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Impact of monopolar and bipolar pulsed electric fields on the quality of Tinta Roriz wines

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Abstract. Considering its capacity to optimize mass transfer processes, Pulsed Electric Fields (PEF) technology holds great promise for the Winemaking industry. This study comapred Control (TRC), Bipolar pulses (TRPEF2), Monopolar pulses (TRPEF1) PEF protocols (W_s≈5kJ/kg and E=2kV/cm), and enzyme-assisted maceration (TRE) was performed. Physicochemical parameters such as pH, Total Acidity, Electrical Conductivity, Tartaric Stability, Total Phenols, and Anthocyanins were monitored, among others. PEF significantly influenced pH, Electrical Conductivity, and Total Acidity during the first three days of maceration, leading to higher extraction rates in PEF-treated musts. Total Phenols in TRPEF1 and TRPEF2 increased by 29% and 23-33%, respectively, at Stage 0 (Grape Reception) and Stage 3 (3 days after grape reception). Anthocyanins increased by up to 120% in PEF-treated groups at Stage 0. Optimal results were obtained for TRPEF2. QDA sensory analysis indicated minimal differences in sensory profiles, with CATPCA confirming similar outcomes for bipolar PEF and enzymatic treatment. Additionally, 68.8% of panellists preferred PEF-treated wines, assigning them higher scores. These findings support PEF as a sustainable, economic alternative to reduce the maceration period by up to 3 days and enhance wine quality, making it a valuable tool for winemakers.

1. Introduction

Pulsed Electric Fields (PEF) technology presents substantial opportunities for the Agrifood industry, considering its ability to disrupt cellular membranes, leading to pore formation - a phenomenon known as *electroporation*. This *electropermeabilization* PEF-induced depends on the chosen treatment protocol (i.e., pulse shape, electrical field strength, specific energy) and the matrix's characteristics (i.e., cell radii and size, pH, electrical conductivity) [1–5]. This allows for the modulation of the process and the capacity to achieve different outcomes, making this technology a versatile tool capable of meeting pre-established objectives. Additionally, PEF is a purely physical, non-thermal (depending on the protocol used), scalable and sustainable technology. In light of this, a great promise is held for the winemaking industry, where the focus has been on two main applications: microorganism inactivation and mass transfer optimization [6–9]. In fact, the use of PEF to assist in the extraction of phenolics and aromatic precursors in red musts and optimize yield in white wine has already been approved by Organization of Vine and Wine in 2020 (Resolution OIV-OENO 634-2020) due to the efforts of several research teams[10].

While being considered a novelty by us, given the lack of published information, we believe the extrapolation of bipolar PEF protocols to this application to be of major relevance. The main reason is its ability to induce additional stress through a more homogenous distribution in the bilipidic membranes [11]. Furthermore, its adoption holds significant benefits from an equipment point of view. It contributes to reduced energy consumption, lowers the risk of food electrolysis, minimizes solid deposition on the electrodes, and decreases the potential for contamination with metal ions [12].

However, there are still gaps of information due to the intrinsic nature of the grape matrix, dependency, which impact the cellular structures and chemical composition [13]. This underscores the necessity of further research and development in this field. Optimizing PEF protocols for each variety according to the oenologist's desired outcome might be essential. This, combined with the scarcity of investigation at pilot and industrial scales and the non-existence of studies published regarding the application of bipolar square waves to improve the maceration of red wines, led to the urgency of developing this study

2. Materials and methods

2.1. Grapes & vinification

Two tonnes of var. Tinta Roriz grapes (*syn.* Tempranillo, Aragonês) were meticulously handpicked and sorted from a previously selected vineyard parcel at APPACDM Viseu Pedagogical Farm in the Dão Region (Portugal). They were then transported to EnergyPulse Systems Pilot-Plant Winery (LInDAAA) in Gouveia. Upon arrival, the vinification process was initiated immediately.

After careful weighing, the whole volume was separated into 12 similar batches, which were randomly assigned to four different groups, each consisting of three subjects:

- TRC, the control group;
- TRE, treated with commercial-grade pectolytic enzymes (2g/100kg of grapes);
- TRPEF1, a group under the influence of a pre-treatment consisting of Monopolar PEF;
- TRPEF2, pretreated with Bipolar PEF.

