Effect of rising time of rectangular pulse on inactivation of staphylococcus aureus by pulsed electric field

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Abstract. Pulsed electric field (PEF) is a novel non-thermal food processing technology that involves the electric discharge of high voltage short pulses through the food product. In PEF study, rectangular pulses are most commonly used for inactivating microorganisms. However, little information is available on the inactivation effect of rising time of rectangular pulse. In this paper, inactivation effects, electric field strength, treatment time and conductivity on staphylococcus aureus inactivation were investigated when the pulse rising time is reduced from 2.5 μs to 200 ns. Experimental results showed that inactivation effect of PEF increased with electric field strength, solution conductivity and treatment time. Rising time of the rectangular pulse had a significant effect on the inactivation of staphylococcus aureus. Rectangular pulses with a rising time of 200 ns had a better inactivation effect than that with 2 μs. In addition, temperature increase of the solution treated by pulses with 200 ns rising time was lower than that with 2 μs. In order to obtain a given inactivation effect, treatment time required for the rectangular pulse with 200 ns rise time was shorter than that with 2 μs.

1. Introduction
Pulsed Electric Field (PEF) is a newly developed technology in non-thermal food processing, and is based on a pulsing power delivered to the product placed between a set of electrodes confining the treatment gap of the PEF chamber. The basic definition of PEF relies on the use of high intensity pulsed electric field (10-50 kV cm⁻¹) for cell membrane disruption where induced electric fields perforate microbial membranes by electroporation. Conventional pulse width applied in PEF technology is in microsecond level, thus very little energy will be consumed during the process and the temperature increase of food can be kept at very low. Therefore, PEF is a non-thermal processing technology with low energy consumption and is capable of keeping food’s flavors and nutrition, particularly suitable for foods processing which can be easily affected by temperature.

Because PEF technology has the advantages of protecting physical & chemical characteristics of foods with low energy consumption and temperature rise, it has been considered as a non-thermal processing technology and has a good industrial application prospect. The degree of inactivation

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strongly depends on several constraints. The lethality factors contributing to the effectiveness of PEF technology include technical (electric field strength, shape and width of the pulse, frequency, and treatment time), media (pH, electrical conductivity and temperature) and biological factors. Typical voltage waveforms of PEF are exponential or rectangular pulse. The rectangular pulse is now a focus in researches because of its higher energy efficiency and better inactivation effect compared with the exponential voltage.

Effects of the pulse width, amplitude and frequency of rectangular pulses on the inactivation have been detailed studied in previous studies. However, little information is available on the effect of pulse rising time of the rectangular pulse. In this paper, we have studied the inactivation effect of the rising time of rectangular pulse on staphylococcus aureus inactivation.

2. Instruments and experimental equipments

2.1 Experimental Equipment
In this study, a high voltage PEF equipment PEF-3 (shown in figure 1) recently developed by Graduate School at Shenzhen, Tsinghua University was used. The pulse generator could output a bipolar rectangular pulse in the range of 2-10 $\mu$s in width and frequency in the range of 0-1 kHz. The electric field strength between the two electrode of the treatment chamber is in the range of 0~45 kV cm$^{-1}$. Normally, the rising time of rectangular pulse is in the range of 2.0-2.5 $\mu$s, and is reduced to about 200 ns by adding the optimization unit.

![Figure 1. Photograph of THU-PEF3.](image)

Typical characteristic of the equipment is that pulse parameters including frequency, width, number and polarity can be precisely adjusted through the upper-control personal computer (PC). The general structure of the pulse generator is shown in figure 2.

![Figure 2. Diagram of THU-PEF3 system.](image)

Co-field treatment chambers, which have a lower temperature increase and a lower probability of electric breakdown used in this study. The structure of the treatment chamber is shown in figure 3. The
inner diameter of the electrode is 4 mm and the distance between electrodes is 4 mm. Equivalent resistance of the chamber depends on the electrical conductivity of the solution treated.

