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Microorganism Inactivation by Nanosecond Pulsed Electric Fields: Full-wave Analysis and Experiment

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Abstract. This paper describes the application of nanosecond pulsed electric fields on microorganism inactivation of food products. Inside custom-designed chamber, liquid and semi-solid samples were subjected to ultrashort high-intensity electrical pulses with a maximum energy input of 75 $kJ.kg^{-1}$. An efficient treatment environment provided by the chamber was preliminarily confirmed through simulated field distributions and specific absorption rates for its field homogeneity and high energy absorption. Observations through statistical analyses showed that nanosecond pulsed electric fields caused as high as 5-log reduction of the total microbial population in liquid medium ($P < 0.05$). An effective microbial destruction and a minimal rise in temperature together provide this treatment an alternative food pasteurization method.

Keywords: nanosecond pulsed electric field treatment; energy dosimetry; total microbial count

1. Introduction

An increasing interest and preference of consumers towards health-concerned nutrition and fresh-like taste of food have led producers to develop preservation technologies preventing the growth of food pathogens responsible of undesired food degradation without having negotiation the initial qualities of food. The most preferred technique is a treatment at temperatures less than those of typical heat pasteurization. The original nutritional and sensory qualities of food, accordingly, are slightly disturbed by heating effects while extending the shelf life of food products is plausible. The development of a widely available, highly effective, less thermal technique that provides a relatively high level of preservation is of great interest.

Application of pulsed electric field (PEF) has been found as a new treatment for food preservation where short electrical pulses are applied momentarily to samples through conductive electrodes in direct contact with samples. Major portion of research efforts on PEF has been focusing on a reduction of microbial fractions in liquid food [1]-[3] or semi-solid food [4]-[7]. This process causes structural damage to microbial membranes at less significant energy levels when compared to typical heating process. As part of the response to an increasing voltage across samples, the resultant transmembrane potential causes the cell membrane to lose its impermeability beyond the critical value, owing to the pore formation induced on the membrane surface leading to the disintegration of the cell membranes [8]-[9]. Studied factors include differences among microorganisms, bacterial growth phase, food pH, food ionic strength, food conductivity, and food water activity. In addition, an effectiveness of PEF on microorganism inactivation has been extensively studied over the past decade through a number of influencing parameters, including electric field strength and pulse profiles, i.e., polarity, duration, repetitiveness, and rise time [10]. Of all pulse profiles,

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monopolar exponential decaying and monopolar and bipolar square wave pulses are commonly used in such energy efficient applications.

Methods to control the pulse width in the nanosecond regime have been recently studied in practical applications in the areas of medicine and biology. The manipulation of inner structures of biological cells, e.g., animal, plant and microbial, can be accomplished while maintaining considerably low thermal absorption through a delivery of pulses in the range of nanoseconds with relatively large electric field intensities in kilovolts per centimeter. Applications of a nanosecond pulsed electric field (nsPEF) with nanosecond pulse duration and electric field strength going up to several hundred kV/cm have been occupied for the response of mammalian cells [11]-[12]. However, little has been reported on investigation of engaging nanosecond pulses for microorganism inactivation in foods. Exploring this new concept of food hygiene applications would offer great benefits without substantially affecting the nutrition and taste of food products.

Preliminary attempts to inactivate microorganisms in liquid and semi-solid samples by PEF in nanoseconds would therefore suggest nsPEF treatment a feasible approach of food pasteurization while remaining small heating effects within the samples. Thus, the goal of this work was to investigate an effectiveness of high-intensity nanosecond pulses on microbial inactivation for a variety of samples in both liquid and semi-solid forms. We, therefore, report through this paper a microbial reduction as results of nsPEF treatments using laboratory-assembled batch treatment chamber. All experiments were carried out with fixed field strength and exposure duration. Following the introduction, Section 2 reports the configurations of the static exposure chamber through mathematical equations and three-dimensional full-wave simulations. The remaining of this section details treatment method and preparation. The results are analyzed and discussed in Section 3 and concluded in Section 4.

