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Application of Pulsed Electric Field for Microorganisms Inactivation in Date palm Fruits

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Abstract Pulsed electric field (PEF) processing of liquid and semisolid food is a nonthermal technology alternative to traditional thermal preservation method with preserving nutritional and sensory values of food. In this study, the components of a laboratory scale prototype of PEF system were designed and constructed. Evaluating the performance of the prototype was carried out to inactivate the existing microorganisms (mesophilic aerobic bacteria, Yeasts and Molds) in semi-solid pitted date palm fruits, which was defined in terms of applied intensity of electric field and pulses number at a constant duration of pulse (40 µs) and (1 Hz) pulse frequency. The microbial count was decreased with the increase in intensity of electric field and pulses number. The electric field intensity of 10.82 kV/cm and 120 pulses lead to reduced total microbial counts of 1.18×10^4 cfu/g to less than 10 cfu/g of mesophilic aerobic bacteria in most treated samples. The electric field intensity of 8.84 kV/cm and 90 pulses lead to reduce total microbial counts of 3.27×10^3 cfu/g of yeasts and molds to less than 10 cfu/g that meet Saudi standards requirements. Non-detectable levels of yeasts and molds in most treated samples were observed when 10.82 kV/cm of electric field intensity and 60 of pulses number were applied. The current results indicated that PEF technology is promising as a non-thermal method for inactivation of microorganisms on date palm fruits processing.

Keywords: *pulsed electric field, date palm fruits, microbial contamination, preservation, non-thermal technology*

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1. Introduction

Dates are considered among the most important and more prevalent type of fruit in the Arab world. The global production of fruit from the date palm tree (Phoenix dactylifera L.) was estimated at 7.75 million tons worth US $3.82 billion in 2010. Saudi Arabia is ranked as the second largest date producer after Egypt in the world, with an annual production of 1.078 million tons of dates produced from over 24 million date palm trees [1]. During harvesting and handling processes, dates are subject to microbial infection, such as the potential spoilage microorganisms include yeasts, molds and lactic acid bacteria, whereas potential pathogens include *Staphylococcus aureus* and yeasts such as *Candida pelliculosa* [2]. Additionally dates are stored under refrigeration for a long time to supply a national or international market. Hence, storage of fresh fruits is accompanied by evaporation of water, changes of physiological characteristics and microbial spoilage. Therefor a heavy losses reaching up to 40% in the producer countries [3]. Recently, many technologies have been investigated that have the ability of inactivating the spoilage and pathogens microorganisms at lower temperatures than typically used in treatments of conventional heat [4]. These technologies such as pulsed electric field (PEF), high pressures, modified atmosphere packaging, and edible coatings [3]. PEF is one of the most important technologies due to its short time of treatment, minimize heating effects, gentle food processing, and environmental friendly. Moreover, a several advantages including of a wide range of microorganisms inactivation with minimal changes with preserving nutritional and sensory values of food and can be used to develop a new methods for semi-solid products not feasible through conventional methods of thermal processing [5,6,7]. The main components of PEF processing system consists of: a) high voltage direct current power supply, b) signal generator, c) capacitors for energy storage, d) continuous or static treatment chambers with two parallel or several electrodes, high voltage switches, e) measurement devices, and f) central control unit. The basic principle of the PEF technology is the application of high electric field pulses of 1-50 kV/cm with microsecond duration delivered to the food placed between two parallel electrodes confining the treatment gap of the PEF chamber. The high intensities of electric field are achieved through storing the electric energy from a direct current power supply in storage capacitors. Total time of processing is calculated by multiplying the effective pulse duration with the times of pulses number [8,9,10,11,12]. Treatment chamber is one of the most important components in the PEF processing system is the. Treatment chambers mainly operate in a

batch for handling of solid or semisolid foods or continuous manner for liquid foods [13]. Many studies were conducted with successful results for PEF applications on foods processing such as on particulate pea soup with plastic beads [14] liquid food such as orange juice [15]. Most of study showed that the important factors affecting the microbial inactivation during PEF treatments include: a) intensity of electric field, b) numbers of pulses applied, c) treatment time, d) temperature of treatment, e) shape of pulse, and f) type and growth stage of microorganism [6,7,16,17,18,19,20,21]. The study aimed to use PEF as a modern and promising technology for control microbial contamination on semi-solid pitted date fruits in a non-continuous system through designing and constructing the components of a laboratory scale prototype of PEF treatment system. Evaluating the performance of the PEF prototype by define the inactivation of mesophilic aerobic bacteria, yeasts and molds in terms of applied electric field intensity and pulses number. Until now, there are a few reports on the application of PEF on solid and semi-solid agricultural products and based on our knowledge this study is the first on the application of PEF on date palm fruits processing.

