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Optimization and Intensification of Bioactive Components and Antioxidant Activity of Extracts from Date Fruit (*Phoenix dactylifera* L.) Using Pulsed Electric Fields (PEF) Technology and Thermal Processing

Ikbel Souli [1,2,*], Nizar Chaira [2], Monia Jemni [3], Samir Tlahig [2], Ali Ferchichi [2] and Jean-Louis Lanoisellé [4]

[1] Faculty of Sciences of Tunis, Department of Biology, University of Tunis El Manar, Tunis 2092, Tunisia
[2] Dryland Farming and Oasis Cropping Laboratory (LR16IRA02), Medenine 4119, Tunisia
[3] Regional Research Center in Oases Agriculture of Degache, Laboratory of Dates Technologies, Km1 Street of Tozeur, Degache-Tozeur 2260, Tunisia
[4] University Institute for Technology, University of Bretagne Sud, UMR CNRS 6027, IRDL, F-56300 Pontivy, France
* Correspondence: ikbel.souli@fst.utm.tn

Abstract: The objective of this study was to assess the impact of pulsed electric field (PEF) treatment on the extraction of polyphenolics and antioxidant activity from downgraded date palm fruits. The PEF pretreatments (frequency: 30 Hz, time: 50 µs, pulse number: 240, the electric field strengths were found to be 1, 2, and 2.5 kV.cm^{-1}, and methanol (50%) and temperatures (20, 40, 50 °C)) were optimized and applied before extraction to produce an extract of date fruits with a high content of bioactive compounds. The extracts obtained immediately after pretreatments were analyzed for total polyphenolic content, antioxidant activity, and phenolic profiles. The results revealed that the PEF-assisted extracts at 2.5 kV.cm^{-1} at T_{50} °C exhibited higher polyphenol content (+27%) and antioxidant activity (+31%) and notably improved phenolic profiles compared to untreated extracts. PEF treatment processing significantly enhanced the bioactive components and antioxidant activities of date fruits over time, regardless of the treatment applied and the extraction's temperature. Hence, the application of PEF combined with thermal processing can be an appropriate alternative treatment for a better extractability of bioactive compounds from fruit of dates and food byproducts. These biomolecules could be consumed as new food technology, incorporated as food additives, and nutraceuticals products.

Keywords: *Phoenix dactylifera* L.; PEF; Thermal treatment; Polyphenolics and Antioxidant capacity; Phenolic profile; Peleg's model

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1. Introduction

The date (*Phoenix dactylifera* L.) fruits are an important part of the diet of many countries and are consumed fresh or at various processed forms because of their exceptional nutritional, biochemical and physicochemical characteristic. In Tunisia there are more than 250 varieties of dates, but the variety Deglet Nour predominates in a number of trees and production [1].

The production of dates is increasing every season, but losses during harvesting; postharvest handling and marketing are very high due to the incidence of physical and physiological disorders, pathological diseases and insect infestation [1]. These losses can achieve 30% of the total production in Tunisia [2]. In addition, the main activity of Tunisian date processing stations is date packaging and export. For that, tons of dates unfit for human consumption are rejected by date processors and only a small proportion is recycled as plant fertilizer or goes to animal feed [3]. Due to their richness of important phytochemical and nutritional properties; containing carbohydrates, fat, protein, dietary

fiber, and vitamins [4], some studies have been carried out to valorize these dates and to develop new products such as syrup, james, oils, vinegar, alcoholic . . . [3]. In addition, the date fruits are considered a source of bioactive compounds such as polyphenols, exhibt antioxidant, anti-mutagenic, anti-inflammatory, anti-allergic or anti-microbial activity, have an effect in human infertility, medicinal properties and a substrate to the production of a new metabolites such as ethanol [5–8]. Several studies reported that bioactive components and antioxidant activities of the date fruits extracts had been suggested as a source of by-product for production of natural antioxidants, or as ingredients for functional foods etc [9].

Recently, there is a developing demand of novel extraction techniques with rapid, and effectual potential than the traditional extraction process. Various researchers have estimated the capacity of some new physical methods, including, pulsed electric field (PEF) technology [10,11] and high pressure [5], microwaves [12], have shown their efficiencies for the extraction of biomolecules from different substrate.

Newly Pulsed electric field (PEF) is one of the most developed technologies. It is a nonthermal technology that consists in the application of an electric field, exposing the sample to repetitive short voltage pulses of relatively low energy and moderate intensity [13]. It has increased the efficiency and yield of the extraction process, such as the extraction of sugar from beetroot, juice from grapes or apple, and bioactive compounds [14].

Interestingly, PEF treatment has been shown to been couraging as an effectual conventional alternative techniques of cell disintegration by constructing these pores in the cell membrane via the phenomen on named electroporation [15,16]. Thus, these pores are accelerated their lease of intracellular contentment's, and that enhances the yield and the purity of the extracts. Several research revealed that PEF processed extraction has a beneficial impact on producing most important compounds from nutriments and nutritional food by-products and assists to reduce the extraction time and the solvent consumption and/or nether extraction temperatures [9,17].

It also PEF processing method helping to better results for the extraction of some bioactive components in fruits and vegetable juices for instance Grape Juices [18], Onion [19], Orange Peel [20] and Borago Officinalis leaves [21]. Furthermore, the process does not influence the quality and nature of the extracted products but improve the extraction yields and rates of diverse main ingredients.

For recuperation of polyphenols and various functional compounds, solid-liquid extraction is universally used in the food industry. In a previous work a solid-liquid extraction and identifications of polyphenolics s of 10 varieties of dates, the results prove that the methanolic extracts of date fruits are a rich source of phenolic compounds. In addition, a positive linear correlation ($p < 0.05$) between the phenolic compounds and antioxidant activity was found. The major phenolic compounds identified and quantified by LC-ESI-MS were the trans-ferulic and syringic acids for the majority of cultivars [22].

For these reasons, this work is focused on the application of a pretreatment using PEF to help the intensification for bioactive molecule extraction from dates fruits and to develop their application in food industry as a source of natural antioxidants in the diet or as natural food additives in benefit of human health.

2. Materials and Methods

2.1. Materials

This trial concerned fresh date palm (*Phoenix dactylifera* L., cv. Allig) fruits, which are a common variety characterized by their low commercial quality. The fresh date palm cv. Allig fruit samples (1 Kg) were randomly obtained from the oasis of Tozeur (Tunisia). The fruits were cleaned and sorted, and the seeds were removed. The samples were then kept at 20 °C until extraction.

2.2. Chemical Standards and Reagents

Methanol, Folin–Ciocalteu phenol reagent, ABTS$^{\bullet}$, gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, 97%), potassium persulfate ($K_2S_2O_8$), and

sodium hydroxide (NaOH) were purchased from Sigma Chemical Company (St. Louis, MO, USA).

Syringic acid, p-coumaric acid, trans-ferulic acid, ocoumaric acid, chlorogenicacid, trans-cinnamic acid, quercitrin (*quercetin-3-O-rhamonoside*), caffeic acid, *4-o*-caffeoylquinic acid, *3,4-di-o*-caffeoylquinic acid, *4,5-di-o*-caffeoylquinic acid, *(+)*-catechin, protocatechuic acid, epicatechin, 1,3-di-o-caffeoylquinic acid, hyperoside (*quercetin-3-O-galactoside*), luteolin-*7-O*-glucoside, apigenin, luteolin, naringenin, naringin, cirsiliol, quinic acid, and rutin of a purity > 98% were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Methanol and formic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Ultra-pure water used for HPLC (High-Performance Liquid Chromatography grade) was obtained from Scharlau (Barcelona, Spain).

