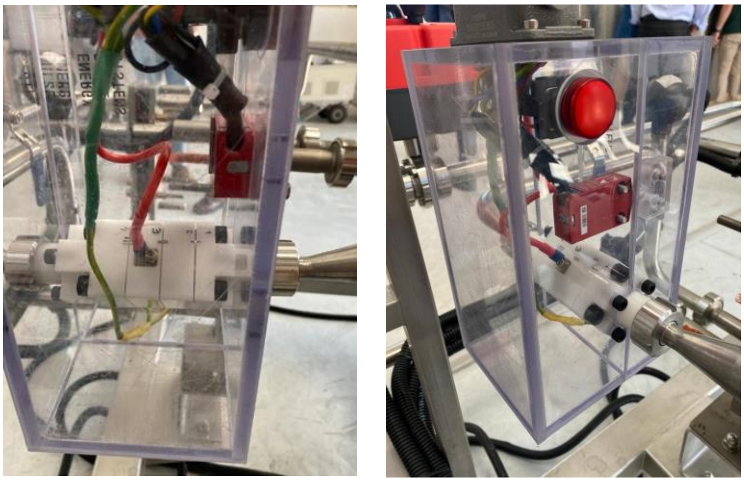


Figure 5. Co-linear pulsed electric fields treatment chamber used for wine stabilization before bottling (PEF2) (designed by EnergyPulse Systems).

2.4. Sensory Assessments

2.4.1. Wine Sensory Characterization

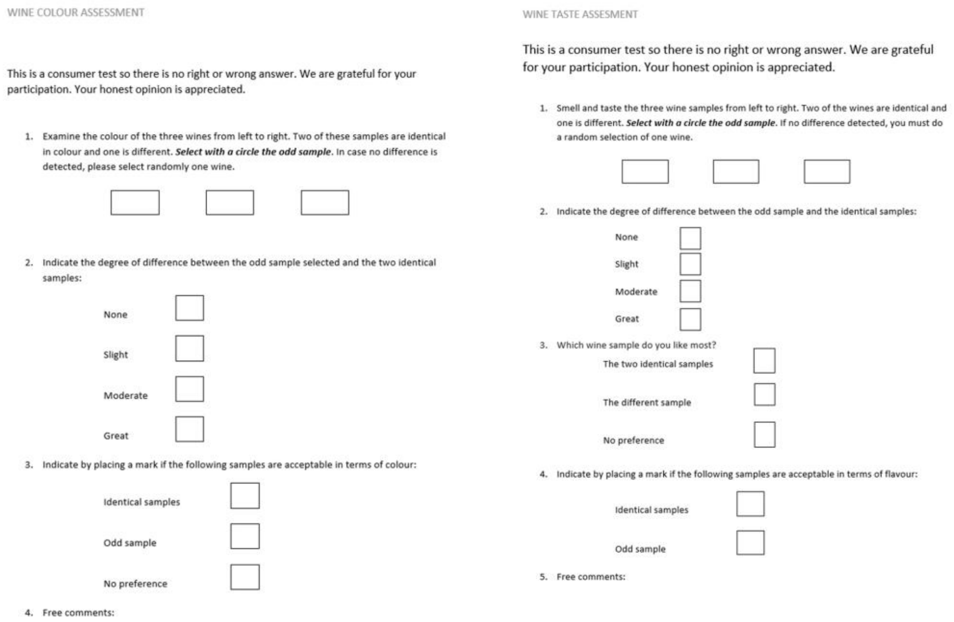
A complete sensory evaluation of the untreated wine with must conventionally extracted (control sample) was performed using a tasting sheet for table wine as a guide ("Ficha Auxiliar de Prova") from the Instituto Superior de Agronomia [22]. First, a visual exam of the wine was carried out, followed by a smell, and lastly a taste examination. The retronasal aroma was also assessed. The exam finished with the classification of global wine quality and the identification of defects, if detected.

2.4.2. Consumer Triangle Test for Colour and Overall Flavour

A sensory panel composed of twenty-four or more panellists with experience in sensory analysis, regular wine drinkers but not wine experts, was chosen to better represent general wine consumers. Following ISO Standard n. 4120 for the triangle test [23], three samples of wine coded with different 3-digit numbers, in which two identical and one different wine samples were presented to each judge. The panellists were asked (and forced) to choose the odd wine sample. A tasting sheet with instructions was presented to each panellist together with the 3 samples of wine (Figure 6). First, a triangle test was performed for wine colour, and then another triangle test was carried out for the flavour (aroma and taste) of the same wines. Briefly, 30–40 mL of wine samples was presented to panellists in ISO-standard wine glasses at 12 °C in isolated tasting booths with a randomised wine presentation order across judges. The first set of wines to compare were the PEF1 sample (PEF extraction of must) and the wine produced with conventional extraction (CE), which did not undergo any treatment. The other sensory tests were carried out to investigate the effect of PEF stabilization (PEF2) on CE and PEF1 samples, so PEF1 was compared with PEF1 + PEF2 (PEF1 wine stabilised by PEF2 before bottling) for both wine varieties. Moscatel CE and CE + PEF2 (CE wine stabilised by PEF2 before bottling) were also assessed and compared.

In addition to the triangle test with the 3 samples presented, the panellists answered a few questions about the 3 wines, namely, to classify the degree of difference between the identical and odd samples (none, slight, moderate, great), then which wine sample the panellist liked best, and finally, a question to obtain information about the wine's acceptability in terms of colour and taste. In addition, there was a section for free comments.

For the analysis of triangle test results, if the % of correct responses is greater or equal to 54% (for $n = 24$, $\alpha = 0.05$), taken from Table A1 of the ISO Standard n. 4120 [23], there is a perceptible difference between the 2 wine samples submitted to different treatments.



WINE COLOUR ASSESSMENT

This is a consumer test so there is no right or wrong answer. We are grateful for your participation. Your honest opinion is appreciated.

1. Examine the colour of the three wines from left to right. Two of these samples are identical in colour and one is different. ***Select with a circle the odd sample***. In case no difference is detected, please select randomly one wine.

2. Indicate the degree of difference between the odd sample selected and the two identical samples:

   None

   Slight

   Moderate

   Great

3. Indicate by placing a mark if the following samples are acceptable in terms of colour:

   Identical samples

   Odd sample

   No preference

4. Free comments:

WINE TASTE ASSESMENT

This is a consumer test so there is no right or wrong answer. We are grateful for your participation. Your honest opinion is appreciated.

1. Smell and taste the three wine samples from left to right. Two of the wines are identical and one is different. ***Select with a circle the odd sample***. If no difference detected, you must do a random selection of one wine.

2. Indicate the degree of difference between the odd sample and the identical samples:

   None

   Slight

   Moderate

   Great

3. Which wine sample do you like most?

   The two identical samples

   The different sample

   No preference

4. Indicate by placing a mark if the following samples are acceptable in terms of flavour:

   Identical samples

   Odd sample

5. Free comments:

Figure 6. Taste sheets used by panellists in the triangle tests carried out for colour and flavour (the 3 blank spaces in number one were filled with the different numbers of 3 digits used for each wine sample identification).