2. Material and Methods

2.1. PEF Treatment System

A pilot scale prototype of PEF treatment was designed and constructed. (Figure 1) shows the general block diagram of the PEF designed system. The PEF system consists of a) high voltage pulse generator (HVPG), b) static treatment chamber, and c) measurement devices.

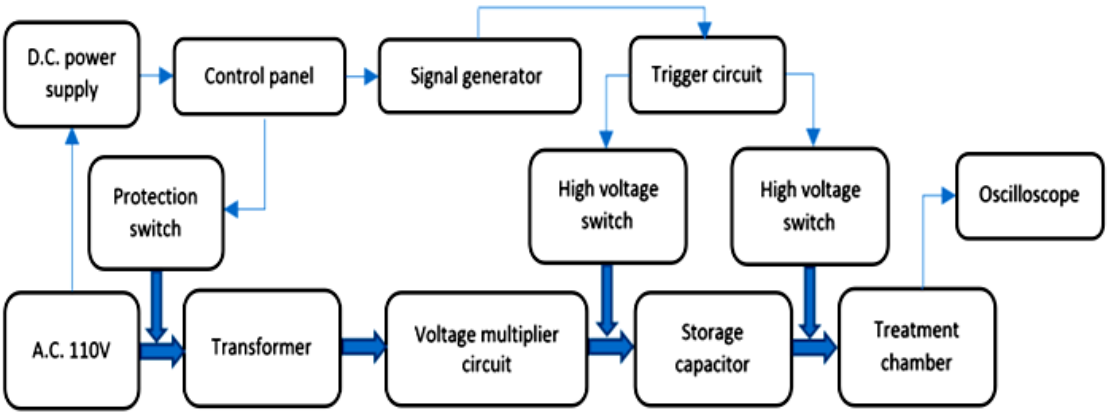

Figure 1. General block diagram of PEF system

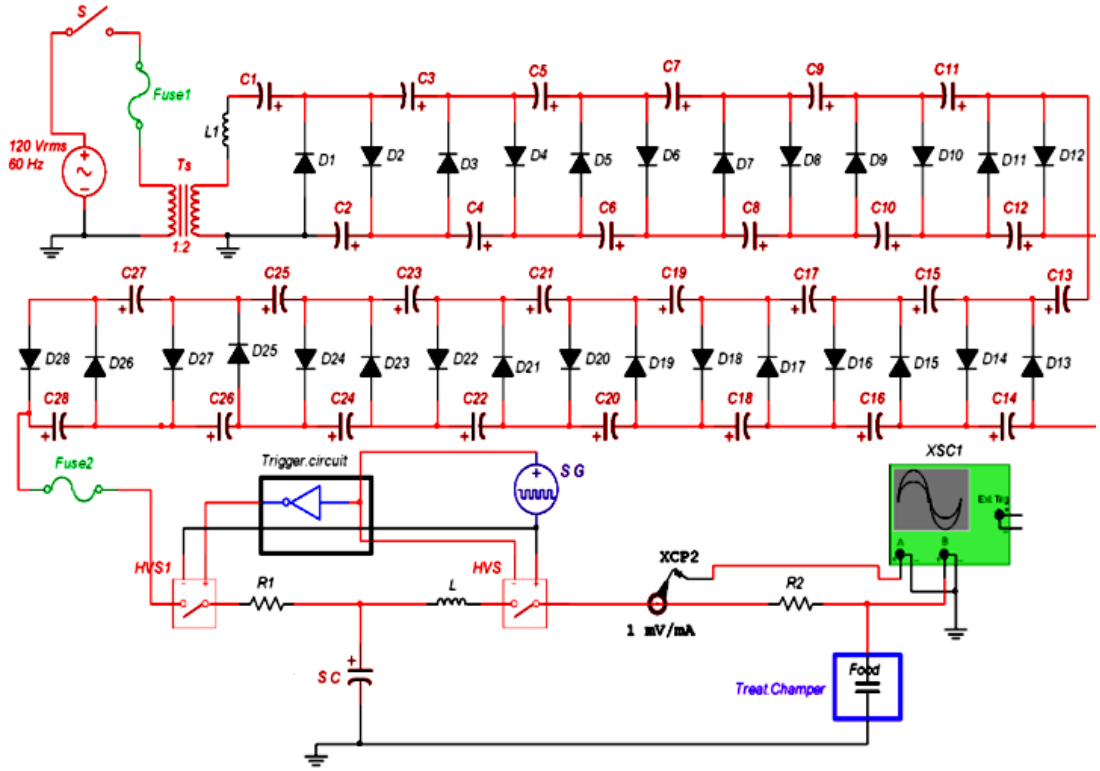

Figure 2. Schematic diagram of (HVPG) electrical circuit

2.1.1. High Voltage Pulse Generator (HVPG)

The components of HPVG consists of a high voltage direct current source (HVDC), switches (S), high voltage switches (HVS), signal generator (SG) and storage capacitor (SC). (Figure 2) shows the design of the (HVPG) electrical circuit. The voltage increases by a transformer

(Ts) from line 110 VAC to 220 VAC and from 220 VAC to high voltage more than 6000 VDC using Cockcroft-Walton (CW) voltage multiplier. The voltage multiplier is a circuit that converts alternating current (AC) from a low voltage to a high direct current (DC) voltage, typically using a network of capacitors and diodes. The input voltage of voltage multiplier circuit has been taken from the secondary of single-phase transformer (110 VAC). A cascade of fourteen-stage constructed the CW voltage multiplier with each stage containing two capacitors and two diodes. The main components used for construction voltage multiplier circuit, it mainly