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Identification of equivalent processing conditions for pasteurization of strawberry juice by high pressure, ultrasound, and pulsed electric fields processing

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ABSTRACT

The objective of this study was to evaluate the effectiveness of high pressure processing (HPP), ultrasound (US) and pulsed electric fields (PEF) for the pasteurization of strawberry juice (SJ). Acid-adapted *Escherichia coli* was used to inoculate SJ prior to treatment with HPP, US, and PEF. HPP was applied at several pressures (200–400 MPa) up to 2 min while US (120 μm, 24 kHz) was conducted at 25, 40, and 55 °C up to 10 min in continuous pulsing mode. In order to avoid excessive use of SJ, PEF was performed using a model solution (MS) basically composed of citric acid (8 g/L), fructose (35 g/L), glucose (35 g/L), Na_2HPO_4 (0.2 M) and NaCl (5%) to simulate the SJ electrical conductivity, pH, and total soluble solid (TSS). A face-centered composite design was conducted for PEF processing at different electric field intensities (EFI) (25–35 kV/cm) and treatment times (5–27 μs). Processing conditions were selected that resulted in 5-log CFU/mL inactivation of *E. coli*. HPP at 300 MPa for 1 min, and US at 55 °C (thermosonication) for 3 min reduced *E. coli* in SJ by 5.75 ± 0.52 and 5.69 ± 0.61 log CFU/mL, respectively. PEF treatment at 35 kV/cm, 27 μs treatment time, 350 mL/min flow rate, and 2 μs pulse width in monopolar mode resulted in 5.53 ± 0.00 log reduction of *E. coli* in MS. Likewise, *E. coli* population in SJ was also reduced by 5.16 ± 0.15 log after applying the same PEF conditions to SJ. No *E. coli* was detected in SJ subjected to conventional thermal pasteurization at 72 °C for 15 s. All technologies reduced the natural microbiota below 2 log CFU/mL in terms of the total aerobic bacteria and yeast-mold counts. Thus, this study identified the equivalent conditions for the SJ pasteurization by three nonthermal processing technologies.

Industrial relevance: Consumers have an increasing interest towards fresh-like food products with desirable nutritional and sensorial attributes. High pressure, ultrasound and pulsed electric field are three relevant novel nonthermal technologies as alternatives to conventional thermal treatments. This study identified the processing conditions of these three nonthermal technologies for the pasteurization of strawberry juice based on equivalent inactivation of acid-adapted *E. coli*. From an industrial point of view, the established processing conditions are useful references for the development of novel berry juices. In addition to microbiological safety, this study on equivalent processing allows direct efficacy and quality comparisons of a given juice pasteurized by the three nonthermal technologies under consideration.

1. Introduction

Consumption of fresh fruits and their products has been related to the reduced incidences of several degenerative diseases. Strawberry fruit has also been reported to promote human health by detoxifying free radicals, participating in the antioxidant defense mechanism, and preventing DNA damage (Giampieri et al., 2015). Strawberry juice (SJ) is one of the products to benefit from the health-promoting properties of strawberries. However, pathogenic outbreaks by *Escherichia coli* O157:H7 and *Salmonella* spp. are a serious concern for the microbial safety of berry juices despite their relatively low pH (3–4.5) (Duan & Zhao, 2009).

Since several outbreaks have been associated with fruit juices (Raybaudi-Massilia, Mosqueda-Melgar, Soliva-Fortuny, & Martin-

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Belloso, 2009), the Food and Drug Administration (FDA) requires at least 5-log reduction of the microorganism of concern to accept processing conditions (FDA, 2001). Accordingly, the pasteurization term was re-defined by the USDA National Advisory Committee on Microbiological Criteria for Foods (NACMCF, 2006). Although thermal pasteurization has been widely applied to provide microbial safety and extended shelf-life for fruit juices, its undesirable effect on nutritional and organoleptic attributes led scientists to explore alternative preservation methods. Therefore, mild pasteurization and minimal processing have received considerable attention due to the growing consumer awareness towards fresh-like, nutritive, and healthy products (Vervoort et al., 2011). Lately, nonthermal processing technologies including high pressure processing (HPP), ultrasonication (US), and pulsed electric fields (PEF) have been increasingly applied for fruit juice preservation.

