

Article

Extraction of Essential Oils from Plants by Hydrodistillation with Pulsed Electric Fields (PEF) Pre-Treatment

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Featured Application: Increase the efficiency from the extraction of Essential Oils from plants by Hydrodistillation with PEF pre-treatment.

Abstract: Essential oils, EOs, are concentrated liquids with complex mixtures of volatile organic compounds that can be extracted from different plant materials. EOs have been used by humans throughout history due to their natural properties: from pleasant fragrances, to anti-bacterial/fungal activities. This work presents the impact of pulsed electric fields, PEF, application as a pre-treatment for the extraction of EOs from eucalyptus, rosemary, and thyme leaves. The initial PEF pre-treatment was first applied to eucalyptus and rosemary leaves two weeks after harvesting, with a 2 kV/cm electric field and a specific energy of approximately 10 kJ/kg, followed by EO extraction by hydrodistillation, HD, with distillation times of 30 and 60 min. The best results were obtained for PEF pre-treated samples and 30 min HD, exhibiting an increasing trend in the average extraction yield of approximately 17% and 11% for eucalyptus and rosemary, respectively, in comparison with no PEF applied. The composition of the EOs extracted from eucalyptus was analyzed for their total phenolic content, TPC, where PEF pre-treated samples showed a higher polyphenol extraction, reaching 30% for 30 min HD. Finally, the optimization of the PEF pre-treatment was also studied, for maximizing the quantity of EO extracted from dry thyme leaves, while aiming for a minimization of energy consumption, for different distillation times. For this study it was observed that, for this plant material, an electric field of 1 kV/cm with 0.4 kJ/kg and an HD time of 30 min, after PEF application, was able to achieve an extraction yield up to 40% higher than the conventional method.

Keywords: essential oil; pulsed electric fields; hydrodistillation; Total Phenolic Content; eucalyptus; *Salvia rosmarinus*; thymus

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1. Introduction

Essential oils, EOs, are concentrated liquids of complex mixtures of volatile organic compounds that can be extracted from leaves, flowers, or seeds of different plants, which have been used by humans throughout history due to their natural properties: from the fragrance to anti-bacterial/fungal activities, among others [1–5].

There are various methods available to extract EOs, steam distillation being the most commonly used in the industry, as it does not require the use of organic solvents and is still capable of achieving a high extraction yield. For research activities, hydrodistillation, HD, is the most used technique for EO extraction [6–8], a traditional method for the extraction of bioactive compounds from plants, where plant materials are packed with

water in sufficient amount in a static compartment and brought to a boil. Alternatively, direct steam may also be injected into the plant sample.

In order to further optimize the extraction of EOs, the application of pulsed electric fields, PEF, can be used as a pre-treatment before HD. The application of an electric field to a biologic cell induces the formation of pores in the cell membrane, named the electroporation effect [9]. Electroporation through PEF application is becoming a mature processing technique in several fields, from agriculture and food [10–12], biotechnology and health [13,14], to waste and environmental applications [11,15,16]. Through PEF, very intense electric fields, in the order of a few to dozens of kV/cm, can be applied without a thermal effect [17].

Several authors have used PEF for increasing the efficiency in essential oil extraction from some plants, using HD, for example 4 kV/cm was applied to *Rosa alba* L. with 30–250 min extraction time by [7] and to *Nepeta transcaspica* with 120 min extraction time by [18], 0.8–2.5 kV/cm was applied to *Marribium vulgare* with 30–60 min extraction time by [19], 10–30 kV/cm was applied to flowers of Damask rose with 30–180 min extraction time by [20], and 1–3 kV was applied to *Artemisia herba alba* with 30–60 min extraction time by [21], from which it was possible to extract higher EO quantities and to which the PEF parameters were optimized.

Considering the potential of PEF, as a pre-treatment before HD, for increasing the EO extraction from plants and reducing the distillation time, the number of studies published, and plants investigated is still very limited [7,18–21]. Hence, this paper aims to contribute to increase the understanding of this application towards a future industrial acceptance of this technology, where three different plant materials were used, with two clear objectives in study: (a) PEF impact as a pre-treatment for eucalyptus and rosemary EOs extraction, using the plant leaves material some weeks after harvesting; (b) optimization of PEF parameters for extraction of thyme essential oil, using dry plant leaves material.

The first study aims to understand the impact of applying PEF, as a pre-treatment, to the extraction of EOs in similar conditions to what is used in the industry. The PEF protocol used was constant, and derived from the bibliography, where the distillation time was varied, to analyze its impact in both quantity and quality of the extracted EO, versus the EO extracted through the traditional method, for the same distillation times. The main goal is to study if PEF application to the plant material succeeds to achieve an EO with similar quality, and lower energetic costs, i.e., the same quantity and quality in less time.

Eucalyptus and rosemary were selected as rosemary's EO is one of the EO of reference in the industry and eucalyptus is used primarily in the paper industry, in Portugal, and its leaves are seen as a waste, thus the extraction of EO from this plant material would contribute to the circular economy. These two plants are used in similar conditions to those used in the EO industry (within two weeks of harvesting), so another important factor to have into consideration was its availability, considering the seasonal cycle of plants in Portugal. In order to compare the quality of the eucalyptus EOs with and without PEF application, the total phenolic content, TPC, of the oils was determined.