2. Treatment Setup

2.1. nsPEF Treatment System

The experiment setup of the high-intensity nsPEF system is shown in Fig. 1 where the main components include a high-voltage DC supply, a 4-stage pulse forming network (PFN), a self-breakdown switch, and a load. The Blumlein PFN is a circuit topology for the generation of high-voltage pulses across the load with fixed pulse duration through the superposition of electrical pulses launched from different directions. Each stage of Blumlein PFN is created from a group of two 16-kV, 0.022 μF metallized polyester capacitors (ASC Capacitors, USA) connected in series in conjunction with the 15-AWG coil (Bangkok Cable, Thailand) wound on an air core. A LT60P33 high-voltage DC source (Glassman High Voltage, USA) supplies the energy to the PFN through the decoupling resistor of 100 kΩ. Under a matching condition, i.e., the value of the load impedance Z_L is twice as much as that of the PFN's characteristic impedance Z_0, the output voltage peak across the load can be estimated by the input voltage peak supplied to the network. The output voltage's pulse duration τ is 500 ns equivalently to a transit time through 4 stages of the network [$\tau = n \cdot \sqrt{L \cdot C}$] where L and C are, respectively, the inductance and capacitance for each stage, n is the number of stages. The generated high-voltage pulse peak and profiles were monitored using a P6015A high-voltage probe (Tektronix, Inc., Beaverton, OR, USA), reading through a TD53O52C digital oscilloscope (Tektronix, Inc., Beaverton, OR, USA). The self-breakdown switch is a spark gap with a few nanosecond rise time. The PFN load is the treatment chamber filled with samples. The measured load voltage is shown in Fig. 2 from 0 to 10 μs where the peak and the width of the main pulse are approximately 15 kV and 500 ns, respectively, while the presence of the successive pulses is due to small impedance mismatch. The associated frequency spectral components from DC to 10 MHz are described by the inset.

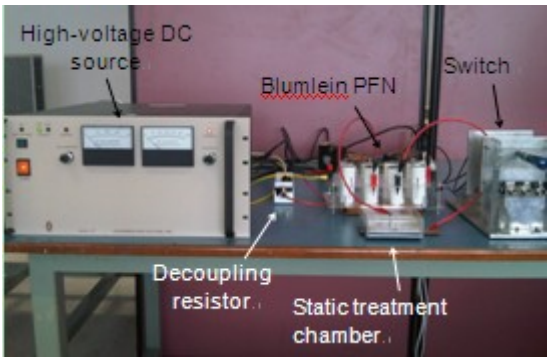

Fig. 1: Experiment setup of the high-intensity nsPEF system.

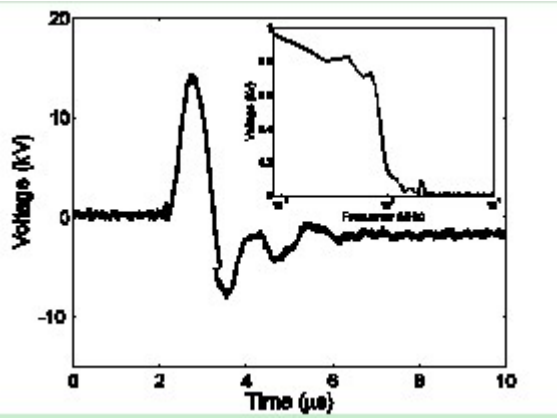

Fig. 2: Measured nanosecond pulse waveform generated across the load and (inset) associated frequency spectral components from DC to 10 MHz.

Cross-sectional view of the static treatment chamber is shown in Fig. 3. The chamber is equipped with electrically conductive plates vertically aligned on the top and the bottom of the chamber volume. For this configuration, the values of the chamber impedances are estimated from the conductivities σ of the samples and the dimension of the chamber, i.e., $Z_L = \sigma^{-1} \cdot L \cdot A^{-1}$ where A is the electrode area. The parameters H and L are 0.5 cm and 10 cm, respectively. Materials of electrodes and insulator sheets are aluminum and Polymethyl methacrylate, respectively, with the same thickness of 2 mm.

2.2. Materials and Methods

The water from stagnant pond located within an urban environment (Salaya, Nakhon Pathom, Thailand) was used as an inoculum in a liquid sample. The 80 g deionized water (Institute of Nutrition, Mahidol University) was contaminated with 10% pond water (v/v) inoculated in tryptic soy broth for 20 hrs. For semi-solid samples, tested variety included 50 g gelatin and 70 g curry paste (70 g) (Theppadungporn Coconut, Thailand). The 0.08% gelatin (w/v) in water was prepared with 3% inoculum of pond water (v/v) whose inoculation process is described previously. Prior to each experiment, 70% ethanol was used as an antimicrobial disinfectant applied to the treatment area. Each sample was then directly poured into the test volume. For all tests, the frequency and the pulse width were maintained at 3 Hz and 500 ns, respectively, providing the sample treatment time of 450 μs. The electric field strength was 15 kV.cm^{-1}. This treatment condition is similar to those applied in the PEF inactivation studies using batch processes of liquid samples, i.e., the electric field strength of 15 – 30 kV.cm^{-1} and total treatment time of 150 – 400 μs, in which the maximum reduction was observed at 4.5 $\log_{10}$ [13] – [15]. Throughout this work, each experiment consisted of at least two groups of treatment replications and two groups of associated untreated controls.

