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Pulsed electric field for *Escherichia coli* inactivation in pumpkin juice and nectar

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ABSTRACT. In this study experiments were performed using a batch pulsed electric field (PEF) system at different electric field strengths with a voltage of 35 kV and pulse frequency of 3 Hz, at 20 and 40°C, with pulse width between 0.8 and 1.0 µs, to evaluate the inactivation of *Escherichia coli* in pumpkin juice and nectar. The performance of the PEF technology can vary as a function of several process parameters and the conditions and procedures applied. The physicochemical characteristics (pH, total soluble solids, electrical conductivity) of the pumpkin juice were also evaluated. The juice showed 5.01 ± 0.01 of pH, 10.70 ± 0.11 (mS cm^{-1}) of electric conductivity and 9.85 ± 0.07 of soluble solids while in nectar, these parameters were changed to 5.11 ± 0.01 of pH, 8.54 ± 0.21 of electric conductivity and 6.40 ± 0.12 of soluble solids. The use of a temperature of 40°C and pumpkin nectar (70:30, juice: distilled water) showed no difference in the bacterial reduction compared to 20°C and using 100% pumpkin juice, since in non-thermal processes it is better to use lower temperatures for less energy expenditure and less possibility of changes in raw material. The data showed that the PEF treatment reduced the microbial load moderately in all experiments, by a maximum of approximately 2.5 - 3 log cycles with 80 J g^{-1} of specific energy and above 26,000 V cm^{-1} of field strength.

Keywords: Non thermal technology; pumpkin; electric field strength; microorganism inactivation.

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Introduction

The maintenance of natural pigments, food safety and achievement of desirable texture and viscosity properties represent a great challenge in the food processing industry. Several factors are responsible for the degradation of food characteristics during the thermal processing, such as changes in the carotenes and *cis-trans* isomers, and in the oxidation and hydroxylation reactions (Dutta, Dutta, Raychaudhuri, & Chakraborty, 2006).

The Pulsed Electric Field (PEF) is considered an economically and environmentally sustainable technology for solid and liquid products, better than conventional heat treatment because it avoids or reduces changes in the sensory and physical properties of the food product while ensuring the microbiological safety of food. Some important aspects are: the generation of high electrical intensities, the chamber design which should provide uniform treatment to the product with a minimal increase in temperature, and appropriate arrangement of the electrodes in order to minimize the electrolysis effect (Toepfl, Heinz, & Knorr, 2007; Gerlach et al., 2008).

Pumpkin juice and puree are intermediate products which are thermally processed to produce jams, jellies, drinks and other products with considerable added value and which are of interest from the nutritional point of view (Provesi, Dias, & Amante, 2011).

Pumpkin processing studies are scarce and predominantly deal with thermal processing. In pumpkin puree and juice there is a risk of microbial contamination due to the nutrient content (carbohydrates, pigments, carotenoids, etc.) and physicochemical parameters (e.g. pH > 4.5). Some pathogenic strains of *Bacillus* and *Clostridium* spp., e.g., form heat resistant spores, surviving temperatures above 100°C for several minutes (Guinebretiere, Girardin, Dargaignaratz, Carlin, & Nguyen-The, 2003).

The elimination of microorganisms applying PEF is dependent on the electric field intensity and time of application. The use of electric pulses of short duration aims to minimize the Joule effect (heat) and thus reduce the undesirable effects of heat on food. The low power consumption of some pulse waveforms is very attractive for the food industry, due to the possibility of preserving foods with low energy (<10 J mL^{-1}) and

consequently low cost (Gerlach et al., 2008). Obtaining safe food implies the elimination of pathogenic microorganisms. As an example, the PEF processing conditions recommended for the inactivation of *E. coli* and other pathogens in blueberry juice are 25-35 kV cm^{-1} for 20-300 µs (Barba et al., 2012). Higher energy pulsing (> 10 kV cm^{-1}) causes permanent membrane disintegration, and therefore the mechanical destruction of the bacterial cells, through irreversible pore formation with breakdown, as in the pasteurization method applied to decontaminate solid and liquid food products (Heinz, Toepfl, & Knorr, 2003; Janositz, Noack, & Knorr, 2011).

The first commercial application of PEF for fruit juice preservation has been reported in the United States, processing 200 L h^{-1} of apple, strawberry, and other juices and showing its potential for an industrial exploitation (Clark, 2006). The PEF technology system is associated with the use of minimum energy and greater energy efficiency than thermal processing. For example, in apple juice treatment, energy utilized in PEF is 90% less than the amount of energy used in high temperature and short time processing methods (HTST). The pulsed electric field technology is mainly used for microbial inactivation, although it can be used for the extraction of biologically active compounds. In orange juice, studies have confirmed that PEF-treated orange juice retains all the physical properties, along with a 97.5% of vitamin C (Clark, 2006; Kumar, Patel, & Kumar, 2014). With PEF technology for fruit juice processing, the contaminating microorganisms are inactivated after being permeabilized.

The main objective of this study was to determine the inactivation behavior of the pulsed electric field technology applied to pumpkin juice and nectar inoculated with *E. coli* in varying specific conditions, such as the pulse width, electric field strength, specific energy intake and juice concentration.