The second study has the objective of optimizing the PEF application conditions, which maximizes the quantity of EO extracted, while aiming for a minimization of energy consumption. For this study, diverse PEF conditions and different distillation times were considered. Thyme was selected because it is also an EO of reference in the industry.

2. Materials and Methods

2.1. Extraction of EOs

2.1.1. Samples and Equipment

Adult eucalyptus and rosemary leaves, harvested from the area of Proença-a-Nova, center of Portugal, and dried thyme leaves from Argelia, were used for this work.

The PEF treatment was applied in batch mode to the treatment chamber shown in Figure 1a, compromising a plastic container with two steel electrodes. Plant leaves were previously chopped in a Bimby® kit (Vorwerk, Wuppertal, Germany) and then introduced in the PEF treatment chamber with a small amount of demineralized water, to allow current passage. The high-voltage pulses were applied by a commercial pulsed generator EPULSUS®-PM1A-10 (from EnergyPulse Systems, Lisbon, Portugal), shown in Figure 1b, which can deliver 1 μ s to 200 μ s positive pulses up to 10 kV and 200 A, with repetition rates of 1 Hz to 200 Hz, at a maximum average output power of 3 kW.

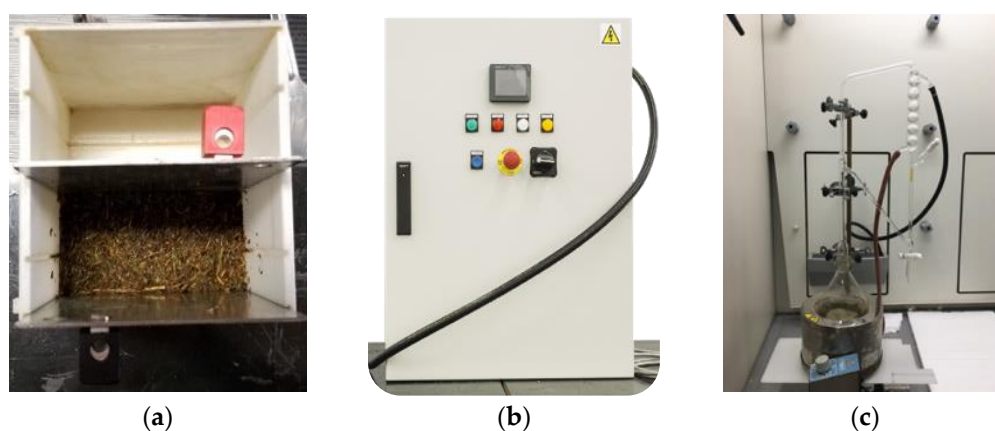


Figure 1. (a) Batch treatment chamber used for PEF application. (b) Pulsed generator used to apply HV pulses to the batch treatment chamber. (c) Clevenger apparatus used for the HD process for EO extraction from plants.

The pulse generator is based on a solid-state Marx generator, where various n capacitors are first charged in parallel from a relatively low voltage U_{dc} , and then connected in series with the load, applying about nU_{dc} [22]. As this generator is based on a direct capacitive discharge, it can deliver almost rectangular pulses, independent of the load impedance, as long as the generator's stored energy is much higher than the pulse energy, which is the case in this application.

In addition, for the HD process a Clevenger apparatus, as shown in Figure 1c, was used, including a 1 L volumetric flask and the heating mantle (R. Espinar, S.L., Raypa, Barcelona, Spain, No 29245, Model XC-1000, 400 W).

2.1.2. Pulse Electric Fields Application

The pulsed electric fields were applied in batch mode using the treatment chamber of Figure 1a, schematically drawn in Figure 2a. A typical voltage and current pulse waveforms, for an operation example, with 10 k and 80 A amplitudes, respectively, for a 10 μ s pulse with 1 Hz frequency, is given in Figure 2b.

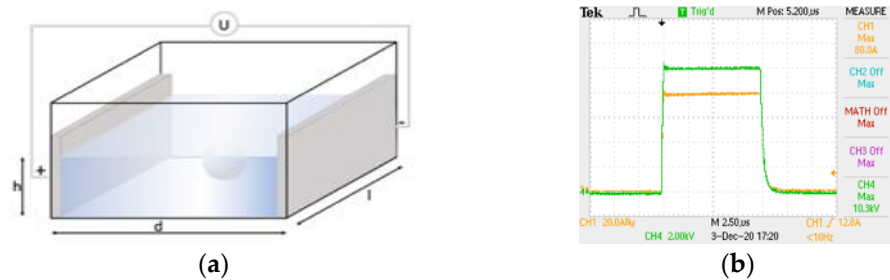


Figure 2. (a) Batch treatment chamber with volume $V = h \cdot d \cdot l$ (cm^3), where h (cm) is the height of the mixture, d (cm) the distance of the electrodes and l (cm) the width of the chamber. The voltage U (kV/cm) is applied between the two electrodes. (b) Voltage and current pulse example during the experiments, (green) voltage 2 kV/div, (yellow) current 20 A/div, 2.5 μs /div.