consisted of voltage transformer (T)s, a column of smoothing capacitors with individual capacity 1000µF/400V (C_2, C_4 ... C_{28}), a column of coupling capacitors (C_1, C_3 … C_{27}), and a series connection of power rectifier diode 1N1190A 600V/40A (D_1, D_2 … D_{28}). The total output voltage of multiplier circuit on the load was less than (2nVmax) where n is the number of stages. A typical measurement of the total output voltage of multiplier circuit is illustrated in (Figure 3). The voltage multiplier circuit charges the storage capacitors (C) until the preset voltage is reached in DC form using (HVS1). Capacitor voltage is transformed to narrow pulses of the treatment chamber by triggering signal using the high voltage switch (HVS) and the pulse generator (SG). In this study, two high voltage switches (HTS 160-500-SCR/16 kV/5 kA-Germany) were used. The first was connected in series from multiplier circuit to storage capacitors and the second was connected in series to a protective resistor and the treatment chamber. Solid-state relays (G3NA SSR) with current ratings up to 50A was used to control and safety circuits. The DC signals (5V) were generated using function generation GF-230. These DC signals were used to define the pulse width and frequency of the PEF system. The storage capacitors were constructed using series and parallel capacitors with total capacitance 6.8µF/8kV. A typical measurement of electric waveform is illustrated in (Figure 4).