2.5. Physico-Chemical Analysis

All analyses were carried out with Arinto and Moscatel Graúdo wine samples at the wine laboratory of the Instituto Superior de Agronomia in two different bottles of the same wine and each bottle wine sample was analysed twice. The average ± standard deviation for the two bottles of each wine sample was calculated.

2.5.1. Electrical Conductivity of Wine from Conventional Extraction (CE) vs. Wine Produced with PEF Assisted Extraction (PEF1)

The electrical conductivity of wine samples was measured with a Thermo Scientific Orion Star A212 Benchtop Conductivity Meter (Thermo Fisher Scientific, Indonesia). The electrode was washed with distilled water and calibrated with two standards. The electrode was washed and dried before taking readings of different samples.

2.5.2. Colour Coordinates (CIE-L*a*b*)

The International Commission on Illumination (CIE) developed the L*a*b* colour space in 1976 with the intent of creating a standard for colour communication. The use of this methodology allows the measurement of colour coordinates and differences in colour not perceptible to the human eye. The chromatic characteristics of a wine colour are defined by the colorimetric or chromaticity coordinates: lightness or clarity (L*, 0—black colour to 100—white colour), green/red colour component (a*), and blue/yellow colour component (b*).

A UV–Vis spectrophotometer (Agilent Cary 100 UV-Vis) was used for transmittance measurements at a wavelength of between 300 and 800 nm (measured every 5 nm), with the Illuminant D65 and observer placed at 10°, according to Method OIV-MA-AS2-11 [24]. Sampling must respect all the concepts of homogeneity and representativeness. For this reason, the first step performed was the centrifugation of the different samples to remove any opacity. Next, the cuvette pair for spectrophotometric reading was selected, i.e., cuvettes of 10 mm optical thickness. After obtaining and preparing the sample, the transmittance from 380 to 780 nm was measured every 5 nm, using distilled water as a reference in a cuvette with the same optical thickness, in order to establish the baseline or white line. Cary WinUV software on a computer linked to the spectrophotometer computed the colorimetric coordinates (L*, a* and b*) from the transmittance measurements.

2.5.3. Total Phenols, Flavonoids and Non-Flavonoids

Total phenols can be divided into two categories: Flavonoids and non-flavonoids, which are a large group of different chemical compounds that influence taste, colour and mouth sensation. First, the wine was centrifuged at 5000 rpm for 10 min at 20 °C. Then a decimal dilution of the wine was undertaken by mixing 10 mL of wine in a 100 mL volumetric flask with distilled water. Total phenol content was expressed in mg of gallic acid equivalent per litre of wine (mg GAE/L) and can be determined by measuring the wine absorbance at 280 nm from the calibration line of concentration of gallic acid (mg/L) vs. absorbance [25]. The absorbance reading at 280 nm wavelength was performed on the Agilent Technologies Cary Series UV-Vis Spectrophotometer using distilled water for the blank reading.

The Kramling and Singleton method [26] allows the determination of an estimate of non-flavonoids. The method was adapted with a few modifications as follows: Centrifuge wine samples at 5000 rpm for 10 min at 20 °C (the same procedure used for total phenols), take 10 mL of wine to a test tube containing 10 mL of HCl with a dilution coefficient of 1:4 and then add 5 mL of formaldehyde (8 mg/mL). A quantity of nitrogen was then added to the test tube, which was then closed and vortexed. The test tube was placed in a light-free place for 24 h. This procedure will cause the precipitation of flavonoid compounds through a reaction with formaldehyde under specific conditions at room temperature and low pH. The next day, centrifugation was performed again for 10 min at 3500 rpm, and then 5 mL of wine solution (containing 2 mL of wine) was diluted in a 20 mL volumetric flask with distilled water. Finally, a spectrophotometric reading was taken at 280 nm wavelength and converted to mg of gallic acid equivalents/L using the calibration line. The amount of flavonoids can be obtained by difference (Equation (1)):

$$\text{Flavonoids (mg/L)} = \text{Total phenols} - \text{Non flavonoids} \tag{1}$$

2.5.4. Turbidity

Turbidity is an optical effect produced by the presence of extremely fine particles scattered in a liquid-dispersion medium. The Hach 2100N IS Turbidimeter (Loveland, CO, USA) was used to obtain an evaluation of the degree of turbidity of the wines under examination using the ISO 7027 method. By measuring the incident light scattered at right angles, the concentration of suspended particles in a liquid sample was calculated. The unit of measurement for turbidity used is the NTU—nephelometric turbidity unit, which is the value corresponding to the measurement of the light diffused by a standard suspension at a 90° angle to the direction of the incident beam. Methacrylate 10 × 10 mm disposable cuvettes were used. These cuvettes allow the passage of a broad range of wavelengths, maximizing the incident light entering the sample, which allows for more accurate turbidity detection. The first step performed was the calibration of the equipment using 4 samples placed inside 4 cuvettes with reference turbidities of <0.1 NTU, 20 NTU, 200 NTU, and 1000 NTU. Each of these cuvettes was placed within the corresponding space in the machine, and the turbidity was calculated for 60 s in "Calibrate" mode. Then the actual turbidity measurement for the different wine samples was carried out by placing the wine inside specific cuvettes. Two of these were the control samples, while the sample treated with pulsed electric field technology for must extraction was identified as PEF1, and the sample treated pre-bottling for wine pasteurisation was identified as PEF2. The measurement was repeated 3 times for each sample for a duration of 60 s each [27].

2.5.5. pH and Total Acidity

The Thermo Scientific Orion Star A211 pH meter (Thermo Fisher Scientific, Indonesia) was calibrated with reference buffer solutions with a pH of 4, 7, or 9. Then, the pH was measured. A tiny beaker was filled with around 10 mL of wine, and the pH was determined at 20 °C. The wine was manually stirred to ensure homogeneity.

For total acidity determination, the OIV-MA-AS313-01 (Type I method) was followed [28]. First, carbon dioxide was subtracted from the 50 mL sample using a vacuum pump for approximately 60 s while continuously agitating the flask containing the wine. After this, a potentiometric titration was carried out with a solution of sodium hydroxide, 0.1 mol/L, in 10 mL of CO_2-free wine, added to 30 mL of boiled distilled water, and 1 mL of bromothymol blue to serve as an indicator. Titration was carried out by adding the sodium hydroxide solution until the colour changed to blue-green. The total acidity (A′) expressed in grams of tartaric acid per litre is given by: $A' = 0.075 \times A$, where A is 10 times (10×) the volume of sodium hydroxide solution 0.1 mol/L added in the titration. The result is given in two decimal places.

2.5.6. Statistical Analysis

First, an ANOVA was carried out with all replicates to investigate if there was any effect of the PEF1 extraction or PEF stabilization on the results. When $p < 0.05$, a Tukey test was conducted to separate the means. In the results, different letters after the parameter averages were used to indicate a significant difference between the means. The average ± standard deviation was calculated and presented in Tables and Figures.