To successfully apply PEF, it is important to know and define some of its parameters, such as the electric field strength, E (kV/cm), treatment time, t_{PEF} (μs), and the specific energy, W_s (kJ/kg), which are also used for comparison purposes with the results from different authors.

The electric field strength can be given by (1), where U (kV) is the applied voltage and d (cm) the distance between the electrodes.

$$E = \frac{U}{d} \quad (1)$$

The electric field is applied in n discrete pulses, with a defined pulse width, t_{on} (μs), corresponding to a treatment time given by,

$$t_{PEF} = n \cdot t_{on} \quad (2)$$

Where each pulse has an energy, W_p (J), of,

$$W_p = U \cdot I \cdot t_{on} \quad (3)$$

considering I (A) is the pulse current amplitude.

The mixture inside the treatment chamber, for this specific study, is water and plant material, with an electrical conductivity, σ (mS/cm), which can change slightly during the treatment time, as the plant material infuses in the water. Because of this slight change during the treatment time, it is important to use an average value for σ , as it will influence the value of the pulse current amplitude, given by,

$$I = \frac{U \cdot A_e \cdot \sigma}{d} \quad (4)$$

where A_e (cm^2) refers to the electrode used area. Then, the total applied energy W_t (J), during the treatment time, is given by

$$W_t = n \cdot W_p \quad (5)$$

and the specific energy applied, can be calculated by,

$$W_s = \frac{W_t}{V \cdot \rho} \quad (6)$$

Considering V (mL) is the volume of the treatment chamber used and ρ (kg/L) is the approximate density of the mixture, where for simplicity it was assumed to be the same as water, 1. From (7), the increase in temperature, ΔT ($^{\circ}\text{C}$), from the application of PEF, can be calculated as,

$$\Delta T = \frac{W_s}{C_p} \quad (7)$$

where C_p is the specific heat capacity (kJ/(kg $\cdot^{\circ}\text{C}$)) of the treated material, which was assumed to be similar to water, 4.18 kJ/(kg $\cdot^{\circ}\text{C}$).

2.1.3. Experimental Procedure

Two different objectives were considered: (a) PEF impact as a pre-treatment for EO extraction; (b) optimization of PEF parameters.

PEF Impact as a Pre-Treatment for EO Extraction in Eucalyptus and Rosemary

Eucalyptus and rosemary were used as plant materials. Both plant materials were used within 15 days of harvesting, and thus, the results obtained were limited to the quantity harvested, as the plant's quality changes over time. The eucalyptus leaves were reduced in size, using the shredder mode of a Bimby®.

Two different protocols were followed: (i) the control (i.e., without PEF), and (ii) the one considering the PEF application. It is important to mention that for each condition considered (i.e., plant material, distillation time, t_{dist} , and specific energy, W_s , applied), the experiments were done in duplicate.

For the control, 50 g of plant material was weighed and introduced into a 1 L volumetric flask alongside with 500 mL of tap water, which was then connected to the Clevenger apparatus, seen in Figure 1c. The mixture was heated up to the boiling point and the distillation would go on for 30 or 60 min. The volume of the EO extracted was measured in the Clevenger apparatus, and then recovered into a dark flask that was covered with Al foil and stored in the fridge, at 4 °C, for a few days. The EO's density was determined, calculated the recovered mass and, finally, the extraction yield, η , was determined by (8).

$$\eta = \frac{\text{Mass of EO extracted}}{\text{Mass of plant material}} \cdot 100 \quad (8)$$

For the PEF experiments, 50 g of plant material was weighed and mixed with 250 mL of tap water in the batch treatment chamber, in order to have a uniform mixture. PEF was applied to achieve a W_s of approximately 10 kJ/kg (i.e., less than 2.5 °C temperature increase expected), with a voltage of 10 kV, frequency of 5 Hz, pulse width of 10 μ s, and field strength of 2 kV/cm. The mixture is then transferred to a 1 L volumetric flask, where an additional 250 mL of tap water was introduced. The volumetric flask was then connected to the Clevenger apparatus, and the same procedure as described for the control was followed.

For clearness, an example of PEF parameters calculation is given in Table 1, for one eucalyptus processing experiment. These calculations were made for all the PEF experiments, using the equations presented in Section 2.1.2.

Table 1. PEF parameters calculation example for one eucalyptus experiment, for, $f = 5$ Hz.

L (cm)	d (cm)	h (cm)	U (kV)	t_{on} (μ s)	# pulses	ρ (mS/cm)	I (A)	E (kV/cm)	W_p (J)	t_{PEF} (μ s)	W_t (kJ)	W_s (kJ/kg)	ΔT (°C)
10	5	6	10	10	312	0.8	96	2	9.6	3120	3	10	2.4

It is important to note that the conductivity, ρ , was calculated, using Equation (4), after applying the first pulse and measuring the pulse current amplitude for a given pulse protocol and batch condition. After this, the number of pulses, n , was calculated in order that the specific energy is approximately 10 kJ/kg.

Optimization of PEF Parameters for Thyme EO Extraction

For this experiment, two different methods were used: (i) the control method; and (ii) the one considering the PEF application. It is important to mention that for each condition considered (i.e., plant material, distillation time, t_{dist} , and specific energy, W_s , applied), the experiments were done only once.

