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Kırmızı Pancar Örneklerinde Darbeli Elektrik Alanın (PEF) Polifenol Oksidaz ve Peroksidaz Aktiviteleri Üzerindeki Etkisi

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Darbeli elektrik alan (PEF), Kırmızı pancar, Polifenoloksidaz, Peroksidaz.

ÖZET

PEF teknolojisi, gıda endüstrisinde çeşitli amaçlar doğrultusunda kullanılan ısısal olmayan gıda işleme proseslerinden biridir. Bu çalışma, darbeli elektrik alan (PEF) uygulamasının kırmızı pancar (*Beta vulgaris* L.) örneklerinde polifenol oksidaz (PPO) ve peroksidaz (POD) aktiviteleri üzerindeki etkisini araştırmaktadır. Bu amaçla, kırmızı pancar örneklerinin PPO ve POD enzim aktivitelerindeki değişimleri gözlemlemek için, 1 kV/cm'lik sabit bir elektrik alanda iki farklı özgül enerjiye (PEF1: 0,5 kJ/kg ve PEF2: 1 kJ/kg) sahip PEF uygulamaları kullanılmıştır. PEF1 uygulaması sonrasında depolamanın 1. gününde, örneklerdeki PPO aktivitesi kontrole kıyasla %45,90 ± 5,10 oranında azalırken, POD aktivitesi depolama süresince sabit seviyede kalmıştır. PEF2 ile indüklenen uygulama sonucunda, pancar örneklerinde elde edilen maksimum PPO ve POD aktivite değerleri sırasıyla 0,82 ± 0,08 U/mg protein (3. gün) ve 0,05 ± 0,01 U/mg protein (6. gün) olduğu belirlenmiştir. Sonuçlar, PEF uygulamasının POD'u uygulanan elektriksel alan şiddetlerinde inaktive edemediğini göstermiştir. Ayrıca, 0,5 kj/kg gibi düşük yoğunluklu PEF ile indüklenen bir ön işlemin ise pancar örneklerinde PPO aktivitesini hücre parçalanmasına bağlı olarak arttırdığı şeklinde değerlendirilmiştir. Bu sonuçlar, PEF işleminin kullanılan işlem parametrelerine bağlı olarak enzimatik aktiviteyi engelleyebileceğini veya uyarabileceğini göstermektedir. Bu nedenle, istenen teknolojik etkiyi elde etmek, işleme ve depolama sırasında gıdanın kalitesini korumak için PEF koşullarının optimize edilmesi gerekmektedir.

Impact of Pulsed Electric Field (PEF) on Polyphenol Oxidase and Peroxidase Activities in Red Beetroot Samples

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ABSTRACT

Pulsed electric field (PEF) technology is one of the non-thermal food processing methods used in the food industry for various purposes. This study investigates the impact of pulsed electric field (PEF) treatment on the activities of polyphenol oxidase (PPO) and peroxidase (POD) in red beetroot (*Beta vulgaris* L.) samples. For this purpose, PEF applications with two different specific energies (PEF1: 0.5 kJ/kg and PEF2: 1 kJ/kg) at a constant electric field of 1 kV/cm were used to observe the changes in PPO and POD enzyme activities of red beet samples. Following the application of PEF1, PPO activity in the samples decreased by 45.90 ± 5.10% compared to the control on the 1st day of storage, while POD activity remained constant throughout storage. As the result of PEF2 application, the maximum values of PPO and POD activity obtained in the beet samples were determined to be 0.82 ± 0.08 U/mg protein on day 3 and 0.05 ± 0.01 U/mg protein on day 6, respectively. The findings indicated that the application of PEF was ineffective in inactivating POD at the applied electric field strengths. Furthermore, a pretreatment induced by PEF at a low intensity of 0.5 kJ/kg was evaluated to increase PPO activity in the beet samples due to cell lysis. These results demonstrate that PEF treatment can inhibit or stimulate enzymatic activity, depending on the processing parameters used. Therefore, in order to achieve the desired technological effect and maintain food quality during processing and storage, PEF treatment conditions need to be optimized.

