Medium for the quantification of microorganisms in sugarcane juice was Czapek-agar medium. Table 1 provides description of the mediums used in this study.

Table 1: Description of Mediums for Microbial Growth

Contents	Quantities of contents for 1 liter of medium (g/l)			
	Czapek (liquid)	**Czapek-agar (solid)**	**Modified Czapek (liquid)**	**Modified Czapek-agar (solid)**
Saccharose	30	30	-	-
Glucose	-	-	5	5
$NaNO_3$	0.3	0.3	0.3	0.3
K_2HPO_4	0.1	0.1	0.1	0.1
KCl	0.5	0.5	0.5	0.5
$MgSO_4$	0.5	0.5	0.5	0.5
$FeSO_4.7H_2O$	0.01	0.01	0.01	0.01
Agar	-	20	-	20

- Well dissolved in 1 liter of deionized water.
- Sterilized at 121^{o}C, 20 min in an Upright Autoclave HV-150 machine (Hirayama, Japan).
- Adjusted to pH 7 and stored at 4^{o}C in refrigerator.

Experimental Setup for PEF Equipment

The design of experimental PEF pasteurization equipment at laboratory scale is described in Figure 1. Fresh sugarcane juice was placed in treatment chamber which has dimensions (mm) of 150 x 40 x 30 (Length x Width x Height). PEF pasteurization equipment was supplied by an ACS (Alternate Current Source) from electrical network (220VAC/50Hz). The electrical field intensity (E) was changed by voltage regulation which was implemented by the power transformer with variable voltage at the output. The direction of electric field (E) was changed continuously with the frequency of 50Hz (Figure 1A) which applied on the two electrodes (ME1 and ME2). The alternative changes of the polarization on cells induces irreversible damage on cell membranes, thus causing microbial inactivation.

The output voltage (U) from the transformer could be set to: 220V, 110V or 100V. The electric field was passed through sugarcane juice in treatment chamber between the two thin metallic electrodes (ME1 and ME2). The distance (d) between ME1 and ME2 was 40 mm (0.04 m) so that the corresponding values of the electrical field intensity (E=U/d) can be set to 5.5 kV/m, 2.75 kV/m or 2.5 kV/m. The frequency of output voltage and the electrical field were always constant, and so was the frequency of electrical network (50 ± 0.1 Hz).

The commands to the driver circuit was sent from the computer via the NIMyRIO–1900 card (National Instruments, USA). The driver circuit included the triac BTA20–20A–600V which played an important role in exact interval time control of the PEF process. The control signal was sent to control gate of the triac via an opto-coupling device to ensure the operational safety for the NIMyRIO–1900 card. The detailed diagram can be found in figure 1B.

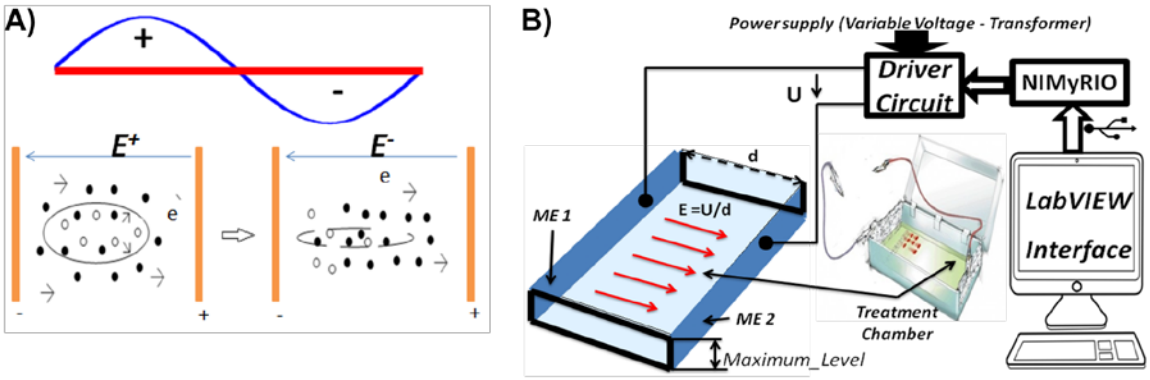

Figure 1. Experimental principle of PEF pasteurization. A) The principle of ACS PEF with two polarizations. B) A diagram of PEF pasteurization equipment at laboratory scale.