For the control, a sample of 10 g of plant material was weighed and introduced into a 1 L volumetric flask, alongside with 220 mL of tap water, which was then connected to

the Clevenger apparatus. The mixture was heated up to the boiling point and the distillation would go on for 30 or 60 min. The volume of EO extracted was measured in the Clevenger apparatus, then recovered into a dark flask, and the mass of EO extracted was weighed. Finally, the flask was covered with aluminum foil and stored in the fridge, at 4 °C, for a few days. Finally, the extraction yield, η , was determined by (8).

For the PEF experiments, a sample of 10 g of plant material was weighed and introduced into a treatment chamber, alongside with 90 mL of tap water. PEF was applied, according to Table 2. The mixture was then transferred to a 1 L volumetric flask, where 130 mL of tap water was introduced. The volumetric flask was then connected to the Clevenger apparatus and the same procedure as described above was followed.

Table 2. PEF protocol applied to the EO extraction of thyme, $f = 10$ Hz, $\tau = 10$ μ s, and $n = 10$ pulses.

Condition	d (cm)	U (kV)	E (kV/cm)	W _s (kJ/kg)
1	2	2	1	0.4
2	2.5	5	2	2.3
3	2.5	10	4	9.4
4	2	15	7.5	21.1

Experimental Parameters Justification

In this paper, we show the influence of PEF pre-treatment in the optimization of hydrodistillation for EO extraction, using parameters that would make sense to the industry, for technology acceptance:

(a) Distillations times,

For a quality oil, the extraction time should not be too long. So, the time of distillation by HD, without PEF, should be chosen in order to extract almost the totality of the essential oil of the aromatic plant. Beyond this duration, only a small quantity of EO should be extracted, but with lower quality and with great losses of energy. Considering the latter, 60 min is a common time used by the industry and used by other authors [19,21]. As mentioned in [8]: “Prolonged distillation produces only a small amount of essential oil but does add unwanted high boiling point compounds and oxidation products.”. The introduction of a PEF pre-treatment has the objective of further reducing the distillation time, e.g., 30 min, by promoting extraction rate and saving energy at the same time.

(b) PEF protocol,

In relation to the PEF protocol used, for mass extraction in plants the parameters are normally: for the electric field <5 kV/cm, and for specific energy <20 kJ/kg. In principle the higher the electric field, kV/cm, the better is the extraction yield, but there is the possibility to extract some unwanted components that could impact the quality of the oil. In addition, other factors should be taken into consideration, such as the scalability of the equipment and the cost associated, namely relative to the increase in the voltage of the equipment. Increasing the electric field two-fold means that the Joule heating increases four times, and the cost of the equipment is higher, as the electrical current increases two-fold also. Higher specific energies, such as 20 kJ/kg, can lead to oil absorption during HD, as described in [7], and in some cases with higher fields, e.g., 3 kV/cm, worst yield results were reported, as described by [21]. Considering the latter and the results of other authors [19,21], 2 kV/cm was chosen and 10 kJ/kg.

2.2. EOs Characterization

2.2.1. Samples and Equipment

To determine the total phenolic content, TPC, the following materials, equipment, reagents, and solvents were used:

- Materials: EOs from eucalyptus, rosemary, and thymus leaves;

- Equipment: UV–Vis Spectrophotometer, Thermo Electron Corporation, Type Helios Alpha, Part No 9423 UVA 1002E, Ser No UVA 150211, made in England; Vortex, Scientific Industries Inc., New York, USA, Model No G560E; Double boiler, Thermo electron, Type003-2859 1200700108006, made in Germany;
- Reagents and solutions: saturated solution of sodium carbonate; gallic acid monohydrate, 98+%; methanol, 99.8+%; Folin and Ciocalteu's phenol reagent, 2M, SIGMA, 47641 500 mL, Lot No BCBN0326V; N-Hexane, 99%, MERCK, Lot No 298067; deionized water.

2.2.2. Experimental Procedure

The determination of the TPC was carried out using the Folin–Ciocalteu method adapted from [23]. Gallic acid was used as a standard to obtain calibration curves. In this way, a stock solution of 5 g/L of gallic acid was prepared, dissolving 0.25 g of the reagent in 5 mL of methanol and diluting to a final volume of 50 mL with deionized water. A set of ten standards were prepared, using different volumes of the stock solution in the concentration range between 12.5 and 500 mg/L.

Then, reactional standard solutions were obtained mixing 50 μ L of these standards with 3.95 mL of deionized water. Next, 250 μ L of Folin–Ciocalteu reagent was added and, after 2–3 min, 750 μ L of a saturated Na_2CO_3 solution was also mixed. The reactional standard solutions were heated at 40 $^\circ\text{C}$ in a double boiler for 30 min. After cooling, the absorbance of these solutions was measured in the spectrophotometer at 765 nm.

Blank solutions were also prepared using a similar procedure but replacing the standards by an equal volume of deionized water.

To ensure the results viability, three repetitions of the calibration curve were made. In each working day the validity of the previous calibration curves was assessed, by analysing reactional standard solutions prepared daily.