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INTRODUCTION

Beetroot (*Beta vulgaris* L.) belongs to the Chenopodiaceae family and originates predominantly from Asia and Europe. The red beetroot variety (*Beta vulgaris* subsp. *vulgaris* (conditiva)), a cultivated form of the plant, is widely used around the world. It is considered a functional food due to its bioactive and health-promoting components, which include vitamins, betalains, carotenoids, fibres, inorganic nitrate, and minerals (calcium, sodium, potassium, phosphorus, magnesium, copper, iron, zinc, and manganese). In addition, all parts of this plant have great interest from researchers and consumers due to different medicinal uses, like antioxidants, anti-depressant, anti-microbial, anti-fungal, anti-inflammatory, diuretic, expectorant and carminative [1-2].

In addition to the enzymes used for various purposes in the food industry, the active enzymes present in foods even after harvest cause unwanted changes in their nutritional and organoleptic properties. In particular, the control of polyphenol oxidase (PPO) and peroxidase enzymes (POD) which are responsible for enzymatic browning reactions in fruit and vegetable is a significant issue in food technology. Therefore, researchers are still interested in non-chemical, non-thermal, and minimal processing methods that can eliminate or minimize the activities of these enzymes in fresh fruits and vegetables [3-5].

Pulsed Electric Field (PEF) treatment is a non-thermal process that employs short pulses of high electric field intensity, applied to a sample positioned between two electrodes. The process induces permeability variation of food membranes by forming pore structure. It is mainly used to extend the shelf life of food products with fresh-like. The process can preserve or minimize the loss of sensory and health-promoting qualities of food products while it can affect the structure of amino acids, proteins and polysaccharides and their weak bonds [6-7]. It follows from the literature; PEF application has also been evaluated as an alternative technology toward sustainable food processing in terms of environmental friendliness [8].

Electro technologies not only effectively inactivate microorganisms and increase processing efficiency at lower processing intensities (e.g., pressing, pretreatment for drying and extraction, etc.), but also preserve the nutritional, functional, and sensory properties of processed foods. PEF technology has been identified as a promising alternative to, or complement to, conventional thermal pasteurization, primarily due to its ability to achieve high efficiency in a relatively short treatment time. This technology has also been reported to be applicable for targeted and controlled enzymatic treatments [9]. Although most of previous studies on PEF have been about reducing microbial activity to increase shelf life or facilitating the applicability of subsequent processes to food sample [10], there are a limited number of studies on its effect on enzymatic activity in food. Those are mostly concerned on inactivation of enzymes by PEF technology [11-12]. Enzyme activities are directly related to the food product's end quality. Furthermore, the effect of PEF on cell rupture has a significant impact on processing times, particularly in the case of dried fruits and vegetables. Within this study, it is aimed to determine the effects of different specific energies of PEF treatment on the PPO and POD activities that are responsible for spoilage of whole red beetroots which are popular raw materials for freeze dried or other dried products as healthy snacks.

MATERIALS AND METHODS

This section includes raw material preparation, PEF application process, analyses of PPO and POD activities of beetroot samples.

Raw Materials

Red beetroot samples were procured from the local market in Karaman, Türkiye, and meticulously washed with tap water to eliminate any foreign matter such as mud and soil. This process was conducted on the day prior to the implementation of PEF treatment. Subsequently, the samples, for which the diameter specifications are specified in Table 1, were stored at a temperature of 4°C until processing.

Table 1
PEF treated samples' information

Sample/Treatment Name	Horizontal diameter (cm)	Vertical diameter (cm)
Control	11.67±0.76^{a}	11.17±0.76^{a}
A1 (PEF1)	8.67±0.67^{bc}	8.67±0.29^{ab}
A2 (PEF1)	8.50±1.61^{bc}	7.13±0.46^{b}
A3 (PEF1)	7.07±0.21^{cd}	7.63±0.81^{ab}
B1 (PEF2)	9.17±0.72^{b}	9.33±0.31^{ac}
B2 (PEF2)	7.17±0.38^{cd}	8.43±0.21^{abc}
B3 (PEF2)	6.43±0.49^{d}	8.07±0.60^{abc}

$^{a-d}$: Groups marked with differing letters in the same column are statistically significant relative to the control group ($p \leq 0.05$).

Chemical Materials

Polyvinylpyrrolidone (PVP40), TritonX-100, catechol, pyrogallol, hydrogen peroxide are the chemicals used for enzymatic activity assays. All chemicals utilized in this study were purchased from Sigma Aldrich.