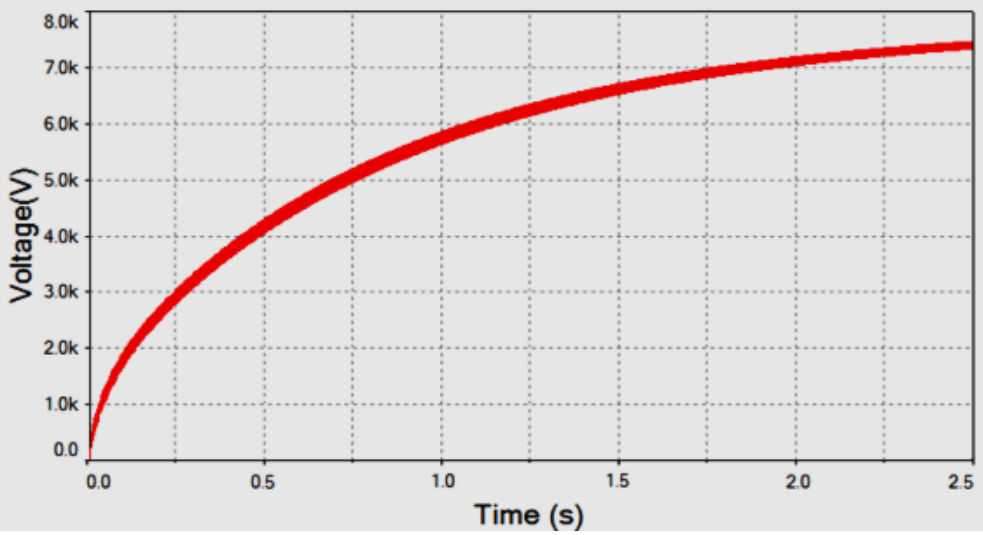

Figure 3. Output voltage of multiplier circuit

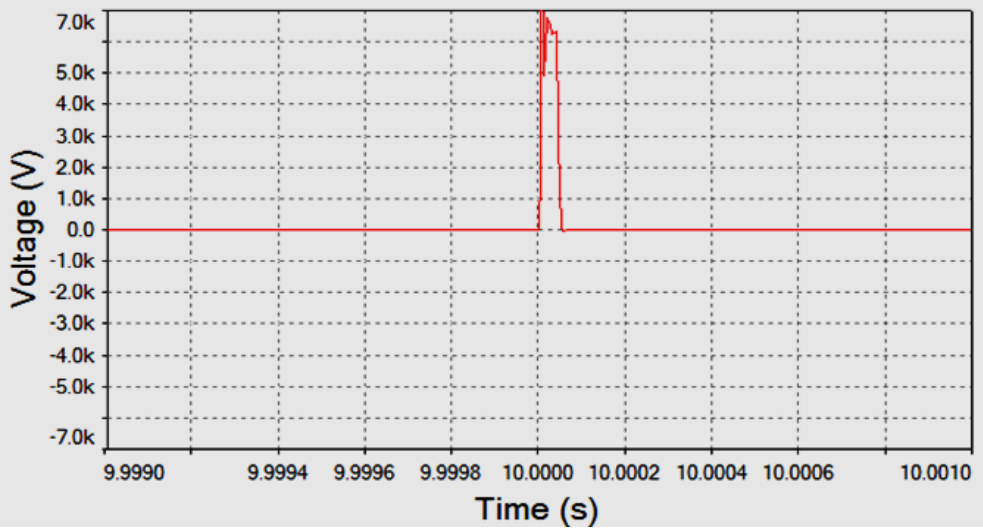

Figure 4. Pulse waveform applied in the PEF system

2.1.2. Treatment Chamber

The static treatment chamber was used to keep the treated date fruits inside during pulsing and to transfer high voltage pulses into PEF. The static treatment chamber has been constructed in outside dimensions of 254*254*252mm with two parallel polished stainless steel electrodes with thickness 2mm by Teflon insulator and plastic supports as shown in (Figure 5). The electrode diameter was 100mm and there was a variable gap ranged

from 0.5 to 2cm between the electrodes insulated by Teflon. The insulator presents internal cylindrical cross section cavities in which the two electrodes were placed. The pitted date fruit was placed in order to pass through the electrodes of this chamber. The microbial inactivation was happen following this process.