The red wine vinification schematics was followed as shown in Figure 1.

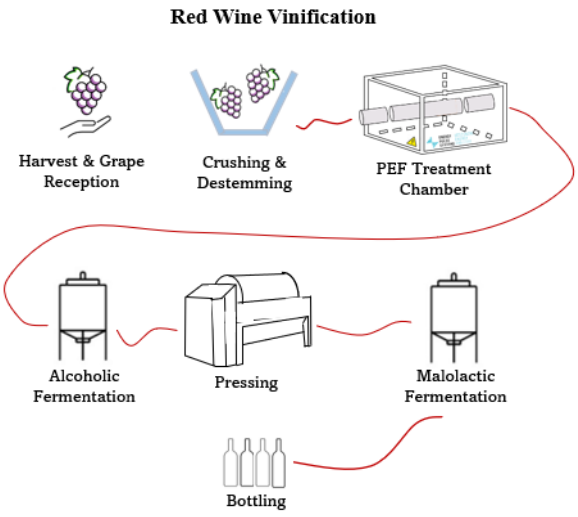

Figure 1. Schematics of the Winemaking Protocol.

Grapes were crushed and destemmed using a Lugana 1 equipment (CMaA, Coridonia, Italy). The grape must was then pumped by a PPC 200 peristaltic pump (CME, Campagnola Emilia, Italy) through a system of DN50 hoses at a rate of 4.5tonnes/hour to the respective fermentation tanks. A continuous collinear treatment chamber is integrated into the hose system (Figure 2).

Figure 2. EPULSUS® BM3B-15 interior & front (Courtesy of EnergyPulse Systems).

This system allows the application of pulses only when necessary (TRPEF1 & TRPEF2 subjects), remaining inactive during the processing of TRC and TRE. Enzymes were added for TRE at this moment. Free SO_2 was added to a concentration of 20mg/L. All tanks were co-inoculated with 25g/hL of *S. cerevisiae* (Merit™, Chr Hansen, Denmark) and 20g/hl of yeast nutrient (Fermaid O, Lallemand, Montreal Canada), and with lactic bacteria *O. oeni* (Viniflora® CH11, Chr Hansen, Denmark), after a 24h period. Alcoholic Fermentation (AF) was carried out at 26°C±1. Must density and temperature were monitored twice per day. AF was considered terminated when density presented values <1000 g/dm³ and the content of D-Glucose+D-Fructose <2g/L. Samples were collected daily during AF, before and after pressing (XPro 5, Bucher Vaslin®, Chalonnes-sur-Loire, France), and 3 months after vinification to ensure the completion of Malolactic Fermentation (MLF) (<0.1g/L of malic acid). Exceptionally, samples were also collected six months after vinification to assess tartaric stability of the wine under study. Free SO2 was corrected to 35mg/L at the end of FML. Four months after vinification, the final wines were subjected to sensory analysis.

2.2. PEF Equipment & Parameters

PEF was applied using a colinear treatment chamber (TC) constituted by polyoxymethylene (POM) insulators and three 316 stainless steel electrodes, with a diameter of 50mm (DN50) and a distance (*d*) between electrodes of 5cm (Figure 3). TC was connected to a high-voltage solid-state Marx generator (EPULSUS® BM3B-15, EnergyPulse Systems, Lisbon, Portugal), with 15 kV/400 A and 9 kW average power (Figure 2). This equipment can deliver nearly perfectly square-shaped pulses and operate both in bipolar and monopolar modes [14].

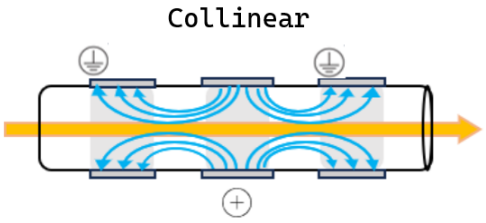

Figure 3. Treatment Chamber Configuration.

The selection of PEF parameters was supported by extensive research from numerous published studies and undisclosed internal findings to assess the most viable protocol to optimize polyphenolic extraction. However, this was not the only question taken into account: it also considered the trade-off between the cost-effectiveness of the equipment design requirements (higher electrical field strength = higher number of electrical components) that could limit the technology application in industry, energy requisites and the benefits of using PEF for red wine vinification [6,15,16].

