Process Optimization for Microbial Reduction in Durian Juice by Using Pulsed Electric Field

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Abstract

Pulsed electric field (PEF) is an interesting non-thermal process for microbial reduction in liquid foods/materials. In this study, a PEF system was set up and characterized for some limitations in its use. The PEF system could generate a supply voltage in a range of 10-40 kV/cm with a frequency range of 10-30 kHz. The flow rate of the liquid food was in the range of 500-1000 mL/min with a continuous flow. The PEF was used for microbial reduction in durian juice. The process optimization was conducted by response surface methodology (RSM) for three parameters: voltage level, frequency of the PEF system and the flow rate of durian juice through a PEF chamber. It was found that the PEF system possessed the most effective functioning on microbial reduction at the voltage of 40 kV/cm at the frequency of 10 kHz. The optimum flow rate of durian juice was at 500 mL/min. The microbial content in the durian juice was reduced for 4.13 log CFU by the optimum condition. The results suggested that the set up PEF system and the obtained condition could be effectively used to reduce the microorganisms contaminating the tested foods.

Keywords: durian juice, microbial contamination, non-thermal processing, pulsed electric field (PEF), response surface methodology (RSM)

Introduction

Durian, the king of fruits in South East Asia, is an important tropical fruit in Thai agriculture with a unique characteristic of having a thorn-covered husk and strong odor[1]. Since 1999, more than 900,000 tons of durian have been produced by over 100,000 hectares of planted lands. Around 98% of total annual output of durian is domestically consumed while the rest is mainly exported to Hong Kong, Singapore, Taiwan and China[2]. Durian flesh is easily microbial deteriorated which leads to lose marketable value. Therefore, the processing of durian flesh is required to produce other products obtained from durian such as durian paste, durian ice-cream, freeze dried durian, and durian chips[3].

A microbial reaction process is necessary to improve the microbiological safety in food products. In several, instances the thermal processes such as pasteurization or sterilization have been done mainly to meet safety purposes. An adverse effect on sensory and nutritional qualities of the food products by those thermal processes is possible[4]. Therefore, many researchers have extended an effort to develop novel non-thermal processes, such as pulsed electric fields (PEF), ohmic heating, high pressure, membrane filtration, etc.[4,5,6]

PEF is an emerging processing technology that has demonstrated the potential to inactivate some vegetative cells of pathogenic bacteria and spoilage bacteria by using short bursts of electricity. PEF processing provides flesh-like safe foods with minimal loss in sensory qualities and nutritional quality[4,7]. In past decades, PEF has gained increasing interest in the mass transfer operation of food technology and biotechnology[8,9,10]

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especially in extraction of bioactive compounds[11,12], food shelf-life extension by sterilization and enzyme inactivation[13], maintaining physicochemical and nutritional quality of foods[12], and degradation of some pesticides contaminated in foods[14]. PEF has been used to reduce the microbial contamination in apple juice, coconut water, carrot-orange mixed juice, liquid eggs, mangosteen puree, milk, orange juice, and strawberry juice[7,11,13,15,17].

PEF processing is an application of external electric fields to induce the electroporation of cell membranes or membrane permeabilisation. The phenomenon enhances the diffusion of solutes inside the food systems. The electrical break down of a cell membrane is undergone by the exposure to a sufficiently intense electric field (above a critical value) in a short duration (milliseconds to microseconds). The PEF may also cause a sub-lethal injury in some microbial population, thus some of them can recover when they are in the optimal condition[16,17]. The membrane permeabilisation can be achieved at moderate electric fields (<10 kV/cm) and low specific energies (<10 kJ/kg)[18]. The lethality and the cell disruption are dependent to the microorganism and the environmental conditions[17]. Therefore, the effectiveness of the microbial inactivation also depends on the processing parameters applied that may cause either transiently or permanently permeability on the cytoplasmic membrane disintegration.[4] reported that the PEF treatment of 35 kV/cm and 300 msec could inactivate 5 $\log_{10}$ cycles of *Salmonella Typhi murium* STCC878 and *Escherichia coli* O517:H7 was reduced 2 $\log_{10}$ cycles and 4 $\log_{10}$ cycles at pH 3.5 and 5.5, respectively[4]. It is essential to know the influence of those factors individually and their concurrent effect in order to evaluate the potential of PEF technology in microbial elimination in food systems.