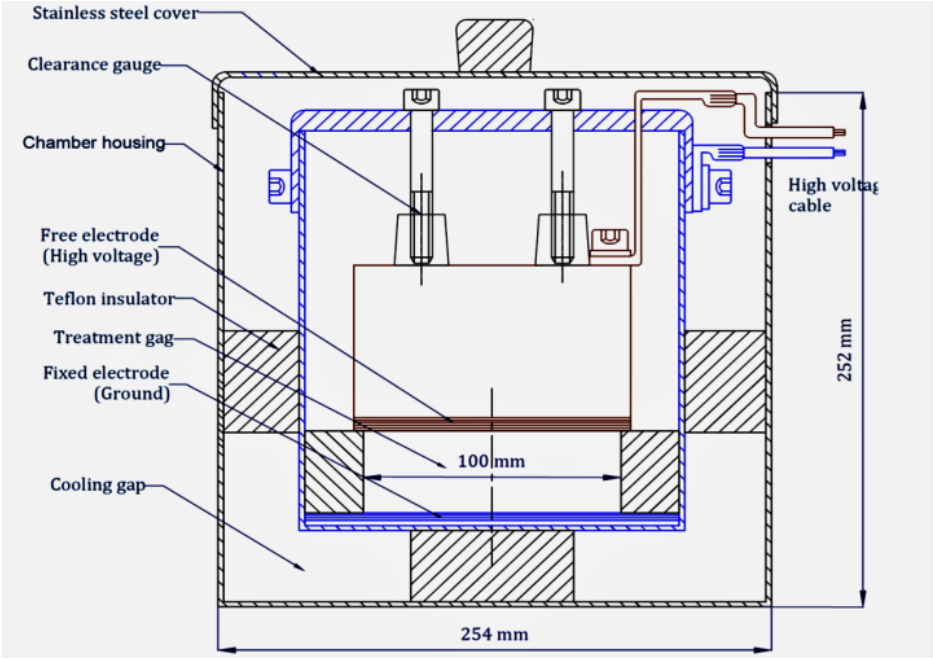

Figure 5. Schematic diagram of the laboratory-scale static treatment chamber

2.1.3. Electrical Measurements

The intensity of electric field (E, kV/cm) was calculated based on the gap distance between the parallel electrodes of the treatment chamber (cm) and the high voltage across the treatment chamber during PEF treatment (V, kV) measurements, using the following formula:

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

The distance between electrodes estimated by digital Vernier caliper with accuracy of 0.01mm. Voltage across the treatment chamber during PEF treatment, waveform (shape), Pulse duration (t, µs), and Pulse frequency (Hz) were measured continuously using a Tektronix high voltage probe (P6015A 1000X -75 MHz). Current (I, A) across the treatment chamber was captured continuously using a Tektronix current probe (TCP303 AC/DC-15 MHz/150 A). The voltage probe and current probe were attached to a digital EZ Digital oscilloscope (DS-1250-250MHz).

2.2. Microbiological Analysis

Total microbial count after and before PEF treatment was determined using the method explained by [22]. Date samples were aseptically destoned using sterile forceps and microbial loads calculated for the flesh. Flesh samples (10g) were weighed into sterile stomacher bags, to which 90 mL of sterile peptone water (Oxoid, CM0009) was added. The mixture was homogenized in a stomacher (Lab- Blender 400, Seward Medical, England) for 45 seconds and aliquots (1.0 mL) plated out in duplicate as 10- fold dilutions in peptone water. Aerobic mesophilic bacteria were counted on Plate Count Agar (PCA Oxoid, CM0325) dishes using the pour plate method. The plates were incubated at 30˚C for 2-3 days and the counts expressed as colony forming units per (cfu/g) of the sample. Yeasts and molds were cultured on potato dextrose agar plates (PDA Oxoid, CM0139) incubated at 20-30°C for 3 to 7 days.

2.3. Microbial Log Reduction

Microbial Log reduction (cfu/g) was assessment according to the following equation:

$$Microbialreduction = Log\frac{No}{N} = LogNo - LogN \quad (2)$$

Where:
No= Microbial count without PEF treatment (cfu/g); and
N= Microbial count after PEF treatment (cfu/g).

2.4. PEF Treatment

PEF treatments were performed by introducing the sample of pitted date in the treatment chamber at room temperature. Before PEF treatments on date fruit, the seeds were removed from the fruits, the sample was immersed in an aqueous medium in treatment chamber resulting in a composite resistance of a system formed by the date material, and of the surrounding water; then sample was pressed to eliminate air gaps. After each PEF treatment, the microbial load was determined. The experiment was designed as factorial trials in completely randomized design with five replicates and two tested factors affecting microbial inactivation (intensity of electric field and pulses number) at the following levels:

1. Electric field intensity was (4.76, 6.82, 8.84, and 10.82kV/cm)
2. Number of pulses was (60, 90, and120 pulse).

PEF treatments carried out using square wave pulse with 40 µs duration and 1 Hz frequency, which were chosen according to previous preliminary studies.

