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Design of a treatment chamber for low-voltage pulsed electric field sterilization

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ABSTRACT

In this paper, different microchips were simulated by the finite element method (FEM) with the aim to clarify the effect of topological parameter on inactivating microorganisms vaccinated in blueberry juice under low voltage for 50–250 μs and 20–100 pulses. For the planar comb teeth microchips in the first generation, only a 1.01 $\log_{10}$ of *Escherichia coli* (*E. coli*) was achieved at 25 °C in all the solutions, then, with the aim to obtain a more uniform electric field distribution, an interdigitated electrode structure are used in the second-generation microchip which improved the inactivation effect of *E. coli* up to 2.85 $\log_{10}$ reduction. At the end, by considering the problems presented by the two aforementioned microchips, the structure of a parallel plate as the third-generation microchip was designed and the best experimental platform was built. This microtreatment chamber exhibited excellent inactivation effect on *E. coli*, *Saccharomyces cerevisiae*, and *Staphylococcus aureus* of 5.32, 5.42, and 4.77 $\log_{10}$ cycles respectively. These results will provide a meaningful guidance in inactivate microorganisms by low-voltage PEF.

1. Introduction

Spoilage, which is mainly caused by the activity of microorganisms, influences the edible quality of food and poses a direct threat to human health. Thus, sterilization is a crucial procedure in food processing. Although traditional thermal sterilization processes are economical, reliable, and effective in inactivating microorganisms, high temperatures also affect the taste, color, and nutritional quality of food (Li, Zhang, & Fang, 2003). With the improvement in living standards, consumers are becoming increasingly concerned with the color, smell, and taste of food in addition to its safety (Morris, 2000). This situation has promoted the development of non-thermal food processing technology.

Pulsed electric field (PEF) technology is currently one of the most popular non-thermal food processing technologies in the world. The color, smell, and taste of food are better preserved via PEF than via thermal sterilization (Barbosa-Cánova & Sepúlveda, 2004; Elez-Martínez, Aguiló-Aguayo, & Martín-Belloso, 2006; Giner, Gimeno, Palomes, Barbosa-Cánovas, & Martín, 2003; McAuley, Singh, Haro-Maza, Williams, & Buckow, 2016). Researchers from all over the world have conducted considerable research on PEF sterilization since Sale and Hamilton (1967) first discovered that PEF exhibit good sterilization effect under normal temperature. The results of some studies have shown that bacteria suspended in various types of liquids can be inactivated under electrical field strengths ranging from 20 kV/cm to 80 kV/cm for tens of microseconds (Cortizo & Fernández Lorenzo de Mele, 2003; Lebovka, Praporscic, & Vorobiev, 2004; Vorobiev & Lebovka, 2010). Some researchers have suggested that PEF can effectively kill target bacteria (Gelaw, Espina, Pagán, García-Gonzalo, & De Lamo-Castellví, 2014; Guo et al., 2014; Liu, Lebovka, & Vorobiev, 2013; Zulueta, Barba, Esteve, & Frígola, 2013). However, microbial inactivation generally requires a strong electric field generated by a very high voltage ranging from several kilovolts to tens of kilovolts (Huang, Jiang, Wang, Gai, & Wang, 2014; Mattar et al., 2014). This process leads to high costs, electrolysis-prone area around electrodes, and destruction of solution composition. In addition, given the huge size of a PEF machine, accurately controlling parameters is difficult. To address the aforementioned problems, effective sterilization at low voltage while avoiding shortcomings in the traditional processing chamber has become a popular research topic in PEF.

With the development of microfabrication, wherein the space between two electrodes is short, low voltage can produce high electric field strength. To date, several laboratories have developed microchips with germicidal function (Sanchez-Moreno, De Ancos, Plaza, Elez-Martinez, & Cano, 2009). To achieve the same field strength, a voltage that is 25 times higher than those of the microchips is required in the developed PEF processing system at laboratory scale. Microchips have

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been widely used to damage the structure and function of cells. Moreover, microorganisms are forced to move into zones with high electric field where they can be captured and accumulated through the dielectric electrophoresis effect, which kills microbes (Pacioni, Cerretani, Procida, & Cichelli, 2014). The structure of microchips is the major factor that affects electric field distribution (Liu, Lebovka, & Vorobiev, 2013; Liu, Zhao, et al., 2013; Movahed & Li, 2013). Microchip technology is currently applied in cell fusion (Wang & Lu, 2006; Zhao et al., 2006). However, only a few studies have been conducted on its bactericidal effect. In addition, no example of juice sterilization by microchips has yet been reported. Existing microchips have a relatively short channel and result in minimal juice mobility, which is not applied to juice sterilization. Thus, designing new microchips for juice sterilization is necessary. Theoretical simulation (Hao, Chen, Sun, Liu, & Wu, 2016) has become an important research tool when direct measurement by the experimental methods is difficult, such as the analysis of a relatively complex electric field distribution. By considering the actual structure of the microchip, the present study uses the finite element analysis (Wang, Liu, & Liang, 2016; Wang, Liu, & Yang, 2015; Zimmerman, 2004) software by COMSOL (Brezmes & Breitkopf, 2015) for analysis, which provides strong support for the design of the microelectrode.