2.2.3. EOs Sample Preparation

The different eucalyptus EO samples extracted were diluted in n-hexane, using 30 μ L of EO in 5 mL of final volume. Using these solutions, reactional mixtures were prepared following the same steps as the reactional standard solutions, presented before, but adding 50 μ L of sample instead of the standard solution.

The TPC, in mg of polyphenols per gram of eucalyptus leaves, was determined through the TPC in sample reactional solution calculated from the calibration curves, $C_{average}$ (mg/L) according to (9) and its standard deviation, $STDev_{TPC}$ by (10).

$$TPC_i = \frac{C_{average} \cdot \frac{5}{0.03} \cdot V_{EO}}{1000 \cdot m_{EO}} \quad (9)$$

$$STDev_{TPC} = \sqrt{\frac{\sum \left(TPC_i - \frac{\sum(TPC_i)}{N} \right)^2}{N - 1}} \quad (10)$$

where V_{EO} (mL), m_{EO} (mg), are the volume and mass of the essential oil extracted, respectively, and the factor 5/0.03 takes into account the eucalyptus EO dilution presented before. For each sample of extracted EO, a dilution was done, at least once. Each dilution was analysed considering up to seven repetitions (represented by N), resulting in a total of approximately 21 collected points for the analysis, i.e., 30- and 60-min HD with and without PEF applied.

3. Results

The essential oils of three different plants (eucalyptus, rosemary, and thyme) were extracted and characterized in order to fulfil the two objectives set. While working with plant materials, the number of repetitions of different samples were directly connected to

the quantity of the plant harvested, as the composition of the plant changes over time and location. This limitation had an impact in the limited number of repetitions from HD, with and without PEF applied.

3.1. PEF Impact as a Pre-Treatment for EO Extraction in Eucalyptus and Rosemary

This experiment considers the analysis of different plant materials, under different PEF and HD time conditions; therefore, it was important to establish proper code names to easily know the conditions each sample referred to. The code names are defined by “condition.plant material_HD duration_replica”, as described in Table 3. For example, for a sample of eucalyptus, with PEF application, 30 min of HD, and the first replica of this condition the code name would be “P.E_30_1”; when indicating the average results of different samples, under the same conditions, the “replica” code is not shown: for the average of the samples of eucalyptus, with PEF application and 30 min of distillation would be “P.E_30”.

Table 3. Description of the code name of the different samples.

Condition	Control	C
	PEF	P
Plant Material	Eucalyptus	E
	Rosemary	R
Distillation Time	60 min	60
	30 min	30
Replica	1, 2	

All the different EOs were extracted by HD, during either 30 or 60 min, and the recovered volume, V , of EO was measured. The density of the extracted EOs, ρ , was determined to calculate their mass, m , that will allow for the determination of the extraction yield, η , through (11). The average results are summarized in Table 4.

$$\Delta\eta = \frac{\eta_{PEF} - \eta_{Control}}{\eta_{Control}} \cdot 100 \quad (11)$$

Focusing first in the results from the eucalyptus EO shown both in Table 4 and Figure 3, there is evidence of a PEF effect for the HD during 30 min, having an average increase in the extraction yield of approximately 17%, when compared with the extracted without the pre-treatment. For the 60 min HD time, the average extraction yield using PEF is similar to the control assay. The results are based on two repetitions per condition, hence for the dispersion analysis, only the amplitude of the results was calculated, as seen in the vertical lines of Figures 3 and 4.

Table 4. Summarized results for the extraction of eucalyptus and rosemary EOs.

Plant	Sample	V (mL)	m (g)	ρ (g/mL)	η (%)	$\Delta\eta$ (%)
Eucalyptus	C.E_30	0.65	0.60	0.93	1.20	17.1
	P.E_30	0.78	0.71	0.91	1.41	
	C.E_60	0.90	0.83	0.93	1.67	2.1
	P.E_60	0.93	0.85	0.92	1.70	
Rosemary	C.R_30	0.30	0.29	0.96	0.57	10.6
	P.R_30	0.35	0.32	0.91	0.63	
	C.R_60	0.38	0.34	0.91	0.68	6.7
	P.R_60	0.40	0.36	0.91	0.73	

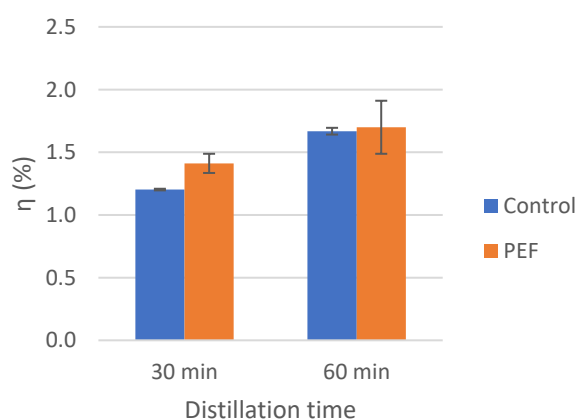


Figure 3. Results of yield of eucalyptus EO extracted with (PEF) and without PEF application (Control), and different HD times. Vertical lines indicate the dispersion, calculated from the amplitude of the results.