Method

Raw material

Butternut pumpkins (*Cucurbita moschata* butternut) in consumption maturation conditions stage were obtained from a local supermarket in Berlin (Edeka Zentrale AG & Co KG – Berlin, Germany).

Production of pumpkin juice

The pumpkins were cleaned with a neutral detergent and washed to remove surface residues. The samples were cut into slices of about 5 cm^2 and then boiled for 5 min. in steam from an autoclave (Varioklav Steam Sterilizer 500E, H+P Labortechnik GmbH, Oberschleiβheim, Germany). The sections were cooled and peeled and the seeds were removed before. The puree was prepared in a kitchen blender (Braun K3000, Kronberg, Germany) and homogenized using an Ultra Turrax disperser (Ultra-Turrax, IKA, Staufen, Germany). The puree produced were placed in glass bottles with screw caps, and sterilized by autoclaving at 121°C for 15 min. This was followed by physicochemical analysis and sample preparation for PEF tests. The juice was produced by centrifugation (Eppendorf, Centrifuge 5804 R - Hamburg, Germany) for 15 min. at 4,500 g to obtain the pumpkin juice and facilitate the subsequent analysis steps. The juice samples were then autoclaved at 121°C for 5 min. Under some of the conditions tested, a nectar sample was made diluting 70% of juice in 30% of distilled water (70:30 juice:distilled water) in order to decrease the electrical conductivity of the sample increase performance of PEF.

Characterization of the pumpkin juice

The centrifuged and sterilized pumpkin juice samples were evaluated for total soluble solids content (°Brix) (RFM 80 Digital Refractometer, Research Equipment Engineering, Hannover, Germany), pH value (Digital pH meter Knick, Berlin, Germany) and electrical conductivity (WTW Conductivity Meter – Weilheim Cond 3110, Berlin, Germany), all in triplicate.

Pulsed electric field (PEF) treatment

The PEF treatment was performed in a batch treatment system, which consisted of a high voltage generator (HCK 800M-20000, fug, Rosenheim, Germany), a capacitor bank with 1.91 nF (Ceramite Y5U 6800Z, Behlke, Kronberg, Germany), a HTS 160-500 SCR switching unit (Behlke, Kronberg, Germany), a discharge circuit with a 2.5 Ohm protective resistor (Stervice, France) and a treatment chamber with a parallel electrode configuration. Pulses of 0.8 - 1 µs (determined at 37 % of peak voltage) were applied at a frequency of 3 Hz, at

different electric field strengths (with a maximum of 35 kV cm^{-1}) and treatment times and at 20 and 40°C. The distance between electrodes in the cuvette was 0.2 cm. A detailed description of the treatment chamber and the numerical simulation procedure can be found in Toepfl, Heinz, & Knorr (2007).The temperatures are adjusted by sensor on the treatment chamber which regulates the internal temperature of the chamber as configured, described by Toepfl, Heinz, & Knorr (2007). After leaving the PEF treatment chamber, the sample was placed in iced water.

The calculated specific energy input ($W_{specific\ calculated}$) was considered as a function of the applied voltage (V), the capacity (C) the pulse number (n) and the mass of sample (m) according to Equation 1.

$$W_{specific\ calculated} = \frac{0.5 \cdot V^2 \cdot C \cdot n}{m} \quad (1)$$

Microbiology analysis

Working cultures of *E. coli* K12DH5a (Hygiene Institute Hamburg, Germany) were prepared from stock cultures 24h before each experiment by inoculating 50 mL of Standard I Nutrient Broth (Oxoid, Basingstoke, UK) which was used as culture medium as explained in Toepfl, Heinz, & Knorr (2007). The methodology of plating on nutrient agar was used to determine the total counts (CFU mL^{-1}). All microbial analysis was carried out in duplicate. Cells were incubated for 24h at 37°C to obtain cultures in the stationary growth phase. The counts for microbial colonies grown in standard agar at 37°C for 24 h were expressed as CFU mL^{-1}. The levels of inactivation, log N N_0^{-1}, were evaluated for each test. The initial count number was 1.86 x 10^8 CFU mL^{-1} in control sample.

PEF tests

The operating conditions for the application of the PEF treatment to the pumpkin juice with exponential decay waveform with field strength ranging from 13,000 to 34,000 V cm^{-1}, was: temperature of 20 and 40°C, electrode distance of 0.2 cm, mass samples of 0.40 to 0.46 g, capacity of 1.91.10^{-08} F, frequency of 3s^{-1}, and time (τ) of 0.8 to 1.0 µs. The tests were performed in duplicate and data for each point are expressed as mean ± standard deviation.

Samples of pumpkin juice (centrifuged) with an inoculum of *E. coli* K12DH5a (Hygiene Institute Hamburg, Germany) in acrylic cuvettes with 2 electrodes were passed through the electric current. Approximately 450 µL of sample was placed in the cuvettes for each test. The samples were placed in an ice bath immediately after each treatment. Samples of 70 % and 100 % pumpkin juice were treated and the electrical conductivity was evaluated for each case in sequence.