3. Results and Discussion

3.1. Sensory Evaluation of Arinto and Moscatel Wines

3.1.1. Characterization of Arinto and Moscatel Graúdo Wines

The Arinto wine was limpid and characterised by a yellow citrine colour. With respect to the olfactive exam, the wine presented an intense smell with fine sufficient persistence. Major attributes detected with smell were complex, fresh, floral, fruity (banana, green apple, peach, lemon peel), characteristic odours from the Arinto variety, and no smell defects. With respect to the taste exam, it has a dry sweetness, very high acidity, is alcoholic-warm, and has a light body. Taste also revealed a new-age wine that was not balanced but pleasant. However, the global appreciation of the wine was reasonable and without defects.

With respect to Moscatel table wine, the visual exam revealed a citrine yellow colour, very similar in the two samples, limpid, and without relevant particles, even if the wine produced with PEF-assisted extraction of must had a slightly higher turbidity. From the olfactory exam, the wine resulted in sufficient quality, high intensity, and persistency, in line with what could be expected from the Moscatel variety. General characters were associated with floral, fruity, and varietal aromas. More precisely, some litchi and pineapple smell attributes were present. From a tasting point of view, four main parameters were taken into account, namely sweetness, alcoholicity, body, and acidity. Wine was soft, sweet, and light-bodied, with a high acidity, while alcohol was not so perceptible. Overall, it appeared balanced and quite pleasant. Retro-nasal aromas were sufficient, intense, and persistent. In sum, the CE and PEF1 wines were very similar, except for a slight off-flavour in the PEF-extracted wine (PEF1 sample). However, this defect could not be directly associated with the treatment.

3.1.2. Triangle Test to Compare Finished Wines with Musts Produced by Conventional Extraction (CE) vs. Pulsed Electric Field Extraction (PEF1): Effect of PEF Extraction on Colour and Flavour

For both Arinto and Moscatel Graúdo wines, there was not a significant difference in colour and flavour (aroma and taste) between the wine produced from conventional extraction (CE) and the wine produced from PEF extraction of must (PEF 1), which means the PEF extraction did not significantly affect the wine colour and flavour sensory characteristics (Figure 7).

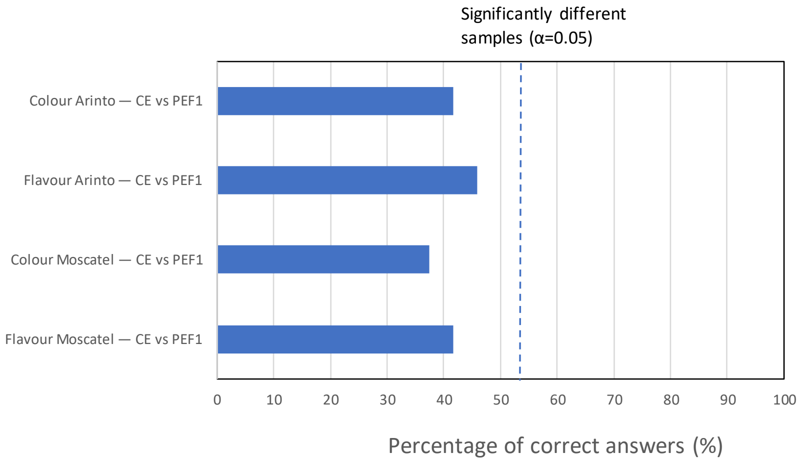


Figure 7. Results of triangle test to compare the colour and taste of white wines (Arinto and Moscatel Graúdo) vinified from must extracted by pulsed electric fields (PEF1) and conventionally extracted (CE). Blue bars crossing the significance line mean the wine samples are statistically different (n = 24 panellists).

With respect to the visual exam of Arinto, 9 panellists found no difference, 13 found a slight difference, 1 found a moderate difference, and 1 found a large difference between the samples. In addition, both wines were acceptable in terms of colour. Regarding the assessment of the smell and taste of Arinto, 11 panellists gave the correct answer, while 13 circled the wrong answer. For the Arinto flavour examination, 2 panellists found no difference, 18 found a slight difference, 3 found a moderate difference, and 1 found a great difference. In general, both Arinto samples were acceptable for the panellists. For flavour assessment, a question about preference was also included, and 9 panellists had no preference, while 9 tasters liked the CE sample better and 6 liked the PEF extracted sample better, with a total of 9 people expressing no preference. In addition, some comments include: "The wine samples do not present any defect or significant alteration in terms of appearance, smell and taste."

Regarding Moscatel, the majority of participants found the colour difference negligible (52%) or slight (38%), while a small part (10%) found it moderate. Furthermore, those who chose well the different wine sample among the 3 wines presented, found the difference slight in 62% of cases and moderate in 13%, while 25% of panellists found no differences between wine treated with PEF before pressing (PEF1) and wine without PEF extraction application (CE) (Figure 7). According to the results, just one-third of panellists were able to detect the different wine sample. Furthermore, relevant comments from those who chose correctly the odd sample were related to the difficulty of the choice because the samples had a similar appearance. These results showed no relevant difference or slight difference between the two samples, as reported by the choice of panellists. However, a slight preference for the CE sample was registered. Detailed results for Moscatel flavour showed that most of the panellists found a slight difference (50%) or moderate (22%). A minority of the panellists ticked the box with none (18%) or a great difference (9%). However, in many cases, they were not able to identify the different samples, although they were forced to make a choice, even if it was random. Furthermore, those who chose the odd sample, found the difference to be slight in 66% of cases and moderate in 11%, while 23% of panellists found no differences between wine treated with PEF before pressing and wine without PEF extraction application. Similar to the colour triangle test assessment, panellists had difficulty choosing the different wine sample, even if the 66% of panellists who picked the right sample, identified a slight difference in terms of smell and taste. For the flavour test, panellists were also asked about wine preferences. Panellists expressed a preference for PEF-treated wine, but without any additional comment.

In agreement with these results, Comuzzo et al. (2018) [29], analysing the aroma composition of Garganega cv. Wine, reported an overall increase of varietal precursors.

However, these values always remained below the odour threshold. This confirms the possibility of applying PEF to the extraction of must from white grapes.

3.1.3. Triangle Tests to Compare Finished Wines Stabilised with Pulsed Electric Fields (PEF2) with Wines Not Submitted to PEF2: Effect of PEF Stabilization on Colour and Flavour

The second triangle test was used to compare the wine produced from PEF extracted must (PEF1) with the same wine submitted again to PEF for pre-bottling stabilization (PEF1 + PEF2). For both Arinto and Moscatel Graúdo wines, there was no significant difference in colour and flavour (aroma and taste) between PEF1 wine and PEF1 + PEF2 wine (Figure 8). For Arinto colour, 13 panellists detected no difference between the three wine samples, 14 detected a small difference in colour, and no panellist ticked the box for considerable or large differences in colour (n = 27). Furthermore, the panellists found the colour of both Arinto wines acceptable. With respect to flavour, 7 panellists found no differences in taste or smell; 14 found a small difference; 6 found a moderate difference; and no one found a great difference. Answers to the inquiry about wine preference diverged; 9 panellists preferred the PEF1 sample, while 9 preferred the PEF1 + PEF2 wine sample, leaving a total of 9 panellists who had no preference.