2.3. Pulsed Electric Field Treatment

2.3.1. Treatment by PEF

The treatment chamber was composed of a Plexiglas® cylinder (6 cm in height) along with two steel electrodes (inner diameter of 6.5 cm). These two electrodes were attached to the PEF system, which comprised a high-voltage power supply (2.5 KV.cm^{-1}, 0.24 A) (SR2.5-P-600, Technix, France) and a pulse generator (TGP 110–10 MHz Pulse Generator-TTI ThurlbyThandar Instruments, France), used to control the protocol of PEF processing by regulating the pulse duration t_i (10^{-5}–10^{-4} s) and the frequency (24–240 Hz). The distance between the electrodes (delectrodes) was set to 1.2 cm and determined by the height of the substrate. To explore the treatment protocol, an oscilloscope (OX 8022-20 MHz Differential, Metcix, France) was used. The high-voltage power supply and the pulse generator were connected to a modulator (AHTPM 2.5, Effitech, Pau, France) which associated the generated high voltage to the pulse protocol to procure the needed PEF. The proceedings of the electrical conductivity, before and after the treatment, were controlled using an LCR meter (U1733C, Agilent, Les Ulis, France).

The impacts of various PEF treatments which cause tissue damage were predicted using the disintegration index (Z), which determines the difference between electrical conductivity values before and after PEF processing [23].

$$Z = \frac{\sigma_m - \sigma_i}{\sigma_d - \sigma_i} \quad (1)$$

where σ_m is the measured electrical conductivity value (S·m^{-1}) after the pause duration (Δ_t) between trains, and σ_i and σ_d refer to the conductivity (S·m^{-1}) of the control (initial) and totally electroporated substrate, respectively. The date fruit (100 g ± 0.1) was inserted between the electrodes.

Equation (1) uses Z = 0 for an intact sample and Z = 1 for a completely damaged tissue. Rectangular monopolar trains (n = 240 pulses) with a $50{\cdot}10^{-6}$ s pulse duration (t_i) for a frequency *f* of 30 Hz were utilized, and the number of trains (N) was 14 for Z_1. Thus, the time of PEF application is:

$$t_{PEF} = n \times N \times t_i \quad (2)$$

Immediately after the PEF treatment, the methanolic extraction of dates (*Phoenix dactylifera* L.) was obtained using the following procedure; a sample of 100 g of date palm fruit was applied for extraction in an orbital shaker using a combination of 100 mL methanol-water at the ratio of 1:1 (*v*/*v*). Next, the extraction was conducted at different temperatures: 20, 40, and 50 °C. After extraction, the final date fruit extract was purified through a microfilter paper (0.45 µM, VWR, France) and centrifuged at 5000 rpm at 20 °C for 20 min in a centrifuge (3-16P, Fisher Bioblock, France), and the supernatant was stocked at −20 °C until analysis.

2.3.2. Solid–Liquid Extraction Kinetics

To evaluate the extraction rate of the polyphenolics, Peleg's model (1988) was proposed as reported by Boussetta et al. [24]. The general form of Peleg's model can be written as:

$$C\ (t) = C_0 + \frac{t}{K_1 + K_2\ t} \quad (3)$$

where C (t) is the polyphenolics content (mg GAE.100 g^{-1} FW) at time t, t is the time extraction (min), C_0 is the polyphenolics content (mg GAE.100 g^{-1} FW) at time t = 0 (mg.100 g^{-1}), K_1 is Peleg's rate constant (min mg GAE.100 g^{-1} FW), and K_2 is Peleg's capacity constant (mg GAE.100 g^{-1} FW).

2.4. Analysis

2.4.1. Determination of Total Polyphenolic Content

The polyphenolic content was determined by applying a Folin–Ciocalteu assay [25] modified by Boussetta et al. [24]. 200 µL of the extract was mixed with 1000 µL of Folin–Ciocalteu reagent and 800 µL of $Na_2\ CO_3$ (75 g/L) solution (VWR, Fontenay-sous-Bois, France). The mixture was incubated in the dark for 10 min at 50 °C. After incubation, the absorbance was measured at 750 nm against a blank by the UV-vis spectrophotometer (Libra S32, Biochrom, Lagny-sur-Marne, France). In order to determine the total polyphenolic content (TPC), The standard curve was prepared using different concentrations of gallic acid (Sigma–Aldrich, St-Quentin Fallavier, France), and the results were expressed as mg of gallic acid equivalents per 100 g of fresh weight (mg GAE.100 g^{-1} FW).

2.4.2. Determination of Antioxidant Activity Using $ABTS^{\bullet+}$ Radical Scavenging Assay

The antioxidant activity was measured using ABTS assay in accordance with the method developed by Re et al. [26]. The ABTS radical cation ($ABTS^{\bullet+}$) solution was prepared by the reaction of 7 mM ABTS and 2.45 mM potassium persulphate, and left to incubate in the dark for 13–16 h. Furthermore, the mixture of $ABTS^{\bullet+}$ was diluted with methanol (80%) to obtain an absorbance of 1.1 at 734 nm. 975 µL of $ABTS^{\bullet+}$ solution was added to 25 µL of extract of date fruits or methanol for blank or Trolox solution as standard. The absorbance was taken using a spectrophotometer at 734 nm, using a UV/vis Thermo Multiskan Spectrum spectrophotometer. The scavenging free radical capacity was expressed as a millimolar Trolox equivalent (mM TEAC.100 g^{-1} FW). All measurements were performed in triplicate.

$$\text{ABTS radical scavenging activity}\ (\%) = \left(1 - \frac{A_{sample}}{A_{blank}}\right) \times 100 \quad (4)$$

2.4.3. Chromatographic Determination of Phenolic Compounds

The individual phenolic compounds of date palm fruit were extracted and quantified using an analytical LC-MS grade. The phenolic compounds were identified compared with the standard of each compound using the retention time and UV spectra as well as by examining the extracts after the addition of pure standards. The quantification of phenolic compounds was carried with a calibration curve obtained with the standards. Then, results were expressed as mg.100 g^{-1} FW. All measurements were performed in triplicate.

a. Preparation of extracts for LC-ESI-MS analysis

Date fruit was dissolved in LC-MS grade methanol (50%) to make 10 mg.mL^{-1} sample mixtures. The supernatant was decanted after centrifugation at 4 °C, at 1000× *g* for 15 min, and all samples were purified with a filter paper (0.45 µm) before LC-MS analyzes.

b. LC-ESI-MS (Liquid Chromatography-Electrospray Ionization Mass Spectrometer) Analysis

The quantification and characterization of polyphenol compounds in date fruits were performed by an LC-ESI-MS-type mass spectrometer. The sample to be analyzed was

pushed by a liquid (called the mobile phase) through a column filled with a stationary phase. The flow rate of the mobile phase is high, which leads to an increase in pressure in the system. This high flow decreases the time required to separate the components along the stationary phase. The extract was filtered through a 0.45 µM membrane filter before being injected into the HPLC system.

