Application of Pulsed Electric Field for Inactivation of Yeast *S. cerevisiae* in Apple Juice

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Abstract. Pulsed electric filed (PEF) is a non-thermal food preservation method as alternative to traditional thermal method. The effect of pulsed electric field (PEF) on inactivation of yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) in apple juice containing different sugar concentration was studied. The results of this study have shown that, using PEF, the yeast could be inactivated at room temperature (20°C). The inactivation effect of PEF was dependent on pulse number, field strength, and pulse width as well as sugar concentration. Pulse numbers less than 20 pulses (at 12.6kV/cm) had no or very less effect on yeast inactivation. Increasing the pulse number up to 400 pulses resulted about 3 logs yeast inactivation. The most important factor for yeast inactivation was the field strength (10 to 30 kV/cm). Whereas, at 10.2 kV/cm and 100 pulses, less than 2 logs yeast inactivation could be achieved, was the yeast inactivation at field strength of 30kV/cm and 100 pulses about 5 logs. At given field strength and pulse number, longer pulse width (1 to 2.5 µF) affected positively the inactivation of microorganism. In contrast, increasing the sugar concentration in apple juice higher than 20% negatively affected the inactivation of yeast during PEF treatment at given treatment conditions.

1. Introduction

Although conventional thermal pasteurization assures the safety of food products, it can also negatively affect sensorial acceptances. Therefore, alternate pasteurization processes are intensively investigated. The application of electrical fields to biological cells induced electrical charges on the cell membrane. Exceeding membrane potential a critical value of 1 V caused membrane breakdown in many cellular systems. For example, an external electric field of about 10 kV/cm can cause cell disruption for *E. coli* [1]. Pulsed electric field (PEF) processing involves the application of very high voltage (field strength in the range of 10 to 80 kV/cm). Food placed between two parallel electrodes treated at these high voltage and very short pulse duration in the range of micro or nano second. PEF technology is considered to be superior to traditional heat treatment because of less thermal effect on food components compare to conventional thermal pasteurization and higher retention of sensory and physical properties of foods [2].

Microorganism inactivation using pulsed electric field (PEF) can be achieved at room or moderate temperatures avoid the harmful effect of heat on sensory and nutritional value of liquid foods. Many microorganisms including bacteria, moulds, and yeast can be inactivated using PEF at temperature distinct lower than thermal pasteurization. The process parameters such as electric field strength, pulse number, pulse width, specific pulse energy, treatment time and temperature are important for effective microorganism inactivation using PEF method. Sitzmann [3] reported up to 3 logs reduction of the native microbial flora of freshly squeezed orange juice at electric field strength of 15 kV/cm without
significantly affecting its quality. Zhang et al. [4] reported 3 to 4 logs of aerobic counts in reconstituted orange juice after PEF treatment at 32 kV/cm. Both thermal pasteurized and using PEF pasteurized juices have a shelf-life of more than 5 months at 4 °C. PEF treated juice has lower vitamin C losses and better color compared to the heat-treated ones up to 90 day at 4 °C. The shelf life of raw skim milk (0.2% milk fat) treated with PEF at 40 kV/cm (30 pulses, 2 µs, exponential decay pulses) was studied by Fernandez-Molina et al. [5]. They found that the shelf life of PEF treated milk was 2 weeks at 4 °C. The inactivation of Listeria innocua suspended in skim milk using PEF treatment combined with nisin was investigated by Calderon-Miranda [6]. They observed the synergistic effect of combined PEF and natural antimicrobial for inactivation of Listeria innocua. Whereas, PEF treatments at 30, 40 or 50 kV/cm can reduce L. innocua up to 2.5 logs, was the L. innocua reduction after PEF treatment and subsequent exposure to 10 IU nisin/ml up to 3.4 logs. The microbial population of L. innocua was reduced by 2.5-logs after PEF treatments at 30, 40 or 50 kV/cm. Pulsgnulda et al. [7] studied the inactivation of yeast (Saccharomyces cerevisiae) and lactic acid bacteria in the wine using PEF method. They found that the inactivation of investigated microorganisms followed first order kinetic. Increasing field strength and pulse frequency increased the microorganism inactivation using PEF. Furthermore, they calculated the D and Z values for PEF treated investigated microorganisms for different field strength and pulse frequency. The effect of heat assisted PEF for inactivation of S. cerevisiae (isolated from spoiled beverage) was reported by Montanari et al. [8]. They found that preheating of sample at 50 °C is one of the main factors for inactivation of yeast during PEF treatment. Adding citral in to the sample increased the yeast inactivation during heat – assisted PEF treatment. They postulated that the synergistic effect of Preheating, PEF and citral were maybe die to the increased membrane permeability during PEF treatment which improve the solubility of citral in the cell membrane causing cell inactivation.