Pulsed Electric Field Application

The same procedure of Tekin et al. (2026) was applied for PEF treatment with small modifications [13]. A pilot-scale batch PEF unit (PEF PilotTM) (Elea GmbH, Germany), which can deliver voltages of up to 28 kV via monopolar and exponential decay pulses was used for that purpose (Figure 1). The pulse number was subjected to variation, ranging from 7 to 33, in accordance with the mass amount in the treatment cell, in conjunction with the requisite specific energy (kJ/kg). The pulse width was maintained at a constant value of 0.5 μs. The distance between the electrodes, which were composed of stainless steel, was measured to be 28 cm. Weighed whole beetroot samples were determined and then placed into the treatment cell between two parallel electrodes. Subsequently, tap water (σ = 907 μS/cm and T = 18.8 °C) was added until the samples were completely covered. While the total mass of the product was 1.2-1.5 kg (that corresponds to weight of 3 beetroots with high diameter), 6 L of distilled water was used. When the total mass of the product was 0.9-1 kg (equivalent to the weight of three small beetroots), 4 L distilled water was used for the reason that it completely covered the beetroot samples. Two different specific energies of 0.5 and 1 kJ/kg (PEF1 and PEF2 respectively) with the constant field strength of 1.0 kV/cm were chosen to investigate their effects on PPO and POD enzymatic activities. All treatments were performed in triplicate. PEF application parameters are summarized in Table 2. PEF untreated beetroots were used as control. All samples (PEF treated and untreated) were cut into four pieces and placed directly into refrigerator bags to be stored at 4 °C. Then, each piece in every package was separately subjected to enzyme activity analysis at different storage days to minimize the repeatedly cutting effect.

Table 2

PEF application conditions

Sample and Treatment Name	Pulse number	Voltage [kV]	Field Strength [kV/cm]	Mass in Cell [g]	Energy [J]/ pulse	Energy [J/kg]/ pulse	Specific Energy [kJ/kg]	Volume of Water (L)
A1 (PEF1)	11	28	1	8240	392	47.57	0.52	6
A2 (PEF1)	11	28	1	8540	392	45.90	0.51	6
A3 (PEF1)	7	28	1	5285	392	74.17	0.52	4
B1 (PEF2)	22	28	1	8537	392	45.92	1.01	6
B2 (PEF2)	23	28	1	8599	392	45.59	1.05	6
B3 (PEF2)	13	28	1	4929	392	79.53	1.03	4

Figure 1

The schema of PEF application process

Measurement of Enzymatic Activities

PEF pretreatment for beetroot samples was evaluated in terms of its capability to avoid the activation of degradative enzymes such as POD and PPO that consequently provoke pigments and nutrients loss.

Polyphenol Oxidase Activity Assay

The PPO activities of food samples were evaluated spectrophotometrically at 420 nm in a UV spectrophotometer (Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer, USA) with some modifications made in the method described by Rocha and Morais (2001) [14]. Control and PEF-treated beetroot samples were subjected to PPO activity analysis on the 1st, 3rd and 5th days of the storage period. For the purposes of analysis, 5 g of food sample was homogenized in a mortar on ice bath with a mixture of 10 ml of cold sodium phosphate buffer (0.2 M; pH 6.8), including 1% PVP40 and 0.25% Triton X-100. Following centrifugation (9418 g/20 min) at 4 °C, the resultant upper layer was designated as the enzyme solution. The reaction mixture containing 14 µL of enzyme solution and 186 µL of catechol (0.16 mol/L) as a substrate was used to determine PPO activity of the samples. Subsequently, the change in light absorption was measured at 15-second intervals for a period of 60 seconds. The resulting data, in the form of a curve representing the relationship between time and the change in light absorption, was utilized to calculate the activity of PPO (U/mL). The phosphate buffer served as the negative control.