The pasteurization system was operated via custom-designed Human Machine Interface (HMI) which was programmed in LabVIEW (Laboratory Virtual Instrumentation Engineering Workbench) environment, version 2014 (National Instrument, USA) (Figure 2). The pasteurization time could be preset via the "Numeric Control" box on the HMI after the source was connected to circuit driver and before the OFF/ON button switch was turned to the "ON" status. The pasteurization process was automatically stopped by the control device after preset interval time for each experiment.

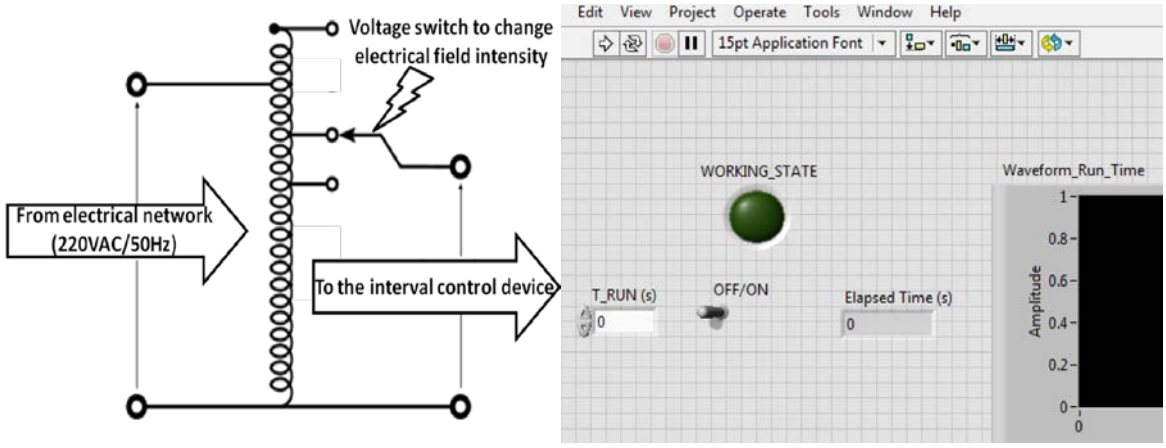

Figure 2. Variable voltage source to change electrical field intensity and Human-Machine Interface to control pasteurization interval time via computer.

PEF of Sugarcane Juice and Quantitative Measurements of E. coli and Sugarcane Juice's Microorganisms

A modified Czapek medium containing suspended *E. coli* cells at a density of 10^4 cells/ml (or fresh sugarcane juice) was filled up the treatment chamber up to maximum ¾ of the volume of the chamber. Petri dishes containing modified Czapek-agar medium and Czapek-agar medium for the cultivation of *E. coli* and native microorganisms of sugarcane juice, respectively, were prepared in advance. After PEF treatment, 100 µl of *E. coli* suspension (or sugarcane juice) was taken out by micropipette and placed on prepared petri dish where it was distributed equally over the medium's surface.

Each treatment was repeated at least three times. The dishes were then placed in the incubator at 37°C for 2 days for the formation of microbial colonies. The number of colonies formed in each dish was counted and the final microorganism density was ten-folds of that number (CFU-colony forming unit/ml).

Results and Discussion

Effect of PEF Pasteurization's Treatment Time on Temperature and pH of Medium

It has been well known that when passing through mediums, electric fields will induce heat due to the internal resistances of the mediums. Conventional pasteurization methods using electric fields thus are referred to as ohmic pasteurization. In our experiments, we wanted to separate the effects of pulsed electric fields and heat for preventing heat-induced loss of valuable contents in the mediums. Therefore, treatment conditions should be selected in order to control the temperature of mediums below 60°C, the temperature that most of microorganisms can still tolerate. We tested on modified Czapek medium with two output electric voltages: 220V and 110V, which correspond to the two powers: 5.5 and 2.75 kV/m, respectively. Treatments was conducted up to 12 and 40 s for the voltages of 220V and 110V, respectively. Figure 3 indicates the fluctuations of the temperature and pH of the medium while treating with the two voltages. It showed that the medium's pH was not changed too much and was kept between 6 and 7 in both cases, while the temperature of the medium gradually increased. In the case of 220V, the temperature increased quickly after 5 s and went over 60°C at 9 s. The temperature in the case of 110V, on the other hand, slowly increased during the first 20 s and reached 55°C at 30 s. After 30 s of treatment, the temperature of the medium increased quickly to 64°C at 35 s. Considering the output power and the ease and convenience of system control, we chose the output electric voltage of 110V with treatment time up to 30 s for our next experiments.

