LWT - Food Science and Technology 90 (2018) 180–185

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Application of high voltage electrical discharge plasma for the inactivation of *Escherichia coli* ATCC 700891 in tangerine juice

Sudheer Kumar Yannam[a], Pooya Estifaee[a], Shane Rogers[b], Selma Mededovic Thagard[a,*]

[a] Department of Chemical and Biomolecular Engineering, Clarkson University, Potsdam, NY 13699, USA
[b] Department of Civil and Environmental Engineering, Clarkson University, Potsdam, NY 13699, USA

ARTICLE INFO

Keywords:
Escherichia coli
High voltage
Inactivation
Plasma
Tangerine juice

ABSTRACT

A tangerine juice inoculated with *Escherichia coli* ATCC 700891 was treated using direct-in-liquid electrical discharge plasma. The reactor system used a point-plane electrode configuration with both a high voltage electrode and a grounded electrode submerged in the juice. The effectiveness of the treatment was assessed at different applied voltages (17–30 kV), discharge frequencies (20–80 Hz) and temperatures (15–35 °C). At optimum operational parameters (30 kV, 40 Hz, and 25 °C), *E. coli* was reduced by 4.8 Log_{10} CFU/mL after a plasma exposure of 2 min and below the detection limit of > 1 Log_{10} CFU/mL within 3 min. Scanning electron microscopy (SEM) revealed changes in cell morphology following the treatment suggesting that *E. coli* might be inactivated by cell wall damage caused by shock waves produced by the plasma. Physicochemical characteristics of the tangerine juice were not altered by the plasma treatment when compared to the untreated tangerine juice. In contrast, there were significant changes observed in total acidity, ascorbic acid and total phenol content in pasteurized tangerine juice. The results obtained in this study promote the further development of this technology for the inactivation of foodborne pathogens and extended shelf-life of fruit-based beverages.

1. Introduction

One of the most widely adopted technologies used for shelf-life extension and preservation of fruit juices is conventional thermal processing. However, the demand for nutritious foods, which are minimally processed at low temperature, has led to an interest in non-thermal technologies (Tiwari, O'donnell, & Cullen, 2009). While thermal processing adversely affects the nutritional quality of food, non-thermal processing technologies have the potential to preserve their natural characteristics as well as their flavor, color, and taste (Barbosa-Cánovas and Zhang, 2001; Caminiti et al., 2011).

One food group that is extremely popular as it contains antioxidants, vitamins and minerals which are essential for human growth is that of fruit juices and fruit-based drinks. Unfortunately, nutrients in fruit juices can support the growth of several types of microorganisms, such as bacteria, yeasts, and molds that are also the most common causes of the spoilage of these products. The most common foodborne pathogens such as *Escherichia coli* and *Salmonella* can survive the acidic environment of fruit juices and may lead to foodborne disease outbreaks associated with the consumption of unpasteurized fruit juices (Aneja, Dhiman, Aggarwal, Kumar, & Kaur, 2014). Controlling these foodborne pathogens has been done with conventional thermal pasteurization; however, over the last decade, pasteurization has been minimized with the use of different non-thermal food preservation techniques including high hydrostatic pressure, ultraviolet radiation technology, ultrasound and pulsed electric field (Surowsky, Fröhling, Gottschalk, Schlüter, & Knorr, 2014).

Low-temperature plasma is another emerging technology that has been shown to inactivate pathogens at moderate temperatures and short treatment times in water, milk, and fruit juices (Barbosa-Cánovas and Zhang, 2001; Gurol, Ekinci, Aslan, & Korachi, 2012; Korachi and Aslan, 2011). For example, atmospheric plasmas formed above water have been successfully used to inactivate *Staphylococcus aureus* and *Escherichia coli* to concentrations below the limit of detection (< 1 CFU/mL)(Perni et al., 2007; Xiaohu, Feng, Ying, Jing, & Jianjun, 2013). Direct-in-liquid electrical discharges have also been used with similar effectiveness to inactivate various microorganisms in water (Abou-Ghazala, Katsuki, Schoenbach, Dobbs, & Moreira, 2002; Son et al., 2014; Vukusic et al., 2016). Raw milk was treated by a low-temperature gas plasma, and the system was capable of 3 Log_{10} CFU/mL reduction of *E. coli* without significantly altering the milk properties (Gurol et al., 2012). Surowski and co-authors used a cold plasma argon-oxygen jet with 480 s plasma exposure to reduce *Citrobacter freundii* cells in apple juice by about 5 Log_{10} CFU/mL (Surowsky et al., 2014).

