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Inactivation kinetics of *Escherichia coli* in cranberry juice during multistage treatment by electric fields

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ABSTRACT

The inactivation of *Escherichia coli* inoculated in cranberry juice by processing with radio frequency electric fields was studied. *E. coli* ATCC 35218 was chosen among three non-pathogenic strains based on its ability to survive in low pH cranberry juice. Studies were conducted by measuring the survival population when changing the electric field strength between 2.2 and 13.2 kV cm^{-1}, number of treatment stages from 1 to 6 and flow rates between 13 and 25 L h^{-1} at moderate temperatures of 20, 30 and 40 °C. A minimum inactivation of 5-log reduction, as requested by the Food and Drug Administration (FDA), can be achieved by increasing the number of treatment stages, temperature or both. At 40 °C and 6 treatment stages, 6.57 $\pm$ 0.02 log CFU ml^{-1} reduction in the initial population of *E. coli* (ATCC 35218) was obtained. At a constant electric field, increasing the temperature produced higher microbial inactivation, consuming lower radio frequency energy input, than increasing the number of treatment stages. Furthermore, a primary model that accounts for the combined effect of time and electric field is proposed. The model represented the sigmoidal curve composed of shoulder, log-linear and tailing sections as observed when changing electric fields. A secondary model that accounts for the effect of temperature and flow rate on the primary model constants is also proposed. The combined primary and secondary models were found to fit the data well with a high coefficient of determination (R^2 = 0.965). The proposed model can be extended to kinetic models for pulsed electric fields.

1. Introduction

Fluid food processing by applying high intensity electric fields has been researched as an alternative to thermal pasteurization, such techniques seek to retain the key quality and nutritional parameters of products such as fruit juices while enabling microbiological safety (Trujillo & Geveke, 2014). Pulsed electric fields (PEF) and radio frequency electric fields (RFEF) can achieve microbiological safety at sub-pasteurization temperatures due to the membrane electroporation induced by high intensity electric fields (Geveke & Brunkhorst, 2004; Masood, Razaeimotlagh, Cullen, & Trujillo, 2017). PEF and RFEF can be distinguished by how the electric fields are generated and applied to food products. Sinusoidal electromagnetic waves are used in RFEF whereas pulses are used in PEF. When electric fields are applied to conductive liquids heat is generated due to joule heating. PEF controls heat generation mostly by pulsating while RFEF, which applies continues sinusoidal electric fields, controls it by increasing the flow rate, and/or cooling the liquid food before entering into the treatment chamber. Advantages of RFEF over PEF include a lower capital cost of RF generators, compared to PEF generators (Jeyamonkondan, Jayas, & Holley, 1999; Masood et al., 2017; Trujillo & Geveke, 2014), and the reduction of undesirable electrochemical reactions, due to the frequency on the kHz range compared to PEF frequency on the Hz range. Reyns, Diels, and Michiels (2004) stated that the application of PEF in grape juice resulted in the generation of mutagenic compounds due to the induced electrochemical reactions. RFEF may reduce the likelihood of these undesirable electrochemical reactions due to its higher frequency.

The effects of RFEF on microbial inactivation have been studied on liquid foods such as apple juice (Geveke & Brunkhorst, 2004), orange juice (Geveke, Brunkhorst, & Fan, 2007; Uemura & Isobe, 2003), apple cider (Geveke & Brunkhorst, 2008), and also on saline water (Masood et al., 2017). Microbial inactivation is mostly affected by factors such as electric field strength, treatment time, temperature, and frequency (Geveke et al., 2007; Geveke & Brunkhorst, 2008; Geveke, Gurtler, & Zhang, 2009; Geveke, Kozempel, Scullen, & Brunkhorst, 2002). Higher inactivation can be obtained by increasing the number of treatment stages as the approach increases the treatment time proportionally (Geveke & Brunkhorst, 2004). Also, food characteristics such as pH (Aronsson & Rönner, 2001) and conductivity (Vega-Mercado,

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Pothakamury, Chang, Barbosa-Cánovas, & Swanson, 1996) can affect electric field processing. Lower pH juices allow for an effective electric field treatment at milder processing conditions while high conductivity juices facilitate dielectric breakdown and pose problems to control joule heating (Gachovska, Subbiah, Thippareddi, Marx, & Williams, 2013). Furthermore, microbial species, size of the cells, the construction of the cell wall, and growth stage of the microorganisms affect inactivation (Aronsson & Rönner, 2001; Hülsheger, Potel, & Niemann, 1983).

Different treatment chamber designs such as parallel plate, colli-near, and coaxial have been proposed for RFEF and PEF (Huang & Wang, 2009; Trujillo & Geveke, 2014). Recently, Masood et al. (2017) proposed a novel Steinmetz design consisting of two perpendicular cylinders, one to accommodate the electrodes and the second the fluid. Computational modeling was utilized to optimise the treatment chamber design, including filleting the electrodes, to provide a homogeneous electric field. Masood et al. (2017) performed experimentations in saline water inoculated with *E. coli* (ATCC 25922) yielding a microbial reduction of 3.6 ± 0.3 log CFU ml^{-1} at electric field strength of 26.5 kV cm^{-1}, a treatment time of 900 µs, and outlet temperature of 45 °C.