Peroxidase Activity Assay

The determination of peroxidase activity was adapted from the method employed by Chance and Maehly (1984) [15]. Control and PEF-treated beetroot samples were taken into the activity assay on the 2nd, 4th and 6th days of the storage period. For the process, approximately 1 g of food sample was thoroughly homogenized in a mortar on ice with 5 mL of 0.1 M sodium phosphate buffer (pH 6.0). Centrifugation (9418 g/20 min) was performed at 4 °C. The upper phase was utilized as the enzyme solution. The following mixture was used for the measurement of light absorption in a UV spectrophotometer: 140 µL of ultrapure water, 21 µL of sodium phosphate buffer, 21 µL of a 5% pyrogallol solution and 11 µL of hydrogen peroxide (0.5%). The mixture was stored in a dark environment for a period of 10 minutes. Subsequently, a quantity of 7 µL of enzyme solution was added to the mixture. The absorbance at 420 nm was measured over a period of three minutes, with a 15-second interval between each reading. For the negative control, the process was repeated, with the enzyme solution substituted by 7 µL phosphate buffer. The calculation of POD activity (U/mL) as a function of time was performed using three repetitions of different absorbance measurement results.

Protein Analysis

Pierce™ BCA Protein Assay Kit (Catalog Number: 23227, Thermo Scientific™, USA) was used to determinate the amount of protein in enzyme solutions. The values were measured as mg/mL and used to calculate enzyme activity as U/mg protein.

pH Value Evaluation

Approximately 1 g of beet root sample was homogenized with ultrapure distilled water (1:1, w/v). The pH of the homogenate was determined by using a calibrated digital pH meter (Mettler Toledo, USA) with the measurement of at least three repetitions.

Statistical Analysis

Analysis of each test (PEF1, PEF2 treatments, PPO and POD activity assays) was performed in triplicate. Statistical analysis was performed using SPSS version 20.0 (IBM SPSS Statistics, Chicago, IL, USA) and significant difference between means was verified by Duncan test in One Way Anova at $p \leq 0.05$. All results are expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Changes in PPO Activity

To study the effect of pre-treatment on the PPO activity of fresh beetroot samples, the biomass was treated with PEF. It follows from the PEF1 and PEF2 treatment results, enzymatic activities at the end of storage period were not different than the control sample (Table 3). Figure 2 shows that PEF2 caused relatively high activity compared to control in the first and third days of the storage. Due to the cell rupture, the enzymatic activities increased at PEF1 and PEF2 treated samples on the 3rd day. Previous studies show that PEF is generally associated with a decrease in enzyme activity due to its capability of provoking alterations of the three-dimensional enzyme structure, preventing compatibility with active site and avoiding the conversion into products [16-17], there are also some new studies which demonstrate its capacity to increase enzyme activity. In a previous study, PEF treatment of grape, orange and tomato juices preserved most of the quality properties by providing >90% inactivation on pectin methyl esterase, PPO, and lipoxygenase enzymes. Also, it was emphasized that enzyme inactivation increased with increased energy [18].

In contrast, Herrara-Lavados et al. (2025) reported that PEF treatments at 15 and 20 kV/cm enhanced flavourzyme (FV) activity by modifying the protein's tertiary structure, reducing its surface hydrophobicity and intrinsic fluorescence, and increasing the hydrolysis degree and peptide yield from 9.6% to 16.6% and 10.6% to 18.7%, respectively [19]. PEF is well known as an emerging technology which enhances pectinase, β-galactosidase, peroxidase, protease, glucoamylase, invertase, enolase, lysozyme and pepsin activities [20]. This is generally associated with PEF causes increase at the size of active sites or creation of new ones in related enzyme molecule [21]. Furthermore, it is known that PEF-induced activation or inactivation of enzymes mostly depends on parameters such as electric field strength, pulse specifications (number, duration, frequency, shape, width, polarity), electrical energy density, treatment chamber geometry and shape, treatment mode (batch or continuous), physicochemical properties of the enzymes, initial temperature and media factors, etc. [12]. It means that the described PEF conditions showed these results for the beetroot samples with mentioned features. The results may change due to the different parameters of PEF treatment to be applied. Therefore, comprehensive research related to PEF effect on PPO should be carried out to provide the most effective conditions for target food before PEF treatment.

Table 3

PEF application effects on PPO activity of beetroot samples during the storage time

Applications	Enzyme Activity (U/mg protein)		
	1st day	3rd day	5th day
Control	0.29±0.04[a]	0.25±0.03[a]	0.37±0.03[a]
PEF1	0.16±0.02[b]	0.45±0.01[b]	0.38±0.07[a]
PEF2	0.75±0.01[c]	0.82±0.08[c]	0.39±0.04[a]

[a-c]: Groups marked with differing letters in the same column are statistically significant relative to the control group ($p \leq 0.05$).