Therefore, this research aimed to optimize the PEF operation in microbial reduction in durian juice with emphases on three parameters: the pulse frequency, the supplied voltage and the flow rate of the durian juice. The response surface methodology (RSM) was used to design the treatment matrix for the process optimization.

Experimental

1. Raw Material

Frozen durian flesh was prepared from Mon Thong cultivar (*Durio zibetbinus* Murr.) obtained from the local market in Chanthaburi Province, Thailand. The sample (450-500 g) was separately frozen and kept in a polypropylene freeze bag at -20°C, until use.

2. Sample Preparation

Two-hundred grams of the frozen durian flesh was mixed with sterile water 250 mL. The sample was blended using a sterile wire blender (at a speed level 1) for 1 min. The sample was then filtered through a triple layer of sterile cloth-sheet and then kept in a sterile bottle embedded in ice to maintain the temperature of 0-4°C.

3. PEF system

The PEF equipment used in this investigation was designed and setup[15] The PEF system comprised a stainless steel chamber, a pulse generator and a pivotal system. The set up PEF has a capacity in the range of 10-30 kHz. The voltage is applied to the PEF chamber in the range of 10-40 kV. The voltage supply could also be determined by the space of the electrodes in the treatment chamber, the flow rate of fluid through the pipe was in the range of 500-100 L/min[15].

4. Experimental Design

The response surface methodology was used to evaluate the effect of the three treatment parameters at two levels (low-high) including pulse frequency (10-30 kHz), pulse intensity (10-40 kV) and flow rate of the fluid foods (500-1000 mL/min) on the responses of the survival of the microbial content and the rate of microbial reduction. The obtained data, after the sample treated with different conditions, were modeled with the following second order polynomial equation (1)[18]:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i + \sum_{i>j}^{k} \beta_{ij} X_i X_j \qquad (1)$$

Where,

Y = the response variable to be modeled

X_i and X_j = the independent factors

β_0 = the intercept

β_i = the linear coefficient

k = the total number of independent factors

5. PEF-treatment on microbial reduction in durian juice

The sample was mixed with 3 L of the sterile water and then subjected to the PEF chamber as the condition assigned in the (Table 1). In a previous report on this PEF system, the PEF treatment should be run as a two-cycle run to provide the most effective result for microbial reduction. Therefore, all runs were performed as two-cycle runs. In addition, the PEF chamber and pivotal line were washed twice with 5 min circulation of 4 L hot water before running the next cycle.

6. Determination of microbial survival content

The samples were decimal diluted in sterile 0.85% NaCl before spreading on a plate to count agar (PCA) medium and then cultivated at 37°C for 12-24 h[19]. After that, the survival rate (equation 2) and the microbial reduction rate (equation 3) were calculated based on the model linearity.

The survival rate $= \frac{1}{\sqrt{\eta}} = \frac{1}{\sqrt{TVC}}$ (2)

The microbial reduction rate

$$= \mu_{red} = \frac{\left(\frac{TVC_0 - TVC_t}{TVC_0}\right)}{t} \quad (3)$$

Where η = Survival microbial content (CFU/mL)

μ_{red} = Rate of microbial reduction (min^{-1})

TVC = Total viable count (log CFU/mL)

TVC_0 = Total viable count of PEF-untreated sample (log CFU/mL)

TVC_t = Total viable count of PEF-treated sample (log CFU/mL)

t = treatment time (min)

7. Statistical analysis

The experiments were done in triplicate and the central composite design (CCD) and the corresponding analysis of the data were carried out by using the software package Design-Expert (Trial Version). The parameters of the models were tested in the regression and interaction at the statistical significance at *P<0.05*.

Results and Discussions

According to the results, there were two responses: the function inverse of the survival rate ($h^{-1/2}$) and the rate of microbial reduction (m_{red}). These were obtained from the total viable count (TVC) but had a different account of the treatment time (t). (Table 1) shows 16 runs by the treatment matrix of central composite design (CDD). By the statistical analysis, the survival rate was suggested to take a power of -0.5 to be fitted as linearity behavior (*F-value = 8.50*, *P = 0.0027*). The rate of reduction was calculated based on the treatment time, thus finding fitted linearity (*F-value = 6.54*, *P <0.0001*). By the fitted linear modeling, two models of the microbial survival (equation 4) and the microbial reduction rate (equation 5) were suggested.