LC-ESI-MS analysis was performed using a LC-MS-2020 quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) equipped with an electrospray ionization source (ESI) and operated in negative ionization mode. The electrospray source was combined online with an ultra-fast liquid chromatography system consisting of a LC-20AD XR binary pump system, SIL-20AC XR autosampler, CTO-20AC column oven, and DGU-20AS degasser (Shimadzu, Kyoto, Japan). An Aquasil C18 column (Thermo Electron, Dreieich, Germany) (150 mm × 3 mm, 3 µM) preceded by an Aquasil C18 guard column (10 mm × 3 mm, 3 µM, Thermo Electron) were applied for analysis. Then, the mobile phase was composed of A (0.1% formic acid in H_2O, *v*/*v*) and B (0.1% formic acid in methanol, *v*/*v*) with a linear gradient elution: 0–45 min, 10–100% B; 45–55 min, 100% B. Re-equilibration duration was 5 min between individual runs. The flow rate of the mobile phase was 0.4 mL.min^{-1}, the column temperature was maintained at 40 °C, and the injection volume was 5 µL. Spectra were monitored and processed using Shimadzu Lab Solutions LC-MS software. High-purity nitrogen was used as the nebulizer and auxiliary gas. The mass spectrometer was operated in negative ion mode with a capillary voltage of −3.5 v, a nebulizing gas flow of 1.5 L.min^{-1}, a dry gas flow rate of 12 L.min^{-1}, a DL (dissolving line) temperature of 250 °C, a block source temperature of 400 °C, and a voltage detector of 1.2 v.

2.5. Statistical Analysis

Each experiment was repeated three times. Means ± standard deviations (SD) of the data and medians ± ranges (minimum value–maximum value) were measured. The error bars in all figures correspond to the standard errors. Duncan's test was applied to determine multiple comparisons between means at a significance level of $p < 0.05$. Statistical analyses were performed using SPSS 26.00 Software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism v.9. After testing the normality of the dataset with the Kolmogorov–Smirnov and Shapiro–Wilk tests ($p > 0.05$), an analysis of the variance (two-way ANOVA, $p < 0.05$) was performed to compare the effects of treatments, temperature, and their interaction on the variation in polyphenol content and ABTS antioxidant activity. Post hoc comparisons of means were computed by the Bonferroni test ($p < 0.05$).

3. Results and Discussion

3.1. Effect of PEF Treatment on Tissue Permeabilization (Z)

First, preliminary experiments were completed to optimize the protocols of the pulsed electrical pretreatment of the date fruits. The PEF treatments produced damage in the cells of the date tissue. The electroporation of the tissue and resultant mass transfer process is a complex function of the interaction among the electric field and the substrate properties.

The Figure 1 shows the variance of cell disintegration index Z, calculated by Equation (1), as a function of the number of trains for various studied treatments of date fruit tissue. The increase in Z with t_{PEF} resulted from a dramatic improvement in conductivity as a consequence of the electroporation of the cellular membrane. Accordingly, the PEF processing was consistent in opening pores in the cell membrane and in the efflux and influx of bioactive compounds.

The development of the cell permeabilization (Z) with the treatment time was a direct consequence of cell-tissue damage caused by PEF processing, resulting in the release of ionic intracellular compounds into the extracellular medium [17]. For instance, the lowest value of Z (0.48) was observed for a PEF treatment at 1 kV.cm^{-1}. When the electric field intensity increased to PEF 2 and 2.5 kV.cm^{-1}, the value of Z improved to 0.91 and 0.94, respectively.

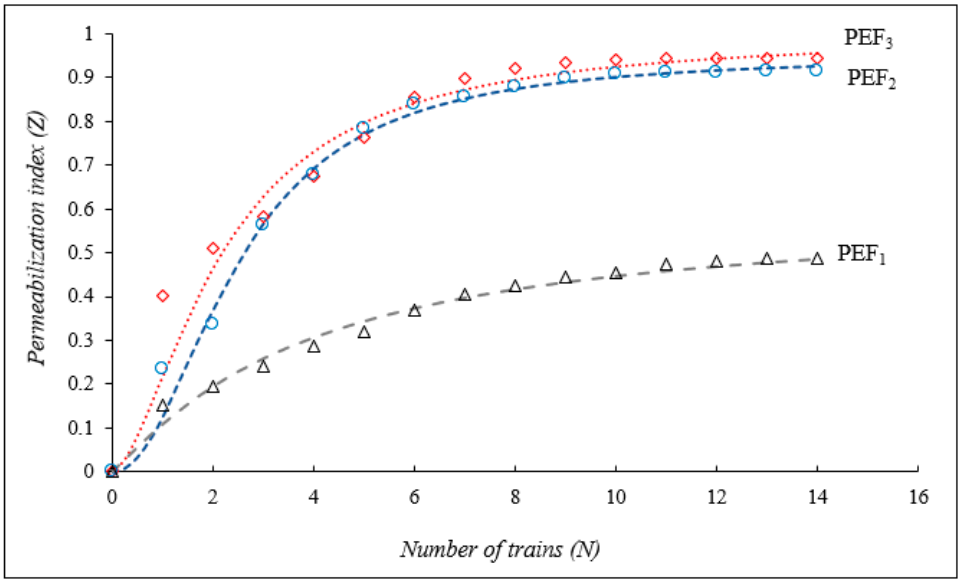

Figure 1. Permeabilization index Z as a function of number of trains (N) at different electric field strengths (PEF_1 = 1 KV.cm^{-1}, PEF_2 = 2 KV.cm^{-1}, and PEF_3 = 2.5 KV.cm^{-1}).

Hence, the results collected during this work showed that the Z value improved significantly ($p < 0.05$) while the electric field intensity increased. Nevertheless, the stage of permeabilization depended mostly on the electric field strength, and seemed to be more pronounced when the maximum field strength was used. The increase in Z values with the augmenting intensity of the PEF treatments was confirmed by the previously recorded data for various substrates tissues such as purple-fleshed potato and blueberry fruits [19,27].

3.2. Preliminary Study

The application of PEF in association with different temperatures accelerated the extraction of the phenolic compounds and their individual phenolic contents. As a result, it was proved that higher temperatures support the extraction and, consequently, the increase in diffusion coefficient.

Furthermore, according to the study of Aguilera et al. [28], the heating changes the characteristics of the cell membranes and improves the cell permeability. When PEF was accomplished at higher temperatures, the electrically produced damages were further pronounced. This consequence previously showed that the application of the pretreatment of the two-stage method (PEF + supplementary aqueous extraction at 50 °C) permitted a significant improvement in the rate and the antioxidant activity of the extracted components from papaya peels [29].

3.3. Extracts Analysis

Evidently, the extraction process, temperature, ratio (solvent/samples), solvent, and their concentrations are considered the main characteristics which significantly affect the extraction yield and phytochemical compounds such as the phenolic content [30,31].

3.3.1. Intensification of Polyphenol Extraction Using PEF Treatment

The extraction temperature is another processing variable and that was optimized in this work. The total polyphenol content of the extracts collected through PEF-assisted and untreated extraction at 20, 40, and 50 °C is shown in Figures 2–4. The experiments were executed with an alcoholic solvent (MeOH) for 95 min.