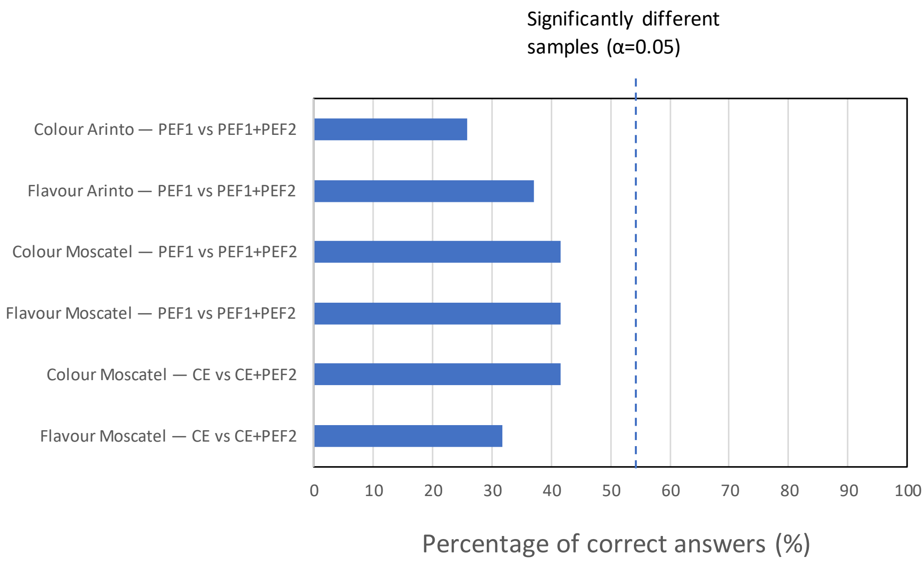


Figure 8. Results of triangle test to compare the colour and taste of white wines (Arinto and Moscatel Graúdo) stabilised with pulsed electric fields (PEF2) with non-stabilised wine samples (CE—conventional extraction, PEF1 must extracted with PEF during vinification). Bars crossing the significance line mean the wine samples are statistically different (n = 24 panellists).

For the Moscatel colour triangle test with PEF1 and PEF1 + PEF2 wine samples, most of the panellists detected none (37%) or slight differences (33%). Furthermore, among those who selected the different wine sample, the difference was slight in 33% of cases, and moderate in 33%. For taste tests, most of the panellists found a slight (45%) or moderate difference (37%). However, in many cases, they were not able to identify the different sample.

An extra triangle test was performed to compare Moscatel CE and CE + PEF2 wines. Similar results, with no significant differences between CE and CE + PEF2, were obtained for colour and flavour. For colour assessment, panellists found a slight difference (58%) or none (21%), while for flavour, panellists detected slight (33%) or moderate (38%) differences.

In summary, PEF stabilization treatment had no effect on Arinto and Moscatel colour and flavour, as assessed by the sensory panel.

A study carried out with Cabernet Sauvignon submitted to PEF (46.8 kV/cm, 20.5 µs treatment time) and stored for 1 year revealed no difference in overall sensory quality [30].

Another study conducted with red wine and 50 trained panellists investigated the effect of PEF (up to 31 kV/cm) on various visual, smell, and taste parameters (cloudiness/clearness, dullness/brightness, red colour density, particle status, density, odour/flavour, wine taste, bitter taste, sour taste, and after taste), and showed no significant difference between conventional and PEF-stabilised wines [31].

3.2. Physico-Chemical Analysis of Arinto and Moscatel Wines

3.2.1. Effect of PEF Assisted Grape Juice Extraction on Electrical Conductivity of Finished Wines

The PEF-assisted extraction of grape juice increased the electrical conductivity in Arinto from 1.585 $\pm$ 0.007 mS/cm to 1.615 $\pm$ 0.007 mS/cm. The electrical conductivity of Moscatel wine was higher and was also increased with PEF-assisted extraction from 1.875 $\pm$ 0.007 mS/cm to 2.010 $\pm$ 0.000 mS/cm. This increase in electrical conductivity with PEF extraction was expected, as more ionic compounds from the grape skins were extracted with PEF.

3.2.2. Analytical Colour Assessment (CIE L*a*b* Colour Coordinates)

The results of the instrumental assessment of 3 colour coordinates for Arinto wine samples are shown in Table 1. The Arinto wine clarity L* value was not affected significantly by the PEF extraction (PEF1) or PEF stabilization (PEF2) treatments, confirming the colour results obtained with the sensory panel. The PEF extraction treatment (PEF1) caused a slight decrease in a* colour coordinate and a significant increase in b* colour coordinate, although no significant change in colour a* and b* was detected between the same wines stabilised by PEF: Samples CE + PEF2 and PEF1 + PEF2. The comparison of CE and PEF1 Moscatel Graúdo wine samples revealed that PEF extraction decreased L*, and increased a* and b* colour coordinates, although those differences were not detected after stabilization of the same wines (CE + PEF2 vs. PEF1 + PEF2) (Table 2). A study conducted with Garganega white variety showed the PEF extraction of grape juice produced a more intense colour than wine produced from conventional extraction [29].

Table 1. CIE L*a*b* colour coordinates values in conventionally extracted (CE), pulsed electric fields extracted (PEF1) finished Arinto wine samples, and corresponding PEF stabilised (PEF2) Arinto wine samples (CE + PEF2, PEF1 + PEF2) [1].

Wine Treatment	L*	a*	b*
CE	98.5 ^{a}	-1.1^{a}	6.0 ^{a}
PEF1	97.6 ^{a}	-1.4^{b}	8.2 ^{c}
CE + PEF2	99.1 ^{a}	-0.9^{a}	6.7 ^{ab}
PEF1 + PEF2	98.6 ^{a}	-1.0^{a}	7.4 ^{bc}

[1] For each colour parameter (column), wine treatments with different letters present significantly different colour.

Table 2. CIE L*a*b* colour coordinates values in conventionally extracted (CE), pulsed electric fields extracted (PEF1) finished Arinto wine samples, and corresponding PEF stabilised (PEF2) Moscatel wine samples (CE + PEF2, PEF1 + PEF2) [1].

Wine Treatment	L*	a*	b*
CE	98.0 ^{a}	-1.0^{a}	6.4 ^{a}
PEF1	93.1 ^{b}	-0.5^{c}	8.9 ^{b}
CE + PEF2	93.5 ^{b}	-0.7^{b}	9.2 ^{b}
PEF1 + PEF2	93.3 ^{b}	-0.7^{b}	9.3 ^{b}

[1] For each colour parameter (column), wine treatments with different letters present significantly different colour.