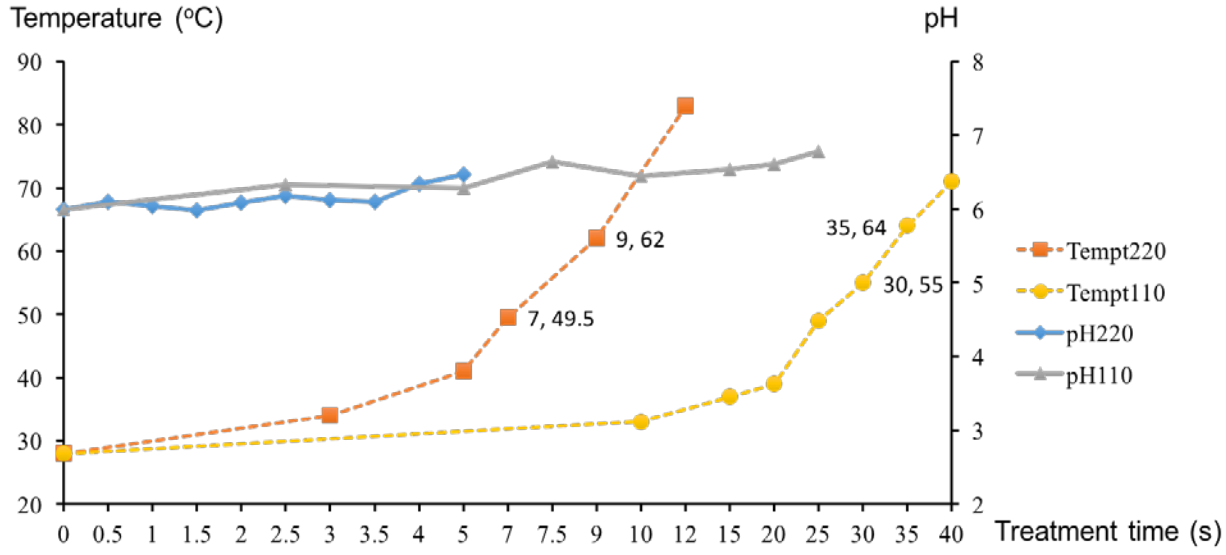

Figure 3. Effect of the power of electric pulses and treatment time on the physical conditions of liquid medium. Tempt220, tempt110 and pH220, pH110 denote fluctuations in temperature and pH of the medium while pulsed at the voltages of 220 V and 110 V, respectively.

In order to validate the above setup conditions for PEF pasteurization, non-sterilized Czapek medium was used. At certain time points during the PEF treatment, 100 µl of medium was taken out from the treatment chamber to perform quantifications of total microorganisms following the procedure described in previous section. As indicated in Figure 4, PEF pasteurization was effective in inactivation of microorganisms in non-sterilized Czapek medium. At 30 s of PEF treatment where the medium's temperature is still below 60ºC, the microbial content reduced 1.42-log cycle compared to non-treated medium. PEF pasteurization for 40 s could inactivate almost microorganisms of the medium.