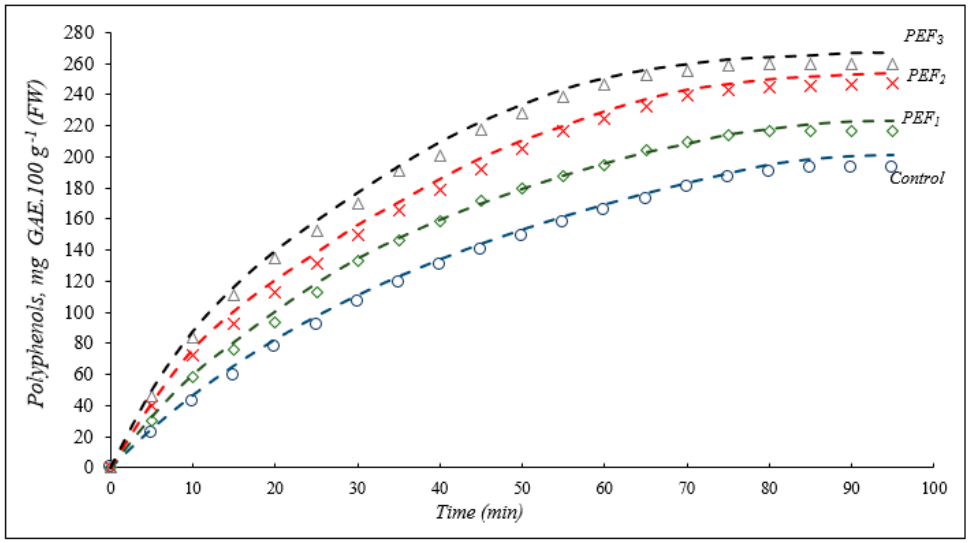

Figure 2. Effect of different treatments of PEF (PEF_1 = 1 KV.cm^{-1}, PEF_2 = 2 KV.cm^{-1}, and PEF_3 = 2.5 KV.cm^{-1}) on the polyphenolics extraction during diffusion at 20 °C (Each experiment was repeated three times).

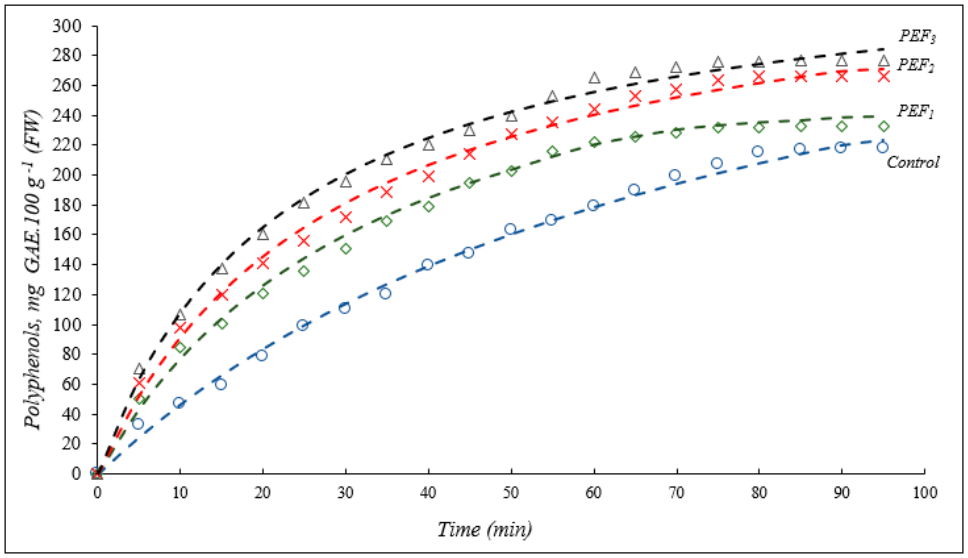

Figure 3. Effect of different treatments of PEF (E_1 = 1 KV.cm^{-1}, E_2 = 2 KV.cm^{-1}, and E_3 = 2.5 KV.cm^{-1}) on the polyphenolics extraction during diffusion at 40 °C (Each experiment was repeated three times).

Figures 2–4 present the progression of the polyphenolic content extraction for the PEF method and control treated at various temperatures. An improvement in polyphenol content could be attained at supportable temperatures. With an augmentation of temperature from 20 to 50 °C, a significant enhancement in the extraction of the aforementioned compounds was detected for the PEF-treated samples compared with the untreated ones. As shown in Figure 5, the maximum polyphenolic contents reached 259.86 ± 1.45, 276.12 ± 1.52, and 285.70 ± 1.13 mg GAE.100 g^{-1} (FW), respectively, treated at the intensity field strength (1, 2 and 2.5 kV.cm^{-1}) at 50 °C.

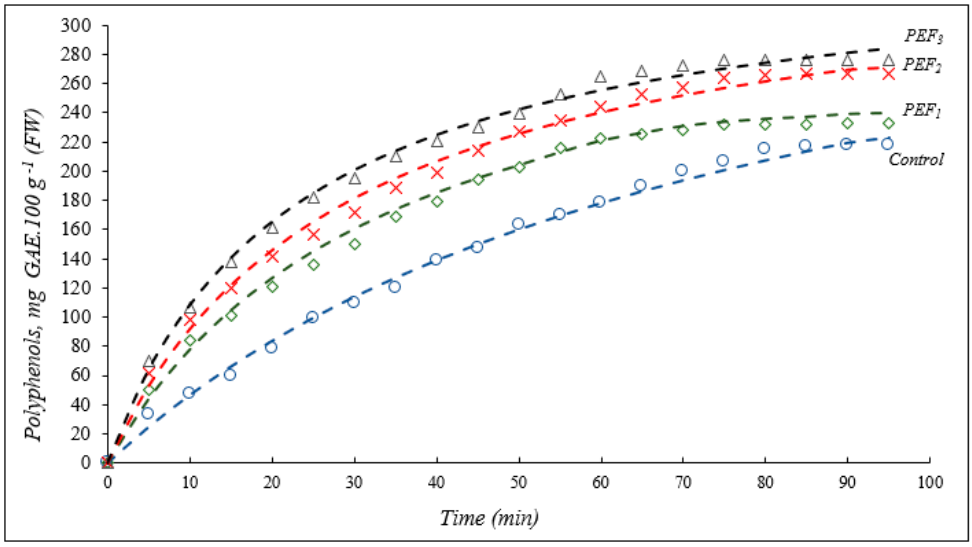

Figure 4. Effect of different treatments of PEF (PEF_1 = 1 KV.cm^{-1}, PEF_2 = 2 KV.cm^{-1}, and PEF_3 = 2.5 KV.cm^{-1}) on the polyphenolics extraction during diffusion at 50 °C (Each experiment was repeated three times).

The initial concentration of the polyphenolic content in date fruit extract were 192.86 ± 1.54, 217.51 ± 1.91, and 225.12 ± 1.59 mg GAE.100 g^{-1} (FW), respectively, treated at temperatures 20, 40, and 50 °C. Preceding findings proposed that the phenolic compound values of date fruits extracted using the conventional method are mostly varied, from 98.64 to 124.12 mg GAE.100 g^{-1} (FW) [22]. Immediately after processing, the results demonstrated a significant increase in the polyphenol content of date fruit extract (PEF_3 > PEF_2 > PEF_1) in comparison with untreated extract. The corresponding increase in polyphenolic contents for the different PEF treatments were: T_{20} (PEF_1 = 17%, PEF_2 = 29%, and PEF_3 = 33%), T_{40} (PEF_1 = 13%, PEF_2 = 22%, and PEF_3 = 27%), and T_{50} (PEF_1 = 15%, PEF_2 = 22%, and PEF_3 = 27%), respectively, compared to untreated extraction (Figure 5A–D). Overall, total polyphenolic contents ranged from 259.86 to 285.70 mg GAE.100 g^{-1} (FW). The assessed extracts exhibited highly significant differences ($p < 0.001$) under the effects of treatment, temperature, and their interaction, resulting in 77.02%, 22.39%, and 0.39% of the total variances, respectively.