2. Materials and methods

2.1. Sample preparation

The yeast Saccharomyces cerevisiae DMS 70451 was used for inactivation experiments. For preparing the yeast, the sample was added to potato dextrose nutrient and incubated for 24 at room temperature with shaking (125 rpm). After that, the sample solution was centrifuged at 6000 rpm and the sediment (concentrate yeast) was added to sterile apple juice (Apple juice, Aro, Thailand). The prepared apple juice containing yeast was applied for PEF and thermal treatments.

2.2. Pulsed electric field treatment

For PEF treatment of yeast was PEF equipment with max. voltage of 30 kV applied. The capacity of capacitance was 25 nF. 1 to 2 ml of yeast containing apple juice was injected in the cylindrical treatment chamber (gap = 10 to 40 mm variable, electrode surface area= 0.706 cm²) using a sterile siring and subjected to PEF treatment at different pulse number, field strength, and pulse weight. After PEF treatment the sample was removed using a sterile siring and conducted for microorganism counting.

2.3. Thermal treatment

5 ml of with yeast inoculated apple juice was inserted in a sterile plastic bag and seal it. The bag was immersed in water bath (at temperature 50, 60, 70, and 80 °C) for 2 min. After that the bag was immediately immersed in water bath at room temperature to cool the sample.

2.4. Microorganism counting

Before and after each treatment viable counts of the surviving yeasts were determined by standard plating technique. Every sample was serially diluted in sterile 0.5 % saline solution (dilution 1:10), plated in Petri dishes containing Potato Dextrose agar (HIMEDIA, India) and incubated at 30 °C for 3 days before counting. The results are expressed as Log N₀/N, were N₀ is the initial count of microorganism/ml and N is the microorganism count after treatment/ml sample. Each experiment was carried out three times at least and the arithmetic mean was reported as result.
3. Results and discussions

3.1. Thermal treatment
Thermal treatment of apple juice containing yeast *S. cerevisiae* has indicated that at temperature up to 60 °C (2 min treatment) very less inactivation (about 2 log) could be achieved (figure 1). Increasing the temperature up to 80 °C resulted up to 4 logs yeast inactivation.

![Figure 1. Thermal inactivation of yeast (S. Cerevisiae).](image)

3.2. Pulsed electric field treatment (PEF)

3.2.1. Effect of pulse number. In figure 2 is the effect of pulse number during PEF treatment at 12.6 kV/cm field strength on the yeast inactivation demonstrated. Pulse number equal or less than 20 have no or very less (less than one log) effect on yeast inactivation. With increasing the pulse number higher than 50 to 400 pulses increased the microorganism inactivation drastically. Up to 3 logs yeast inactivation could be observed after 400 pulse treatments. Grahl [9] have investigated the inactivation of *S. cerevisiae* in milk and alginate solution. An inactivation of up to 5 log after 5 pulse at 8kV/cm and 5μF was reported. The lower inactivation effect of PEF on yeast observed in our investigation is maybe because of lower energy per pulse (smaller capacity of capacitors of 25 nF) compare to applied capacitance (5000 nF) during his investigation. Qin et al. [10] have reported an *S. cerevisiae* inactivation in apple juice up to 3 log after PEF treatment at 30kV/cm, 200nF and 5 pulses. Grahl et al. [11] reported the influence of pulse number in microbial inactivation of E. coli. They were able to reduce populations of E. coli in UHT milk by 1-, 2-, and 3-log cycles when 5, 10, and 15 pulses (22 kV/cm) were applied. Qin et al., [10] achieved more than a 6-log cycle reduction in E. coli suspended in simulated milk ultra filtrate (SMUF) using electric field intensity of 36 kV/cm with a 5-step (50 pulses) PEF treatment.