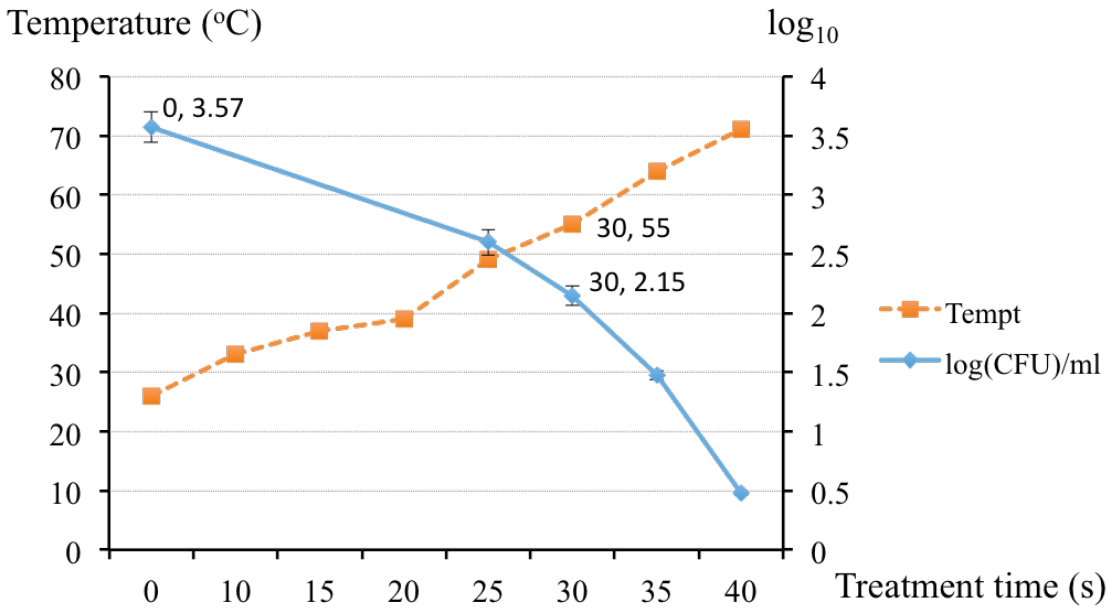

Figure 4. Fluctuations of temperature and microbial content with time, in non-sterilized Czapek liquid medium during PEF pasteurization. Data presented as mean ± standard deviation.

Inactivation of Suspended *E. coli* in Modified Czapek Medium

In this experiment, we tested the efficiency of our PEF pasteurization equipment in the inactivation of the bacteria *E. coli* suspended in modified Czapek medium, where glucose was used as a substitute for saccharose, providing better use of *E. coli* for proliferation. The liquid medium was prepared and sterilized in advance at 121ºC, 20 min. After it was cooled down to room temperature, *E. coli* cells were added into the medium to the density of 10^4 cells/ml. The suspension was then filled in the treatment chamber for PEF pasteurization. Cell counting was performed at different time points following quantitative procedure for bacterial content described above. The data shown in Figure 5 suggests that our PEF equipment and the setup conditions were efficient in removing most of *E. coli* cells in the medium at the temperature lower than 60ºC. It showed a higher efficiency in the reduction of microbial content in this experiment, compared to previous experiment with non-sterilized Czapek medium. A possible explanation for this case is that the medium contained only *E. coli* while the medium in previous case contained wide range of microorganisms including fungus, yeasts and other bacteria. Due to differences in membrane structure of cells, some may be more susceptible to PEFs than others.

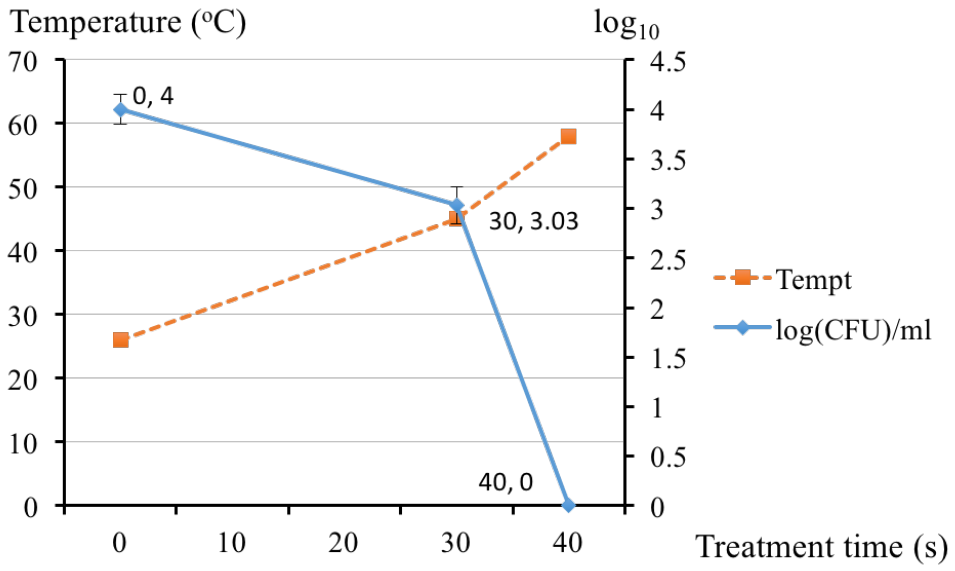

Figure 5. The inactivation of suspended *E. coli* in modified Czapek medium by PEF pasteurization. Data present as mean ± standard deviation.

A previous report by Zgalin and colleagues [14] on the effect of microsecond and nanosecond pulsed electric fields on the inactivation of *E. coli* in water samples also suggested a reduction in bacterial counts when using microsecond pulses, but not any detectable effect with nanosecond pulses. The reduction in bacterial count was only achieved when nanosecond and microsecond pulses were combined. It should be noted that, in most previous cases of study with PEF pasteurization, high intensity (3 – 30 kV/cm) and very short pulses were applied which might require high investment in equipment for pulse generation and control. The system used in our study is considered a simple setup which provided efficient inactivation of *E. coli* in bacterial culturing medium, thus indicating an advantage for a wide-range of applications.