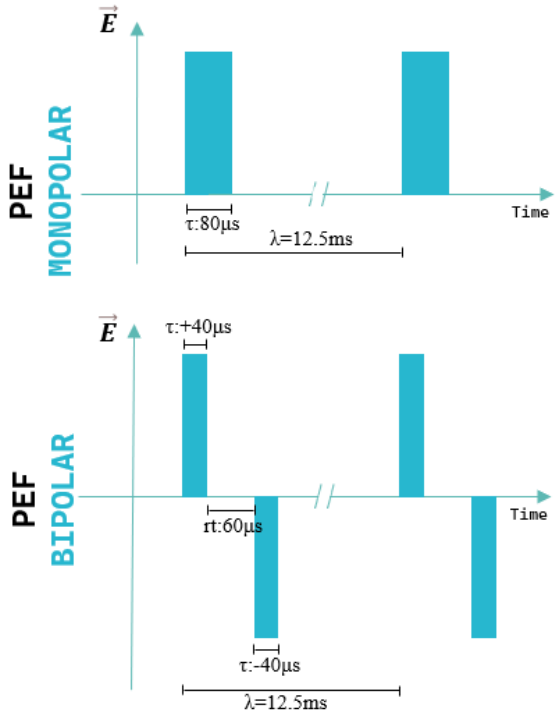

Figure 4. Visual Representation of Monopolar vs Bipolar Protocol.

The final selected PEF protocol consisted of an electrical field strength E=2kV/cm, by applying a voltage (U) of 10kV ($E=V/d$). Regarding the monopolar protocol, the selected pulse width was of τ= 80µs. In contrast, the bipolar protocol was constituted by both a positive and a negative pulse of τ= ±40µs, separated by a relax time of r_t=60µs (Figure 4). Both protocols were applied at a frequency of f=80Hz, meaning the grape must was subjected to n_p=12 pulses.

This selection guarantees that the only difference between protocols that should be considered is the polarity of the pulses, while maintaining the same energy delivery and electric field strength, respectively, W_s≈5kJ/kg and E=2kV/cm.

2.3. Analytical Assessment

2.3.1. Physicochemical Analysis

Samples were collected daily during AF, after 3 months (guaranteeing the conclusion of Malolactic Fermentation (FML), and, exceptionally, 6 months after the end of vinification to assess the impact on Tartaric Stability and Total Tannins. Each sample and analysis were performed in triplicate (n=9).

Sample collection took place at seven key moments:

- Stage 0: at grape reception, immediately after processing.
- Stage 1: the 1st day after the reception, representing the beginning of alcoholic fermentation (≈1080g/dm^3);
- Stage 2: when musts presented a density value between 1060-1070 g/dm^3;
- Stage 3: at 1040-1050 g/dm^3;
- Stage 4: where the samples were collected from tanks with densities of 1020 g/dm^3;
- Stage 5: representing the end of AF, being the sample collected immediately before pressing (<1000 g/dm^3)
- Stage 6: wine samples collected immediately after pressing,
- Stage 7: samples collected from inox tanks, after 3 months of aging.

Both pH and Electrical Conductivity (*EC*) were assessed through direct measurement using a SesION+ pH31 pH meter (Hach, Loveland, USA) and a portable conductivity meter Dist 4 HI98304 (Hanna, Woonsocket, USA). Total Acidity (*TA*) was determined by employing the titration methodology described in Method OIV-MA-AS313-01. Turbidity (*T*) was measured 3 months post-AF, resorting to a 2100Q portable turbidimeter (Hach, Loveland, USA).