Results and discussion

The centrifuged juice samples had a pH of 5.01 ± 0.01, electric conductivity of 10.70 ± 0.11 (mS cm^{-1}) and soluble solids (°Brix) of 9.85 ± 0.07. For the pumpkin nectar (70:30, juice:distilled water) after centrifugation the pH was 5.11 ± 0.06, electric conductivity was 8.54 ± 0.21 (mS cm^{-1}) and soluble solids (°Brix) was 6.40 ± 0.12.

The soluble solids of pumpkin juice of this work are aligned with Gliemmo, Latorre, Gerschenson, and Campos (2009) who found 10.5 ± 0.5 °Brix for pumpkin puree, Ahmed, Al-Foudari, Al-Salman, and Almusallam (2014) who found 10.1 ± 0.2 °Brix, and Nawirska-Olszanska, Biesiada, Sokol-Letowska, and Kucharska (2014) who showed 11.9 ± 0.02. Dutta et al. (2006) found 7.2 ± 0.3 of soluble solids also for pumpkin puree. Conti, Villari, Amico, and Caruso (2015) evaluated the quality of pumpkin (*C. moschata* duch.) over 240 days and found that on the first day, the soluble solids content was 10.7 ° Brix, while at the end, it decreased to 6.0 ° Brix, according to the authors, expected results.

Pulsed electric field is more effective for inhibiting microbial in food with lower electrical conductivities since it increases the difference in electrical conductivity between the medium and the microbial cytoplasmic membrane and weakens the structure of the membrane of microorganisms due to an increase in the flow of ionic substances through the membrane during PEF-Treatment. This increases the difference in electrical conductivity. A lower electrical conductivity caused the largest increase intensity of the electric field and thus resulted in more efficient microbial inactivation. This is one of the reasons why this work chose to experiment with the centrifuged sample diluted (70:30) with distilled water (nectar). Furthermore, it is important that high electrical conductivity values of a treatment medium decreases inhibition of microorganisms at a

constant energy input. (Vega-Mercado, Pothakamury, Chang, Barbosa-Canovas, & Swanson, 1996; Raso, Calderón, Góngora, Barbosa-Cánovas, & Swanson, 1998; Jin, Guo, & Zhang, 2015).

Figure 1 (A) shows the decrease of *E. coli* (log N N_0^{-1}) in 100% pumpkin juice, as a function of specific energy intake - $W_{specific\ calculated}$ (J g^{-1}), following the PEF treatment applied with a field strength of 14,000 to 30,000 V cm^{-1} and energy of 0 to 120 J g^{-1}. Increasing field strength resulted in a better inactivation rate, as shown in Figure 1 (A). Under these conditions, a reduction of over 2 log cycles can be obtained with a specific energy of 120 J g^{-1} and field strength above 22,000 V cm^{-1}.