2.5. Statistical Analysis

Analysis of variance (ANOVA) was performed to detect differences in effects of applied treatments on microbial inactivation. The analysis was carried out using statistical package (SPSS) version 20 (SPSS Inc., Chicago, USA). Duncan's multiple range test (DMRT) at significance of differences (P<0.05) was used to compare between means.

3. Results and Discussion

3.1. Effect of PEF Parameters on Mesophilic Aerobic Bacteria Inactivation

The average of mesophilic aerobic bacteria total count in the samples before PEF treatments was 1.09×10^4 cfu/g ranged from 71.14×10^4 cfu/g and $1.19x10^4$ cfu/g. Effects of electric field intensities and number of pulses on total count of mesophilic aerobic bacteria (log value) are shown in (Figure 6). Total count of mesophilic aerobic bacteria (log value) progressively was decreased at increasing electric field intensity. The effect of increasing number of pulses was much more evident when the electric field intensity was increased from 6.82 to 8.84 kV/cm. A greater reduction in microbial counts was observed when intensity of electric field was increased from 8.84 to 10.82kV/cm. About 3.70±0.35 log cycles (cfu/g) reduction in counts were observed at electric field intensity of 10.82 kV/cm and 120 of pulse number. While the lowest average value of reduction was 0.04±.02 log cycle 60 pulse and 4.76 kV/cm electric field intensity. Reduction of mesophilic aerobic bacteria ($log_{10}N/No$) was significantly reduced (P<0.05) at all levels of electric field intensity and pulses number as shown in Table 1. No previous studies have been conducted in order to explain the effect of PEF processing on different types of microorganisms in dates fruit. In general, many researchers mentioned the bacterial rate killing by PEF has depended on the field intensity, time and number of pulses and the width of pulses. The microbial inactivation depends on the stage of microbial growth, microorganism type, the initial microbes amount, and the ionic concentration and suspension conductivity. Microorganisms Inactivation increases with intensity of electric field after exceeding the critical transmembrane potential of the cell, which corresponds to the critical electric field intensity *(E_c)* [23]. The critical electric field intensity value depends on the size and shape microorganisms' cell and the medium characteristics. This critical value is in the range of 4 to 14 kV/cm [24]. There is a linear relationship between the log inactivated number cells and the permeabilized percentage of cells up to at least a 3.6 log reduction when 12 or 15 kV/cm of electric field intensity applied [25]. At lower electric field intensity, there was very little inactivation and less membrane permeabilization. Microbial inactivation by PEF increases with treatment time. Which depends on number and width of pulses. The electric field intensity and time of treatment are the two main process parameters influencing microbial inactivation by PEF [26, 13].