![Image](image_url)

**Figure 3.** Treatment chamber.

### 2.2 Material and method

#### 2.2.1 Preparation of apple juice

Huiyuan 100% pure apple juice (i.e. the volume fraction of this apple juice is 100%) was used and kept at 4 °C in refrigerator. Unfreeze the juice at room temperature for two days before the experiment, and then adjust the pH value and the conductivity using NaOH and NaCl of 1 mol L⁻¹ respectively. Finally, inactive the juices at 120 °C for 20 min, and then cool it to prepare for inoculating microorganism.

#### 2.2.2 The culture of microorganism

Staphylococcus aureus (CGMCC1.1861, bought from the China General Microbiological Culture Collection Center) was used as target microorganism

Activate the freeze-dried powder of staphylococcus aureus and then preserve it on inclined plate. Put the strains into an appropriate amount of nutritional broth medium. Centrifuge the bacteria liquid after culturing at 37 °C for 18 h and pour away the clear-water. Add some sterile water and then take out the bacteria after centrifuging the bacteria liquid again. Finally, dilute by equivalent inactivation apple juice to make the number of microorganisms fall in 10⁶~10⁸ cfu mL⁻¹.

#### 2.2.3 PEF treatment method

In this paper, we have individual studied the effect of electrical conductivity and treatment time on inactivation. Hence, the specific experiment methods were slightly different.

For studying the effect of electrical conductivity, a constant equivalent treatment time of 75 μs was used. The juice solution was pumped into the treatment chamber by gear pump at a constant flow speed of 6.4 mL min⁻¹. The electrical conductivities of the solution were 1.5 mS cm⁻¹, 2.0 mS cm⁻¹ and 2.5 mS cm⁻¹, respectively. Pulse voltage was kept at 10 kV, 12 kV and 14 kV, corresponding electric field strength is 25 kV cm⁻¹, 30 kV cm⁻¹ and 35 kV.cm⁻¹, respectively. The rising time of the rectangular pulse was reduced from 2 μs to 200 ns after optimization, as shown in figure 4. Solution temperature was measured at the inlet and outlet of the treatment chamber.

For studying the effect of equivalent treatment time, juice solution with an electrical conductivity of 2.0 mS cm⁻¹ was selected. The equivalent treatment time was changed by adjusting pulse frequency at a constant pump flow rate of 6.4 mL min⁻¹. The pulse width was kept at 5 μs, while the pulse frequency was set at 8 Hz, 10 Hz, 12 Hz, 14 Hz and 16 Hz, respectively. For each treatment time, juice solution was treated at a pulse voltage of 12 kV and 14 kV respectively, and the corresponding electric field strength is 30 kV cm⁻¹ and 35 kV cm⁻¹.
2.2.4 Detection of microorganism

The plate count method was adopted to detect the microorganisms. Dilute the apple juice by 10 time’s gradient dilution method with the NaCl solution (0.85% of volume fraction). Pour 1 mL of the diluted solution and 15 mL of the nutrient agar medium onto each plate, and then put the plate into the incubator at 37 ℃ for 24 h. Count the microorganisms after culturing and take an average from three Parallel specimens. The inactivation effect can be expressed by residual rate $S$, calculated by following formula:

$$\log S = -\log \frac{N}{N_0}$$

Where N is the number of residual microorganisms after treating by pulse electric field, and its unit is cfu mL$^{-1}$; $N_0$ is the number of microorganisms before processed by PEF, cfu mL$^{-1}$; the residual rate S represents the decline of the microorganisms calculated by logs.