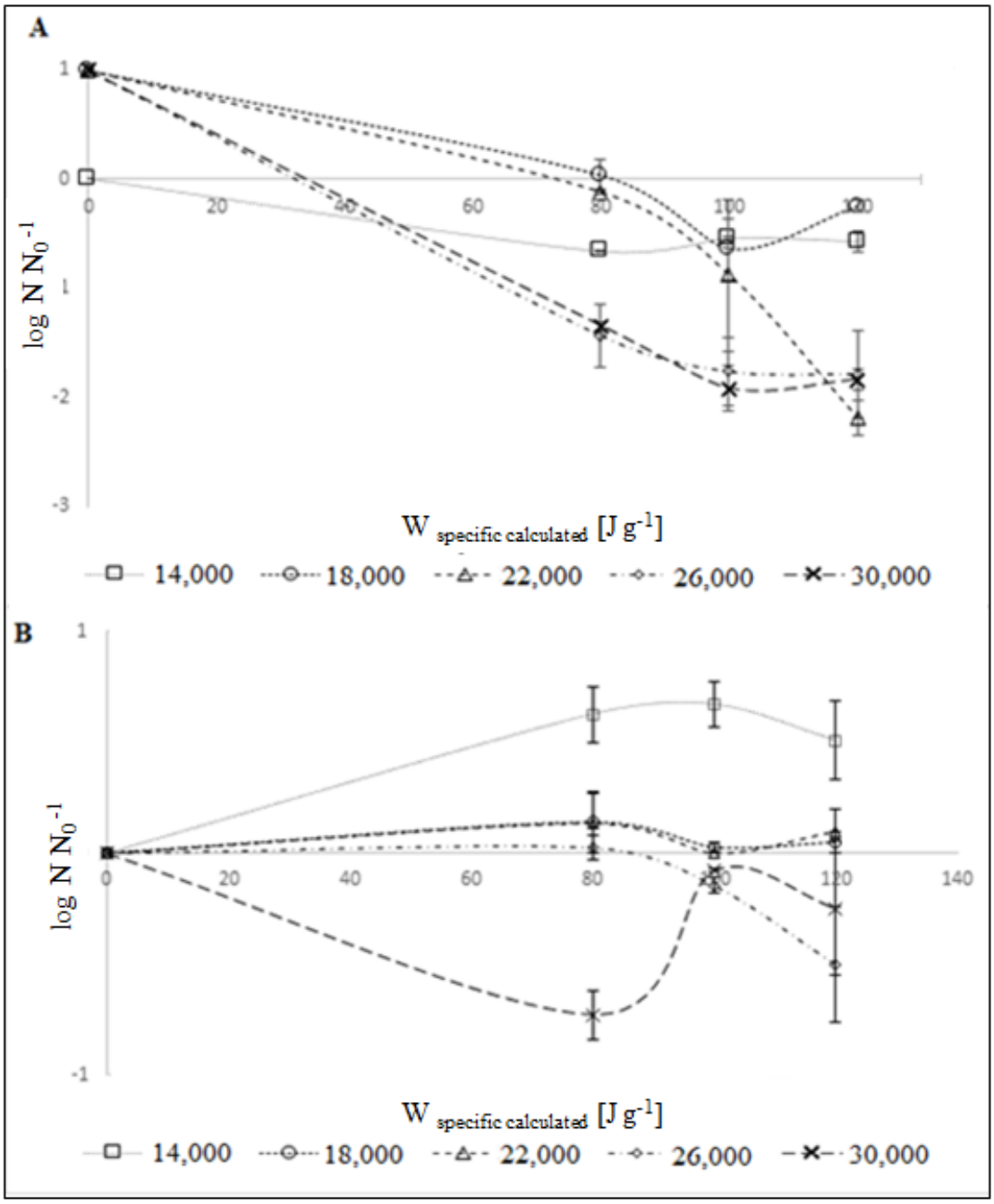

Figure 1. Decrease of *E. coli* (log N N_0^{-1}) in 100% pumpkin juice as a function of specific energy - $W_{specific\ calculated}$ (J g^{-1}) following PEF treatment with field strength (A) 14,000 (□), 18,000 (○), 22,000 (Δ), 26,000 (◊) and 30,000 (×) (V cm^{-1}). (B) 14,000 (□), 18,000 (○), 22,000 (Δ), 26,000 (◊) and 30,000 (×) (V cm^{-1}), using pumpkin nectar (70:30 juice:distilled water). Results are means based on data from two experiments at 20°C and standard deviations are shown by error bars.

In Figure 1 (B), in pumpkin nectar (70:30) with a field strength of 13,000 to 30,000 V cm^{-1} and energy of 0 to 120 J g^{-1}, it can be observed that a reduction of around 0.80 log cycles can be achieved with a specific energy of 80 J g^{-1} using 30,000 V cm^{-1} of field strength. Under these conditions the pumpkin

nectar (70:30) was used in order to decrease the electrical conductivity of the medium which, in this case, was 7.31 mS cm^{-1}. The decrease in conductivity achieved through the dilution in distilled water may also have contributed to the difference in the results. The conductivity of liquid food shows a linear relation with temperature. However, even when the temperature is constant during the electrical discharge, the temperature rises depending on the specific energy, leading to changes in the medium conductivity and electric field distribution (Toepfl, Heinz, & Knorr, 2007). It was observed that when field strength of 13,000 V cm^{-1} was used, there was no effect on the inactivation of *E. coli*. According to Toepfl, Heinz, and Knorr (2007), large cells are more susceptible to electric fields. For small cells, such as Gram-negative bacteria like *E. coli*, at least 15,000 V cm^{-1} is predicted to be sufficient to lethally damage most of the organisms in a bacterial suspension exposed to PEF. The values for the inactivation were lower and the behavior differed from that shown in Figure 1 (A).

Some factors, such as the agglomeration of cells or insulating particles like fat globules, can strongly reduce the lethality of the PEF process, as the required membrane potential for electroporation is not achieved (Toepfl, Heinz, & Knorr, 2007).

The degree of microbial inactivation by PEF is strongly dependent on the process parameters such as field strength, specific energy and treatment temperature as well as the properties of the food matrix. Pulse wave shape, treatment chamber design and the precipitation of biological macromolecules by solubilized metal ions of the electrode, also play a role. The condition of the microorganism is another possible determinant factor in relation to the effect of the PEF treatment (Heinz, Toepfl, & Knorr, 2003).

Sale and Hamilton (1968) were among the first authors to report microbial inactivation by PEF and they applied 10 pulses of varying electric fields to several varieties of microorganisms, including *E. coli*. The reductions of one to two log cycles were observed, depending on some variables. Using field strengths of up to 30 kV cm^{-1}, the process was shown to be effective for the inactivation of approximately 4 log cycles (except spores) in liquid foods. Microbial death raised with increasing electric field strength from 5 to 25 kV cm^{-1} and weaker effect was observed when the pulse width was increased from 2 to 20 µs, although not all microorganisms responded in the same way to the electric field (Sale & Hamilton, 1967).

Another significant factor in PEF is the treatment time (t), which is the length of time the liquid is exposed to it. This variable is expressed as the product of n (pulse number) and τ (pulse width). Generally, τ is fixed, since the duration is set by both the system circuit and the resistivity of the material during treatment. The n is thus increased to obtain longer treatment times and the effect of its variation is dependent on other factors. Previous studies have indicated that increasing n raises the extent of cellular injury (Hodgins, Mittal, & Griffiths, 2002).

According to Mittal, and Griffiths (2005), the initial pulses may cause catastrophic failure of the cell due to pore formation or the loss of the pH gradient and subsequent pulses may produce additional cycles of stress on the membranes. However, they classified n as a secondary and limited effect, due to the fact that the highest death rates do not correlate as well with n as they do with field strength. Pulse frequency also plays a small role in determining the lethality of the treatment.