All analysis involving spectrophotometric techniques, except for Total Tannins, were conducted using a U-2900 Spectrophotometer (Hitachi, Japan). Total Phenol (TP) concentration was determined according to internal protocol (*MI22 - Revisão 4, 2020*), while colorimetric parameters, namely Colour Intensity (CI), Tonality (TON), and the Colour Compound %Ye were obtained by the application of Glories methodology, as described in the OIV's *Compendium of International Methods of Analysis* (OIV-MA-AS2-07B:R2009). %Ye was used as a simplified index for the evaluation of browning in wines, indicative of oxidative reactions [17] Anthocyanins were evaluated by Internal Method (MI21 –Revisão 4, 2020) [18,19]. Total Tannins were determined using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan), following the protocol described by Zamora [20]

Total Dry Extract (TDE) and Volatile Acidity (VA) were measured three months post-vinification, following the procedures published by OIV (OIV-MA-AS2-03B and OIV-MA-AS313-02).

Tartaric Stability was assessed 6 months after vinification, with a protocol based on the DIT test (*degree d'instabililité tartrique*) [21].

Total Dry Extract (TDE) and Volatile Acidity were assessed 3 months after the end of AF, at the end of FML, using OIV procedures (OIV-MA-AS2-03B & OIV-MA-AS313-02).

2.3.2. Sensory Analysis

Quantitative Descriptive Analysis (QDA) was conducted on the finished wines, after a four-month interval post-vinification. Descriptive attributes were selected based on references detailing general wine descriptors, and those commonly associated with Tinta Roriz [22–24].

Sixteen descriptors were selected and categorized into three groups:

- Visual (Clarity, Colour Intensity, Viscosity):
- Aroma (Freshness, Intensity, Metallic/Pneumatic, Fruity (general), Red Berries, and Spices).
- Mouthfeel (Sweetness, Acidity, Astringency, Body, Balance, Persistence, Intensity).

All sensory attributes were assessed using an ordinal numerical scale, from 1 to 5. A score of 1 indicated a weak or null presence or intensity, while a score of 5 indicated a strong presence.

In addition, the panel was asked to attribute a score on a scale of 1 to 20 (Likert Scale) as Global Evaluation of the wines understudy.

The blind tasting session occurred late in the morning, at the sensory evaluation laboratory of University of Trás-os-Montes-e-Alto-Douro (UTAD), with a tasting panel comprised of 26 trained panellists.

2.3.3. Data Analysis

Considering the large amount of collected data regarding physicochemical analysis, critical moments were selected based on three criteria to perform statistical analysis: 1st Multivariate Analysis of Variance (MANOVA), 2nd on visual analysis of graphical representation of data, and 3rd, from a technical point of view. After identifying the critical stages, MANOVAs and ANOVAs, were computed as appropriate. If statistically significant differences were found, follow-up ANOVAS and *post* hoc Bonferroni tests were performed (p=0.05).

Partial Eta-squared (η_p^2) was used to measure effect size, indicating the proportion of variance between subjects attributable to the type of treatment. Sensory Evaluation results were examined through Categorical Principal Component Analysis (CATPCA). Statistically significant effects were assumed at a 5% significance level.

All data was subjected to statistical analysis, using IBM SPSS Statistics, Version 28.0.1.0 (SPSS Inc., Chicago, IL, USA).

Graphics were generated using GraphPad Prism, Version 8.0.2 (GraphPad Software Inc, San Diego, CA, USA), being a visual characterization of Mean ± SD; each data point represents the mean value of each replicate tank (e.g. TRC1, TRC2, TRC3). The radar chart was created using Microsoft® Excel®, for Microsoft 365, version 2312, Build 16.0.17126.20132 (Microsoft Corporation, Redmond, USA).

3. Results & discussion

3.1. Physicochemical Analysis

3.1.1. pH, Total Acidity & Electrical Conductivity

Given the importance of pH and TA from an oenological perspective, these parameters were analysed daily during AF and after 3 months, at the end of FML[18]. Likewise, EC was also monitored at the same moments, considering its relevance as an indicator of the bilipid membrane damage caused by electroporation due to the leakage of intracellular species, such as ionic species (e.g. K^+ and Na^+) and mineral salts [25]. pH and Total acidity were significantly influenced by PEF in early maceration stages, likely due to the increased extraction of K+ and Ca^{2+} (Table 1). For instance, immediately after grape processing (Stage 0), statistically significant differences were found amongst all subjects, with a very large effect size for pH. The different extraction protocols explain 93.4% of the differences found (p<0.001; η_p^2=0.934).

Table 1. Results pH and Total Acidity parameters for Stages 0, 1, 6 and 7.