Following the completion of the chamber design, three-dimensional full-wave analyses were carried out to observe the energy dosimetry of the medium inside the chamber subjected to nanosecond electrical pulse given in Fig. 2. Simulation analyses were achieved by the commercial electromagnetic solver (CST Microwave Studio, Darmstadt, Germany) based on the finite integration technique (FIT) [16]. The frequency of interest was 50 kHz, corresponding to the maximum amplitude spectrum of the signal shown in Fig. 2.

The evaluation of the activity of microorganisms was then determined by counting total aerobic bacteria populations using Petrifilm aerobic count plates (3M, Microbiology Products, St. Paul, MN, USA). Multiple dilutions of controls and treated samples from 10^{-1} to 10^{-4} were used to ensure the count within appropriate range of colony forming units each plate. The plates were incubated at 35 °C for 24 hrs, and the colonies were counted afterwards. The inactivation effect was expressed as the viability of the bacteria with and without treatments by $\log_{10} N_0/N$ where N_0 and N are the numbers of bacterial survival counts before and after treatments in CFU/ml. For statistical significance tests, the P values were determined with all replication results together through one-tailed paired sample t-test using a Microsoft Excel 2003 spreadsheet running on Windows XP. Conventionally, if $P < 0.05$, the result is statistically significant, and if $P < 0.01$, the result is highly significant with more confidence of true effect. The error bar for each replication was determined from the standard error (SE).

3. Results and Discussions

3.1. Initial Observations by Specific Rate Absorption

Preliminary dosimetry examinations were done through simulations of specific absorption rate (SAR) where energy dose distributions on the liquid sample subjected to pulsed electric fields is shown in Fig. 4 for the *xz* and *yz* planes. The sample's electrical properties were simply modeled using water's ($\sigma = 10^{-2}$ S.m^{-1}, $\varepsilon_r = 81$). The amount of energy absorption as high as 44.5 W.kg^{-1} is observed around the center area and decreases uniformly outward from the center. The field absorption characteristics of the medium could attribute to the survival of microorganisms.

3.2. Inactivation Effects of nsPEF

Fig. 5 compares the average total microbial counts of nsPEF-treated and non-treated media for liquid and semi-solid samples. The inactivation data were plotted against the sample variety. The figure shows that, with the treatment with the electric field strength of 15 kV.cm^{-1} for 450 µs, the inactivation effect is apparent in liquid sample [see Fig. 5(a)] in which a maximum reduction of 5.15 $\log_{10}$ cycles in the average number of total microbial populations ($P < 0.05$, one-tailed paired sample t-test) compared to the control can be observed. A rise in temperature from 30ºC to 60ºC after the treatment was also reported. It was found that nsPEF treatment achieves the same lethal effect as does PEF treatment (15 kV.cm^{-1}, 15 µs/pulse, +5.5°C/pulse, 5 - 20 pulses [14]) at somewhat lower temperature. Therefore, the applications of pulsed electric fields in nanosecond regime could be used as an alternative for food pasteurization with low heating effects. On the other hand, only small changes in total microbial populations after treatments for all semi-solid samples were observed with a maximum reduction of only 0.4 $\log_{10}$ cycles [see Figs. 5(b) and 5(c)]. In fact, eliminating microorganisms in semi-solid media even with conventional PEF treatments were inconclusive where mixed results have been reported. Manus et al. (34 kV.cm^{-1}, 1 .7 µs/pulse, 80 pulses) [9], Zhang et al. (40 kV.cm^{-1}, 64 pulses) [10] and Ravishankar et al. (15 – 30 kV.cm^{-1}, 3.25 µs/pulse for up to 20 pulses) [11] inactivated microbial populations with 2 $\log_{10}$ cycles in fish eggs in semi-solid suspension, 5 $\log_{10}$ cycles in potato dextrose agar and 3 $\log_{10}$ cycles in water-based gellan gum gel, respectively, whereas Bolton et al. [12] reported only a reduction of 0.6 $\log_{10}$ cycles in beef burgers. The ineffective effect could be attributed to the material properties, such as the water activity and the pH level. Lowering the level of water activity as is seen in normal semi-solid food preservation seems to protect *E. coli* and *S. cerevisiae* from PEF inactivation reported by Aronsson and Ronner [17]. Additionally, in the case of acidified curry paste, Alvarez et al. [18] showed that decreasing the pH level caused *Salmonella* to gain a value of nsPEF resistance.