HPP utilizes intense pressures rather than heat to inactivate spoilage microorganisms and harmful pathogens (Balasubramaniam, Farkas, & Turek, 2008). Highly homogenous products can be obtained due to isostatic pressure transmission independent of size, shape, and food composition (Deliza, Rosenthal, Abadio, Silva, & Castillo, 2005). Foods are processed in batch or semi-continuous systems under pressures ranging from 50 to 1000 MPa at low or mild process temperature, while the time ranges from seconds to 20 min. Thereby, HPP increases the shelf-life of the product by maintaining its physicochemical and nutritional characteristics (Balasubramaniam et al., 2008; Tokusoglu, 2016). Ultrasound refers to sound waves beyond the audible frequency range (in general, > 20 kHz). As ultrasound travels through the liquid medium, the interaction among the ultrasonic waves, liquid, and dissolved gas leads to the generation and collapse of micro-bubbles that further result in localized temperatures of up to 4000 K and pressures of up to 1000 atm (Rastogi, 2011). This phenomenon is called cavitation (Leighton, 1995) which enables microbial inactivation by disrupting the cell membrane (Bermúdez-Aguirre & Barbosa Cánovas, 2011). PEF processing applies very short electric pulses (ms or μs) of high electric field strengths (kV/cm) and moderate temperatures (Martín-Belloso & Elez-Martínez, 2005) to destroy the functionality of the cell membrane due to electroporation (Deeth, Datta, Ross, & Dam, 2007). Formation of irreversible pores causes disintegration of the membrane and loss of cell viability when the applied electric field is higher than the critical value (Saulis, 2010).

Many studies related to the application of HPP, US, and PEF for different types of fruit juices by either individual or combined treatments can be found in the literature. However, comparison of the effects of different processes on microbial safety, quality, and shelf-life of food products should be conducted under equivalent processing conditions that provide an equivalent effect (*i.e.,* equivalent degree of microbial inactivation) (Vervoort et al., 2011). Therefore, the purpose of the present work was to identify HP, US, and PEF processing conditions for pasteurization of SJ resulting in equivalent degrees of microbial inactivation that satisfy the FDA's 5-log reduction requirement. The objectives of this research were (i) to determine the target microorganism for SJ by comparing the resistance of different nonpathogenic surrogates to the acidic environment; (ii) to investigate the inactivation efficiency of HPP, US, and PEF on the target microorganism in multiple processing conditions; and (iii) to evaluate the impact of the selected equivalent processing conditions on the SJ natural flora.

2. Materials and methods

2.1. Preparation of strawberry juice and model solution

Strawberries (*Fragaria × ananassa*) were purchased from a local grocery store (Pullman, WA, USA) at their commercial maturity. Fruits stored at −30 °C prior to use were defrosted overnight at ambient temperature in the dark. Then, the green parts were discarded and the whole fruits were directly homogenized using a blender (Model K, Regal Ware, Inc., USA). To remove the suspended particles, centrifugation (Beckman J2 HS centrifuge, GMI, MIC Group, Inc., MN, USA) at 5111.5 g and 4 °C for 5 min using a Fiberlite F14 6 × 250 rotor (Piramoon Technologies, Inc., USA) and subsequent filtration through double-layer cheese cloth were performed.

A model solution (MS) was developed to avoid use of an excessive amount of SJ for PEF processing. The MS was composed of citric acid (8 g/L), fructose (35 g/L), and glucose (35 g/L) to simulate the SJ electrical conductivity, pH, and total soluble solid (TSS). The pH was adjusted to 3.4 by 0.2 M Na_2HPO_4, and an identical electrical conductivity was obtained to mimic the SJ (*i.e.,* 3.9 mS/cm) by using 5% NaCl. The MS was further used for inactivation of the target microorganism by PEF treatments.