With respect to the effect of PEF stabilization treatment applied to conventionally extracted (CE) Arinto wine, L*a*b* coordinates were not affected, confirming sensory assessments. The PEF stabilization of Arinto wine previously extracted by PEF (PEF1) only affected a*, which increased slightly. A harsher PEF treatment of 45 kV/cm for 70 µs (real treatment time) increased the colour density of Sauvignon Blanc and Pinot Gris wines [32]. Regarding PEF stabilization (PEF2) of Moscatel, a significant difference was found in the lab colour coordinates of CE and CE + PEF2 wine samples, although no difference in the L* and b* coordinates was found between the PEF1 and PEF1 + PEF2 wine samples.

3.2.3. Total Phenols, Flavonoids and Non-Flavonoids

Phenolic compounds are important components in determining the quality of wine and influence the colour, mouthfeel, and aging ability of wines. PEF promotes the extraction of phenolic compounds from the grapes into the must [33]. Figure 9 presents the results of total phenols in Arinto wine samples ranging from 165 to 260 mg gallic acid/L. The PEF extraction increased total phenols from 165 to 259 mg gallic acid/mL in CE and PEF1 samples, respectively. Comuzzo et al. also observed a higher level of total phenols in Garganeja white wine produced from PEF extracted must [29]. In another study in which traditional winemaking from red grapes was carried out, authors found that only a part of grape skin compounds was transferred to the must—about 40% of anthocyanins and 20% of tannins [34]. PEF electroporates grape flesh and skin cells, enhancing not only the extraction of grape juice/must production but also the compounds contained inside the grape skin cells. PEF extraction did not affect total phenols in Moscatel Graúdo wine (Figure 9).

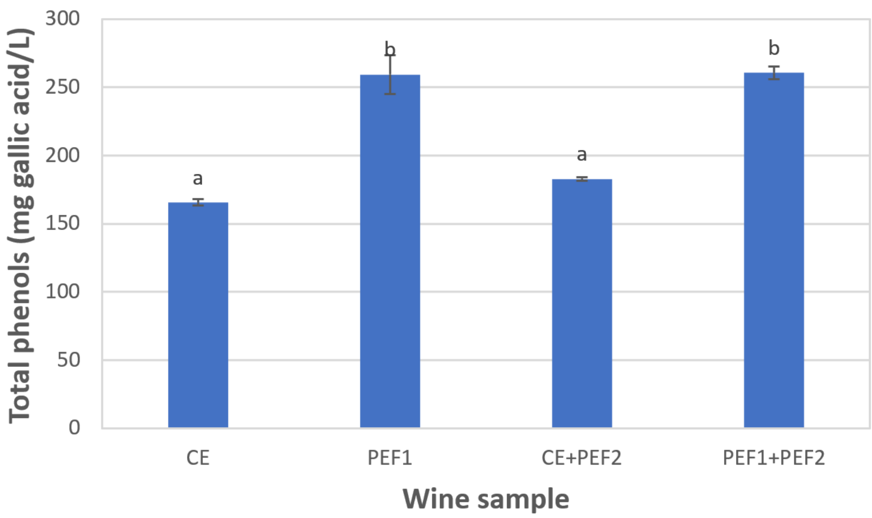


Figure 9. Total phenols (mg gallic acid/L of wine) in Arinto wine from conventionally extracted must (CE) and pulsed electric fields extracted must (PEF1), and the same wines stabilised by PEF before bottling (CE + PEF2, PEF1 + PEF2) (wines with different letters are significantly different).

PEF stabilization treatment of CE and PEF1 wine samples had no effect on their total phenol content. Van Wyk et al. (2021) obtained similar results with Sauvignon Blanc and Pinot Gris wines, as total phenols were not affected by PEF stabilization treatment (45 kV/cm, 70 µs) in both wines [31]. PEF stabilization treatment caused a slight decrease in the total phenols of Moscatel wine (Figure 10), probably due to some precipitation.

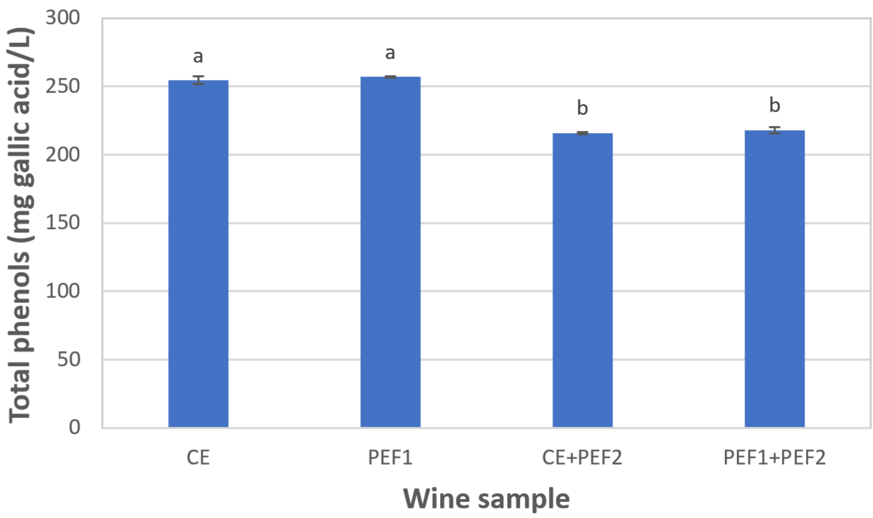


Figure 10. Total phenols (mg gallic acid/L of wine) in Moscatel Graúdo wine from conventionally extracted must (CE) and pulsed electric fields extracted must (PEF1), and the same wines stabilised by PEF before bottling (CE + PEF2, PEF1 + PEF2) (wines with different letters are significantly different).

Table 3 shows the phenols, non-flavonoids, and flavonoids. Most of the phenols in the wine samples from both grape varieties are non-flavonoids (159 to 252 mg GAE/L), representing 85 to 98% of total phenols. Flavonoid content is low, within 4 to 40 mg GAE/L. Kramling and Singleton [26] also registered 91% of non-flavonoids, obtaining 190 mg/mL of non-flavonoids and 208 mg/L of total phenols in Chardonnay wine. The fact that Arinto and Moscatel wines were produced from free-run must obtained at very low pressure (1 bar) can also explain the low content of flavonoids registered.

Table 3. Phenols non-flavonoids and phenols flavonoids in conventionally extracted (CE), pulsed electric fields extracted (PEF1) finished wines of Arinto and Moscatel Graúdo, and corresponding PEF stabilised (PEF2) wine samples (CE + PEF2, PEF1 + PEF2) [1].

Wine Sample	Non-Flavonoids mg/L	Flavonoids mg/L	Total Phenols mg/L
Arinto CE	159 (96%)	6 (4%)	165
Arinto PEF1	219 (85%)	40 (15%)	259
Arinto CE + PEF2	162 (89%)	20 (11%)	183
Arinto PEF1 + PEF2	244 (93%)	17 (7%)	261
Moscatel CE	247 (97%)	8 (3%)	255
Moscatel PEF1	252 (98%)	5 (2%)	257
Moscatel CE + PEF2	212 (98%)	4 (2%)	216
Moscatel PEF1 + PEF2	212 (97%)	6 (3%)	218

[1] The percentage of non-flavonoids and flavonoids within total phenols is shown in parenthesis.