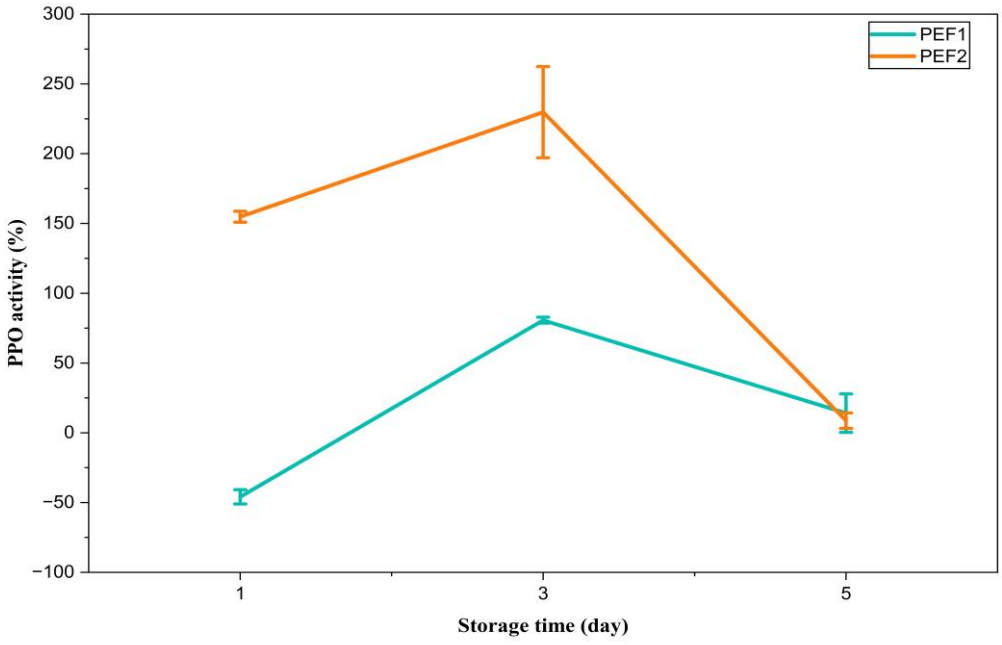

Figure 2

PPO activity changes during the storage time

Changes in POD Activity

The results of POD enzyme activity in this study are as given in Table 4 and Figure 3. While PEF2 significantly caused the maximum POD activity with 0.05±0.01 U/mg protein at the end of storage time, PEF1 did not change the activity during the storage period. PEF2 decreased POD activity a little bit on the 4th day of storage but it was not statistically significant. It is known that the application of PEF not only inactivates enzyme activity but, in some cases, has also been seen to increase the activity due to cell rupture. When the applied electric field strength exceeds the critical value, pulsed electric field (PEF) treatment can induce the refolding of denatured enzymes. This can increase their activity and reduce the activation energy of enzymatic reactions by orienting substrates and enzymes towards each other due to cell rupture [22]. Additionally, enzyme activity may differ in the outer layers of the food from that in the inner core, depending on environmental factors and cellular structure [23]. In our study, we tried to prevent this difference by taking samples from almost every part of the food for analysis.

Table 4

PEF application effects on POD activity of beetroot samples during the storage time

Applications	Enzyme Activity (U/mg protein)		
	2nd day	4th day	6th day
Control	0.01±0.00[a]	0.03±0.01[a]	0.03±0.00[a]
PEF1	0.01±0.00[a]	0.03±0.00[a]	0.03±0.00[a]
PEF2	0.02±0.00[a]	0.03±0.00[a]	0.05±0.01[b]

[a-b]: Groups marked with differing letters in the same column are statistically significant relative to the control group ($p \leq 0.05$).