3. Results and discussion

3.1 Influence of rising time

The influence of rising time on the inactivation effect of the solution was shown in figure 5. As shown in figure 5, for all the solution conductivities, inactivation effect of PEF increased as the electric field increased. In addition, for a constant applied electric field, higher inactivation effect was achieved for 200 ns rising time rectangular pulse than 2 μs. Generally, survival ratio of the microorganisms could be improved by 0.5~1 logs in all the experiments, indicating that short rising time was favorable to improve the inactivation effect in PEF process.
Meanwhile, we had compared the temperature increase of treated solutions with three different conductivities before and after the PEF treatment, as shown in figure 6. With the increase of the electric field strength, temperatures of the treated solutions increased under all conductivities. However, the temperature increase of the sample treated by the short rising time pulse of 200 ns was lower than that of 2 \( \mu \)s. For example, temperature increase of juice solutions (2.5 mS cm\(^{-1}\)) treated by the short rising time pulse of 200 ns was 14.2 °C, 18.2 °C and 25.5 °C, correspondingly the electric field strength was 25 kV cm\(^{-1}\), 30 kV cm\(^{-1}\), 35 kV cm\(^{-1}\) respectively. However the temperature increase was 16.7, 19.8 and 31.5 °C when the 2 \( \mu \)s rising time pulse was used. The maximum temperature difference of the solution even reached 6 °C at the 35 kV cm\(^{-1}\)(solution conductivity is 2.5 mS cm\(^{-1}\)). It should be noted that the lower temperature increase has a significant benefit for ensuring the non-thermal characteristic of PEF technology.

![Figure 6. Temperature increase before and after treatment by PEF.](image)

3.2 Influence of treatment time

Figure 7 showed the effect of equivalent treatment time on survival ratio under the electric field strength of 30 kV cm\(^{-1}\) and 35 kV cm\(^{-1}\). It could be concluded that survival ratio of the microorganisms reduces with the increase of the treatment time. No obvious difference can be found in the survival ratio treated by the two kinds of waveforms when the treatment time is less than 55 \( \mu \)s. However, when the equivalent treatment time was longer than 55 \( \mu \)s, the advantage of inactivation by the shorter rise time rectangular pulse becomes obvious, when the maximum treatment time 75 \( \mu \)s was reached, the survival difference can even be enlarged by more than 1 log.

![Figure 7. The relationship between equivalent treatment time and survival ratio under the electric field strength of 30 kV cm\(^{-1}\) and 35 kV cm\(^{-1}\).](image)
4. Conclusion
   a) For the present PEF treatment with rectangular pulse, better inactivation effect can be achieved at larger electric field strength, longer treatment time and higher solution conductivity.
   b) The rising time of the rectangular pulse has significant effect on the inactivation of staphylococcus aureus. Rectangular pulse with a rising time of 200 ns has better inactivation effect than that with 2 μs.
   c) Temperature increase of the solution treated by pulse with 200ns rising time is lower than that with 2μs.
   d) In order to achieve a given inactivation effect, lower treatment time was required when the rising time of the rectangular pulse decreases, indicating that less energy consumption are needed to obtain the same inactivation effect.

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References
[1] Beveridge J R, MacGregor S J, Marsili L, et al. 2002 IEEE Trans. Plasma Sci. 30 1525
[2] Castro A J, Barbosa-Canovas G V and Swanson B G 1993 Microbial inactivation of foods by pulsed electric fields (Food Proc Preserve)
[3] Chen J, Zhang R B, Xiao J F, Li J, Wang L M and Guan Z C 2010 IEEE Trans. Plasma Sci. 38 1935
[4] Redondo L M, Margato E and Silva J F 2002 IEEE Trans. Power Electr. 17 196
[5] Redondo L M, Silva J F and Margato E 2007 IEEE Trans. Magn. 43 1973
[6] Chen J, Zhang R B, Xiao J F, Wang L M and Guan Z C 2011 IEEE Trans. Dielect. El. In. 18 1163
[7] Zhang R B, Chen J, Xiao J F, Liao X J, Wang L M and Guan Z C High Voltage Engineering 03 2011
[8] Wang L M, Mo M B, Zhang R B, Chen J, Li J, Xiao J F, et al. High Voltage Engineering 04 2010
[9] Chen J, Zhang R B, Wang X Q, Luo W, Mo M B, Wang L M, Guan Z C Spectroscopy and Spectral Analysis 01 2010