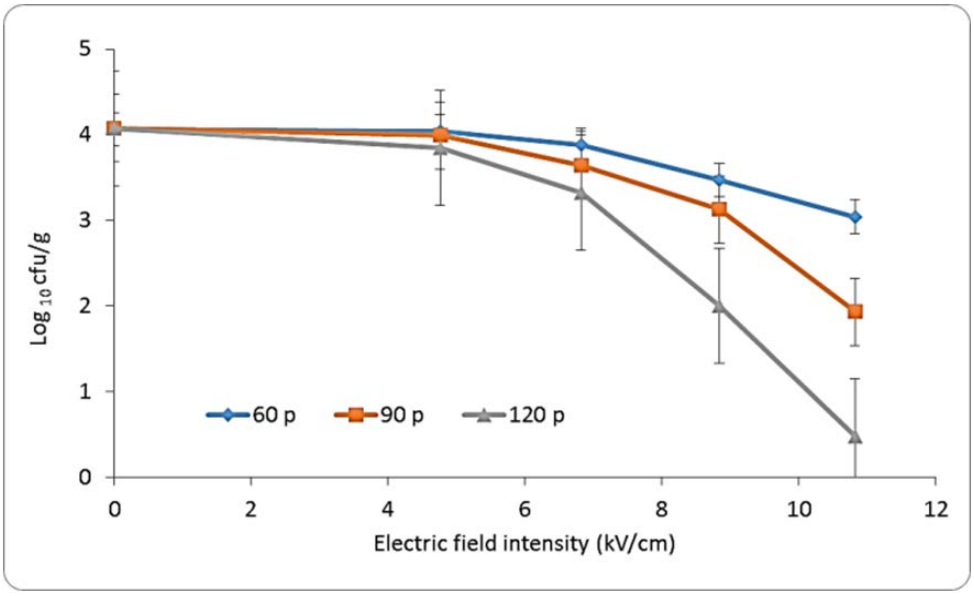

Figure 6. Effect of electric field intensity on total mesophilic aerobic bacteria count of date palm fruits. P indicates number of pulses at out using square wave pulse with 40 µs duration and 1 Hz frequency

Table 1. Effect of electric field intensity and pulses number on Log values of microbial reduction (Mesophilic aerobic bacteria) of date palm fruits

Microbial reduction of mesophilic aerobic bacteria, Log(N_o/N), average and stander deviation					
Pulses Number	Intensity of electric field (kV/cm)				
	0	4.76	6.82	8.84	10.82
60	0^{i}	0.04^{i} ±0.02	0.18^{gh} ±0.03	0.59^{e} ±0.07	1.02^{c} ±0.07
90	0^{i}	0.09^{hi} ±0.02	0.43^{f} ±0.06	0.95^{c} ±0.05	2.14^{b} ±0.05
120	0^{i}	0.23^{g} ±0.06	0.75^{d} ±0.05	2.07^{b} ±0.06	3.70^{a} ±0.35

$^{a\,i}$, means with a similar lower case letter(s) in column and row are not significantly different at 95% level for interaction effect. Values are mean (n=5) ±standard divisions.

LSD at 0.05: Intensity of electric field = 0.07and Pulses number = 0.06.

3.2. Effect of PEF Parameters on Yeast and Molds Inactivation

Contamination with molds and yeasts was found in all untreated samples ranged from $2.83x10^3$ cfu/g to 3.27×10^3 cfu/g. The log reduction of yeast and mold increased as electric field intensity increased at all level of number of pulses. On the first stage of PEF treatment at using 4.76 to 8.84 kV/cm of electric field intensity, the microbial inactivation increased linearly, but progressively decreased at 8.84 to 10.82 electric field strength, and microbial count curves became concave as shown in (Figure 7). Increasing the number of pulses from 60 to 120 at lower value of electric field intensity of 4.76 kV/cm, resulted in a slightly greater reduction in microbial count. The effect of increasing pulses number was much more evident when the intensity of electric field was increased from 6.82 to 8.84 kV/cm. The PEF treatment at 10.82 kV/cm of electric field intensity and 120 of pulses number reduced average value of count load of 3.09×10^3 to non-detectable levels in the most treatment sample. Analysis of data showed the inactivation effectiveness markedly increased when higher electric field intensity were applied regardless of the pulse number. The maximum inactivation of molds and yeast, corresponding to reduction of 3.91 log cycles was achieved at 10.82 kV/cm and pulse number 90 pulse. Table 2. showed the significant effect of increasing the electric fields of 4.76 and 10.82 kV/cm number of pulses from 60 to 90 ($P<0.05$). On the contrary, doubling the pulses number from 60 to 120 pulse did not show significant differences in the effect on the compressive strength of microbial count ($P<0.05$). No work on treatment of date palm fruits with PEF was found in the literature cited. Electrical breakdown of microorganisms by PEF explained by [5] as follows: When the cell is exposed to an external electric field with high intensity with short duration, the cell membrane is charged due to charge movement, then a corresponding membrane potential is induced. If the total membrane potential exceeds a critical electrical value of 1V, reversible electrical breakdown occurs. The intensity of critical external field required for inactivation of microbial is dependent on the size of cell as well as the orientation of field [20]. Cells of yeast are known to be more susceptible to pulsed electric fields than bacteria due to their larger size of cell [27]. Intensity of electric field and time of treatment are the major factors determining inactivation of microorganisms at PEF processing. 39 and 49 ms of treatment time caused 3.3-log reduction in total counts of molds and yeasts [28,29].