The fitted equation of microbial survival:

$$\frac{}{\sqrt{\eta}} = 0.068 - 2.16\times10^{-3} f - 1.44\times10^{-3}\nu - 1.44\times10^{-4}\Phi - 1.87\times10^{-4} f\nu + 5.21\times10^{-6} f\Phi - 3.96\times10^{-6}\nu\Phi + 4.79\times10^{-5} f^2 + 2.26\times10^{-4}\nu^2 + 1.11\times10^{-7}\Phi^2 \quad (4)$$

The fitted equation of microbial reduction rate:

$$\mu_{red} = 9.47 - 0.44 f + 0.03\nu + 3.79\times10^{-3}\Phi - 4.12\times10^{-3} f\nu + 1.27\times10^{-4} f\Phi - 6.64\times10^{-5}\nu\Phi + 9.72\times10^{-3} f^2 + 3.61\times10^{-3}\nu^2 - 7.3\times10^{-6}\Phi^2 \quad (5)$$

Where

η = Survival microbial content (CFU/mL)

μ_{red} = Rate of microbial reduction (min^{-1})

f = Pulse frequency (kHz)

ν = Supplied voltage (kV/cm)

Φ = Flow rate of the sample (mL/min)

The model of microbial survival (equation 4) had lower reliability to use for the prediction of the PEF efficiency indicated by the statistical analysis (*Model, F-value = 10.93, P = 0.0044; LOF, F = 27.38, P = 0.0111*). The model of microbial reduction rate was fitted (*Model, F-value = 33.67, P = 0.0002; LOF, F-value =3.65, P = 0.1579*).

Table 1 Central composite design (CCD) matrix and responses for the microbial reduction in PEF-durian juice (predicted and experimental values).

Run	f (kHz)	ν (kV/cm)	Φ (ml/min)	Predicted value		Experimental value	
				Survival rate$^{-0.5}$	Reduction rate (min^{-1})	Survival rate$^{-0.5}$	Reduction rate (min^{-1})
1	10 (10)*	28 (30)*	800 (800)*	0.0310	6.09	0.0449	6.50
2	30 (30)*	40 (40)*	680 (700)*	0.1000	7.25	0.0737	7.05
3	10 (10)*	40 (40)*	500 (500)*	0.1700	10.87	0.1769	10.84
4	17 (20)*	40 (40)*	1000 (1000)*	0.1410	5.21	0.1160	5.04
5	10 (10)*	40 (40)*	500 (500)*	0.1700	10.87	0.1769	10.84
6	15 (15)*	18 (20)*	660 (700)*	0.0125	6.22	0.0037	5.44
7	30 (30)*	20 (20)*	1000 (1000)*	0.0600	3.71	0.0447	3.52
8	22 (25)*	29 (30)*	800 (800)*	0.0270	4.75	0.0289	4.84
9	30 (30)*	10 (10)*	500 (500)*	0.0100	5.85	0.0167	5.99
10	10 (10)*	10 (10)*	1000 (1000)*	0.0110	3.05	0.0258	3.39
11	22 (25)*	10 (10)*	800 (800)*	0.0125	3.89	0.0318	4.26
12	10 (10)*	10 (10)*	1000 (1000)*	0.0260	3.76	0.0258	3.39
13	30 (30)*	10 (10)*	500 (500)*	0.0125	6.21	0.0167	5.99
14	30 (30)*	40 (40)*	1000 (1000)*	0.0600	4.48	0.0883	4.71
15	10 (10)*	10 (10)*	500 (500)*	0.0156	6.59	0.0057	6.66
16	22 (25)*	28 (30)*	500 (500)*	0.0310	5.82	0.0225	6.17