	Parameter	TRC	TRE	TRPEF1	TRPEF2	p	η_p^2
Stage 0	pH	3.55^{a} ± 0.01	3.57^{b} ± 0.01	3.65^{c} ± 0.01	3.62^{d} ± 0.01	<0.001	0.934
	TA	4.52^{ab} ± 0.12	4.43^{a} ± 0.06	4.57^{b} ±0.10	4.60^{b} ±0.09	0.004	0.336
Stage 1	pH	3.64^{a} ± 0.03	3.63^{a} ± 0.01	3.71^{b} ± 0.05	3.72^{b} ± 0.02	<0.001	0.659
	TA	4.67^{a} ± 0.08	4.87^{ab} ± 0.04	5.00^{b} ± 0.27	4.80^{ab} ± 0.32	0.019	0.264
Stage 6	pH	3.71 ± 0.03	3.65 ± 0.01	3.67 ± 0.08	3.71 ± 0.06	0.058	0.206
	TA	6.23^{a} ± 0.33	6.75^{b} ± 0.19	6.56^{ab} ± 0.39	6.32^{a} ± 0.27	0.004	0.338
Stage 7	pH	3.76^{a} ± 0.04	3.71^{b} ± 0.05	3.75^{ab} ± 0.02	3.76^{ab} ± 0.04	0.013	0.283
	TA	5.09 ± 0.15	5.18 ± 0.13	5.06 ± 0.12	5.03 ± 0.05	0.052	0.212

NOTE: Different letters assigned to the means indicate statistically significant differences (p<0.05) (Stage 0: After Grape Reception; Stage 1: 1st day, beginning of AF (1080g/dm^3; Stage 6: End of AF/After pressing; Stage 7: 3 months post-AF/End of MLF)

The same was observed for EC (p<0.001; η_p^2=0.859). This influence diminished as AF progressed. Notably, EC was higher in PEF-treated wines during the initial stages, supporting the pH observations (Figure 5).

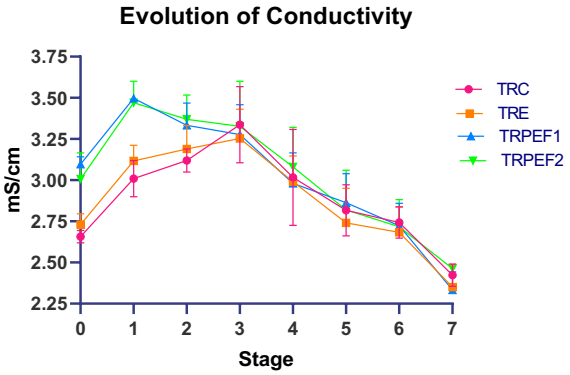

Figure 5. Evolution of Electrical Conductivity.

3.1.2. Spectrophotometry

Analysis of Total Phenols revealed distinct trends between TRC-TRE and TRPEF1-TRPEF2 during the first stages (TRPEF1 & TRPEF2: Stage 0: +29% TP, Stage 3: +23-33% TP), suggesting the ability to accelerate the maceration process with PEF treatments (Figure 6).

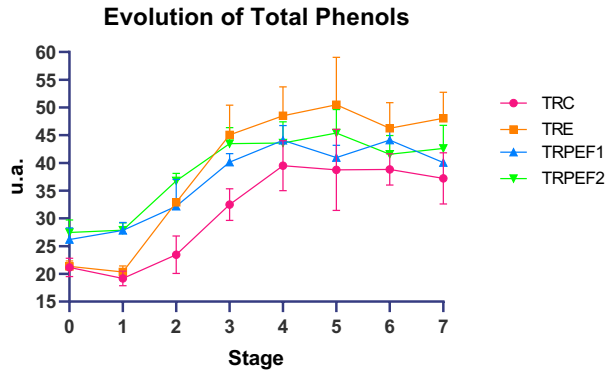

Figure 6. Evolution of Total Phenols.

However, this trend shifted mid-alcoholic fermentation, with TRE exhibiting the highest concentration of Total Phenols (+19% relative to TRC), by the end of AF. In contrast, TRPEF1 and TRPEF2 had 7 and 13.7% more TP than control.

