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Effect of cell membrane fatty acid composition of *Escherichia coli* on the resistance to pulsed electric field (PEF) treatment

Zhi-Wei Liu [a, b], Xin-An Zeng [a, *], Michael Ngadi [b], Zhong Han [a]

[a] College of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510641, China
[b] Bioresource Engineering Department, McGill University, 21111 Lakeshore Rd, Ste-Anne-de-Bellevue, QC H9X 3V9, Canada

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ABSTRACT

The underlying relationships between growth temperature, membrane fatty acid composition (mFAC) and PEF resistance of *Escherichia coli (E. coli)* were explored. Results indicated the PEF resistance of *E. coli* at stationary stage was directly related to mFAC. For the cells at stationary stage, as growth temperature decreased from 37 °C to 15 °C, the PEF (23.3 kV/cm, 1.2 ms) lethality of *E. coli* was increased 1.5 log10, reached 3.2 log10. Meanwhile, the proportion of unsaturated fatty acid (UFA) was increased from 20.07 to 47.65%. And the fluidity index (FI) of the cell membrane was also increased from 0.42 to 1.42. It revealed that the PEF resistance of *E. coli* at stationary stage was determined by the mFAC and fluidity of the cell membrane. Those conclusions were also confirmed by cytomembrane fluidity determined by fluorescence probe (DPH) and the different PEF-induced electro-permeabilization of dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) vesicles. On the other hand, for the cell at exponential stage, the PEF resistance of *E. coli* was rarely affected by mFAC. The PEF (23.3 kV/cm, 1.2 ms) lethality of cells was almost the same (3.2 log10) at different growth temperature.

1. Introduction

PEF processing as a non-thermal sterilization technology has been fully studied in the last two decades for preservation of fruit juices and milk (Aadil et al., 2015; Dymek, Dejmek, Galindo, & Wisniewski, 2015). It has become one of the most interesting alternatives to traditional thermal sterilization (Aguilo-Aguayo, Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2009; Odriozola-Serrano, Soliva-Fortuny, & Martín-Belloso, 2009).

Although large number of studies have demonstrated that PEF treatment can successfully inactivate different microorganisms in various liquid foods (Monfort, Saldana, Condon, Raso, & Alvarez, 2012), the mechanisms are still not well understood. It is generally acknowledged that cytomembrane damage induced by PEF treatment is related to microbial inactivation (Meyer et al., 2000). Loss of the physical integrity of the cytoplasmic membrane has also been demonstrated by the leakage of ATP or UV-absorbing material from bacterial cells after PEF treatment (Aronsson, Ronner, & Borch, 2005). Membrane breakdown induced by PEF is referred to electroporation or electro-permeabilization (Teissie & Tsong, 1981). And occurs when a PEF-induced transmembrane potential reached a critical value of approximately 0.2–1 V (Kinosita et al., 1988).

Although bacteria were killed effectively by PEF treatment in liquid food, its efficiency was discrepant for different microorganisms, even for the same bacteria at different growth environment and growth stage. Ohshima, Okuyama, and Sato (2002) reported that *E. coli* grew at 20 °C were easily inactivated by PEF treatment than those of growth at 37 °C. *Listeria monocytogenes* grew at 4 °C was more sensitive to PEF treatment than the cells grew at 35 °C (Álvarez, Pagán, Raso, & Condón, 2002). Moreover, for *Staphylococcus aureus*, PEF resistance was increased significantly after the cells were exposed to heat (45 °C) and alkaline (pH = 9.5) for 2 h (Cebrian, Raso, Condon, & Manas, 2012). The reasons for these phenomena maybe attributed to the changes in the mFAC of microbial cell membrane under different growth environment. It is well recognized that microorganisms in response to the changes of growth temperature, pH and osmotic stresses can adjust their mFAC to maintain the ideal membrane fluidity required for proper enzyme activity and substance transportation in cell membrane (Beales, 2004). Therefore, in order to reveal the underlying mechanism of PEF resistance of bacteria, the exploration of the effect of the mFAC of cell membrane on the efficiency of PEF treatment should be done. To the best of our knowledge, although there were a few studies focused on PEF resistance of microorganism at different condition (Arroyo, Cebrián, Pagán, & Condón, 2010), there was no detail reports about the relationship between cell mFAC and

* Corresponding author.
E-mail address: xazeng@scut.edu.cn (X.-A. Zeng).

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PEF resistance of microorganism.

Vesicles as a simulation of biomembrane which consisted by synthetic phospholipids have been employed to explore membrane electro-permeabilization under PEF treatment (Teissie & Tsong, 1981). In our previous studies, the effect of cell size and cell membrane fluidity on the electro-permeabilization of cell membrane under PEF treatment was confirmed by using different size and membrane composition vesicles (Liu, Zeng, Sun, & Han, 2014). To some extent, vesicles are a simple and reliable model to elucidate the mechanism of PEF-induced electro-permeabilization of cell membrane.

The objective of this study was to obtain a better knowledge about the role of mFAC played in the PEF resistance of microorganisms. The effect of growth temperature on the PEF resistance of *E. coli* at different growth stages was investigated. mFAC was analyzed by gas chromatograph-mass spectrometer (GC-MS). Cytomembrane fluidity of *E. coli* was determined by using 1, 6-diphenyl-1, 3, 5-hexatriene (DPH). What's more, the PEF-induced electro-permeabilization of DOPC and DPPC vesicles were employed to confirm the relationship between growth temperature, mFAC and PEF resistance.