As shown in Figure 2, a reduction of about 2.5 log cycles can be obtained with a specific energy of 80 – 100 J g^{-1} and between 26,000 and 30,000 V cm^{-1} of field strength. In this test the volume was slightly above those used previously (0.45 g) to avoid arcing effect. The pulse width (τ) was increased to 1 µs due to a decreased electrical conductivity and the process was applied to pumpkin nectar (70:30). A relatively high microbial reduction was only possible up to 26,000 V cm^{-1}. These results are similar to those found in Figure 1 (A) obtained by increasing the pulse width. The most significant microbial influence factors on the PEF-treatment are cell size and gram behavior (Schottroff et al., 2019), but other parameters may have influenced the increase in microorganism count above 30,000 V cm^{-1} of field strength as reported in this work. Microbial resistance by PEF has been reported to depend on many factors, including process and product parameters, microbial characteristics etc. (Wang et al., 2018). Also, stress adapted microorganism is likely to become less sensitive to subsequent applications of the same stress. Zhao, Yang, Shen, Zhang, and Chen (2013) reported that the PEF technology does have some limitations, for instance, vegetative cells like *E. coli* strains who are resistant to PEF under certain conditions and can tolerate the currently applied PEF treatment.

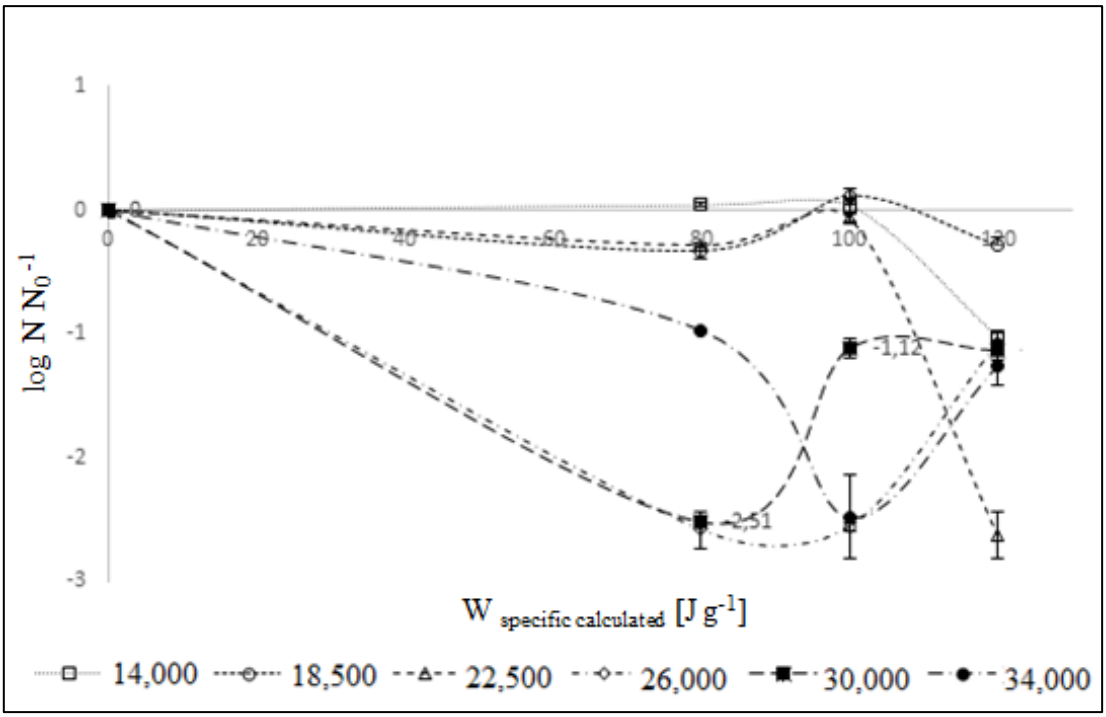

Figure 2. Decrease of *E. coli* (log N N_0^{-1}) as a function of specific energy - $W_{specific\ calculated}$ (J g^{-1}) following PEF treatment with field strength 14,000 (□), 18,500 (○), 22,500 (δ), 26,000 (◊), 30,000 (■) and 34,000 (●) (V cm^{-1}), using pumpkin nectar (70:30 juice:filtered distilled water). In this test τ was 1 μs. Results are means based on data obtained from the two experiments at 20°C and standard deviations are shown by error bars.

Figure 3 shows the results obtained for 100 % pumpkin juice. Under these conditions, a reduction of more than 2 log cycles could be obtained above 26,000 V cm^{-1}. Thus, these conditions were less efficient than those applied to obtain the results shown in Figure 2, with pumpkin nectar, and similar to the behavior reported in Figure 1 (B). In contrast, as it can be seen in Figure 1 (A), at below 26,000 V cm^{-1} the behavior for the three treatments differed considerably, even under similar conditions.