* Corresponding author. Department of Chemical and Biomolecular Engineering, Clarkson University, 8 Clarkson Avenue, Potsdam, NY, USA.
E-mail address: smededov@clarkson.edu (S.M. Thagard).

https://doi.org/10.1016/j.lwt.2017.12.018
Received 12 May 2017; Received in revised form 21 September 2017; Accepted 9 December 2017
Available online 12 December 2017
0023-6438/

Almeida and co-authors achieved complete bacterial inactivation in orange juice using atmospheric cold plasma at 70 kV and 20 s for direct plasma (in juice) and 45 s for indirect plasma (over juice) treatments (Almeida et al., 2015). Indirect cold plasma has also been successfully applied in the treatment of pomegranate and grape juice (Kovačević et al., 2016; Pankaj, Wan, Colonna, & Keener, 2017).

The effectiveness of the direct-in-liquid plasma treatment has been attributed to a variety of processes occurring directly in the plasma, immediately surrounding the plasma and in the bulk liquid and may include physical processes such as electric field, plasma-induced UV light and shockwaves as well as chemical processes involving stable molecules (e.g., H_2O_2) and short-lived active species including OH, H, O, HO_2 and O_2^-. Because of this direct exposure of the bulk liquid to the plasma reactive species, electric field and UV photons, inactivation rates for direct-in-liquid plasmas are frequently higher than those of indirect plasmas wherein only the top liquid layer is exposed to the plasma reactive species, with the rest of the bulk liquid remaining more or less untreated (Laroussi, 2009; Ziuzina, Patil, Cullen, Keener, & Bourke, 2013).

Although several studies have demonstrated the potential for low-temperature gas plasma treatment technologies in replacing conventional technologies, there is only a handful of studies that have assessed the applicability of direct-in-liquid electrical discharge plasmas for microbial inactivation of liquids other than water. As a result, the suitability of the plasma process for the treatment of liquids such as fruit juices and the effect of the treatment on organoleptic properties of the juice is not known. The present study investigates the effectiveness of direct-in-liquid electrical discharge plasma for the inactivation of *E. coli* in tangerine juice and reports the impacts of temperature, applied voltage and discharge frequency on inactivation rates. Physicochemical properties of the tangerine juice before and following the treatment were characterized and compared to pasteurized tangerine juice.

2. Materials and methods

2.1. Fruit juice and microbial culture

Tangerine juice (Cuties 100% tangerine juice, Califia Farms, Bakersfield, CA) was obtained from a local grocery store and stored at 5 °C until use. The sugar content of the juice was determined to be 11.5 °Brix. The pH and electrical conductivity of the juice were 3.8 and 4.4 mS/cm, respectively.

E. coli ATCC 700891 was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). The culture was stored in the tryptic soy broth (Becton, Dickinson, and Company (BD), Franklin Lakes, New Jersey, USA) growth medium containing 20% glycerol at −70 °C. Viable counts were performed according to standard methods on tryptic soy agar plates (TSA, Thermo Fischer Scientific, USA) and expressed in Log_{10} CFU/mL. For pasteurized juice, thermal pasteurization was completed at 80 °C for 2 min.