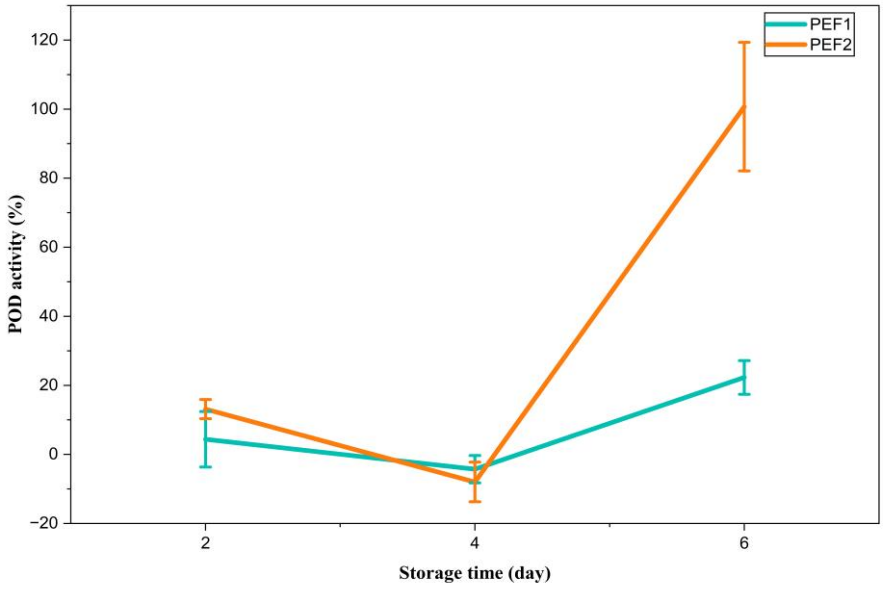

Figure 3

POD activity changes during the storage time

When all enzyme activity tests are performed are examined, there is an increase in enzyme activity in direct proportion to the increased specific energy. Among treatments, only PEF1 application reduced half-fold PPO activity relative to the control sample (Figure 2). It is on the 1st day of the storage time. In other cases, PPO and POD activities generally could not be reduced compared to control. Some studies published in the last two decades show that PEF reduces PPO (76.2% inactivation of mushroom PPO in buffer was obtained after a longer PEF treatment of 744 microseconds at 25 kV/cm) and POD (97% inactivation after PEF treatment of tomato juice at 35 kV/cm for 1500 microseconds using four microseconds of bipolar pulses at 100 Hz) activities in some cases [11],[2]. Consistent with the literature, the results proved that PPO activity was reduced in some cases. In a study related to the effect of PEF on POD and PPO activities of both carrot and apple juice, Mannozzi et al. (2019) also reported that POD was reduced from 50% to 90% by PEF+60°C and PEF+80°C, whereas PPO activity decreased significantly with PEF at room temperature compared to the control group. These results were evaluated against the hypothesis that elevated temperatures would increase the internal energy of the enzymes, thereby causing the bonds that determine their three-dimensional structure to break [24]. On the other hand, POD activity could not be significantly diminished by PEF treatment. Although the exact mechanism of how electro technologies initiate the (in)activation of enzymes is not yet fully known, these processes are thought to trigger the association or dissociation of functional parts in proteins, causing charged separation, destabilization or denaturation of globular enzyme structures [9]. Despite the absence of an effect on the inactivation of certain enzymes under specific treatment conditions in some studies, a comparison between studies is precluded by disparities in equipment, treatment parameters and medium. Optimization and/or combination of different applications with PEF parameters through the related enzymes and target products is necessary to be able to supply an effective treatment.

Morphological Evaluation of Beetroot Samples

To evaluate the effect of PEF treatment, Figure 4 shows the appearance of the beetroots at the end of the storage period. PEF-treated samples exhibited a waterier structure compared to the control samples after storage with the cell rupture effect of PEF. The appearance of pre-treated food samples deteriorated faster than the control group. In accordance with this result, Alkanan et al. (2024) have stated that the use of PEF in the production of juices enhances the amount of juice extracted from fruits and vegetables [25]. This indicates that there is more damage to the plant cell membrane due to the increased specific energy. The changes in enzyme activity can be conducted with the effect of PEF processing on the native structure of enzymes [26]. After PEF treatment, pH values of control, PEF1 and PEF2 treated samples at the end of storage day were 5.86±0.04, 6.19±0.24, 6.59±0.77, respectively. No significant difference was observed among the pH values. The pH value of food is an important indicator of enzymatic activity. Although no difference was found between these values in our study, it has been reported that shifts in pH values during and after the PEF process can be related to understanding PPO inactivation [27]. Some studies have demonstrated that PEF treatment according to the electric field strength could be used not only to prevent the undesirable effects of enzymes in foods [28, 29]; it may be used to activate some enzymes in foods. For example, Zhang et al. (2017) have been reported that a PEF application at 12 kV/cm and a flow rate of 80 mL/min was the reason for the maximum pectinase activity, which was 21.89 ± 1.67% higher than the untreated enzyme [20]. In some cases, activation of enzymes is needed in food processing. However, activation of deteriorative enzymes like PPO and POD are generally undesirable in food industry due to its possible adverse effect on food quality.