2.2. Characterization of strawberry juice and model solution

The SJ and MS were characterized considering physicochemical, thermal, and rheological properties. A digital hand-held pocket refractometer (PAL-BX/RI, Atago Co., Ltd., Japan), a pH meter (FE20 FiveEasy, Mettler Toledo Columbus, OH, USA), and a benchtop pH–electrical conductivity meter (Orion 4 star, Thermo Scientific, USA) were used for the measurement of TSS, pH, and electrical conductivity, respectively. Specific heat capacity (C_p) was measured by a differential scanning calorimeter (DSC) Q2000 Series (TA Instruments, Inc., USA) at a temperature rate of 10 °C/min from 20 to 80 °C (Zainal, Rahman, Ariff, Saari, & Asbi, 2000). The viscosity of the SJ and MS at 20 °C was measured by a rheometer (MCR 300 rheometer, Anton Paar GmbH, Germany) under the controlled stress mode using 27-mm inner diameter concentric cylinder geometry (CC27). Shear stress data were plotted *versus* shear rate (0.18–3.33 s^{-1}), and viscosity was calculated from the slope of the graph.

2.3. Selection of target microorganism

Although *E. coli* O157:H7 is one of the most common causal agents in fruit juices associated with several outbreaks of foodborne illnesses (Raybaudi-Massilia et al., 2009), on the first attempt, the growth and the survival potential of the selected nonpathogenic microorganisms in SJ were monitored by taking into account the likely contamination at the industrial level. *E. coli* (ATCC 11775) (Moody, Marx, Swanson, & Bermúdez-Aguirre, 2014), *Listeria innocua* (ATCC 51742) (Pokhrel et al., 2019), and *Enterococcus faecium* (ATCC 8459) (Zhou et al., 2018) were used as the surrogates of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* Enteritidis, respectively, due to the biosafety in pilot plant and laboratory. Frozen stock cultures of *E. coli* 11775 and *E. faecium* in glycerol solution (20%, v/v) were activated in 100 mL of nutrient broth (NB) and Tryptic Soy Broth (TSB), respectively, by incubating at 37 °C for 18 h. Frozen stock culture of *L. innocua* was melted and reactivated in TSB containing 0.6% of yeast extract at 37 °C for 5 h. *E. coli* was also adapted to the acidic environment by gradually lowering the medium pH according to a slightly modified procedure (Koutchma, Paris, & Patazca, 2007). Briefly, *E. coli* cells were grown in NB at 37 °C for 18 h, and sequentially transferred to a new NB medium adjusted to the lower pH (6.0, 5.5, 5.0, 4.5, 4.0) as the cells reached to the early stationary phase.

Before inoculation of the surrogates, SJ was thermally pasteurized at 72 °C for 15 s to eliminate the existing microbiota. The pasteurized SJ (100 mL) was inoculated with the individual surrogates at an approximate concentration of 10^6 CFU/mL. To determine the most resistant microorganism, the survival of each surrogate in SJ was monitored during 48 h at 25 °C by placing the inoculated SJ in a shaking water bath (100 rpm). Appropriate dilutions of the collected samples were plated on MacConkey Agar (MCA), Tryptic Soy Agar (TSA), and TSA containing 0.6% yeast extract (TSAYE) for the enumeration of *E. coli*, *E. faecium*, and *L. innocua*, respectively. All the media used for microbial growth were from BD Diagnostic Systems (Sparks, MD, USA).