Inactivation of Native Microorganisms in Fresh Sugarcane Juice

To prove the efficiency of the PEF equipment with natural mediums, we tested the system with fresh sugarcane juice. The treatment chamber was filled with sugarcane juice up to ¾ of the maximum volume of the chamber. An electric field with the voltage of 110V was passed through the juice in the chamber producing an electric power of 2.75 kV/m. At certain time points during the treatment, total microorganisms were counted, following the quantitative procedure described in previous section. As shown in Figure 6, for sugarcane juice, the PEF treatment could be prolonged to 70 s while the juice's temperature was still kept below 60°C. At 70 s of the treatment, the native microbial content was reduced 1.2-log cycle, compared to that of the original juice before treatment. This efficiency was lower than that in the *E. coli* case.A possible explanation could be that the microbial structure of sugarcane juice contains different types of microorganisms including fungus, yeasts and bacteria, which express different susceptibilities to PEFs. Nevertheless, the proposed PEF pasteurization equipment was efficient at the inactivation of native microorganisms in fresh sugarcane juice. Controlling the temperature of the juice by submerging 3/4 of the chamber in cold water could prolong the time of treatment for higher efficiency in reduction of microbial growth of the juice.

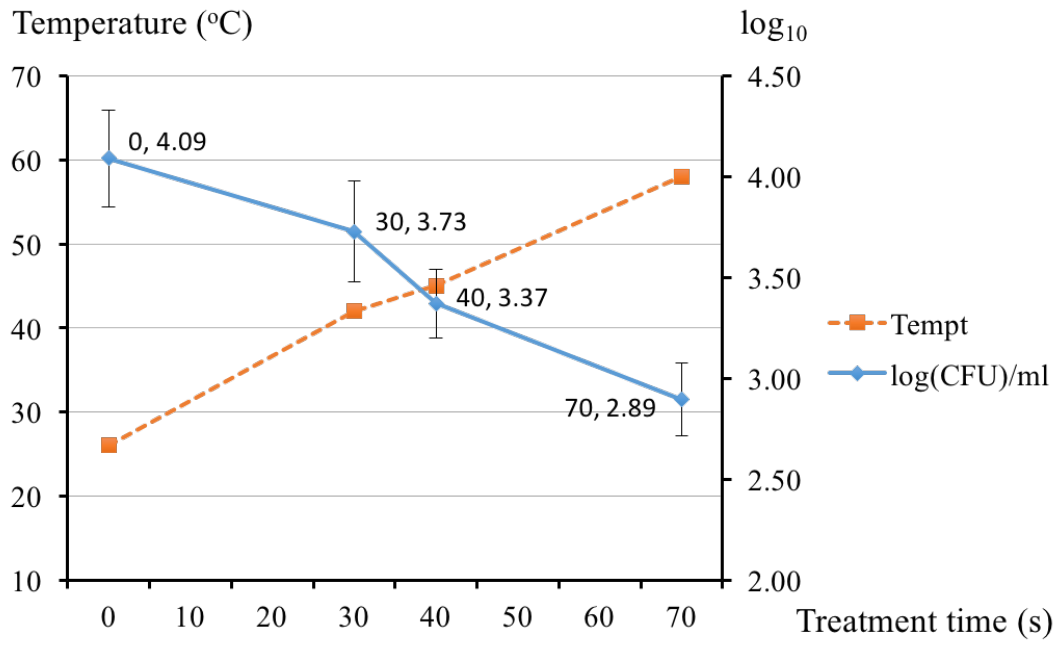

Figure 6. The inactivation of native microorganisms in fresh sugarcane juice by PEF pasteurization. Data present as mean ± standard deviation.

Conclusions

We proposed a lab-scale PEF pasteurization equipment that is simple, utilizes common output electric voltages (220V and 110V), with easy-to-find spare parts and materials, and a simple designed Human-Machine Interface control. The equipment has proved efficient in the inactivation of suspended *E. coli* in modified Czapek medium, and native microorganisms in Czapek medium and fresh sugarcane juice. Although there are still many technical issues that need to be overcome to improve the efficiency and to bring the system to higher scale, the study suggests the potential of this equipment for small scale or family applications. In the future, we are going to study the design of a compact system, which possibly allows continued treatment of fluids as well as finding suitable treatment conditions for different juices.

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