Blueberries does not only have high nutritional values (Barba et al., 2012; Molan, Lila, Mawson, & De, 2009; Nindo, Tang, Powers, & Singh, 2005; Petzold, Moreno, Lastra, Rojas, & Orellana, 2015; Turan, Cengiz, & Kahyaoglu, 2015), but also exhibit a wide range of functional characteristics, such as delaying cranial nerve aging, improving vision and body functions, as well as anticancer, and anti-aging activities (Borges, Degeneve, Mullen, & Crozier, 2009; Castrejón, Eichholz, Rohn, Kroh, & Huyskens-Keil, 2008; Oliveira, Amaro, Pinho, & Ferreira, 2010; Papandreou et al., 2009; Phillips et al., 2010; Zafra-Stone et al., 2007). As the standard of living rises, the unique flavor and health functions of blueberry juice have attracted the attention of researchers.

Therefore, the processing medium used in this study was blueberry juice, whereas the processed objects were *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), and *Saccharomyces cerevisiae* (*S. cerevisiae*), because of *E. coli* is a typical gram-negative bacterium, *S. aureus* is a gram-positive bacterium which is commonly found in the gut of humans and animals, it is also used as target bacterium in food hygiene detection. The yeast can easily grow in an acidic environment meanwhile it is the main microorganism that cause the spoilage of fruit juice.

In this paper, different types of microchips were produced through design, simulation, and manufacture; these microchips were encapsulated in a microtreatment chamber. With the aim to solve the problems exposed by sterilization experiments, the optimized design of new microchips was successfully produced in the end. The objectives of this study were to set up an optimal experimental platform of PEF in microsystems and to investigate the effect of sterilization on the object microbes under low-voltage PEF in blueberry juice. A new sterilization technique could be developed based on this system.

2. Materials and methods

2.1. Materials

The blueberries were provided by Organic Food Co., Ltd. (Dandong City, Liaoning Province, China). The fruits were ripened and refrigerated at − 80 °C. *E. coli*, *S. aureus*, and *S. cerevisiae* were purchased from the China General Microbiological Culture Collection Center (CGMCC, Beijing, China). The species numbers were CGMCC 1.90 (*E. coli*), CGMCC 1.1861 (*S. aureus*), and CGMCC 2.604 (*S. cerevisiae*).

2.2. Design and improvement of the microtreatment chamber

The area of high-electric field region is essential to applications of microchips in sterilization. In this section, the procedure involved designing, improving and encapsulating microelectrodes. At present, the structures of interdigital comb teeth (ICT), planar comb teeth (PCT), and symmetrical comb teeth (SCT) have attracted extensive attention due to their unique physical properties to gather cells in the high-electric field region based on dielectrophoresis effect (Fox, Esveld, Luttge, & Boom, 2005). However, whether this effect is beneficial for sterilization or not still remains uncertain. Thus, the design and process of the microelectrode is started from those above-mentioned structures. The abstained new microelectrode will be encapsulated in a microtreatment chamber as a core part of the sterilization experimental platform.

COMSOL software was used to simulate microelectrode structures to optimize the first-generation microchip, and electric current is chosen as the physics mode.

The first- and second-generation microelectrodes comprise a three-layer structure. The basal layer is the insulating glass basement membrane. The surface of this layer is covered with a copper (Cu) electrode array. Finally, a gold (Au) film is plated on the outermost layer. A multi-electrode array is composed of two electrode arrays, namely, the positive and the negative arrays, which are connected to the main lead. The specific microchip processing steps are illustrated in Fig. 1.

After the microchips were manufactured, the next step was to encapsulate them in a microtreatment chamber for electric sterilization. Polydimethylsiloxane (PDMS) (Hu et al., 2009) was selected as the encapsulation material because of its good chemical inertness, non-toxicity, and good flexibility.

The main factors consider in the material selection for the third-generation microchip is the safety, stability and electrical conductivity. Then, by contrast, stainless steel (Walke et al., 2016) was used for the third-generation microchip because compared with other conductive material, while maintaining strong corrosion resistance (stability), stainless steel can also exhibits good electrical conductivity meanwhile it will not release toxic substances in extreme environment (safety) (Geng et al., 2007). Moreover, stainless steel can quickly disperse ohmic heat generated during the experiment, and thus, effectively prevent the electrolysis problem at the electrode.

2.3. Setup of the experimental platform

An Gemini X2 cell electroporation signal generator (American BTX Company) was used as the power system in the current study. The square wave pulse is selected as the experimental pulse waveform.

In order to describe the pulse conditions clearly, Fig. 2 shows the square wave diagram and the waveform measured by the oscilloscope. Where v_u is the pulse voltage, t_1 to t_2 is the pulse width, t_1 to t_3 is the

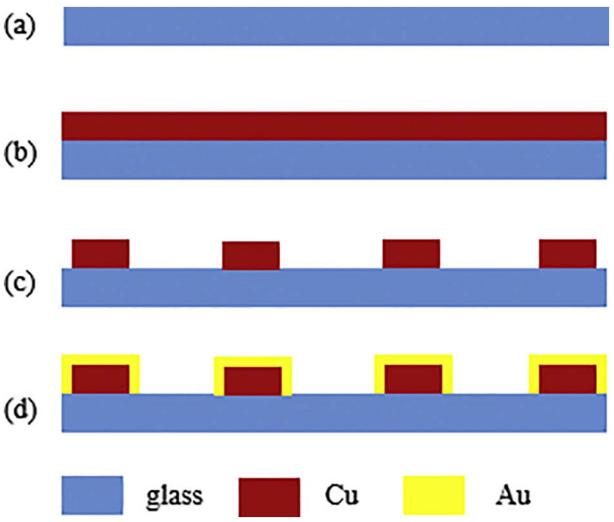

Fig. 1. Manufacturing processes for the microchips.