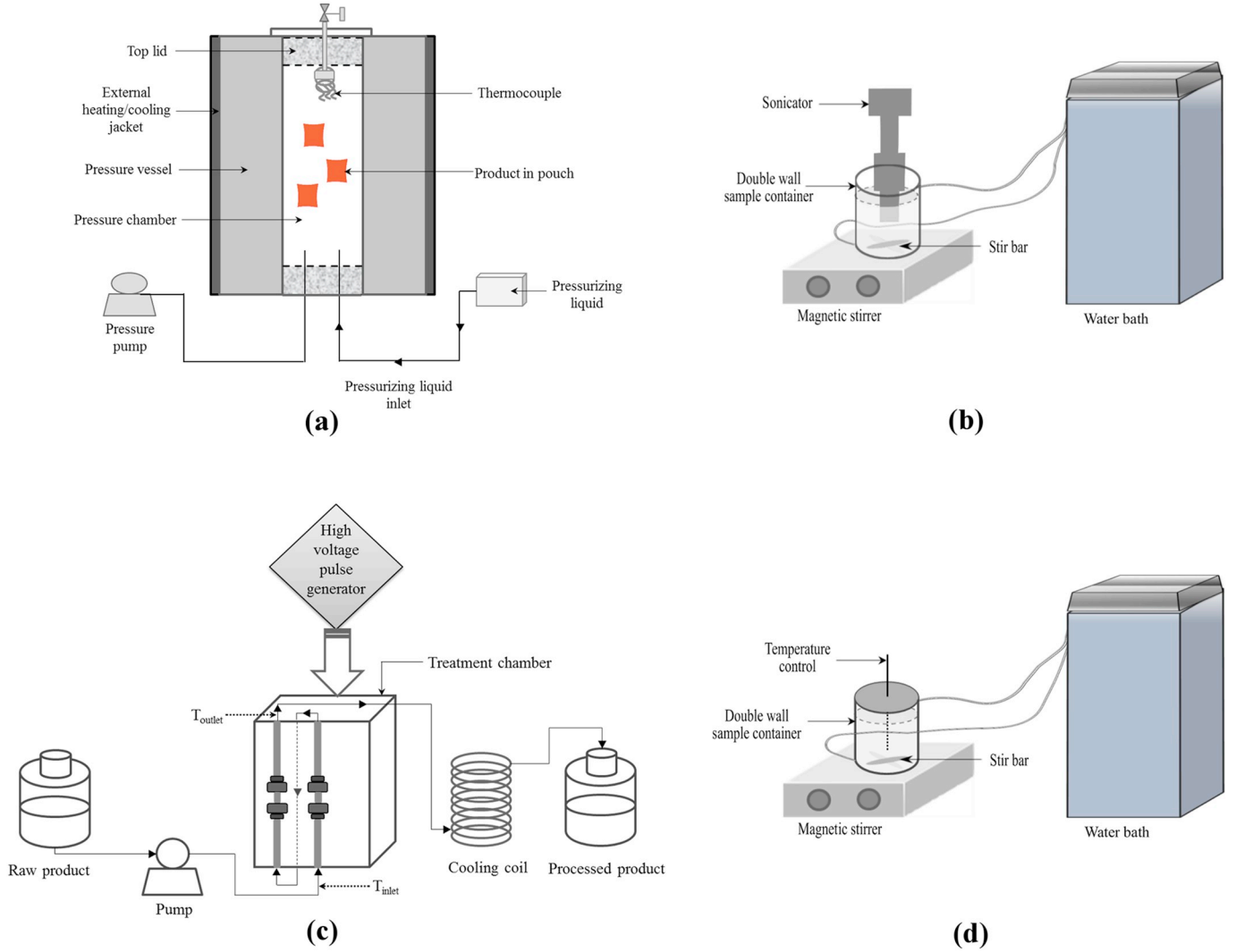

Fig. 1. Schematics of the following systems: a) high pressure processing (HPP), b) ultrasound (US), c) pulsed electric fields (PEF), d) thermal processing.

2.4. Inoculation and processing of strawberry juice

Based on the study of target microorganism selection, SJ was inoculated with acid-adapted *E. coli* cells at the early stationary phase to provide an initial inoculum level of approximately 10^6 CFU/mL, and subsequently subjected to HPP, US, PEF, and thermal pasteurization. The use of acid-adapted *E. coli* was in accordance with the study of Patil, Bourke, Kelly, Frías, and Cullen (2009) where stress or acid-adapted *E. coli* was exposed to nonthermal treatments.

2.4.1. High pressure processing

SJ (200 mL) was inoculated with 1 mL of acid-adapted *E. coli*, and thoroughly mixed on a magnetic stirrer for 1 min. Afterwards, 20 mL of inoculated SJ was aseptically transferred to nylon/PE-type plastic pouches (5 cm × 7 cm) (3 MIL, UltraSource, Kansas City, MO, USA). The pouches were sealed using a hand-operated sealer and then exposed to HPP treatments.

A high hydrostatic pressure unit (Engineering Pressure Systems, Inc., Haverhill, MA, USA) with a cylindrical chamber vessel (0.1-m internal diameter, 0.25-m internal height) was used to treat the SJ (Fig. 1a). The plastic pouches containing SJ inoculated with acid-adapted *E. coli* were placed in the chamber vessel filled with pressurizing liquid (5% mobil hydrasol 78 in water). An electrohydraulic intensifier pump (Hochdruck-Systeme GmbH, AP 10-0670-1116, Sigless, Austria) was used to apply different pressure levels (200, 250, 300, 350, 400 MPa) for treatments times up to 2 min where these treatments were conducted in triplicate. Since preliminary results (data not shown) indicated that pressures < 200 MPa required longer holding times from the industrial point of view, HPP trials started from 200 MPa. The temperature increase during HPP was 2.6 °C per 100 MPa due to the compression heating of water in accordance with the study of Rasanayagam et al. (2003).