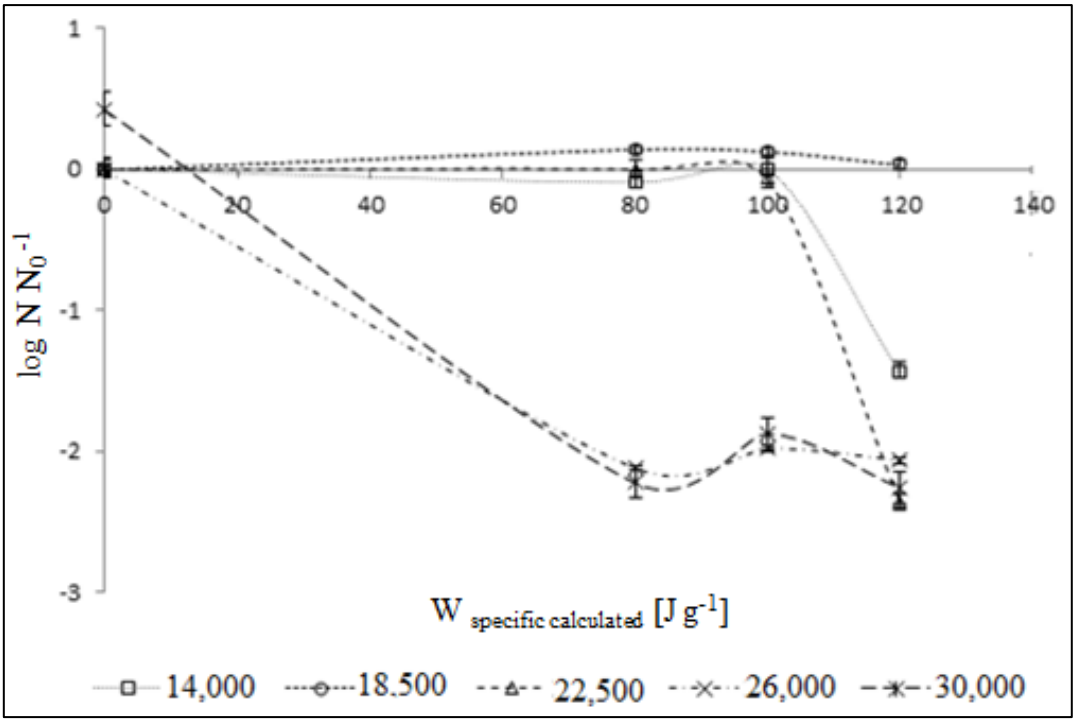

Figure 3. Decrease of *E. coli* (log N N_0^{-1}) as a function of specific energy - $W_{specific\ calculated}$ (J g^{-1}) following PEF treatment with field strength 14,000 (□), 18,500 (○), 22,500 (Δ), 26,000 (×) and 30,000 (ж) (v cm^{-1}), using 100 % pumpkin juice. Results are means based on data obtained from the two experiments at 20°C and standard deviations are shown by error bars.

Figure 4 shows the results for the samples analyzed with a difference in temperature (20 and 40ºC), to verify the influence of this parameter on batch PEF treatments of the pumpkin liquids. At 20°C it was possible to achieve a relatively high microbial reduction, reaching around 2.5 log cycles with a specific energy of 80 J g^{-1} and field strength of 26,000 V cm^{-1} and higher. However, at 40°C the reduction was slightly above 1.5 log cycles, applying the same specific energy and field strength. This result does not match with that expected, since an increase in temperature is an important factor in relation to microbial reduction. Moreover, the optimum temperature for the growth of *E. coli* is 37°C, and thus this behavior was expected. The total soluble solids content of the 100 % pumpkin juice was around 10 %. The principal compounds in these solids were carbohydrates of varying molecular weight, suggesting that insufficient microbial reduction may be related to higher availability of nutrients in pumpkin. In addition, it may have been a protective effect on *E. coli* at 40ºC and higher specific energy at this temperature. This is not a high temperature, but it can contribute to the evolving of the *E. coli* cells in this process.