In Arinto, similar to results obtained with total phenols, PEF extraction increased the non-flavonoids and also increased the proportion of flavonoids from 4 to 15%. With respect to Moscatel Graúdo wines, the non-flavonoid content did not change with PEF extraction, following the trend registered with total phenols. PEF stabilization treatment did not affect Arinto non-flavonoids and decreased non-flavonoids in Moscatel, in line with total phenol results.

3.2.4. Turbidity

The PEF extraction treatment applied to the Arinto grapes significantly increased the turbidity of the finished wine from 1 NTU (CE) to 32 NTU (PEF1) (Figure 11). A similar difference in turbidity was also registered in the same samples after being submitted to PEF stabilization treatment (PEF2). This effect is certainly related to the greater extraction of solutes due to the electroporation of the grape cells during the PEF treatment applied for grape juice extraction. The PEF treatment applied for stabilization did not affect Arinto's turbidity. In Moscatel wine, a slight increase from 4.9 to 9.0 NTU was registered with PEF extraction.

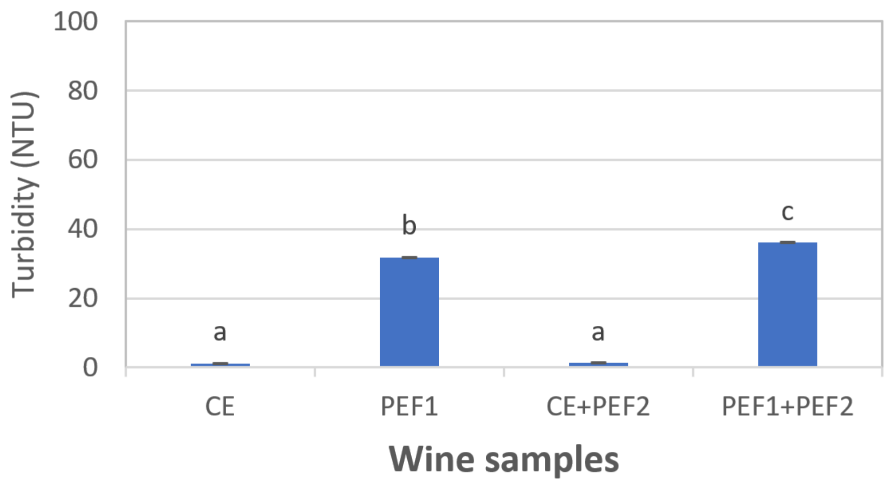
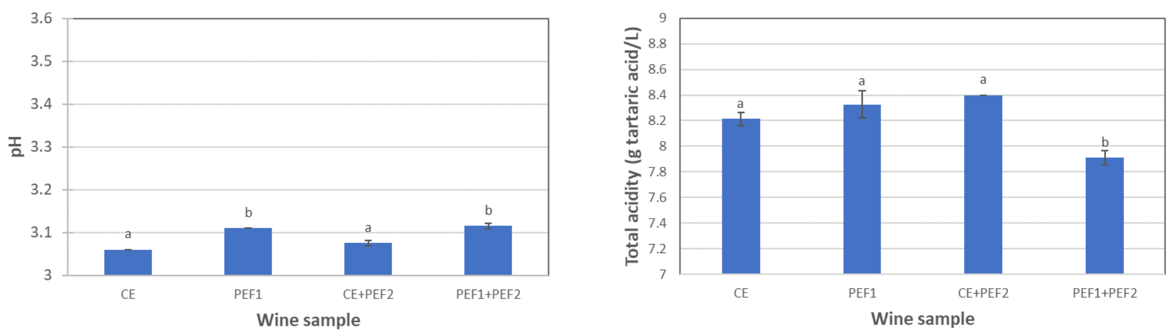


Figure 11. Turbidity expressed in NTU in Arinto wine from conventionally extracted must (CE) and pulsed electric fields extracted must (PEF1), and the same wines stabilised by PEF before bottling (CE + PEF2, PEF1 + PEF2) (wines with different letters are significantly different).

3.2.5. pH and Total Acidity

The pH of Arinto increased slightly with the PEF extraction of the must from 3.06 to 3.11 but was not affected by the PEF stabilization treatment, whereas total acidity did not change significantly with the PEF extraction treatment, with values of 8.2 g tartaric acid/L for CE wine and 8.3 g tartaric acid/L for PEF1 wine (Figure 12). Similar results were obtained with the pH of Moscatel Graúdo (Figure 13). The increase in pH with PEF extraction means there is an increase in the concentration of free hydrogen ions in the wine, possibly from an increased dissociation of wine acids. The total acidity of Moscatel was not affected by PEF extraction. However, an increase in total acidity was registered in the PEF1 wine sample submitted to PEF stabilization (PEF2). A study carried out with Chardonnay wine subjected to 15 kV/cm PEF stabilization treatment for 177 microseconds (97 kJ/kg) revealed no change in pH and a slight increase in total acidity from 5.67 to 6.12 g/L [35].



(**a**) (**b**)

Figure 12. pH (**a**) and total acidity (**b**) in Arinto wine from conventionally extracted must (CE) and pulsed electric fields extracted must (PEF1), and the same wines stabilised by PEF before bottling (CE + PEF2, PEF1 + PEF2) (wines with different letters are significantly different).

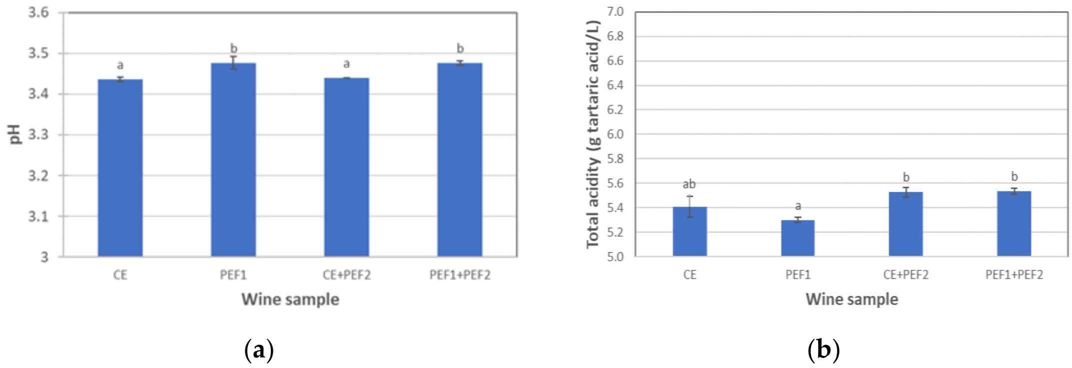


Figure 13. pH (**a**) and total acidity (**b**) in Moscatel Graúdo wine from conventionally extracted must (CE) and pulsed electric fields extracted must (PEF1), and the same wines stabilised by PEF before bottling (CE + PEF2, PEF1 + PEF2) (wines with different letters are significantly different).