2.4.2. Ultrasonication

An ultrasonic device (UP400S Hielscher USA Inc., Ringwood, NJ, USA) equipped with a probe of 22-mm diameter (sonotrode H22) and a water bath (Viscotherm VT 10) was used (Fig. 1b). The probe was immersed (2 cm) into 400 mL of SJ placed in a double-wall sample vessel where the temperature of SJ was controlled by circulating water through the walls. In order to evaluate the effect of sonication with or without mild heat, sonication was performed at sub-lethal (25 and 40 °C) and lethal (55 °C) temperatures. The temperature of the circulating water was adjusted to 4, 15, and 25 °C to keep the SJ at the desired corresponding temperatures of 25, 40, and 55 °C, respectively. Actually, different temperatures were preliminarily tested for circulating water to minimize the temperature increase during sonication of SJ (data not shown). 4, 15, and 25 °C were selected for circulating water since these temperatures led the SJ to achieve the desired processing temperature and also avoided the excessive temperature increase in SJ during sonication. Once the initial temperature of the SJ reached the

desired values after running the sonicator, acid-adapted *E. coli* cells were added into the juice. Subsequently, samples were collected during sonication (24 kHz, 120 μm amplitude in continuous pulse mode) up to 10 min and analyzed to determine the processing time necessary for the required level of inactivation. The acoustic power was calculated considering Eq. (1).

$$\text{Power (W)} = m \times C_P \times \frac{dT}{dt} \quad (1)$$

where m is the mass of the juice (g), c_p is the specific heat of the SJ (3.71 J/g·K), and dT/dt is the change in temperature over time (K/s). Then the acoustic energy density (AED) was calculated based on Eq. (2) (Tiwari & Mason, 2012).

$$\text{AED (mW/mL)} = \frac{P}{V} \quad (2)$$

where P is the power obtained by Eq. (1), and V is SJ volume.

2.4.3. Pulsed electric fields processing

A pilot plant-scale Powermod™ PEF system manufactured by Diversified Technologies Inc. (DTI, Bedford, MA, USA) was used. The continuous processing line was composed of sample containers, a peristaltic pump, pipes, a treatment chamber containing four electrodes, a high voltage pulse generator, and a cooling coil (Fig. 1c). The treatment chamber was equipped with two pairs of co-field electrodes with a diameter of 0.50 cm and gap distance of 0.65 cm.

PEF treatment conditions were identified by using MS that simulated SJ in terms of pH, TSS, and electrical conductivity. The MS (6 L) was inoculated with 12 mL of acid-adapted *E. coli*, and then subjected to PEF at a flow rate of 350 mL/min based on response surface methodology. A face-centered central composite design (CCD) was constructed considering electric field intensity (EFI) (25–35 kV/cm) and treatment time (5–27 μs) as independent variables. The ranges or the levels of these variables were selected according to the operating conditions of the PEF equipment. Factorial and axial points were duplicated and three central points were added to check the reproducibility of the results. EFI, frequency, and pulse width were adjusted by the control panel in the modulator cabinet. A constant pulse width of 2 μs was applied in monopolar square waveform, and an oscilloscope (Hewlett Packard 54520A) was used to monitor the shape and width of the electric pulses. The number of pulses per transit (per treatment chamber) and frequency were calculated using Eqs. (3) and (4), respectively.

$$\text{Number of pulses per transit} = \frac{t}{N \cdot w} \quad (3)$$

$$\text{frequency}\left(\frac{1}{s}\right) = \frac{t \bullet \frac{\dot{V}}{A}}{w \bullet N \bullet L} \quad (4)$$

where t is the treatment time (μs); N is the number of treatment chambers, which is 4; and w is the pulse width (μs); $\dot{V}$ is the flow rate (cm^3/s); A is the chamber area (cm^2); and L is the length of the chamber (cm).