All treatments did not affect Tonality; however, treatments significantly improved Color Intensity during AF but were only sustained for TRE three months post-AF (Table 2). %Ye was used as a simple browning index, did not differ between subjects. Thus, one can conclude that neither PEFs nor enzymes affected the antioxidant capacity (Stage 6: $F_{(3, 32)}$=2.774, p=0.057, η_p^2=0.206, Stage 7 $F_{(3, 32)}$=2.860, p=0.052, η_p^2=0.211).

Table 2. Results of Color Intensity and Tonality for Stages 0, 3, and 7.

	Parameter	TRC	TRE	TRPEF1	TRPEF2	p	η_p^2
Stage 0	Color Intensity (u.a)	2.267^{ab} ± 0.434	3.179^{a} ± 1.263	2.049^{b} ± 0.194	2.562^{ab} ± 0.537	0.014	0.278
	Tonality (-/-)	1.250^{a} ± 0.143	0.997^{b} ± 0.140	1.005^{b} ± 0.059	1.082^{b} ± 0.090	<0.001	0.473
Stage 3	Color Intensity (u.a)	7.041^{a} ± 0.850	10.128^{b} ± 1.221	9.535^{b} ± 0.140	9.743^{b} ± 0.721	<0.001	0.707
	Tonality (-/-)	0.429^{a} ± 0.030	0.379^{b} ± 0.003	0.381^{b} ± 0.011	0.380^{b} ± 0.003	<0.001	0.657
Stage 7	Color Intensity (u.a)	7.144^{a} ± 1.083	8.943^{b} ± 0.688	7.571^{a} ± 0.209	7.761^{a} ± 0.796	<0.001	0.463
	Tonality (-/-)	0.587 ± 0.018	0.567 ± 0.006	0.577 ± 0.013	0.579 ± 0.020	0.055	0.209

NOTE: Different letters assigned to the means indicate statistically significant differences (p < 0.05). (Stage 0: After Grape Reception; Stage 3: 1060-1070g/dm^3; Stage 7: 3 months post-AF/End of MLF)

The results for Anthocyanins can be found on Table 3. Anthocyanin extraction exhibited a similar behavior to those of pH, Colour Intensity, and Total Phenols – higher extraction rates during the first stages of maceration for TRPEF1 and TRPEF2. Immediately after grape reception, PEF-treated groups presented a concentration of Anthocyanins up to 120% higher (p<0.001, η_p^2=0.824). From there, the difference reduces after the 1st day to 31% (vs TRC). Interestingly, bipolar PEF treatments resulted in higher anthocyanin extraction compared to monopolar counterparts (+7%) on the 2nd day. TRPEF2 was the treatment that presented more similarities to TRE at the end of vinification, considering that both presented ~12% more ANT content than control.

Table 3. Anthocyanin concentration in Stages 0, 1, 2, 6, and 7.

	Parameter	TRC	TRE	TRPEF1	TRPEF2	p	η_p^2
Stage 0	ANT (mg/L)	47.6^{a} ± 13.0	48.1^{a} ± 15.8	95.8^{b} ± 11.0	105.0^{b} ± 11.5	<0.001	0.824
Stage 1	ANT (mg/L)	136.1^{a} ± 8.6	178.7^{b} ± 17.7	254.8^{c} ± 16.5	237.3^{d} ± 4.5	<0.001	0.937
Stage 2	ANT (mg/L)	238.1^{a} ± 51.1	350.7^{b} ± 12.0	332.3^{b} ± 63.2	412.1^{c} ± 22.3	<0.001	0.708
Stage 6	ANT (mg/L)	459.5 ± 29.8	477.0 ± 38.6	486.8 ± 27.2	480.6 ± 28.8	0.308	0.105
Stage 7	ANT (mg/L)	346.2^{a} ± 32.1	387.6^{b} ± 34.6	363.2^{ab} ± 4.1	389.6^{b} ± 25.9	0.004	0.332

NOTE: Different letters assigned to the means indicate statistically significant differences (p<0.05)(Stage 0: After Grape Reception; Stage 1: 1st day, beginning of AF (1080g/dm^3); Stage 2: 1060-1070g/dm^3; Stage 6: End of AF/After pressing; Stage 7: 3 months post-AF/End of MLF)

Total Tannins were assessed six months post-vinification, and it was shown that only the subjects treated with enzymes (TRE) presented a significantly higher concentration in comparison to the other groups ($F_{(3, 32)}$=18.788, p<0.001, η_p^2=0.638).

