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Defining treatment conditions for pulsed electric field pasteurization of apple juice

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ABSTRACT

The influence of temperature and the presence of N^{α}-lauroyl ethylester (ethyl lauroyl arginate, LAE) on the inactivation caused by continuous pulsed electric field treatments (PEF) in *Escherichia coli* O157:H7 suspended in apple juice have been investigated to define treatment conditions applicable at industrial scale that promote an equivalent safety level when compared with thermal processing. In the range of experimental conditions investigated (outlet temperature: 20–40 °C, electric field strength: 20–30 kV, treatment time: 5–125 μs) at outlet temperatures equal or lower than 55 ± 1 °C, the inactivation of *E. coli* O157:H7 treated in apple juice ranged from 0.4 to 3.6 Log_{10} cycles reduction and treated in apple juice supplemented with LAE (50 ppm) ranged from 0.9 to 6.7 Log_{10} cycles reduction.

An empirical mathematical model was developed to estimate the treatment time and total specific energy input to obtain 5 Log_{10} cycles reduction in the population of *E. coli* O157:H7 suspended in apple juice supplemented with 50 ppm of LAE at different electric field strengths and inlet temperatures. Treatment conditions established for *E. coli* O157:H7 were validated with other PEF resistant Gram-positive (*Listeria monocytogenes*, and *Staphylococcus aureus*) and Gram-negative (*Salmonella enterica* serovar Typhimurium) strains. When the treatment was applied to the apple juice, a treatment of 25 kV/cm for 63 μs corresponding with an outlet temperature of 65 °C and input energy of 125 kJ/kg was required to achieve more than 5 Log_{10} cycles in the four strains investigated. The addition of LAE reduced the treatment time required to obtain an equivalent inactivation (>5 Log_{10} cycles) in the four microorganisms to 38.4 μs, the outlet temperature to 55 °C, and the input energy to 83.2 kJ/kg.

1. Introduction

The introduction of innovative preservation technologies for extending food shelf-life and enhancing microbial food safety without compromising the nutritional and sensory characteristics of foods and with a reduction of energetic costs is one of the strategies that may lead to improve the competitiveness of the food industry (Rajkovic et al., 2010). Different technologies that allow killing microorganisms at temperatures below those used in thermal processing have been proposed to achieve this objective. High hydrostatic pressure (HHP) and pulsed electric field (PEF) are the most investigated (Toepfl et al., 2006).

The main advantage of PEF compared with HHP is the possibility of processing liquid foods in continuous flow in a few seconds. The main limitation of studies in which different liquid foods have been treated in continuous flow PEF systems is that the criteria used to establish the applied PEF treatment conditions (electric field strength, treatment time, etc.) were arbitrary and did not demonstrate if these treatments were enough to guarantee food safety. As bacterial spores are resistant to PEF treatments, its main application for food preservation must be focused on pasteurization.

The traditional pasteurization process in the food industry is based on thermal processing. To replace these treatments by PEF, treatment conditions used for evaluating the potential applications of this technology should not only improve food quality but should also promote an equivalent microbial safety, as compared with thermal processing.

Generally, studies to evaluate the application of PEF for microbial decontamination at laboratory scale have been conducted with very long treatments (>100 μs) at very high electric field strengths (>30 kV/cm) (Mosqueda-Melgar et al., 2007; Sampedro et al., 2006; Sepúlveda et al., 2005; Walkling-Ribeiro et al., 2009; Zhao et al., 2008). At commercial scale, technical and economical limitations exist in applying these intense treatments. Long treatments require using several treatment chambers and cooling the food between chambers to maintain the temperature below those used in thermal processing (Min et al., 2003). Consequently, in addition to the high total specific energy required in these long treatments, an extra cost is necessary for controlling food temperature. On the other hand, technical limitations and risks of arching exist for applying electric field strengths above 30 kV/cm in the treatment chambers required for commercial application of PEF technology (Barbosa-Cánovas and Altunakar, 2006).

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Recently, it has been observed that when operating at room temperature, PEF process conditions that would be commercially applicable (30 kV/cm, 100 μs) are not sufficient to obtain substantial inactivation in several PEF-resistant strains of pathogenic microorganisms in the pH range between 3.5 and 7.0 (Saldaña et al., 2010a). The higher inactivation achieved in the population of *Salmonella* Typhimurium 878 and *Staphylococcus aureus* 4459 was around 3 Log_{10} cycles. But in the case of *L. monocytogenes* 5672 and *E. coli* O157:H7, the inactivation reached was generally less than 1 Log_{10} cycles.

An approach to increase the lethal effect of PEF with short treatment times at moderate electric field strengths involves combining this technology with other preservation factors (Raso and Barbosa-Cánovas, 2003). The temperature and the presence of antimicrobials are two factors that influence microbial inactivation by PEF (Wouters et al., 2001). Generally, a temperature elevation above room temperature causes a greater level of microbial inactivation, even at temperatures that are not lethal for the microorganism (Heinz et al., 2003; Saldaña et al., 2010b; Sepúlveda et al., 2005). The presence of naturally occurring antimicrobials such as nisin has been proven effective in increasing the lethality of PEF (Gallo et al., 2007; Liang et al., 2002; Terebiznik et al., 2000). There is a synergistic effect in the inactivation of Gram-positive bacteria when PEF treatments are combined with nisin, but in some Gram-negative microorganisms, this combination is ineffective (Calderón-Miranda et al., 1999; McNamee et al., 2010). In foods, both Gram-positive and Gram-negative microorganisms are generally present, but the design of an effective pasteurization process by combining PEF with an antimicrobial requires searching for antimicrobials effective against both groups of bacteria. Cationic surfactant N^{α}-lauroyl ethylester (ethyl lauroyl arginate, LAE) yields a wide spectrum of activity against Gram-positive and Gram-negative bacteria, yeasts, and molds (Rodriguez et al., 2004). LAE has been generally recognized as safe (GRAS) in the US since 2005, at levels up to 200 ppm and it has been evaluated by the European Food Safety Authority (EFSA) as a preservative to be used in non alcoholic beverages made with fruit juice, energy and sports drinks, and meat products (EFSA, 2007).