Looking at the results from rosemary presented in Table 4 and Figure 4, and similar to what is observed in eucalyptus, there is evidence of an average increase of 11% in the extraction yield upon PEF application and 30 min distillation. For the 60 min distillation time, there still is an average increase in yield when comparing with the traditional method, in the order of 7%.

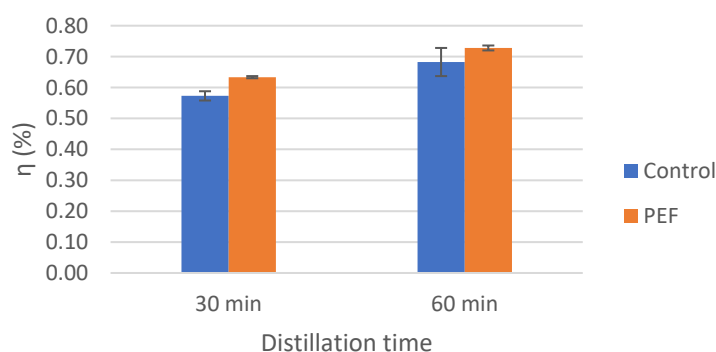


Figure 4. Results of yield of rosemary EO extracted with (PEF) and without PEF application (Control), and different distillation times. Vertical lines indicate the dispersion, calculated from the amplitude of the results.

The reported average yield increase, when using PEF, lead to the following possible explanation for the EO yield variation observed, obtained by HD, with and without PEF pre-treatment:

- In the case of eucalyptus, a more substantial effect with a shorter distillation time, i.e., 30 min, was observed, when PEF is used. Whereas, 60 min of HD is probably sufficient to extract almost all EO available in 50 g of eucalyptus leaves, as the yields obtained with and without PEF are very similar.
- In the case of rosemary, a similar effect with both distillation times, i.e., 30 and 60 min, was obtained, where the yield was not enhanced substantially. Additionally, 60 min distillation might not be enough to extract all EO present in 50 g of rosemary leaves, as there is still some yield variation with and without using PEF. Unfortunately, during the present work, it was not possible to further investigate this possibility due to the reduced material available.

The observed dispersions of the results, seen in the amplitude values, were unable to take a more definitive conclusion.

Considering that PEF induces the formation of pores in the cell membrane, depending on the field strength and cell size, as described in [9], it seems that in the eucalyptus case, PEF induced a bigger cell disintegration than in the case of rosemary, in the cells where EOs are located. For validation of this hypothesis, further work should be developed addressing the mechanism behind the PEF effect on the cells, namely eucalyptus cells.

From the results of EO extraction of the two plant materials discussed, it is possible to conclude that the use of PEF, as a pre-treatment before HD, with a field strength of 2 kV/cm and a specific energy of approximately 10 kJ/kg, is beneficial for increasing the extraction yield, when comparing to the traditional HD extraction. This result could lead to a minimization of energetic costs, as it allows for lower extraction times for the same EO quantity extracted. In fact, for the conditions proposed in this work, one can calculate the amount of energy that can be saved by using PEF. On one hand, the heating mantle used has a 400 W power consumption, corresponding to a 400 Wh energy consumption during the HD process. This means 400 W consumed in a HD with a 60 min duration. On the other hand, PEF application, considering the Table 1 protocol, uses 96 W average power for a 62 s treatment time, i.e., 1.65 Wh. Hence, PEF application together with 30 min HD can represent an energy saving of near 200 W (i.e., from 400 W for 60 min HD to 201.65 W for 30 min HD and PEF pre-treatment). This would happen without compromising the extraction yield of EO.

3.2. Optimization of PEF Parameters for Thyme EO Extraction

The PEF application was done varying different PEF parameters, leading to four different field strengths and specific energies delivery. The different PEF conditions applied are named from 1 to 4, where 1 represents the condition that deliver the lowest energy and 4 the one that delivers the highest energy. The yields obtained from the different extractions are shown in Figure 5.

From these results, it is clear that the application of PEF, despite the energy supplied, has a visible effect, with extraction yields higher than what is achieved with the traditional HD (control).

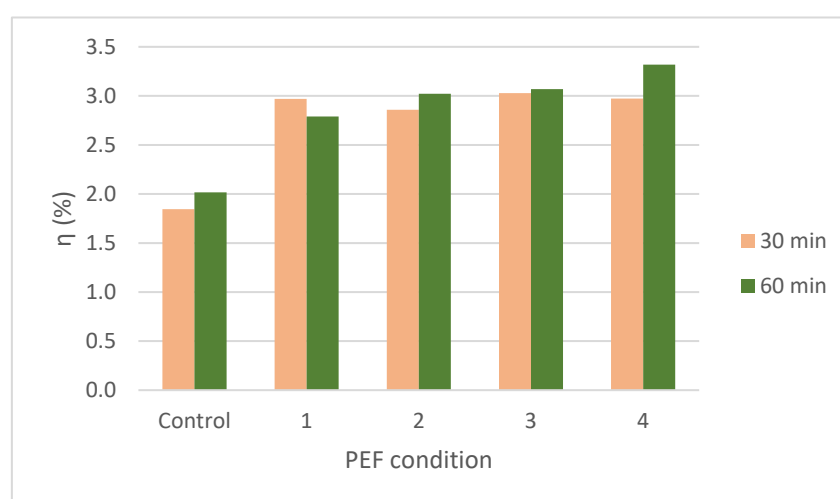


Figure 5. Results of yield thyme EO extracted with and without (Control) PEF application and different distillation times.