3.1.3. Total Dry Extract, Volatile Acidity, Tartaric Stability & Turbidity

Statistically significant differences were not found regarding the impact of the different maceration-aiding techniques on Volatile Acidity ($F_{(3, 32)}$=2.183, p=0.109, η_p^2=0.170). This outcome was expected, as most studies reported similar effects. For example, López *et al.* consistently obtained these results at bench-scale, by applying two different PEF protocols (5kV/cm, 1.8kJ/Kg and 10kV/cm, 6.7kJ/Kg to Tempranillo and a PEF treatment of 5kV/cm and 2.1kJ/kg to Cabernet Sauvignon [26,27] Puértolas and Aguiar-Macedo corroborated these results after vinifying, respectively, Cabernet Sauvignon at microvinification scale, and Arinto, at Pilot-Scale [6,28].

In contrast, only TRE demonstrated a statistically significant higher Total Dry Extract (23.9g/L), compared to the other groups ($F_{(3, 32)}$=8.069, p<0.001, η_p^2=0.431). While TRPEF2 followed with 22.9g/L, curiously, TRPEF1 presented a slightly lower TDE (22.4g/L) than TRC (22.6g/L). This is similar to the results obtained for the white grape variety Arinto [6]. Comuzzo *et al.* observed a similar behavior regarding the effect of monopolar PEF (1.5kV/cm, 10-11kJ/kg) and enzymes in the concentration of TDE. In addition, he also concluded that, by applying shorter pulses (1µs) and consequently reducing the *Ws* to 2kJ/kg, he also observed a TDE reduction of 5.2% compared with control samples [29,30].

Contrary to our previously published results regarding the effects of PEF on the grape variety Arinto, Tartaric Stability was not affected by any of the different treatments: TRC, TRE, TRPEF1 and TRPEF2 ($F_{(3, 32)}$=0.542, p=0.657, η_p^2=0.048). All wine understudy presented a conductivity drop ($\Delta\chi$%) of <3%, which can be interpreted as all being considered stable from a tartaric standpoint [6,21].

Enzymes contributed to a reduction in Turbidity ($F_{(3, 32)}$=33.039, p<0.001, η_p^2=0.756), with no significant differences between PEF-treated and Control wines.

3.2. Sensory Analysis

The sensory profiles of wines resulting from the various macerative pre-treatments – TRC, TRE, TRPEF1 and TRPEF2 – are depicted in Figure 7. Generally, similar sensory profiles were obtained across subjects, with few descriptors posing apparent differences. The most significant difference identified by the tasting panel was *Astringency*, with TRE and TRC displaying the highest and lowest scores, respectively, while both PEF treated subjects presented similar values. This was followed by the alteration regarding the perception of the aromatic descriptor *Metallic/Pneumatic*, which was more present in TRPEF1 and TRPEF2 wines.

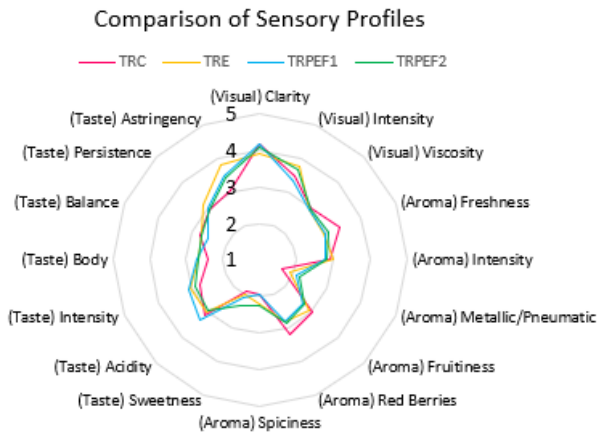

Figure 7. Sensory Profiles of TRC, TRE, TRPEF1 and TRPEF2.