This paper investigates the effects of temperature and the presence of LAE on the inactivation caused by continuous PEF treatments in *E. coli* O157:H7, suspended in apple juice. The objective of this study is to define treatment conditions applicable at industrial scale that promote an equivalent safety level when compared with the commercially available products processed with conventional technologies. Established treatment conditions have been validated with other PEF resistant Gram-positive (*Listeria monocytogenes*, and *S. aureus*) and Gram-negative (*Salmonella* Typhimurium).

2. Material and methods

2.1. Microorganisms and growth conditions

Escherichia coli O157:H7 used in this investigation is a VTEC — (Phage type 34) isolated by Dr. Chapman (Chapman et al., 1993) and the strains of *L. monocytogenes* (CECT 5672), *S. aureus* (CECT 4459) *Salmonella* Typhimurium (CECT 878) were supplied by the Spanish Type Culture Collection (CECT). In a previous study, it was demonstrated that these strains were especially resistant to PEF (Saldaña et al., 2009).

During this investigation, the cultures were maintained on slants of tryptic soy agar (Biolife, Milan, Italy) with 0.6% yeast extract added (TSAYE) (Biolife). A broth subculture was prepared by inoculating a test tube containing 5 mL of tryptic soy broth (Biolife) with 0.6% yeast extract (TSBYE) with a single colony, followed by incubation at 37 °C for 18 h. With this subculture, a flask containing 50 mL of sterile TSBYE was inoculated to a final concentration of approximately 10^6 cells/mL. The culture was incubated under agitation at 37 °C until the stationary growth phase was reached (12 h for the strains of *E. coli* and *S. aureus*, 15 h for the strains of *Salmonella* Typhimurium and 24 h for the strains of *L. monocytogenes*).

2.2. PEF treatments

The PEF unit used in this investigation was previously described by Saldaña et al. (2010a,b). The apparatus generates square waveform pulses of a width of 3 μs. Preliminary studies to investigate the efficacy of the presence of LAE on the inactivation of *E. coli* O157:H7 by PEF at 4, 27 and 50 °C were conducted using a batch parallel electrode treatment chamber with tempered electrodes (Saldaña et al., 2010b). This chamber consists of a cylindrical polypropylene tube closed with two polished stainless steel cylinders of 2.01 cm^2 in surface area and 4 cm in length. The distance between electrodes was 0.25 cm and the volume of the treatment zone was 0.5 mL.

Experimental setup for continuous PEF treatments consisted of a reservoir, a pump, a heat exchanger, and a treatment chamber. An eight-roll peristaltic pump (Ismatec, Glattbrugg, Switzerland) was used to pump the apple juice from the reservoir through silicone tubes to a coiled metal tube ($Ø_{in}$ 2 mm, $Ø_{out}$ 3 mm, 230 cm length) immersed into a heating bath and the treatment chamber. The treatment chamber consisted of two parallel stainless steel electrodes with a gap of 4.5 mm and an electrode area of 1.47 cm^2. Considering the influence of the temperature on apple juice conductivity, the load resistance of the treatment chamber ranged from 100 Ω at 25 °C to 87 Ω a 60 °C. The flow rate was set at 3 L/h, and the calculated mean residence time was 10 and 0.8 s in the heat exchanger and the treatment chamber, respectively. The time elapsed between the exit of the chamber and the taking of the sample in Eppendorf cups immediately placed on ice was 5 s. Temperature of the apple juice was measured with thermocouples located before and after the heat exchanger and just after the PEF treatment chamber.

The specific energy input (W) per pulse expressed in kJ/kg was calculated by the following equation (Eq. 1):

$$W = \frac{1}{m} \cdot V \cdot I \cdot t \tag{1}$$

where m (kg) is the mass of the apple juice contained in the volume of the treatment chamber; V is the input voltage (kV); I is the current intensity (A); and t is the treatment time (μs). The total specific energy was calculated by multiplying the total energy input per pulse by the number of pulses.

2.3. Microbial inactivation experiments in batch

Before treatments, microorganisms were centrifuged at 6000×g for 5 min at 4 °C and re-suspended in a citrate-phosphate McIlvaine buffer of an electrical conductivity of 0.10 ± 0.01 S/m and pH 3.5. LAE (Lamirsa, SA, Terrasa, Spain) was added to the corresponding media to obtain a concentration of 25 or 50 μg/mL. The microbial suspension (0.5 mL) at a concentration of approximately 10^8 CFU/mL was placed into the treatment chamber with a sterile syringe. Two independent experiments were performed for each treatment condition.

2.4. Microbial inactivation experiments in continuous treatments

Before treatments, microorganisms were centrifuged at 6000×g for 5 min at 4 °C and re-suspended in commercial apple juice (Auchan, Alcampo S.A., Spain) of an electrical conductivity of 0.27 ± 0.02 S/m and pH 3.5 ± 0.1. LAE at a concentration of 50 ppm was added to the apple juice contaminated with microorganisms in those experiments in which the influence of this compound on the efficacy of PEF was investigated. The influence of electric field strength, treatment time, and initial treatment temperature was investigated. Electric field strength was set at 20, 25, and 30 kV/cm; the treatment