The PEF treatments induced the permeabilization of the cell membranes of the samples. Nevertheless, methanol was an extracted solvent of some cell components and could advance extraction kinetics. It might be concluded that the destruction of tissue structure by methanol also contributes to increasing the extraction of polyphenolics.

In fact, Siddeg et al. [32] used a combination of water and ethanol (4:1 v/v) extract bioactive molecules from similar fruit (*Phoenix dactylifera* L., cv. Sukkari). The attained results demonstrated that the mean polyphenolic contents under the treatments PFE_1, PFE_2, and PFE_3 were 64.20, 65.90, and 67.35 mg GAE.100 g^{-1} (FW), respectively, compared with control extract (62.50 mg GAE.100 g^{-1} (FW)).

However, the extraction using ethanol contributed to the lowest recuperation of total polyphenolic content compared to the results obtained. This significant variance proves that a change in the solvent polarity could have a significant impact on the phenolic compounds.

Accordingly, it was found that PEF technology remarkably improved the extraction of phenolic compounds from date fruit extracts. According to Prior et al. [33], the Folin-Ciocalteu method for the determination of total polyphenolics is interfered with by many processes, such as the reduction in sugar, soluble proteins, ascorbic acid, and other substances which cause the increase in the concentration of total polyphenolics.

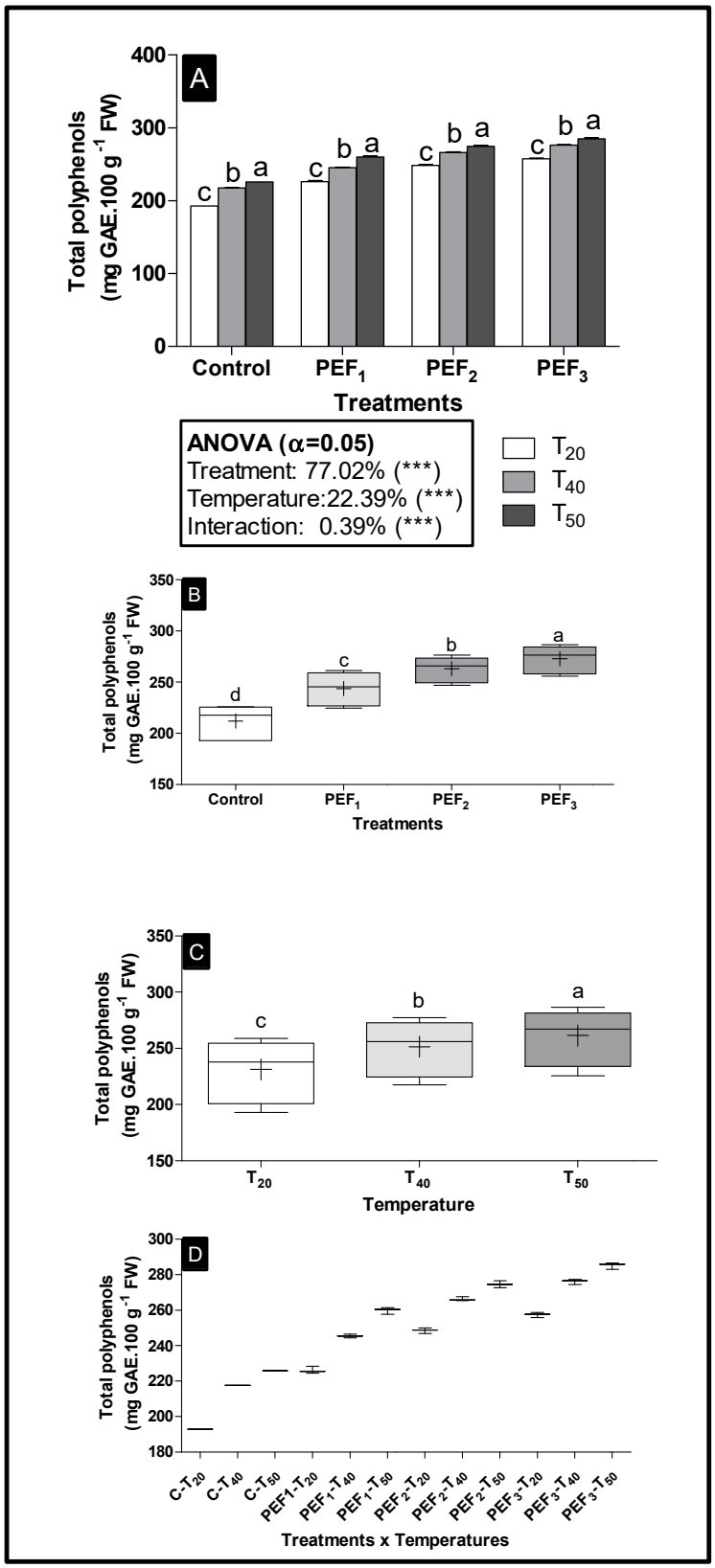

Figure 5. Concentrations of total polyphenolics content for the extracts obtained with untreated and PEF-treated methods at 20 °C, 40 °C, and 50 °C. The tests were performed as triplicates and values are mean ± standard deviation. Different superscript alphabets in a row show significant difference ($p < 0.05$) using Duncan's multiple-range tests.

Hence, this fact can illustrate the variation and increase in phenolic compounds of the date fruit extracts treated by PEF. Moreover, the increment in the extraction with PEF treatment is consistently ascribed to the electropermeabilization of biological cells which promote intracellular compound extraction [23]. It can be deduced that high electric-field intensity and higher temperatures encourage the higher solubility of polyphenols in the solvent, improving the diffusion amount and leading to a promoted mass-transfer rate.

The trend of improvement in the obtained results was comparable to the resulting polyphenolic content of the fruit extracts of the fresh date palm (*Phoenix dactylifera* L., cv. Sukkari) using different PEF treatments (1, 2 and 3 KV.cm^{-1}) [32]. In addition, these results were in accordance with the reported results in the studies of Luengo et al. [20] on orange peels treated by PEF-assisted pressing technology, by Grimi et al. [9] and Jaeger et al. [34] on juices apple, by Grimi et al. [35] and Donsi et al. [9,36] on grapes, and on the waste valorization in industrial tomato processing by Andreou et al. [37].

However, corresponding to Lafka et al. [38], polyphenolics maintained over 60 °C for a long time can sustain thermal deterioration (oxidation) and activity loss. As a consequence, there is an agreement about the functions of time and temperature in the extraction procedures of bioactive compounds. Bobinaitė et al. [27] found that intense processing conditions (>1 kV.cm^{-1}) for fresh blueberries might not be sufficient in liberating the phenolic components and the colorants from the juice. In addition, Morales-De la Peña et al. [39] reported that total polyphenolic and flavonoid content, regardless of the treatment applied, did not present significant diversity in juice-skim milk fruit (FJ-SM) and whole-milk beverages (FJ-WM) after high-intensity pulse electric field (HIPEF) or thermal pasteurization (TP) treatments.