3.2.2. Effect of field strength. The yeast inactivation during PEF treatment was dependent on field strength as it shown in figure 3. Increasing the field strength results increased inactivation effect of PEF on *S. cerevisiae*. Whereas at field strength of 10 kV/cm and pulse number of 100 pulses, only slight yeast inactivation (< 1 log) was achieved, increased the yeast inactivation rapidly at filed strength of 30 kV/cm. The microorganism inactivation at 30 kV/cm and 100 pulses (25nF) was higher than 4 logs (figure 2). Hülsheger et al. [13] and Jayaram et al. [14] have reported that the microorganism inactivation increased exponentially with increasing the field strength. This indicates that the increasing of filed strength is much more effective than increasing of pulse number for inactivation of microorganism. Electric field intensity is one of the main factors that influence microbial inactivation (15],[16]). The microbial inactivation increases with an increase in the electric field intensity, above the critical trans‐membrane potential [12]. This is consistent with the electroporation theory, in which the induced potential difference across the cell membrane is proportional to the applied electric field. Mohammed et al. [17] have reported that the microorganism inactivation in dates palm fruit increased with increasing of electric field strength and pulse number.
They found that the field strength of 10.28 kV/cm and 120 pulses lead to up to 4 log microorganism reduction.

3.2.3. Effect of pulse width. Increasing the pulse width from 1 µs to 2.5 µs at given pulse number (100 or 500 pulses) and field strength (12 kV/cm) lead to increased inactivation effect of PEF treatment on *S. cerevisiae* (figure 4). Up to 1 log higher inactivation effect could be observed if the pulse width increase from 1 to 2.5 µs at given field strength and pulse number. This makes the importance of pulse width for microorganism inactivation obvious. Schoenbach et al. [18] find out that the Pulse width also influences the critical electric field; for instance, with pulse widths greater than 50 µs, critical field strength (*E*<sub>c</sub>) is 4.9 kV/cm. With pulse widths less than 2 µs, *E*<sub>c</sub> is 40 kV/cm.

3.2.4. Effect of sugar content in sample. In general increasing the sugar content in sample increased the resistance of microorganism during PEF treatment (figure 5). This was at sugar concentration of 20 to 50 % obvious. Sugar content less than 20 % didn’t show any protective effect. In contrast with increasing the sugar content from 20 % to 50 % the yeast inactivation during PEF treatment rapidly decreased. At sugar concentration of 50 % nearly no yeast inactivation at 12kV/cm and 1 µs pulse width could be observed.

4. Conclusion
Thermal treatment of apple juice inoculated with yeast *S. cerevisiae* at temperature of 80 °C lead to less than 2 log yeast inactivation. Lower temperature has no or very less inactivation effect. In contrast,
PEF treatment is an effective method to inactive yeast at room temperature. Increasing the pulse number higher than 20 pulses at given treatment conditions (12.6 kV/cm, 25 nF, 1µs) increased drastic the inactivation effect. Up to 3 log yeast inactivation in inoculated apple juice could be observed after 400 pulses. Increasing the field strength showed a positive effect on yeast inactivation. Treatment at 30kV/cm and 100 pulses resulted higher yeast inactivation (about 4 logs) compare to treatment at 12.6kV/cm and 500 pulses. In addition, increasing the pulse width positively affect the yeast inactivation. In contrast, increasing the sugar content higher than 20 % in apple juice showed a protective effect on yeast during PEF treatment. These results indicated that PEF treatment is a suitable non thermal method for inactivation of liquid foods.

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5. References
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