In agreement with the results obtained by Arinto and Moscatel, the pH of Sauvignon Blanc and Pinot Gris wines was not affected by PEF stabilization treatment with a field intensity of 45 kV/cm for 70 µs [32].

4. Conclusions

In this study, continuous PEF was successfully employed in a pilot-scale winery to potentiate the extraction of must from crushed and destemmed grapes of Arinto (1.2 kV/cm) and Moscatel de Setúbal (1.6 kV/cm). A flow rate of 4 tonnes/h was submitted to a 100 Hz monopolar of 50 µs width. The PEF extraction changed the CIE measured a* (green-red) and b* (blue-yellow) colour coordinates in Arinto wine, but L* was not affected, whereas in Moscatel the 3 colour parameters were affected by the PEF extraction treatment. This change in spectrophotometrically assessed colour of Arinto and Moscatel wines was not perceived by the panellists in the sensory triangle tests. Furthermore, PEF extraction did not affect the wine flavour assessed by the sensory panel for both wines. With respect to physico-chemical analysis in Arinto, the following was concluded: Total phenols increased (by 100 mg/L) with PEF extraction; wine turbidity also increased with PEF extraction; the pH increased slightly with PEF extraction of must; total acidity was not affected. With respect to Moscatel, the results were similar to Arinto, except for total phenols, which remained unchanged with extraction.

The impact of PEF stabilization of wine before bottling on wine quality was also investigated using a non-thermal pasteurisation/decontamination treatment. Continuous PEF stabilization was successfully carried out at a pilot-scale winery, processing 200 L/h of wine. The PEF parameters were 10 kV/cm, monopolar pulses of 25 µs width, and 150 Hz frequency, delivering about 60–70 kJ/kg of specific energy. Panellists did not detect colour, aroma, or taste differences in wine submitted to PEF stabilization. CIE colour coordinates in Arinto were not affected by PEF stabilization, confirming the sensory result. The other quality parameters assessed in Arinto (total phenols, turbidity, pH, and total acidity) were not affected by the PEF stabilization treatment applied. Regarding Moscatel, PEF stabilization decreased total phenols, increased total acidity, and had no effect on pH.

The results revealed the good potential of PEF application for Arinto and Moscatel de Setúbal white wines' vinification, as white wines' sensory quality was not affected by the treatments. Further studies applying PEF to other grape varieties are needed, as wine quality produced from different grape varieties can be affected in a different way by PEF extraction and stabilization treatments. In addition, more specific chemical analysis on the effect of PEF extraction on different types of organic acids, polysaccharides, and detailed phenolic profiles can be investigated. Pulsed electric fields used as a physical stabilization method for wine can potentially reduce pre-bottling added SO_2 to wine, the conventional chemical method of wine stabilization in wineries. Storage studies of SO_2-free wine and wines with lower concentrations of SO_2 submitted to PEF stabilization treatments of different intensities are needed to investigate the possibility of reducing pre-bottling SO_2 addition.

Author Contributions: Conceptualization, supervision, original draft writing, F.V.M.S.; experiments and investigation, R.B. and A.G.; writing—review and editing, J.M.R.-d.-S.; experiments, M.A.-M. and L.M.R.; supervision, project administration, funding acquisition, L.M.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Portugal2020 and European Union, grant number CENTRO-01-0247-FEDER-041392 | LISBOA-01-0247-FEDER-041392, Project "PUREWINE—Increasing quality and production capacity of European wine industry through an innovative Pulsed Electric Field-based process applied to vinification". FCT—Fundação para a Ciência e a Tecnologia, I.P., under the project UIDB/04129/2020 of LEAF—Linking Landscape, Environment, Agriculture and Food, Research Unit.

Data Availability Statement: Data are contained within the article.

Acknowledgments: Sensory panel composed by students of Enology and Food Engineering Programmes, and staff volunteers with prior experience in sensory analysis. Thanks to the laboratory technical staff, Joana Granja Soares and Joana Sales, and Joel Santos for help in the physico-chemical analysis.

Conflicts of Interest: EnergyPulse Systems develops and commercializes PEF equipment that can be used for wine vinification. Authors not affiliated with EPSystems declare no conflict of interest.

References

1. Sulaiman, A.; Farid, M.; Silva, F.V.M. Strawberry puree processed by thermal, high pressure, or power ultrasound: Process energy requirements and quality modeling during storage. *Food Sci. Technol. Int.* **2017**, *23*, 293–309. [CrossRef] [PubMed]
2. Van Wyk, S.; Silva, F.V.M. Nonthermal preservation of wine. Chapter 7. In *Preservatives and Preservation Methods for the Beverage Industry, The Science of Beverages Series*; Grumezescu, A.M., Holban, A.M., Eds.; Elsevier: Amsterdam, The Netherlands, 2019; Volume 15, pp. 203–235.
3. Milani, E.A.; Silva, F.V.M. Pasteurization of Beer by Non-Thermal Technologies. *Front. Food Sci. Technol.* **2022**, *1*, 798676. [CrossRef]
4. Silva, F.V.M.; Oey, I. Non-Thermal Technologies for Food Processing. *Appl. Sci.* **2023**, *13*, 7734. [CrossRef]
5. Milani, E.A.; Alkhafaji, S.; Silva, F.V. Pulsed Electric Field continuous pasteurization of different types of beers. *Food Control* **2015**, *50*, 223–229. [CrossRef]
6. Milani, E.A.; Silva, F.V.M. Nonthermal pasteurization of beer by high pressure processing: Modelling the inactivation of *Saccharomyces cerevisiae* ascospores in different alcohol beers. *High Press. Res.* **2016**, *36*, 595–609. [CrossRef]
7. Milani, E.A.; Silva, F.V.M. Ultrasound assisted thermal pasteurization of beers with different alcohol levels: Inactivation of *Saccharomyces cerevisiae* ascospores. *J. Food Eng.* **2017**, *198*, 45–53. [CrossRef]
8. van Wyk, S.; Silva, F.V.M. High pressure processing inactivation of *Brettanomyces bruxellensis* in seven different table wines. *Food Control* **2017**, *81*, 1–8. [CrossRef]
9. van Wyk, S.; Silva, F.V.; Farid, M.M. Pulsed electric field treatment of red wine: Inactivation of *Brettanomyces* and potential hazard caused by metal ion dissolution. *Innov. Food Sci. Emerg. Technol.* **2019**, *52*, 57–65. [CrossRef]
10. Pérez-Porras, P.; Bautista-Ortín, A.B.; Jurado, R.; Gómez-Plaza, E. Combining high-power ultrasound and enological enzymes during winemaking to improve the chromatic characteristics of red wine. *LWT* **2022**, *156*, 113032. [CrossRef]
11. Alzahrani, M.; Silva, F.V.M.; Alkhafaji, S. Non-Thermal Processing of a Protein Functional Beverage Using Pulsed Electric Fields: *Escherichia coli* Inactivation and Effect on Proteins. *Beverages* **2022**, *8*, 68. [CrossRef]
12. Silva, F.V.M.; van Wyk, S. Emerging, Non-Thermal Technologies as Alternative to SO_2 for the Production of Wine. *Foods* **2021**, *10*, 2175. [CrossRef] [PubMed]
13. Delso, C.; Berzosa, A.; Sanz, J.; Alvarez, I.; Raso, J. Microbial Decontamination by Pulsed Electric Fields (PEF) in Winemaking. In *Grapes and Wine*; Morata, A., Loira, I., González, C., Eds.; IntechOpen: London, UK, 2021.
14. Praporscic, I.; Lebovka, N.; Vorobiev, E.; Mietton-Peuchot, M. Pulsed Electric Field Enhanced Expression and Juice Quality of White Grapes. *Sep. Purif. Technol.* **2007**, *52*, 520–526. [CrossRef]
15. Morata, A.; Loira, I.; Vejarano, R.; González, C.; Callejo, M.J.; Suárez-Lepe, J.A. Emerging Preservation Technologies in Grapes for Winemaking. *Trends Food Sci. Technol.* **2017**, *67*, 36–43. [CrossRef]
16. OIV. Treatment of Grapes by Pulsed Electric Fields—(Pef). Resolution OIV-OENO 634-2020. International Organization of Vine and Wine, 2020. Available online: https://www.oiv.int/standards/treatment-of-grapes-by-pulsed-electric-fields-(pef)- (accessed on 15 October 2023).
17. Delso, C.; Martínez, J.M.; Aguilar-Machado, D.; Maza, M.; Morata, A.; Álvarez, I.; Raso, J. Chapter 6—Use of pulsed electric fields in white grape processing. In *White Wine Technology*; Morata, A., Ed.; Academic Press: Cambridge, MA, USA, 2022; pp. 61–71.