According to the obtained results, PEF processing can be considered a potential alternative technology to pretreatment for fruits under relative conditions. It can notably reduce the extraction time and decrease the utilization of solvents.

3.3.2. Effect of PEF Treatment on Antioxidant Activity

An ABTS assay was used to evaluate the antioxidant activity of the date fruits obtained from different PEF treatments. For the assessment of the antioxidant capacity of the samples, many assays vary in terms of their essentials, proceedings, and experimental conditions. For this reason, in a composite matrix, there are various components, and all antioxidants have different roles in the samples' antioxidant potential [40].

Figure 6 illustrates the ABTS assay results of the extracts samples treated by PEF and thermal treatments, and those untreated. The values of the initial concentrations of ABTS in the date fruit extracts were 1651.91, 1863.57, and 2004.55 mM TEAC.100 g^{-1} (FW), respectively, treated at the temperatures 20, 40, and 50 °C. The ABTS results showed a significant increase in PEF-treated samples compared to the extract collected from the control; PEF pretreatments significantly ($p < 0.05$) enhanced the antioxidant activity of the substrate ($PEF_3 > PEF_2 > PEF_1$). According to data obtained, the ABTS results of PEF_1, PEF_2, and PEF_3 were in the range: T_{20} (PEF_1 = 19%, PEF_2 = 28%, and PEF_3 = 36%), T_{40} (PEF_1 = 19%, PEF_2 = 27%, and PEF_3 = 33%), and T_{50} (PEF_1 = 16%, PEF_2 = 28%, and PEF_3 = 31%).

Furthermore, the results demonstrated that the antioxidant capacity increased in electrical strength at different temperature treatments, as shown in the Figure 6. The highest total polyphenol content reached 285.70 ± 1.13 g GAE.100 g^{-1} (FW) with a corresponding antioxidant activity of 2623.3 ± 6.51 mM TEAC.100 g^{-1} (FW). The assessed extracts' antioxidant activity exhibited highly significant differences ($p < 0.001$) under the effects of treatment, temperature, and their interaction, accounting for 68.27%, 30.80%, and 0.63% of the total variances, respectively.

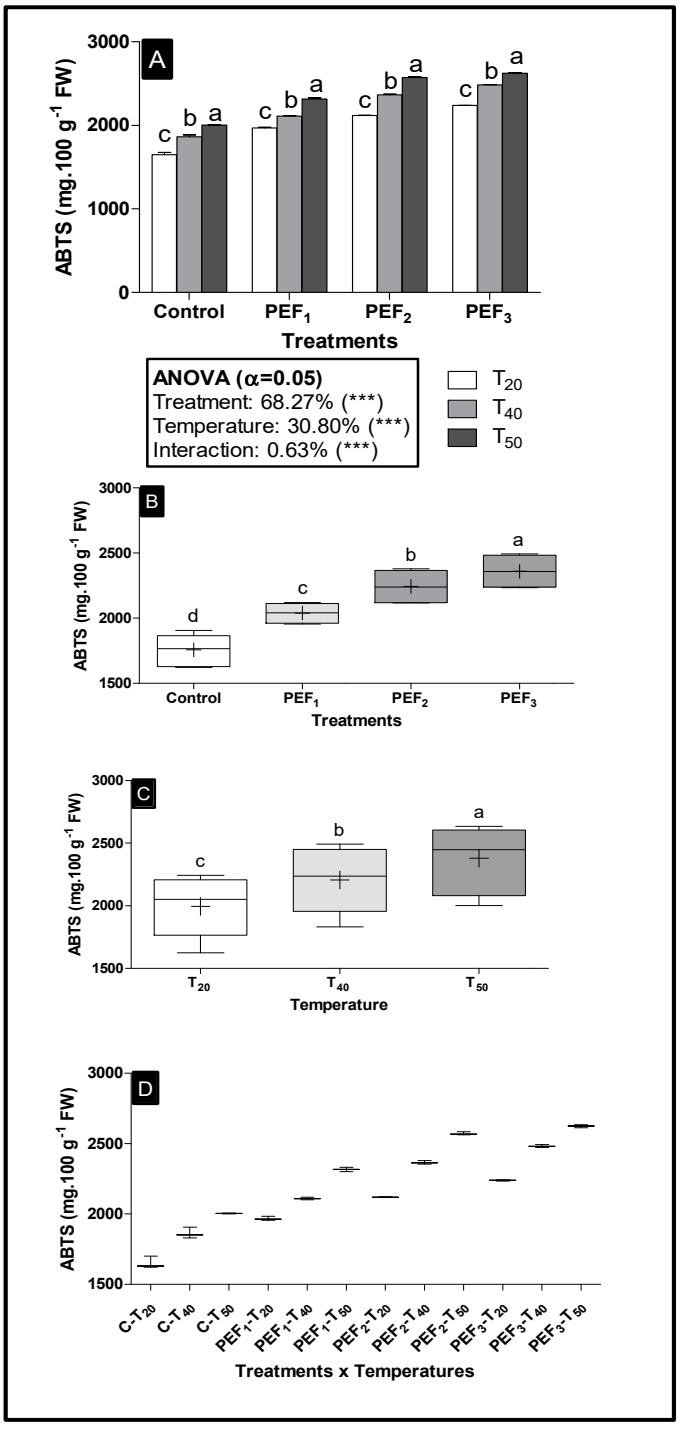

Figure 6. Antioxidant activity (ABTS assay) of the results obtained from untreated and different PEF-treated extracts. The tests were performed as triplicates and values are mean ± standard deviation. Different superscript alphabets in a row show significant difference ($p < 0.05$) using Duncan's multiple-range test.

The results of the DPPH (radical scavenging activity) and RP (reducing power) assay of date palm (*Phoenix dactylifera* L., cv. Sukkari) showed a high antioxidant capacity when treated by PEF (1, 2 and 3 KV.cm^{-1}) and the ethanolic extraction of date fruits [32]. Similarly, Donsi et al. [36] reported that PEF processing improved the antioxidant activity of the wine attained after the 20% PEF treatment on grapes. The same trend was found in apple juice extracts obtained after PEF pretreatment on cut or whole apples,

resulting in an increment in antioxidant activity between 30 and 50% compared with the untreated extract [9]. Corrales et al. [11] found that the antioxidant activity of the grape extract pretreated by PEF was improved four-fold. The antioxidant capacity of the fruit is correlated to the compounds and rate of bioactive components such as phenols, flavonoids, carotenoids, and vitamins [41]. Subsequently, a strong relationship among the total phenolic contents and antioxidant activities could be observed, and it was highly reliant on the extract concentration. The results conformed to the literature data which evidenced that phenolic compounds are in control of the antioxidant activity ([42,43]). Nonetheless, Morales-De la Peña et al. [44] and Plaza et al. [45] reported that high-intensity pulse electric field (HIPEF) treatment did not change the antioxidant capacity of a soymilk juice beverage or orange juice extract with regard to the untreated extract. Similarly, Elez Martinez et al. [46], Odriozola-Serrano et al. [47], and Odriozola-Serrano et al. [48] reported that high-intensity pulse electric fields (HIPEF) or thermal processing on orange juice, gazpacho, and tomato juice resulted in comparable levels of antioxidant capacity. Moreover, Rana et al. [49] considered the significant enhancement when compared with control in both phenolic compounds and antioxidant activity using DPPH test of 0, 5, 10, 15, 20 and 25 kV cm^{-1} PEF-treated grape fruit juice at 40 °C.