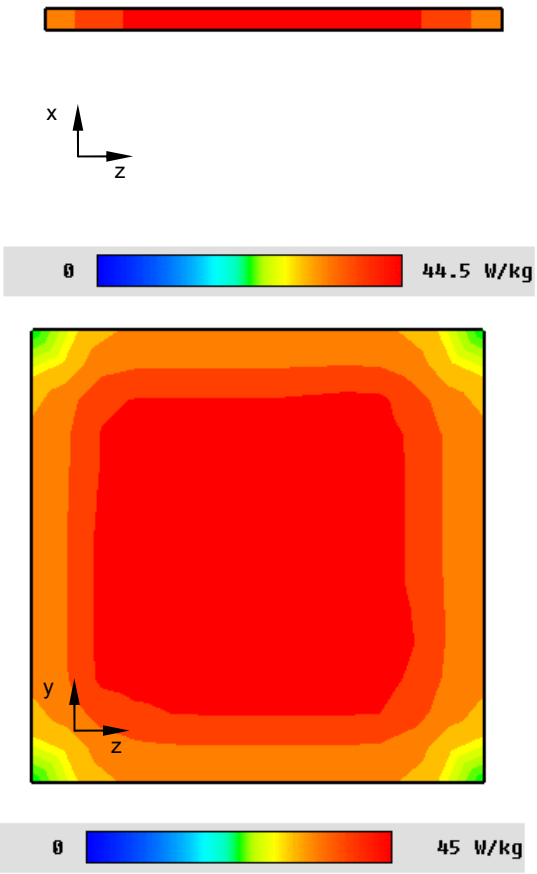

Fig. 4: Energy dose distributions on the *xz* plane (left) and *yz* plane (right) by simulated specific absorption rate subjected to pulsed electric fields on the liquid sample in the treatment chamber.

4. Conclusion

In this work, we have investigated microbial inactivation effects in liquid and semi-solid samples subjected to nanosecond pulsed electric fields. All tests were conducted under the influence of nsPEF, given the same set of pulse width, electric field strength, and treatment time that corresponds to a maximum energy input of 75 kJ.kg^{-1}.

Prior to experiments, simulated field distributions and SAR levels suggested that the custom-designed chamber can provide treated samples an effective inactivating environment with field homogeneity and high energy absorption. Statistical analyses from treating inoculated liquid medium indicate that nsPEF caused as high as 5-$\log_{10}$ reduction in the average number of total microbial population. Besides, a minimal rise in temperature makes nsPEF treatment applicable for using as an alternative food pasteurization method. Additional nsPEF experiments focusing on liquid samples, particularly on optimizations of pulse profiles for a low-thermal absorption mechanism in correlation with other factors such as food pH and food water activity, are recommended.

5. Acknowledgements

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6. References

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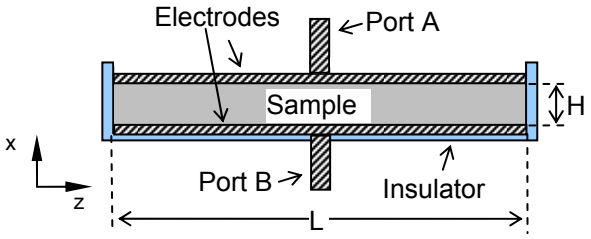

Fig. 3: Cross-sectional view of the static treatment chamber equipped with vertical-aligned conductive plates with *H* and *L* of 0.5 cm and 10 cm, respectively. Pulses are delivered into port A.

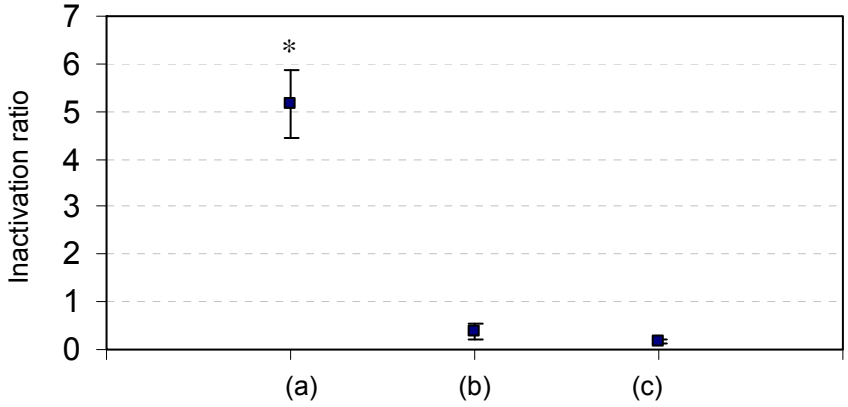

Fig. 5: Inactivation ratios ($\log_{10} N_0/N$) of microorganisms after nsPEF-treatments of (a) liquid samples, and (b) gelatin and (c) curry paste for semi-solid samples (500-ns pulses at 15 kV.cm^{-1} for 450 µs). The asterisk * indicates significant reduction of microbial population for $P < 0.05$.