The MS temperature was measured at the inlet and outlet of the treatment chamber by K-type thermocouples. The PEF-treated MS was pumped to the cooling coil where the MS temperature was reduced to approximately 4 °C. Then the samples were collected and used for enumeration of acid-adapted *E. coli*. The final processing conditions obtained for PEF processing of the MS in this part were then verified for SJ as mentioned in Section 2.5 Validation of processing conditions.

2.4.4. Thermal processing

Thermal pasteurization of SJ was conducted in a double wall sample vessel where hot water at 74 °C was circulated to increase the SJ temperature (Fig. 1d). The sample vessel containing 400 mL of SJ was placed on a stirrer set at 250 rpm and the magnetic stirrer bar was used to increase the heat transfer and to provide a homogeneous distribution of the microorganisms inside the medium. Once the initial SJ temperature increased from 23 to 72 °C, acid-adapted *E. coli* cells were inoculated into the juice. The samples were collected after 15 s of holding time and then used for cultivation and enumeration of any possible surviving *E. coli* cells.

2.5. Validation of processing conditions

Regarding PEF processing, the conditions previously identified for the MS were also tested with the SJ to verify whether the treatment achieved similar reductions of *E. coli* cells when PEF was applied to either MS or SJ. Hence, SJ inoculated with acid-adapted *E. coli* at an initial inoculum level of approximately 10^6 CFU/mL was subjected to PEF conditions that provided at least 5-log reduction of acid-adapted *E. coli* in MS.

Furthermore, the treatment conditions for HPP, US, PEF and thermal processing resulting in equivalent degrees of inactivation of acid-adapted *E. coli* in SJ were evaluated by examining their effect on the SJ natural microbiota (*i.e.,* total mesophilic aerobic count [TMAC] and yeast-mold [YM] count). For this purpose, the freshly squeezed SJ was kept at 4 °C for 5 days to increase its microbial content. Then, this juice was exposed to HPP, US, PEF, and a thermal treatment under the conditions that yielded at least 5-log reduction of acid-adapted *E. coli*.

2.6. Growth conditions and enumeration

Appropriate dilutions of SJ or MS were plated on MacConkey agar, the surviving *E. coli* cells were counted after incubation at 37 °C for 24 h. Samples containing *E. faecium* were plated on Tryptic Soy Agar (TSA) and counted after incubation at 37 °C for 24 h. *L. innocua* cells were counted on TSA containing 0.6% of yeast extract (TSAYE) after incubation at 37 °C for 48 h. Regarding natural flora of SJ samples, total aerobics and YM were plated on Plate Count Agar (PCA) and Potato Dextrose Agar (PDA) acidified with tartaric acid (10%) and counted after incubation at 37 °C for 48 h and 25 °C for 5 days, respectively.

2.7. Data analysis

An Excel worksheet (Microsoft 2010) and Minitab 16 Statistical Software (Minitab Inc., UK) were used to analyze the data. A *t*-test was conducted for characterization of SJ and MS considering a 95% confidence interval. Regarding the survival of different surrogates in SJ, Analysis of Variance (ANOVA) was applied to compare the resistances of the microorganism in SJ. Inactivation of the acid-adapted *E. coli* by different processes was evaluated by monitoring the inactivation kinetics. The equivalent conditions that provided at least 5 log reduction of acid-adapted *E. coli* cells in SJ were determined for each technology. TMAC and YM counts of SJ were also compared by ANOVA. Microbial counts were reported as mean ± standard deviation.

3. Results and discussion

3.1. Characterization of strawberry juice and the model solution

Physicochemical, thermal, and rheological properties of SJ are presented in Table 1. The SJ pH, TSS, and electrical conductivity were 3.40 ± 0.01, 8.05 ± 0.01°Brix, and 3.96 ± 0.01 mS/cm respectively. The SJ pH subjected to several nonthermal treatments was reported to be between 3.1 and 3.7 (Bhat & Stamminger, 2015; Duan & Zhao, 2009; Tiwari, O'Donnell, Patras, Brunton, & Cullen, 2009a; Tiwari, O'Donnell, Patras, & Cullen, 2008) while the TSS content of SJ varied between 9.8 and 11.6°Brix (Duan & Zhao, 2009; Tiwari et al., 2009a). These discrepant results could be due to differences on PEF-processing equipment, fruit varieties, chemical composition, as well as the way the fruit juice was prepared (Chatterjee, Chatterjee, Chatterjee, & Guha, 2004;

Table 1
Selected properties of strawberry juice (SJ) and a model solution (MS).