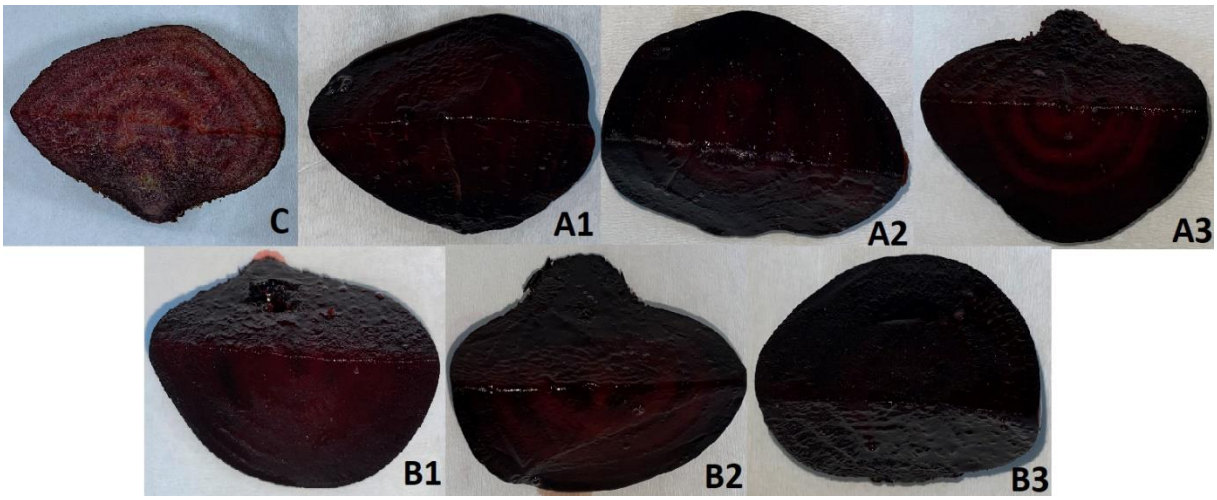

Figure 4

Appearance of beetroot samples end of storage time (C: Control; A1, A2, A3: PEF1 treated samples; B1, B2, B3: PEF2 treated samples).

CONCLUSION

PEF treatment is a non-thermal and non-chemical alternative method to affect PPO activity. However, the applicability of that way needs to be comprehensively investigated according to related food samples properties by changing pulse numbers, application time, sample size, etc. In this study, high intensity at PEF treatment generally caused an increase in PPO and POD activities when compared to control during the storage period of beetroot samples. However, the results showed that PEF-induced treatment supplied considerable inactivation (45.90 ± 5.10%) of PPO activity in beetroot samples by 0.5 kJ/kg but the high intensity of PEF2 (1 kJ/kg) was adversely affecting POD activity. These results suggest that the PEF1 pretreatment process could be used to reduce the PPO and delay the deterioration of beet samples. However, it is ineffective in reducing POD activity.

The study indicates a necessity for further research to comprehensively elucidate the interactions between PEF treatment and enzyme activities. Furthermore, the correlation between the damage to cells of food products following PEF treatment and the enzymes belonging to the oxidoreductase group could facilitate a more profound comprehension of the efficacy of the application. Consequently, the impact of PEF on enzymatic activities is a subject that merits detailed investigation. Moreover, it is possible to combine different hurdle effect technologies with this non-thermal method, thus rendering it more transparent.

Ethical Statement

The present study is an original research article designed and produced by the authors.

Author Contributions

Research Design (CRediT 1) E.T. (70%) – S.E. (30%)

Data Collection (CRediT 2) E.T. (90%) – S.E. (10%)

Research - Data Analysis - Validation E.T. (70%) – S.E. (30%)

Writing the Article (CRediT 12-13) E.T. (80%) – S.E. (20%)

Revision and Improvement of the Text (CRediT 14) E.T. (50%) – S.E. (50%)

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Sustainable Development Goals (SDG)

Sustainable Development Goals: 9 Industry, Innovation and Infrastructure.

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