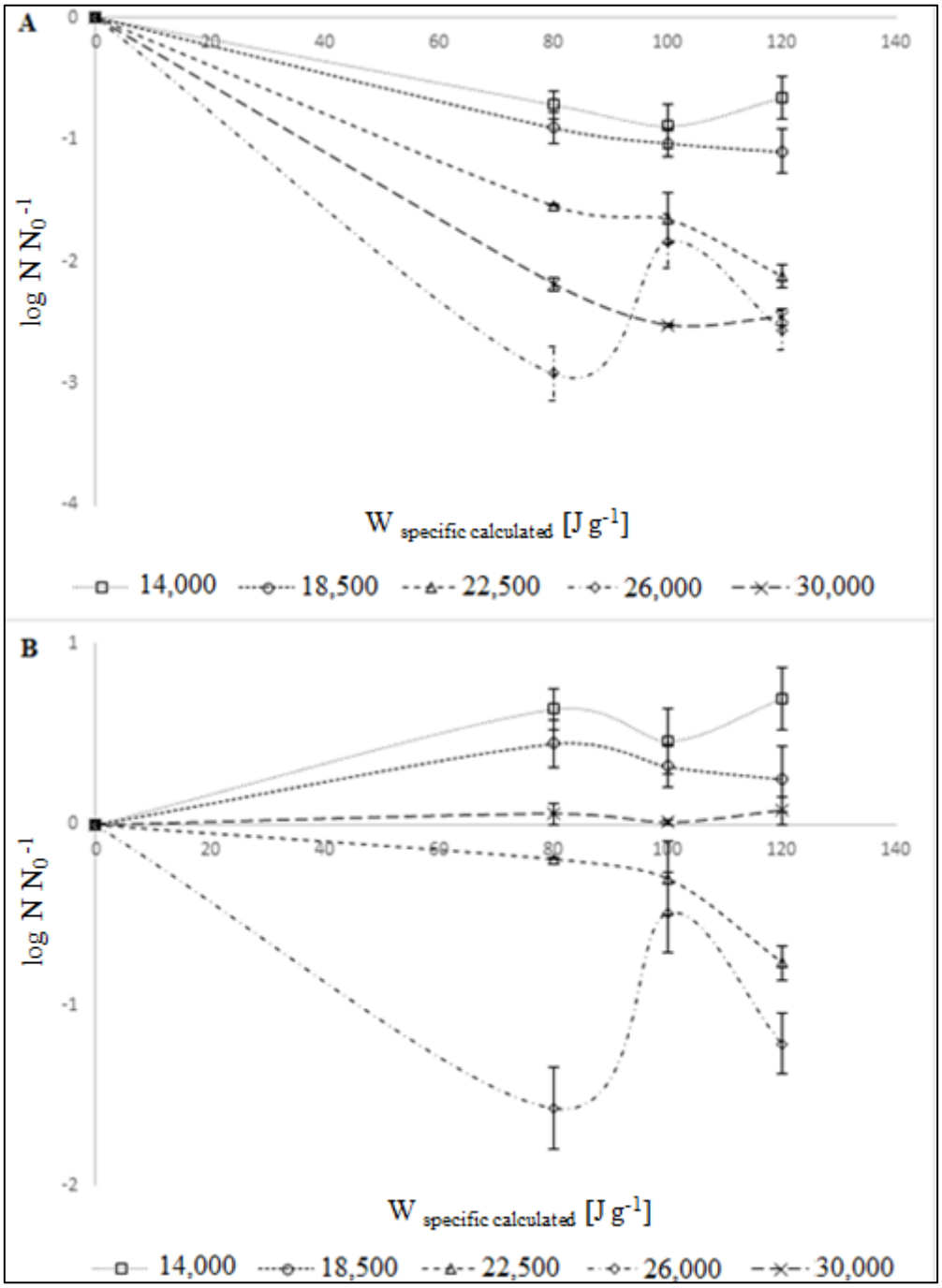

Figure 4. Decrease of *E. coli* (log N N_0^{-1}) as a function of specific energy -$W_{specific\ calculated}$ (J g^{-1}) following PEF treatment with field strength 14,000 (□), 18,500 (○), 22,500 (Δ), 26,000 (◊) and 30,000 (×) (V cm^{-1}), using 100 % pumpkin juice. Results are means based on data obtained from the two experiments at 20°C (A) and 40ºC (B) and standard deviations are shown by error bars.

There are few studies demonstrating that resistance of food-borne pathogenic bacteria to PEF is also influenced by environmental stresses, including growth temperature, pH, Aw and medium composition. Theories have been put forward to explain the membrane-disrupting mechanisms suggesting that PEF causes reorientation and compression of the lipid bilayer, to increase permeability which eventually form reversible and irreversible pores in cellular membrane. However, the viscoelastic property of membrane lipid bilayer against disruptive forces of PEF can change because microbes can adjust their membrane fatty acid composition to maintain proper cell membrane functions and thus affect the balance to make the cells more or less sensitive to the PEF treatment (Sitzmann, Vorobiev, & Lebovka, 2016; Wang et al., 2018; Wang, Wen, Zeng, Han, & Brennan, 2019).

Mosqueda-Melgar, Elez-Martínez, Raybaudi-Massilia, and Martin-Belloso (2008) stated that applied electric field intensities ranges from 20 to 80 kV cm^{-1} depending on the media characteristics, operating parameters and target microorganisms. Schottroff et al. (2019) concluded that at a constant inlet temperature, pH is the most important medium property affecting the inactivation of microorganisms by PEF. This can be explained by the fact that the permeabilization of the membrane by electric field may allow the uptake of acid molecules into the cytoplasm and consequently cause death of the cells. Even the contribution of a low pH in a multi-hurdle inactivation approach involving PEF seems to be species-dependent, still not fully elucidated yet (Saldaña et al., 2010; Arroyo & Lyng, 2017). Saldaña et al. (2009) and Jaeger, Schulz, Karapetkov, and Knorr (2009) also showed that external processing conditions, such as electrical field strength and pH of the treatment medium, played an important role in the occurrence of dead cells or sublethal injuries.

The effect of acid adaptation on microbial PEF resistance has been scarcely studied and the results published are contradictory. A large type of microorganisms can survive or adapt to the acidic nature of fruit juices or nectars (acid-adapted), for instance *E. coli* adapted to acid conditions caused by cross-protection response that resulted in an increased resistance against PEF (Evrendilek & Zhang, 2003). Abeysundara, Dhowlaghar, and Nannapaneni (2019) reported that stressed bacteria probably induce a cross-resistance response that probably change the tolerance to a diverse stress as it may occur with *S. aureus* and *E. coli*.