Although the results from Figure 5 are based only on one repetition per condition test, considering the eight experiments with PEF in comparison to the two without PEF, where there is no significant difference from 30 min to 60 min extraction, the average

values show an increase in EO extraction of approximately 40% with PEF pre-treatment in relation to the conventional method. Therefore, one can assume that, even for the minimal energy delivery (i.e., condition one, 30 min distillation, $E = 1$ kV/cm, $W_s = 0.4$ kJ/kg), it is possible to extract all the EO present in 10 g of dry thyme leaves. Hence, PEF application shows a beneficial effect on the energy efficiency of the process, lowering the extraction time down to 50%, from 60 min to 30 min, and obtaining a maximal EO extraction yield.

3.3. EOs Characterization

The average results of TPC in the extracted eucalyptus EO are presented in Table 5 and Figure 6. The TPC is expressed in mg of polyphenols per gram of eucalyptus. From the results obtained, there are two conclusions that can be taken:

- The longer the extraction time, the higher the TPC obtained in the EO;
- EO extracted with PEF application has a higher average TPC when compared to the traditional method (control).

An ANOVA single factor analysis was done to validate the results obtained, and statistical significance was obtained, showing that for the EO extraction from eucalyptus, the TPC is higher with the application of PEF pre-treatment in comparison with the traditional method.

Table 5. Summarized results for TPC analysis of eucalyptus EO extracted with and without PEF application.

Sample	TPC (mg Polyphenols/g Eucalyptus)	Δ TPC (%)
C.E_30	0.15 ± 0.03 *	+27
P.E_30	0.19 ± 0.04 *	
C.E_60	0.25 ± 0.06 *	+12
P.E_60	0.28 ± 0.06 *	

* Significant at ≤ 0.05 p level.

For the EO extracted after PEF application and 30 min HD, considering the higher average extracted yield, +17%, as shown in Table 4, it was expected that the TPC would also be higher than the one verified for the traditional extraction, +27%. However, for the 60 min distillation time, as there is not a significant yield difference, +2%, as shown in Table 4, it was interesting to observe an increase of +12% in the TPC with the extraction upon PEF application, which suggests that PEF application, on its own, allows for a higher extraction of polyphenols.

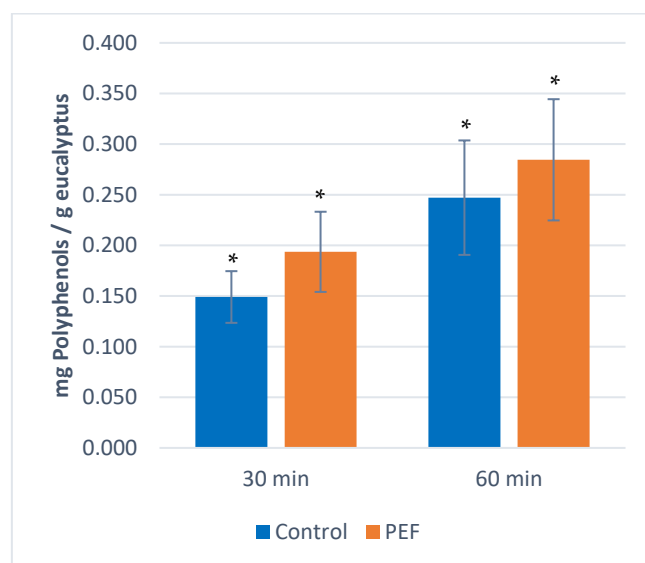


Figure 6. Results for TPC analysis of eucalyptus with (PEF) and without PEF application (Control) and different distillation times, phenols (mg/g eucalyptus). Vertical lines refer to the STDev. * Indicates statistical significance (p -value < 0.05).

4. Discussion

From the experimental results shown previously, there is evidence for a difference between the yield of EO extraction using only the traditional method, i.e., HD, and the extraction with PEF pre-treatment and HD afterwards.

The eucalyptus EOs extracted after the PEF pre-treatment presented more evidence of this positive effect. The EO yield of a 30 min extraction had an average increase of 17% compared to the traditional method, and the 60 min extraction seems to provide the maximum EO to be extracted from this plant material. From this, it is also possible to extrapolate that the maximum EO from eucalyptus could be extracted while lowering the extraction time, due to the effect of the PEF pre-treatment. From the rosemary EO extractions, similar results were obtained.

The lack of more repetitions, due to the reduced amount of plant materials available, in order to get a higher statistic confidence, do not allow to take a more definitive conclusion. For this reason, only the eucalyptus EO extracted both with and without PEF application was analysed for their TPC. In this case, statistical significance was achieved, and the experimental results showed that with PEF a higher TPC is extracted for both HD times. Furthermore, these results seem to show indirectly that, the yield of EO with PEF is higher than the traditional method.