Remark: (*) asterisks represented to the measured values in the experiments

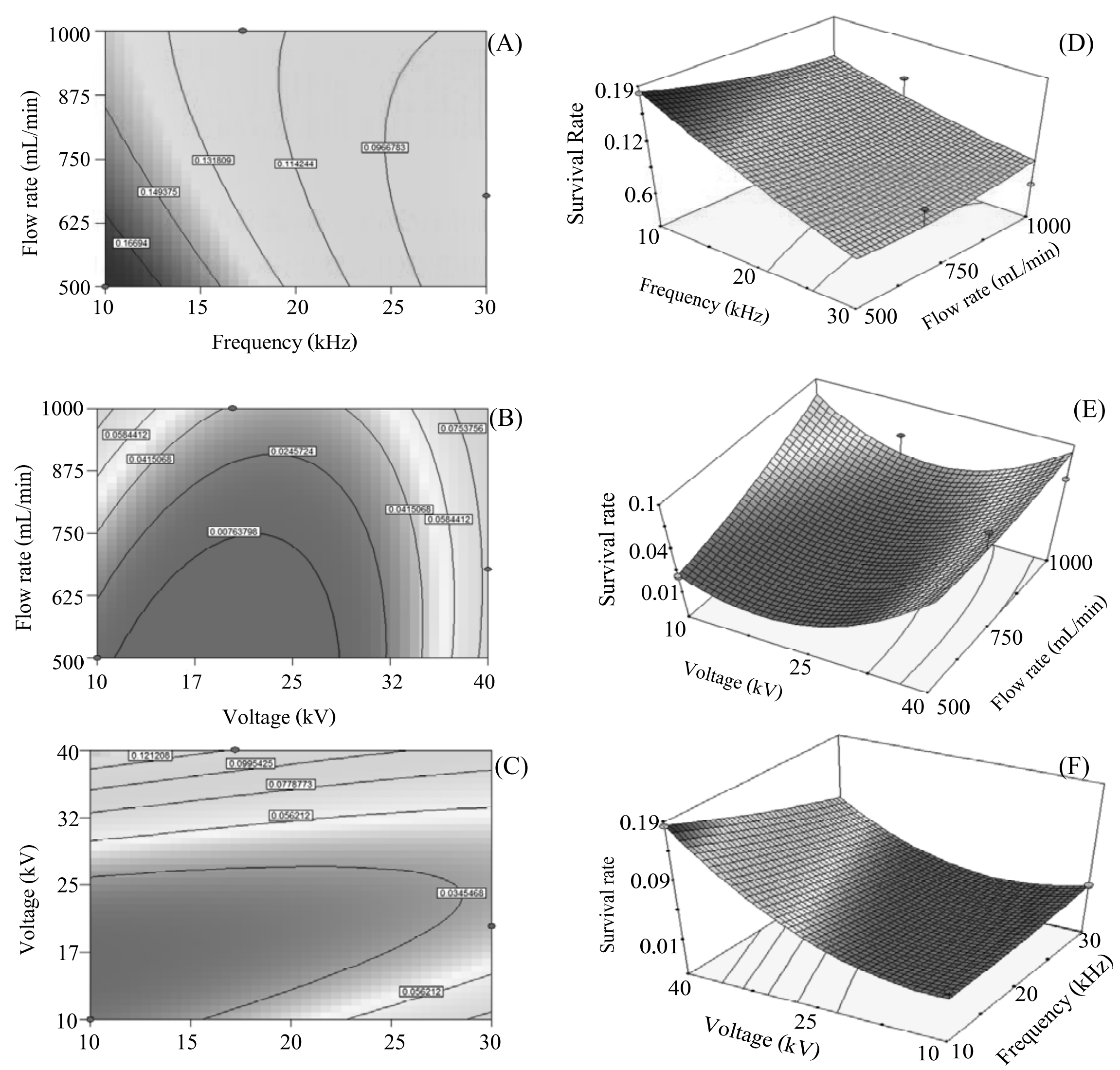

Figure 1 Contour plots (A-C) and response surfaces of the tested parameters (D-F) on microbial survival

From the microbial reduction rate model, it could be implied that the voltage frequency was a domoinant parameter on microbial reduction in the durian juice. The interaction of frequency and voltage supply had a concurrent enhancing on the PEF to reduce microbial content in the liquid foods. The results are similar to the findings of Elez-Martinez et al. (2005) that the increasing of pulse frequency caused decreasing in the PEF effect on microbial reduction[20].