Thus, the fluctuation in the antioxidant capacity might be correlated with the diversity of the phenolic compounds. In addition, the obtained data prove that the application of PEF allows a high level of permeabilization of the cell membrane of dates.

Then, we analyzed the resulting improvement in the polyphenol extracts. Thermal diffusion at 50 °C associated with PEF pretreatment was found to exert the most apparent impacts on the total polyphenolic rate and antioxidant activity. Peleg's equation has been revealed to be acceptable for demonstrating the kinetics of the extraction of phenolic compounds, the use of thermal and PEF processing before hydroalcoholic extraction in the selective extraction of phenolic components and their individual profile.

It can be assumed that the PEF process accelerated the yield of bioactive molecule extraction, which has significant conservation potential as opposed to oxidation, as revealed through ABTS assay. Therefore, we can conclude that PEF technology is encouraging for its future utilization in the valorization of date fruits without using a hydroalcoholic solvent to preserve and maintain the quality and characterization of fresh juices and their nutritional properties.

3.3.3. Individual Phenolic Compounds Profile

The phenolic compounds, including phenolic acids and flavonoids, of treated and control date fruits is shown in Tables 1 and 2. As far as we know, this is the first study regarding the phenolic acid profiles of date fruits treated with PEF. The phenolic content in fruits is highly changeable, and these differences could be affected by many factors such as the variety, maturity stage, cultivation, climatic conditions, and environments factors.

Immediately after PEF processing, the rate of most individual phenolic acids and flavonoids detected in the sample extracts were enhanced or showed no significant changes. Regardless of the treatment applied, the LC-MS analysis of date fruits' methanolic extracts exposed the existence of 27 phenolic compounds: 15 phenolic acids and 12 flavonoids. In agreement with these results, pretreatment by PEF led to a positive result in the individual phenolic compounds. Notably, more phenolic acids with higher rates were found in the samples assisted by PEF. As shown in Tables 1 and 2, quinic acid was the dominant phenolic acid present in the date fruit extracts attained in concentrations of 19.48 ± 0.09 to 31.65 ± 0.55 mg.100 g^{-1} (FW), contributing to 31%, 53%, and 62%, respectively, of the PEF_1, PEF_2, and PEF_3 treatments. While Quercetin (*quercetin-3-o-rhamonoside*) and Rutin were the most abounding flavonoids (0.34 ± 0.01 to 1.04 ± 0.00 mg.100 g^{-1} (FW) and 0.19 ± 0.00 to 1.12 ± 0.01 mg.100 g^{-1} (FW)) present in the untreated and treated samples, it was observed that the content of Rutin was increased about 84%, 147%, and 489%, and the content of Quercetin about 105%, 132%, and 205%, respectively, in the PEF_1, PEF_2, and PEF_3

treatments.Consequently, the total phenolic content was enhanced after PEF processing. This improvement was significantly higher, at PEF3 = 2.5 KV.cm^{-1} ($PFE_3 > PFE_2 > PFE_1$).

Table 1. Concentrations of phenolic compounds (mg GAE.100 g^{-1} FW) of date fruits.

Phenolic Acids	Control	PEF_1	Δ (%)	PEF_2	Δ (%)	PEF_3	Δ (%)
Quinic Acid	19.48 ± 0.09 ^{a}	25.62 ± 0.50 ^{a}	31	29.89 ± 0.72 ^{a}	53	31.65 ± 0.55 ^{a}	62
Trans-cinnamic Acid	0.61 ± 0.02 ^{b}	0.72 ± 0.02 ^{b}	18	0.76 ± 0.04 ^{b}	24	0.86 ± 0.07 ^{c}	41
Trans-frulic Acid	0.44 ± 0.09 ^{c}	0.65 ± 0.02 ^{c}	47	0.70 ± 0.04 ^{c}	59	0.88 ± 0.01 ^{b}	100
p-coumaric Acid	0.25 ± 0.09 ^{e}	0.43 ± 0.02 ^{d}	72	0.46 ± 0.01 ^{f}	84	0.57 ± 0.01 ^{f}	128
o-coumaric Acid	0.33 ± 0.01 ^{d}	0.43 ± 0.02 ^{d}	30	0.53 ± 0.02 ^{e}	60	0.69 ± 0.01 ^{d}	109
Syringic Acid	0.33 ± 0.06 ^{d}	0.42 ± 0.00 ^{e}	27	0.54 ± 0.01 ^{d}	63	0.64 ± 0.01 ^{e}	93
Gallic Acid	0.03 ± 0.02 ^{l}	0.05 ± 0.00 ^{l}	66	0.06 ± 0.00 ^{m}	100	0.07 ± 0.00 ^{m}	133
Protocatchuic Acid	0.11 ± 0.06 ^{g}	0.20 ± 0.01 ^{g}	81	0.20 ± 0.01 ^{i}	81	0.26 ± 0.01 ^{i}	136
Chlorogenic Acid	0.06 ± 0.01 ^{j}	0.07 ± 0.00 ^{j}	16	0.08 ± 0.00 ^{k}	33	0.09 ± 0.00 ^{k}	50
Luteoline-7-*o*-glucosid	0.01 ± 0.02 ^{m}	0.02 ± 0.00 ^{n}	100	0.03 ± 0.00 ^{o}	200	0.04 ± 0.00 ^{p}	300
Caffeic Acid	0.08 ± 0.02 ^{i}	0.09 ± 0.00 ^{i}	12	0.11 ± 0.00 ^{j}	37	0.12 ± 0.00 ^{j}	50
4-o-caffeoylquinic Acid	0.04 ± 0.02 ^{k}	0.05 ± 0.00 ^{l}	25	0.07 ± 0.00 ^{l}	75	0.09 ± 0.00 ^{k}	125
1,3-di-o-caffeoyquinic Acid	0.03 ± 0.02 ^{l}	0.04 ± 0.00 ^{m}	33	0.05 ± 0.00 ^{n}	66	0.06 ± 0.00 ^{n}	100
3,4-di-o-caffeoyquinic Acid	0.23 ± 0.01 ^{f}	0.33 ± 0.02 ^{f}	43	0.45 ± 0.06 ^{g}	95	0.52 ± 0.03 ^{g}	126
4,5-di-o-caffeoyquinic Acid	0.03 ± 0.02 ^{l}	0.04 ± 0.03 ^{m}	33	0.06 ± 0.01 ^{m}	100	0.07 ± 0.01 ^{m}	133

The tests were performed as triplicates and values are mean ± standard deviation. Different superscript alphabets in a row show significant difference ($p < 0.05$) using Duncan's multiple-range test.

Table 2. Concentrations of flavonoid compounds (mg GAE.100 g^{-1} FW) in date fruits.