García et al. (2005) reported that a decrease in treatment medium pH was associated with an increase in PEF resistance. This has not been observed before for any Gram-positive microorganisms and was believed to be a particular feature of some Gram-negative cells. Acid adaptation might lead to an increase in microbial PEF resistance in some conditions that are more likely to occur in the food industry, i.e. when adaptation and inactivation occur in the same matrix, for example in an acid juice. In fact, more investigations are required in order to elucidate if this also happens for other microorganisms of interest for the food industry, including foodborne pathogens (Evrendilek & Zhang, 2003).

Somolinos, García, Mañas, Codón, and Pagán (2010) studied the survival of *E. coli* in orange juice at different pH values in the PEF treatment (20 kV cm^{-1}) and stated that inactivation is higher at pH 7.0 than at pH 4.0. The results showed that the protective effects on *E. coli* cell was mainly due to the presence of organic acids at low pH, and the protective effects of citric acid depend on its concentration; specifically, the sublethal injury was not detectable at pH 5.0 – 7.0 and reached 95% at pH 4.0. These studies indicate that the PEF treatment of acid food may be of particular concern given the potential survival of the cells at low pH. These same authors studied the resistance of *E. coli* to the PEF treatment and found that more than 99% of the sensitive strain survived after the treatment (25 kV cm^{-1}, 50 pulses, exponential waveform, 1 Hz).

A PEF treatment with high energy input at low temperatures results in high operating costs. By using a combination of PEF treatment and heat application, the energy consumption and the maximum temperature can be reduced. In comparison with only heat treatment, a given level of inactivation can be obtained at lower temperatures, resulting in a lower thermal load and this should result in a greater conservation of quality and the fresh-like character of the juice (Heinz, Toepfl, & Knorr, 2003). Timmermans et al. (2019) and Moonesan and Jayaram et al. (2013) concluded that at constant electric field strength, longer pulse width was more effective than a short pulse width on inactivation of microorganisms.

Mendes-Oliveira, Jin, and Campanella (2020) states that specific energy of 60 to 120 J mL^{-1} were necessary to achieve a 1 log cycle reduction of target pathogens, which agrees with range of 1 to 100 J mL^{-1} discussed by Schoenbach, Joshi, Stark, Dobbs, and Beebe (2000). The PEF-technology is different from other nonthermal processing, as so many parameters are involved in a PEF-operation, system and design, since the complexities of PEF-treatment are a challenge for potential users of PEF processing (Hodgins, Mittal, & Griffiths, 2002).

Mok, Pyatkovskyy, Yousef, and Sastry (2019) states that the evaluation of the combined effect of different nonthermal treatments (shear stress + moderate electric field) can disrupt the outer membrane of *E. coli* K12 cell at sublethal temperature (40 – 50°C) in apple juice.

Pakhomova et al. (2012) suggested that cells treated by PEF induce an oxidation stress response in the cells that could increase their resistance to PEF. This oxidation stress results in a feedback that increases the production of glutathione and superoxide dismutase in order to reduce the oxidation stress, resulting in protection against the injury and the recovery of the cells.

Electric field strength in excess of the critical transmembrane potential is required, and this is dependent on the properties of the food matrix and its contents. Electrically insulating gas bubbles which may be produced at the electrode by electrolysis can cause a similar weakening of the effect of the PEF process, which may explain some behaviors when the electric field was higher but not enough to inactivate greater number of microorganisms (Toepfl, Heinz, & Knorr, 2007).

Also, the initial numbers of *E. coli* may have affected the results. When the initial count is higher, it could be attributed to the clumping of cells as a result of the increase of hydrophobicity during the process. The rate of formation of clumps was proportional to the initial numbers. In addition, some studies have shown that *E. coli* is able to release some protective substances when in high density by *quorum-sensing,* which could have occurred in this work including what was shown in Figure 2 after 26,000 kV cm^{-1} of electric field (Bai & Rai, 2011).

Microbial inactivation could perhaps have been enhanced by higher dilutions of the juice in order to reduce electrical conductivity. It should also be emphasized that there are differences in systems in batch, as shown in this work, with continuous systems, as reported in most references in this study, mainly by the isothermal conditions the system batch can keep, unlike the continuous system.

Studies in equipment at least on a pilot plant scale are necessary to confirm that the estimated process parameters from laboratory studies are consistent with the results obtained in plant. We believe that this study will provide valuable information for PEF researchers as well as the juice industry to understand these parameters for future scale-up and successful industrial application of the PEF technology.

Future studies are necessary, aiming at adjustments in the process, as well as the determination of the effects besides the microbial inactivation, on the carotenoids of the juice and nectar pumpkin.

Conclusion

A wide range of process parameters are applied in the batch processing. However, the properties of the suspending medium, process temperature and microbiological conditions are sometimes not reported in the literature. Based on previously reported studies, the microbial reduction rate was found to range from a moderate 1 – 3 log cycles to a highly significant inactivation of 6 – 9 log cycles. The efficacy of the treatment is a function of the various process parameters, conditions and procedures as it can be seen in this work. In this case, the PEF treatment reduced *E. coli* number moderately in all experiments, by a maximum of approximately 2.5 - 3 log cycles with 80 J g^{-1} of specific energy and above 26,000 V cm^{-1} of field strength.

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