Conclusion

The voltage frequency was a major parameter on the microbial reduction in durian juice. In addition, the voltage supply and the frequency use are concurrent in microbial reduction. In this study, the optimal condition for microbial reduction in durian juice was at 40 kV/cm with 10 kHz and the flow rate of 500 mL/min with the microbial reduction rate of 10.84 min^{-1}.

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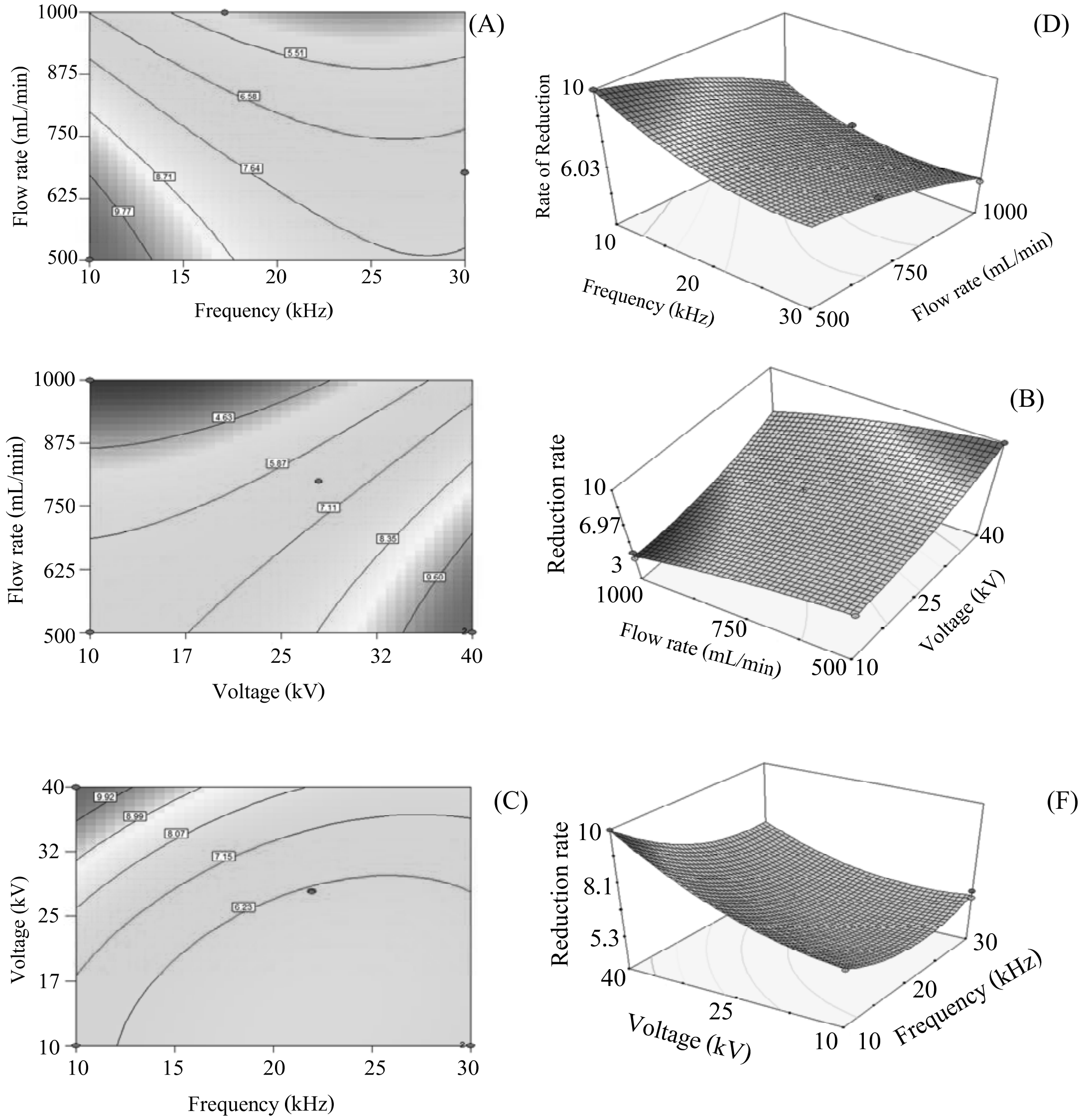

Figure 2 Contour plots (A-C) and response surfaces of the tested parameters (D-F) on the microbial reduction rate

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