Flavonoids	Control	PEF_1	Δ (%)	PEF_2	Δ (%)	PEF_3	Δ (%)
Rutin	0.190 ± 0.005 ^{c}	0.350 ± 0.010 ^{c}	84	0.470 ± 0.010 ^{c}	147	1.120 ± 0.010 ^{a}	489
Quercetin	0.120 ± 0.001 ^{d}	0.130 ± 0.005 ^{e}	8	0.140 ± 0.002 ^{e}	16	0.120 ± 0.072 ^{e}	0
Quercetrin (*quercetin-3-orhamonoside*)	0.340 ± 0.010 ^{a}	0.700 ± 0.030 ^{a}	105	0.790 ± 0.030 ^{a}	132	1.040 ± 0.000 ^{b}	205
Hyperosid (*quercetin-3-o-galactoside*)	0.090 ± 0.002 ^{e}	0.160 ± 0.010 ^{d}	77	0.220 ± 0.010 ^{d}	144	0.520 ± 0.000 ^{d}	477
Naringin	0.060 ± 0.003 ^{f}	0.100 ± 0.002 ^{f}	66	0.120 ± 0.004 ^{f}	100	0.120 ± 0.007 ^{e}	100
kampherol	0.006 ± 0.001 ^{j}	0.007 ± 0.000 ^{j}	16	0.008 ± 0.001 ^{i}	33	0.010 ± 0.001 ^{g}	66
Naringenin	0.008 ± 0.001 ^{i}	0.009 ± 0.001 ^{i}	12	0.009 ± 0.001 ^{h}	12	0.009 ± 0.001 ^{h}	12
Apegenin	0.020 ± 0.001 ^{g}	0.030 ± 0.002 ^{g}	50	0.040 ± 0.002 ^{g}	100	0.050 ± 0.004 ^{f}	150
Luteolin	0.010 ± 0.001 ^{h}	0.020 ± 0.002 ^{h}	100	0.040 ± 0.002 ^{g}	300	0.050 ± 0.003 ^{f}	400
Cirsiliol	0.200 ± 0.005 ^{b}	0.490 ± 0.020 ^{b}	145	0.520 ± 0.003 ^{b}	160	0.530 ± 0.040 ^{c}	165
Catechine (+)	0.030 ± 0.01 ^{l}	0.060 ± 0.00 ^{k}	100	0.070 ± 0.00 ^{l}	133	0.080 ± 0.00 ^{l}	166
Epicatechine	0.090 ± 0.02 ^{h}	0.170 ± 0.01 ^{h}	88	0.220 ± 0.00 ^{h}	144	0.330 ± 0.00 ^{h}	266

The tests were performed as triplicates and values are mean ± standard deviation. Different superscript alphabets in a row show significant difference ($p < 0.05$) using Duncan's multiple-range test.

Hence, this treatment can be applied to enhance the quality and characteristics of date palm fruits and their byproducts such as syrup, wine, juice, and vinegar. However, the obtained results were comparable to those reporting that high-intensity pulsed electric fields (HI-PEF) and thermal pasteurization (TP) processing led to beverages with higher amounts of certain phenolic acids and flavonoids [48]. In addition, it is also visible that PEF pretreatment at 1.5 kV cm^{-1} (10 pulses), applied to Jujube (Ziziphusjujuba Mill), can increase wine quality attributes (such as the dry extract and phenolic contents) and phenolic compound extraction in caffeic acid, morin, and phydroxybenzoic acid [50].

In additional studies focusing on the comparison of MIPEF, HIPEF, and thermal treatments, authors demonstrated that high-intensity pulsed electric field HI-PEF treatments at 35 kV.cm−1 on tomato juices maintained a higher rate of polyphenols than those assisted with thermal treatment [51]. Similarly, Morales-de la Peña et al. [52] found that the concentration of most phenolic compounds in the studied fruit juice–soymilk beverage immediately improved after HI-PEF or thermal treatments.

Morales-de la Peña et al. [52] described that the concentration of Sinapic and Chlorogenic acids in fruit juice-soymilk beverages, treated by HI-PEF or thermal processing, decreased during storage. Del Caro et al. [53] found an increase in the content of Hesperidin with an increase in storage time for minimally processed citrus fruits; however, there was a significant reduction in the flavonoid content of orange juice.

Conversely to our findings, Odriozola-Serrano et al. [54] estimated the variance in total phenolic compounds after HI-PEF or thermal treatments in tomato juice. These authors noticed that there were no significant changes in total phenolic components between the HIPEF of thermally treated and untreated extracts of tomato juices.

The individual phenolic rate of the processed date fruit depended principally of the treatment applied, and the stimulation of the concentration of phenolic acids and flavonoids. In this way, it has been described that through processing, various reactions such as methylation, hydroxylation, isoprenylation, dimerization, and/or glycosylation, which induce modifications between the various phenolic compounds, can develop at different levels [55]. Additionally, we report the existence of some enzymes such as *phenylanine ammonia-lyase* (PAL) and polyphenol oxidase (PPO), which could cause the deterioration or synthesis of other components. PAL is the clef enzyme in phenolic biotransformation, and the incorporation of PAL activity in general corresponds to an increment in the accretion of phenolic compounds [56].

Consequently, the changes found in the rate of individual phenolic compounds of the samples immediately after different PEF treatments can be ascribed to biochemical reactions through them, such as methylation or hydroxylation, and between others which might occur during treatment. Furthermore, it may be possible that PEF processing encouraged favorable conditions that improved the PAL activity, consisting in the intensification of the phenolic rate in the date fruits. Taking into consideration various results described in the literature, it was observed that the concentration of compounds of individual phenolics may improve, reduce, or remain stable due to the extraction solvent, the temperature used, and the intensity of treatment.

Then, taking into account the variation in PEF and thermal treatments, the changes showed in the polyphenolic compounds of the extracts of date fruits could be explained by several of the following causes and effects:

(i) Biochemical reactions might existed during the PEF and thermal processing which lead to the construction of new phenolic compounds;

(ii) A significant impact of temperature on the PEF pretreatment was observed;

(iii) PEF and thermal processing might cause influences on phenolic complexes or in cell membranes with other components, liberating a few free phenolic acids or flavonoids;

(iv) PEF treatment might induce appropriate conditions to enhance PAL activity, resulting in an improvement in the phenolic rate of the methanolic extract of date fruits.

4. Conclusions

The increases in phenolic components as a consequence of the antioxidant capacity of PEF- processed date fruits were perceived after treatments controlled by the electric field strength (1–2.5 kV.cm^{-1}) and temperature. There is a high improvement in the polyphenolic content collected by treating with 2.5 kV.cm^{-1} and 50 °C, enhancing the antioxidant capacity of the date fruits by more than 30%. The obtained results confirm an intensification in the bioactive compounds, which could be assigned to a permeability of the cellular membrane during PEF treatments, which could potentially make the secondary metabolite extraction more efficacious. The PEF process is consequently an excellent extraction technology that produces innovative approaches for the further isolation of bioactive compounds. The most favorable extraction conditions were as follows: methanol (50%), solvent ratio (1/1), extraction temperature (50 °C), and pulsed field strength of 2.5 kV.cm^{-1}.

As a result, the use of PEF and thermal treatments could be suggested as an approach for producing date fruit extracts with a higher rate of phenolic components. This optimized

extraction process could be employed for the further isolation of bioactive compounds from date fruits (food additives, nutraceuticals, and pharmaceutical ingredients).

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Abbreviations

PEF	pulsed electric fields
n	number of pulses
N	number of trains
Σ	electrical conductivity value ($S.m^{-1}$)
t_i	pulse duration, (μs)
t_{PEF}	total time of PEF treatment, (s)
$d_{electrodes}$	distance between electrodes (mm)
T	temperature (°C)
T	time
GAE	gallic acid equivalent
TEAC	trolox equivalent antioxidant capacity

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