Properties	SJ	MS	*p*-Value*
pH	3.40 ± 0.01	3.39 ± 0.01	0.293
Total soluble solids (°Brix)	8.05 ± 0.01	7.9 ± 0.28	0.591
Electrical conductivity (mS/cm)	3.96 ± 0.01	3.90 ± 0.14	0.657
Specific heat capacity at 22 °C (J/g°C)	3.71 ± 0.41	3.75 ± 0.16	0.918
Viscosity at 20 °C (mPa.s)	2.53 ± 0.19	2.19 ± 0.30	0.386

* Two sample *t*-test was applied considering 95% of confidence interval. $p < 0.05$ indicates significant differences between strawberry juice and model solution.

Falguera, Pagan, & Ibarz, 2011; Schols, Intveld, Vandeelen, & Voragen, 1991). Since electrical conductivity is a critical parameter for PEF processing, it must be taken into consideration while preparing MS. The MS pH, TSS, and electrical conductivity were successfully adjusted to those of SJ, and measured as 3.39 ± 0.01, 7.9 ± 0.28°Brix, and 3.90 ± 0.14 mS/cm, respectively (Table 1). Therefore, the formulated MS mimics quite well the SJ, with non-significant differences ($p > 0.05$) in terms of electrical conductivity, pH, and TSS.

In the case of PEF treatment, conversion of electrical energy into heat increases the fluid temperature that can be estimated from the specific energy input and the specific heat capacity of the product (Toepfl, Heinz, & Knorr, 2006). In this study, the MS specific heat characteristic was designed to resemble that of SJ to obtain similar media properties and to justify the term nonthermal by preventing over-heating. The MS specific heat capacity (c_p) (3.75 ± 0.16 J/[g°C]) was not significantly different than that of SJ (3.71 ± 0.41 J/[g°C]) at 22 °C ($p > 0.05$) (Table 1). Although the c_p values found in our study were slightly higher than that of SJ (3.31 kJ/[kg·°C]) reported elsewhere (Tiwari, O'Donnell, Patras, Brunton, & Cullen, 2009b), it is promising that MS can be expected to behave like SJ regarding the energy delivered to the product during PEF treatment. Furthermore, the SJ flow behavior characteristics were well simulated with the MS. Newtonian type of flow behavior was observed for both SJ and MS having viscosity of 2.53 ± 0.19 and 2.19 ± 0.30 mPa.s at 20 °C, respectively, with non-significant differences ($p > 0.05$) (Table 1). Hence, the MS was characterized and validated to be used instead of SJ for the optimization of PEF treatment.

3.2. Selection of target microorganism

Pathogenic microorganisms can survive or adapt to the acidic nature of fruit juices. Therefore, it is important to gain knowledge of the most resistant microorganism likely to survive in the juice because a process effective against the selected target organism can also control the other pathogens (Mazzotta, 2001). The survival of different surrogates (*i.e., L. innocua*, *E. faecium*, *E. coli*, and acid-adapted *E. coli*) in SJ during 48 h of incubation at 25 °C is shown in Fig. 2. The reduction in each surrogate bacteria was monitored to determine the most resistant microorganism in SJ. While the microorganisms were not significantly affected by the SJ pH (3.4) during 12 h, the initial inoculum (approximately 10^6 CFU/mL) of *L. innocua*, *E. faecium*, *E. coli*, and acid-adapted *E. coli* reduced by 2.06 ± 0.45, 2.72 ± 0.03, 1.93 ± 0.27, and 1.54 ± 0.04 log CFU/mL, respectively, after 48 h. In other words, acid-adapted *E. coli* showed the least reduction in the number of inoculated cells; and thereby the highest resistance to the SJ acidic medium. This finding was in agreement with the study of Han and Linton (2004) reporting that 1.3 log cycles of *E. coli* O157:H7 became injured in SJ after three days of cold storage (4 °C). These observations demonstrated that acid-adapted *E. coli* cells could be injured to some extent in SJ due to the juice's pH; however, the surviving cells would still exist in the medium and could cause further microbial outbreaks. Accordingly, Raybaudi-Massilia et al. (2009) identified *E. coli* O157:H7 as a vehicle of foodborne illnesses due to outbreaks in apple cider and apple juice.