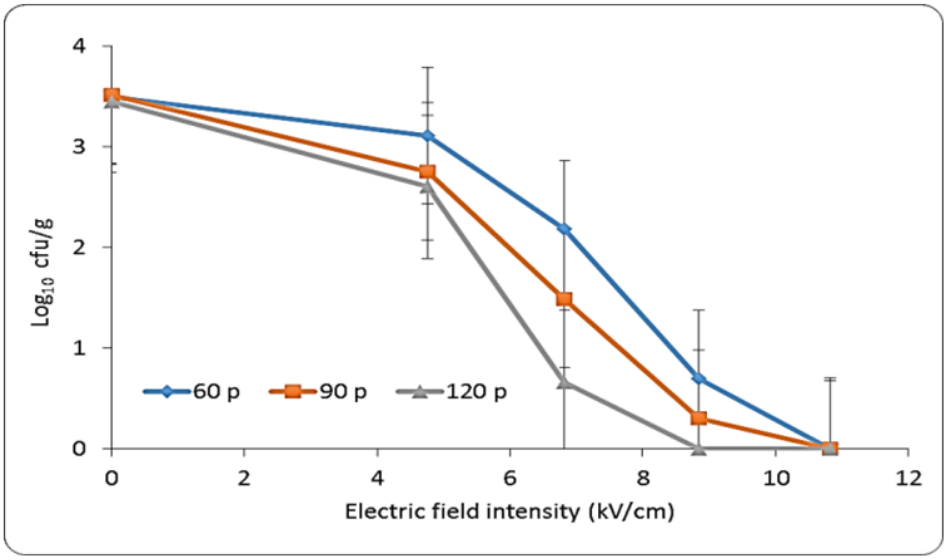

Figure 7. Effect of intensity of electric field on total yeast and mold counts of date palm fruits. P indicates pulses number at out using square wave pulse with 40 µs duration and 1 Hz frequency

Table 2. Effect of electric field intensity and number of pulse on Log values of microbial reduction (Molds&yeast) of Khalas date fruit.

Microbial reduction of Molds and yeast, Log(N_o/N), average and stander deviation					
Pulses Number	Intensity of electric field (kV/cm)				
	0	4.76	6.82	8.84	10.82
60	0^{g}	0.39^{f} ±0.13	1.34^{d} ±0.15	2.87^{b} ±0.23	3.60^{a} ±0.25
90	0^{g}	0.76^{ef} ±0.29	1.99^{c} ±0.23	3.21^{ab} ±0.37	3.91^{a} ±0.24
120	0^{g}	0.85^{e} ±0.25	2.88^{b} ±0.71	3.85^{a} ±0.24	3.85^{a} ±0.27

a i, means with a similar lower case letter(s) in column and row are not significantly different at 95% level for interaction effect. Values are average (n=5) ±standard divisions.
LSD at 0.05: Electric field intensity = 0.20, Number of pulse = 0.15, and Interaction = 0.35

4. Conclusion

PEF treatments were performed by immersed the sample of pitted date fruit in aqueous medium in the treatment chamber resulting in a composite resistance of a system formed by the date material and of the surrounding water; then sample was pressed to eliminate air gaps.

The results showed the impact of the effectiveness of PEF system in microorganisms inactivation through

microbiological tests that have been conducted on the dates after PEF treatments, the inactivation rate of microorganisms increased with increasing intensity of the electric field and the number of pulses. It was found that the intensity of the electric field of 10.82 kV/cm with 120 of pulses number lead to reduced microbial loads of 1.17×10^4 cfu/g to non-detectable levels of bacteria in most treated sample. The intensity of the electric field of 8.84 kV/cm with 90 pulse number lead to reduced loads of 3.27×10^3 cfu/g to non-detectable levels of yeasts and molds in all treated sample.

According to the experimental results, pulsed electric field (PEF) treatment has a promising non-thermal method for microorganisms inactivation in date palm fruits. These preliminary findings may encourage of conducting further studies on PEF in dates processing. These preliminary findings may encourage of conducting further studies on PEF in date palm processing, where there are several areas need further research before the PEF technology is applied commercial.

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