The treatments TRE and TRPEF2 positively impacted *Color Intensity*. This corroborates the results obtained for both spectrophotometric analysis of Color Intensity and Anthocyanin content. Aromatic Freshness was noticeably diminished by all processes used compared to the control. This was also observed, albeit to a lesser extent, for *Fruitiness* and *Red Berries* aroma. Other sensory attributes presenting disparities between subjects were, for taste, *Sweetness* (more perceived for TRPEF2), *Body*, and *Intensity* (slightly higher for TRPEF1).

Following these results, CATPCA was conducted to characterize the sensory profiles. this purpose, two components, CP1 (1st Dimension) and CP2 (2nd Dimension), were considered. The Cronbach's α coefficient, used to evaluate the data's internal consistency and reliability was 0.953 for CP1 and 0.848 for CP2, which is classified as excellent [31]. In addition, the model explains 92.115% (CP1+CP2), of the variability observed in the dataset, which leads to a robust and reliable outcome. A biplot chart was obtained to visualize the relationships between the sensory descriptors (variables) and the wines subjected to sensory analysis (Figure 8).

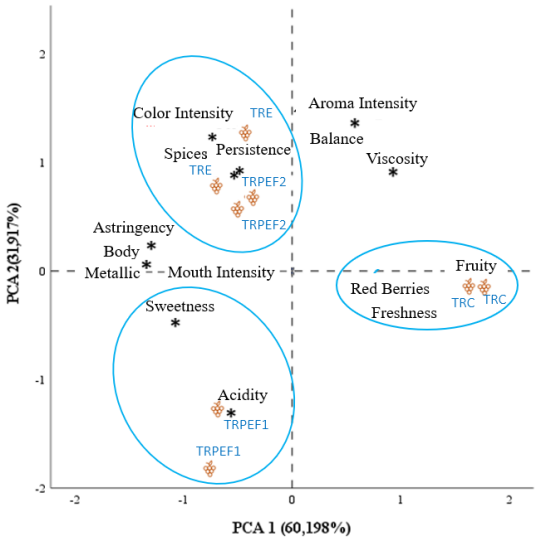

Figure 8. Biplot of the wine samples (TRC, TRE, TRPEF1, TRPEF2).

CATPCA showed that TRPEF1 wine can be mainly characterized by the parameter *Acidity*, and slightly by *Sweetness*. At the same time, TRC differs from all the other subjects due to primarily being mainly characterized by the aromatic fraction, namely *Freshness*, *Fruitiness*, and *Red Berries aroma*. TRE and TRPEF2 seem to be the most similar samples, from a sensory standpoint, considering their proximity in Biplot representation. Both are highly characterized and divergent from the other subjects due to *Color Intensity, Spice aroma*, and *Persistence*. It's interesting to observe that none of the subjects is particularly described by *Metallic/Pneumatic* aroma, *Mouthfeel Intensity* or *Body*. Most of the observations corroborate the results in the sensory profile, apart from *Metallic/Pneumatic* aroma, as these results were contradictory given that higher values were observed for the wines treated with PEF.

In addition, the panel was asked to attribute a score on a Likert scale as a Global Evaluation of the wines understudy. Although statistical analysis showed no significant differences ($F_{(3, 32)}$=0.697, p=0.556), panelist preferences were apparent from the highest grades assigned. PEF-treated wines consistently received higher scores. TRC wines received the highest score from 11.5% of panelists; TRE was preferred by 19.2%, while TRPEF1 and TRPEF2 were rated highest by 26.9% and 42.4% of panelists, totalling 68.8%.

4. Conclusions

The dichotomy observed during the 1st three days of vinification amongst the pairs TRC-TRE and TRPEF1-TRPEF2 supports the idea that PEF can enhance winery efficiency and capacity by accelerating the tank turnover, and reducing production costs, such as labor, energy (as, i.e., thermovinification, pumping) and consumables. In addition, it is demonstrated that, sensory wise, the final wines obtained by PEF application were not negatively impacted. This is supported by CATPCA and the Global evaluation attributed to the wines by the tasting panel, in which 68.8% of the panelists attributed the highest scores to PEF-treated wines. Furthermore, the distinction between the two PEF protocols - based on both physicochemical terms and sensory evaluation - highlights the importance of considering the potential benefits of Bipolar protocols for this specific application.

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