The impact of PEF in the extraction of EO from thyme was more evident than with eucalyptus and rosemary, for the same PEF protocol and HD conditions. This may indicate a different cell disintegration mechanism, depending on the plant material but, also, the fact that the material conditions were different, as eucalyptus and rosemary were used a couple of weeks after harvesting (fresh material) and thyme was completely dry.

Hence, it is possible to say that the application of a PEF pre-treatment to eucalyptus, rosemary, and thyme leaves could result in an overall higher extraction yield of EO or the possibility of a lower extraction time to obtain the same EO quantity from these plants, when using the HD technique, resulting in an important reduction of energy consumption.

From the literature, different studies, with different plant materials, and different PEF conditions suggest that the application of PEF is seen as positive to increase the EO extraction yield. The EO extraction method applied in all studies was HD. In Table 6 are summarized the different PEF conditions and yield increase conditions for different plants described in the literature.

Table 6. Conditions and results of EO extraction yields, after PEF application, in the literature.

Plant Material	E (kV/cm)	W_s (kJ/kg)	Number of Pulses	T_{dist} (min)	Average Yield Increase (%)	Ref.
<i>Rosa alba</i> L.	4	10–20	-	30–150	13–33	[7]
<i>Nepeta transcaspica</i>	4	20	-	120	<67	[18]
<i>Marribium volgar</i>	0.8–2.5	-	150–300	30–60	67–162	[19]
Flowers of Damask rose	10–30	-	4–12	30–180	50	[20]
<i>Artemisia herba alba</i>	1–3	-	100–300	30–60	150	[21]

Considering the extraction of EO from white roses done by [7], the plant material was treated with PEF, with W_s both 10 kJ/kg and 20 kJ/kg. The EO was extracted through HD with a Clevenger apparatus, with t_{dist} between 30 min and 2:30 h (standard extraction time). The results were compared with EOs extracted from untreated plants. The extraction yield was maximized for $W_s = 10$ kJ/kg and $t_{dist} = 1:30$ h. The extracted EOs were characterized by GC–MS, and the results converged to the conclusion that for extractions of at least 1:30 h, there were not significant changes to the EO quality. The overall best results were aligned with the high extraction yield ($W_s = 10$ kJ/kg, $t_{dist} = 1:30$ h), lowering the standard extraction time in 1 h.

For the extraction of EO from catnip leaves studied by [18], the plant material was subject to PEF with $W_s = 20$ kJ/kg, $E = 4$ kV/cm, and $t_{dist} = 2$ h. Differently from the results of the previous paper, the PEF pre-treatment resulted in an extraction yield deficit of 20%, when comparing to the control. Although the quantity of EO extracted differed negatively, the EO quality, analysed by CG, does not present significant changes.

In relation to the extraction of EO from horehound researched by [19], PEF was applied with $E = (0.8–2.5)$ kV/cm, $N = 150$, and 300, and t_{dist} of 30 and 60 min. The condition that maximizes the extraction yield is $E = 2.5$ kV/cm, $N = 150$ (minimum W_s), and $t_{dist} = 30$ min, whereas for the 60 min extraction, after PEF application, there is a significant yield shortage, even comparing to the control. The quality of the EOs extracted in this study was analysed through GC–MS, and the chemical composition did not change significantly.

In relation to the extraction of EO from rose flowers investigated by [20], PEF was applied with $E = 20$ kV/cm and $t_{dist} = 2$ h. No influence on the quality of essential oils was observed, but there was an increase in methyl eugenol percentage, which is a disadvantage from a qualitative point of view. According to the results obtained, the treatment of rose flowers by PEF, before HD, can increase the yield by 50% and reduce the distillation to 120 min with 20 kV/cm.

Finally, the extraction of EO from *Artemisia herba alba* was investigated by [21], PEF was applied with 1, 2, and 3 kV/cm, with 100, 200, and 300 pulses, for HD times of 30 min and 60 min. The higher effect of PEF was achieved with 2 kV/cm, with 30 min HD and 200 pulses, an approximate 150% yield increase. For higher electric fields, the number of pulses, and 60 min HD the results got worse.

According to our results, from the presented studies and experimental results reported by other authors, it is possible to conclude that the application of PEF to the plant materials previously to the distillation step manages to deliver higher EO extraction yields, and does not impact negatively on the EOs composition, when compared to the traditional methods. Nevertheless, it is possible to see, also, in the results reported by other authors some dispersion of the results, where standard deviations are shown, which hinders the conclusions. Hence, more investigation is needed.

5. Conclusions

In conclusion, the processing of leaves, two weeks after harvesting, of eucalyptus and rosemary, but also dry thyme, by pulsed electric fields (PEF) before HD technique, has a beneficial effect on the essential oil yield. An increase in a range of 7%–40% was achieved.

The PEF application led to possible reduction of HD time from 60 min to 30 min, without sacrificing the EO quality. In effect, a higher TPC was obtained in the range of 12% to 27% for eucalyptus with PEF treatment. This can have an impact in the production capacity at the industrial scale, as it allows for a higher number of batches per day, but more importantly, this pre-treatment shows a positive tendency for lower energy consumption, allowing for lower operational costs for the extraction of the EO. However, more research is needed in order to reduce the uncertainties and optimize the results, regarding the correct PEF protocol to be used and distillation times.

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