2. Materials and methods

2.1. Materials

E. coli was provided by Dr. Shi (South China University of Technology, Guangzhou, China). DPPC and DOPC (purity 99%) were purchased from Corden Pharma Co. (Liestal, Switzerland). Supelco 37 component FAME MIX used as the standard of fatty acid was got from Sigma (Sigma-Supelco, Bellefonte, PA, USA). 5 (6)-Carboxyfluorescein (5 (6)-CF), DPH, tryptic soy broth and yeast extract were obtained from Aladdin Chemistry Co. Ltd. (Shanghai, China).

2.2. Culture condition

Sterilized Tryptic Soy Broth with 0.6% of Yeast Extract (TSBYE) prepared in flasks was inoculated with *E. coli*. The cultures were incubated under agitation with 100 rpm/min (HY-5, Jinbo Equipment Industry Co. Ltd, Jiangsu, China) at 15, 20, 30, 37 and 45 °C. The growth curves were measured by a spectrophotometer at 600 nm (UV-1800, SHIMADZU Co. Ltd. Kyoto, Japan). At specified growth stage, *E. coli* was harvested by centrifuging at 4000 g for 5 min at 4 °C (JW-3021HR, Anhui Jiaven Equipment Industry Co. Ltd., An qing, China). After removing the supernatant, the pellet was then re-suspended in 1000 mL distilled water. The conductivity was adjusted to 180 ± 5 μS/cm (DDS-11A, Shanghai Leici-Chuang yi Instrument and Meter Co. Ltd., Shanghai, China) with 3 M KCl solution. And then, samples containing approximately 10^9 CFU/mL cells were ready for PEF treatment. The fatty acids composition of *E. coli* at different growth temperatures and growth stages were analyzed at the same time.

2.3. DOPC and DPPC vesicles preparation

DOPC and DPPC vesicles were prepared by the method described in our previous study (Liu, Zeng, Sun, Han, & Aadil, 2015, 2016). The average diameter of DOPC vesicles was 1265.75 ± 53.4 nm. The average diameter of DPPC vesicles obtained was 1296.23 ± 36.82 nm.

2.4. PEF treatment

A continuous PEF treatment system was used in this study (Liu et al., 2014). The PEF treatment parameters were as follow: titanium alloy electrodes, bipolar square wave, pulse frequency: 1 kHz, pulse duration: 20 μs; electrode distance: 0.30 cm; flow volume in the treatment chamber: 0.02 mL; and sample flow rate: 0.50 mL/s. The applied electric field intensity was 0, 5, 10, 15, 20 and 23.3 kV/cm and 1.2 ms treatment time. For comparison, experiments with control samples were performed using the same procedure without PEF treatment (0 kV/cm). To control the temperature of the sample during PEF treatment, a heat exchanger was used (DLSK-3/10, Zhenzhou Ketai Equipment Industry Co. Ltd. Henan, China). The inlet and outlet temperatures were recorded by a thermocouple thermometer (WRE/E, Xingtai Instrument Co. Ltd., Jiangsu, China). At present study, the initial temperature of all samples was kept at 15 °C. The final temperatures did not exceed 35 °C.

2.5. Determination inactivation

After PEF treatment, Inactivation of *E. coli* was expressed as log reductions of viability. The viability was assayed by counting colony-forming units. A sample of 1 mL was taken from the PEF treated cell and serially diluted with 0.1% peptone solution. The *E. coli* was placed on TSBYE-Plate Count Agar. And then it was incubated at 37 °C for 24 h, and counted afterward.

2.6. Fluorescence measurement

After PEF treatment, the electro-permeability of DOPC and DPPC vesicles was evaluated by the changes of the relative fluorescence intensity of vesicles solution. The 5 (6)-CF release percentage (R (%)) was calculated as follows (Liu et al., 2014):

$$R(\%) = \left[(F - F_{ini}) \Big/ \left(F_{fin} - F_{ini}\right)\right] \times 100\% \quad (1)$$

Where R (%) is the release percentage, F is the fluorescence intensity measured after the PEF treatment, Fini is the initial fluorescence intensity (E = 0 kV/cm), and Ffin is the final fluorescence intensity when the 5 (6)-CF vesicles were completely destroyed by adding 10% Triton X-100 (w/w). The fluorescence intensities F were measured with a spectrofluorometer (LS 55, Perkin Elmer Inc., Bucks, UK) with parameter: excitation light: 490 nm, emission light: 520 nm excitation slit: 5.0 nm and emission slit: 2.5 nm.

2.7. Membrane fatty acid extraction and analysis

The cell membrane fatty acid extraction and methylation were conducted according to the method described by Sasser (2009). About 40 mg (fresh weight) of pellets of *E. coli* was processed by saponification, methylation, extraction and wash. And fatty acids were transferred into fatty acid methyl esters. Fatty acid methyl esters were determined by a GC Agilent 7820A (Agilent Technologies, Wilmington, DE, USA) equipped with a flame ionization detector and a capillary column HP-5 (30 m × 0.32 mm × 0.25 μm, Agilent Technologies, Wilmington, DE, USA) using pure nitrogen as carrier gas at 1 mL/min in a split mode (20:1). The column temperature program: the initial temperature150 °C increased to 170 °C at 10 °C/min. And 170 °C was maintained for 0.5 min. And then, 170 °C increased to 200 °C at 5 °C/min and maintained 1 min. 200 °C increased to 260 °C at 2 °C/min and maintained 2 min. At first, the fatty acid methyl esters were identified by comparing their retention times with the standards (Sigma-Supelco, Bellefonte, PA, USA). And then, the identified fatty acid methyl esters were also confirmed by GC-MS (GC-MS QP2010 Ultra, SHIMADZU, Tokyo, Japan). Results were expressed as relative percentages of each fatty acid, which were calculated as the ratio of the surface area of the peak to the total area of all peaks.