18. Ribéreau-Gayon, P.; Dubourdieu, D.; Donèche, B.; Lonvaud, A. White winemaking. Chapter 13. In *Handbook of Enology Volume 1 The Microbiology of Wine and Vinifications*, 2nd ed.; John Wiley and Sons: New York, NY, USA, 2006; Volume 1, pp. 397–443.
19. Ribéreau-Gayon, P.; Dubourdieu, D.; Donèche, B.; Lonvaud, A. The use of sulfur dioxide in must and wine treatment. Chapter 8. In *Handbook of Enology Volume 1 The Microbiology of Wine and Vinifications*, 2nd ed.; John Wiley and Sons: New York, NY, USA, 2006; Volume 1, pp. 193–221.
20. Aguiar-Macedo, M.; Redondo, L.M.; Pereira, M.T.; Silva, C. Pulsed Electric Fields vs. Pectolytic Enzymes in Arinto Vinification: Effects on Yield and Oenological Parameters. *Appl. Sci.* **2023**, *13*, 8343. [CrossRef]
21. Redondo, L.M.; Silva, J.F. Repetitive High-Voltage Solid-State Marx Modulator Design for Various Load Conditions. *IEEE Trans. Plasma Sci.* **2009**, *37*, 1632–1637. [CrossRef]
22. Laboratório Ferreira Lapa. Ficha auxiliar de prova de vinho elaborada no laboratório de Ferreira Lapa. 2022; Adaptado de I.N.A.O. Fiche: Analyse et vocabulaire de la dégustation—1967 A. Costell.
23. *ISO 4120-2021*; Sensory Analysis—Methodology—Triangle Test. 3rd ed. International Organization for Standardization (ISO): Geneva, Switzerland, 2021.
24. OIV. Method OIV-MA-AS2-11, Determination of Chromatic Characteristics According CIELab. Resolution Oeno 1/2006. In Compendium of International Methods of Wine and Must Analysis. International Organization of Vine and Wine, 2009. Available online: https://www.oiv.int/standards/compendium-of-international-methods-of-wine-and-must-analysis/annex-a-methods-of-analysis-of-wines-and-musts/section-2-physical-analysis/chromatic-characteristics-(type-i) (accessed on 15 October 2023).
25. Somers, C.; Evans, M.E. Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO_2, "chemical age". *J. Sci. Food Agric.* **1977**, *28*, 279–287. [CrossRef]
26. Kramling, T.E.; Singleton, V.L. An Estimate of the Nonflavonoid Phenols in Wines. *Am. J. Enol. Vitic.* **1969**, *20*, 86–92. [CrossRef]
27. *ISO 7027:1990*; Water Quality—Determination of Turbidity. 2nd ed. ISO: Geneva, Switzerland, 1990.
28. OIV. Method OIV-MA-AS313-01 (Type I Method), Total Acidity. In Compendium of International Methods of Wine and Must Analysis. International Organization of Vine and Wine, 2015. Available online: https://www.oiv.int/standards/annex-a-methods-of-analysis-of-wines-and-musts/section-3-chemical-analysis/section-3-1-organic-compounds/section-3-1-3-acids (accessed on 15 October 2023).
29. Comuzzo, P.; Marconi, M.; Zanella, G.; Querzè, M. Pulsed electric field processing of white grapes (cv. Garganega): Effects on wine composition and volatile compounds. *Food Chem.* **2018**, *264*, 16–23. [CrossRef]
30. van Wyk, S.; Farid, M.M.; Silva, F.V. SO_2, high pressure processing and pulsed electric field treatments of red wine: Effect on sensory, *Brettanomyces* inactivation and other quality parameters during one year storage. *Innov. Food Sci. Emerg. Technol.* **2018**, *48*, 204–211. [CrossRef]
31. Abca, E.E.; Akdemir, E.G. Processing of Red Wine by Pulsed Electric Fields with Respect to Quality Parameters. *J. Food Process. Preserv.* **2014**, *39*, 758–767. [CrossRef]
32. van Wyk, S.; Hong, L.; Silva, F.V.M. Non-Thermal High Pressure Processing, Pulsed Electric Fields and Ultrasound Preservation of Five Different Table Wines. *Beverages* **2021**, *7*, 69. [CrossRef]
33. Feng, Y.; Yang, T.; Zhang, Y.; Zhang, A.; Gai, L.; Niu, D. Potential applications of pulsed electric field in the fermented wine industry. *Front. Nutr.* **2022**, *9*, 1048632. [CrossRef] [PubMed]
34. Cerpa-Calderon, F.K.; Kennedy, J.A. Berry integrity and extraction of skin and seed proanthocyanidins during red wine fermentation. *J. Agric. Food Chem.* **2008**, *19*, 9006–9014. [CrossRef] [PubMed]
35. Delso, C.; Berzosa, A.; Sanz, J.; Álvarez, I.; Raso, J. Pulsed electric field processing as an alternative to sulfites (SO_2) for controlling *Saccharamyces cerevisae* involved in the fermentation of Chardonnay white wine. *Food Res. Int.* **2023**, *165*, 112525. [CrossRef]