2.2. Microbial preparation

Fresh cultures of *E. coli* (ATCC 700891) were prepared in 50 mL sterile tryptic soy broth by transfer of cells from the agar slants and incubation at 37 °C for 18 h. Bacteria cultures were pelleted by centrifugation (Beckman Coulter, Indianapolis IN, USA) at 3000 rpm for 10 min at room temperature and washed twice with 1X sterile phosphate-buffered saline (PBS), pH 7.4. The pellet was re-suspended in sterile water, and the bacterial density at t = 0 determined by measuring absorbance at 600 nm with a UV–Vis spectrophotometer (Shimadzu UV-1800, Shimadzu, Japan). For plasma treatment, cell suspension with a concentration of 1×10^7 CFU/mL was prepared in sterile tangerine juice.

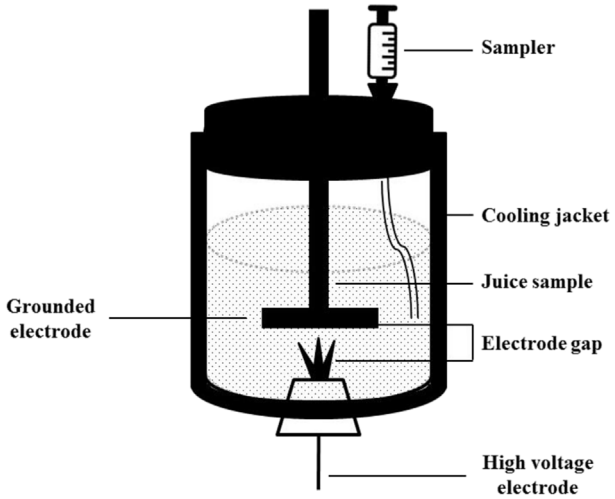

Fig. 1. Schematic view of the plasma reactor.

2.3. Plasma experimental setup

The plasma reactor used in this study was an 18 cm long jacketed cylindrical glass vessel 2.5 cm in diameter. Both the high voltage and the grounded electrode were arranged in a point-plane configuration. The high voltage electrode was a sharpened piece of 0.8 mm diameter nickel-chromium wire. The grounded electrode was a 1.8 cm diameter stainless steel disc (thickness 1.25 cm) welded onto a stainless-steel rod. Both electrodes were submerged under juice sample and placed at a distance of 1 cm (Fig. 1). Electrical discharges were created using a custom-built 0–30 kV, 0–80 Hz, 1 nF high-voltage rotary spark gap power supply (Applied Physical Electronics, Spicewood, TX). The voltage and current in the plasma reactor were measured and recorded using a Tektronix P6015A high voltage probe and a Tektronix P6021 current probe connected to a Tektronix TDS 3032C oscilloscope. Examples of the voltage and current waveforms are shown in Fig. 2.

2.4. Plasma treatment procedure

For each experiment, 10 mL of juice with *E. coli* was transferred to the plasma reactor, where it was treated for 3 min. At 1 min intervals, the treatment was paused and subsamples removed to measure bacterial decimation and juice composition. Experiments were run at six different voltages (17, 20, 23, 25, 28 and 30 kV), four different

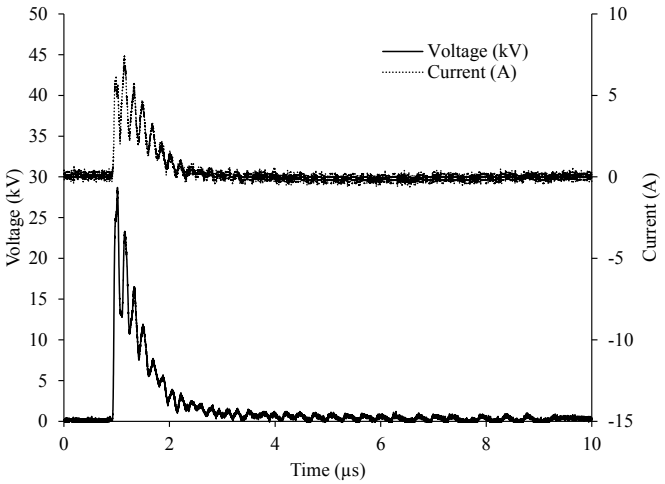

Fig. 2. Voltage and current waveforms for liquid plasma discharge in tangerine juice. The high voltage electrode is an anode (+).