Cranberries, belonging to the *Ericaceae* family of berries, are native fruits from the United States and Canada which produce a wide variety of products such as cranberry juice, concentrate, and sauce (Raz, Chazan, & Dan, 2004). Cranberries have a high antioxidant capacity due to their high phenolic compound (anthocyanins, flavonols, tannins, and phenolic acids) and ascorbic acid content (Skrovankova, Sumczynski, Mlcek, Jurikova, & Sochor, 2015). The bioactive compounds in cranberries have reported health benefits such as preventing cardiovascular diseases and inflammation disorders as well as lowering cancer risks (Skrovankova et al., 2015). Also, cranberry juice can lead to a possible mechanism to prevent urinary tract infections (UTI) (Raz et al., 2004). In industry, cranberry juice is processed thermally which may alter heat sensitive compounds. For instance, bioactive compounds become more susceptible when a combination of oxygen, heat, and light is present; therefore moderate processing conditions are suggested to maintain these compounds at high levels (Odriozola-Serrano, Soliva-Fortuny, Gimeno-Añó, & Martín-Belloso, 2008).

Predictive microbiology uses mathematical models to correlate the effects of intrinsic factors, such as pH and conductivity, and extrinsic factors, such as temperature and energy input, to predict microbial inactivation or growth (Gómez, García, Álvarez, Condón, & Raso, 2005). Microbial inactivation and survival curves due to thermal and non-thermal processing display various shapes that cannot always be represented with log linear models (Geeraerd, Valdramidis, & Van Impe, 2005). In PEF, microbial inactivation kinetics have been correlated with primary models such as first order kinetics, log-logistic and models derived from Weibull distribution (Gómez et al., 2005) for US processors. Although RFEF is similar to PEF, there is no previous RFEF inactivation kinetic model reported in the literature.

The purpose of this work is to firstly, investigate the effect of multiple stage RFEF treatments on microbial inactivation of *E. coli* in cranberry juice, aiming to achieve a 5-log reduction in microbial population as required by FDA (Gupta, Masterson, & Magee, 2005). Secondly, an inactivation model was proposed correlating the inactivation of *E. coli* in cranberry juice with electric field strength, treatment time, temperature, and flow rate.

2. Materials and methods

2.1. Cranberry juice

Commercial cranberry juice (Bickford's, Salisbury South, SA, Australia) containing 25% concentrate was purchased from a local store. Table 1 shows the following analytical characteristics of the juice: electrical conductivity, pH, and Brix, which were measured with a Five-Easy® conductivity meter (Mettler Toledo, Columbus, OH, USA), an Oakton pH meter model 700 (Oakton Instruments, Vernon Hills, IL USA), and an ATAGO pocket refractometer (Tokyo, Japan), respectively.

Table 1
Characteristics of commercial cranberry juice from 25% concentrate.

Characteristic	Quantity
Electric conductivity (S/m)	0.094
pH	2.49
Brix°	13.1°

2.2. RFEF system

A Steinmetz chamber, which was introduced by Masood et al. (2017) was used in this study. It was made from a Rexolite® 1422 block; two cylindrical channels with 2 mm diameter were drilled perpendicularly inside the block. The treatment zone was created at the junction of these cylinders (Masood, Diao, Cullen, Lee, & Trujillo, 2018). Two titanium cylindrical electrodes, of 2 mm diameter, were fitted through the channels leaving a 1 mm separation from each other. The edges of the electrodes were filleted to avoid the formation of high electric fields occurring at sharp corners. Liquid was pumped through the other perpendicular channel. The Steinmetz chamber design allows for a more homogeneous distribution of the electric field in the treatment zone. The RFEF rig, as described by Masood et al. (2017), consists of an AG-1024 T&C power RF generator with a constant frequency of 20 kHz, a Dyne impedance matching transformer, a P60115A high voltage probe, a Pearson 411C current probe, and a Tektronix TDS2004B oscilloscope. Cranberry juice was transported through the system via a Dura-10 peristaltic pump. A pulsation dampener was used in order to make the flow oscillations created by the pump unruffled. Two Madden SC004 heat exchangers were used to adjust the fluid's inlet temperature and to cool it down after RFEF treatment. Heat exchangers were driven by a LAUDA Variocool VC 2000 water circulator. A 4-channel OMEGA fibre optic thermometers system was used to monitor temperature at different points.

2.3. RFEF experimental design

Microbial inactivation experiments were conducted at a fixed frequency of 20 kHz and flow rates varying from 13 to 25 L h^{-1}. The treatment time, which is defined as the ratio of the treatment zone volume and the flow rate (residence time), varied from 540 to 3240 µs. Three temperature levels of 20, 30, and 40 °C and electric fields ranging from 2.2 to 13.2 kV cm^{-1}, estimated by computational simulations from a measured peak to peak voltage as explained by Masood et al. (2017), were chosen for this study. The RFEF rig allowed for connection of a maximum of two chambers in series with a cooling stage between them. Inlet temperatures were adjusted with a heat exchanger to 8.5, 16 and 25 ± 1 °C in order to reach outlet temperatures of 20, 30 and 40 °C respectively. For a single treatment stage, the cranberry juice was cooled down to 4 °C with a second heat exchanger. For 2 treatment stages, two chambers were connected in series. The outlet temperature of each chamber was adjusted to the same value of 20, 30 or 40 °C, which required adjusting the inlet temperature of both chambers with corresponding heat exchangers. Samples were collected by triplicate and placed in the fridge where they were cooled down to 4 °C. Because the rig only allowed for connection of two chambers in series, the effects of multiple treatment stages were studied by recycling the treated product, either once or twice providing 4 and 6 stage treatment respectively.

For the kinetic studies, the effect of the electric field was determined by performing experimental runs on two treatment stages, a constant flow rate of 25 L h^{-1} at three outlet temperatures of 20, 30 and 40 °C.