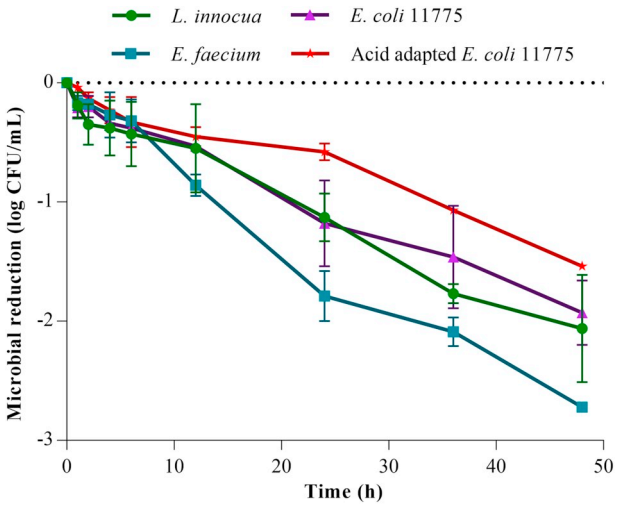

Fig. 2. Survival of tested surrogates in non-treated strawberry juice during 48 h.

To develop a pasteurization process, the NACMCF (2006) recommends determining the most resistant pathogen of public health concern that is likely to survive in the food. Thus, this study led us to identify acid-adapted *E. coli* as the most resistant surrogate that can most likely survive during the first two days of freshly squeezed SJ. Further findings of this study were obtained by using acid-adapted *E. coli* cells for the establishment of the equivalent treatment conditions for HPP, US, PEF and thermal processes.

3.3. Inactivation of acid-adapted E. coli 11775

3.3.1. High pressure processing

The effect of HPP on acid-adapted *E. coli* in SJ at various pressures and processing times are shown in Fig. 3a. HPP applied at 200 and 250 MPa for 120 s was able to reduce acid-adapted *E. coli* by 3.52 and 4.02 log cycles, respectively. On the other hand, HPP treatments at higher pressures resulted in at least 5 log reductions as follows: the treatments at 300 for 60 s; 350 MPa for 30 s; and 400 MPa for 15 s achieved 5.75 ± 0.52, 5.85 ± 0.05, and 6.01 ± 0.35 log reduction, respectively. The findings of this study were in good agreement with the studies on the inactivation of different *E. coli* strains in either buffer systems or juice. For example, the viability loss of *E. coli* O157:H7 (932) was 5.64 log cycles after pressurization of 1% peptone solution at 345 MPa for 5 min at 25 °C (Alpas et al., 1999). A single pulse at 400 MPa achieved 8 log cycles reduction in an *E. coli* (29055) population in apple juice (Ramaswamy, Riahi, & Idziak, 2003). Tahiri, Makhlouf, Paquin, and Fliss (2006) reported that dynamic high pressure at 200 MPa resulted in 5 log reduction and complete inactivation of *E. coli* O157:H7 (ATCC 35150) in orange juice after three and five passes, respectively. Low pH values and subsequent storage conditions could enhance the lethal effect of pressure in fruit juices. For instance, application of HPP at 300 MPa for 5 min caused a small reduction of *E. coli* (ATCC 11775) (*i.e.,* between 1.7 and 3 log cycles in orange, tomato, and apple juice). However, no surviving cells were observed for any type of fruit juice after subsequent storage at 5 °C for 24 h (Jordan, Pascual, Bracey, & Mackey, 2001).

Depending on the type of food product and target microorganism, practical HPP times may be limited to < 20 min from the economical point of view (Farkas & Hoover, 2000). Similarly, Balasubramaniam, Martiinez-Monteagudo, and Gupta (2015) also recommended a processing holding time of < 10 min to develop a commercially viable process. Many studies related to HPP of fruit juices have been applied at processing times of up to 5 min to accomplish 5 log reduction of the target microorganism (Guerrero-Beltran, Barbosa-Canovas, & Welti-

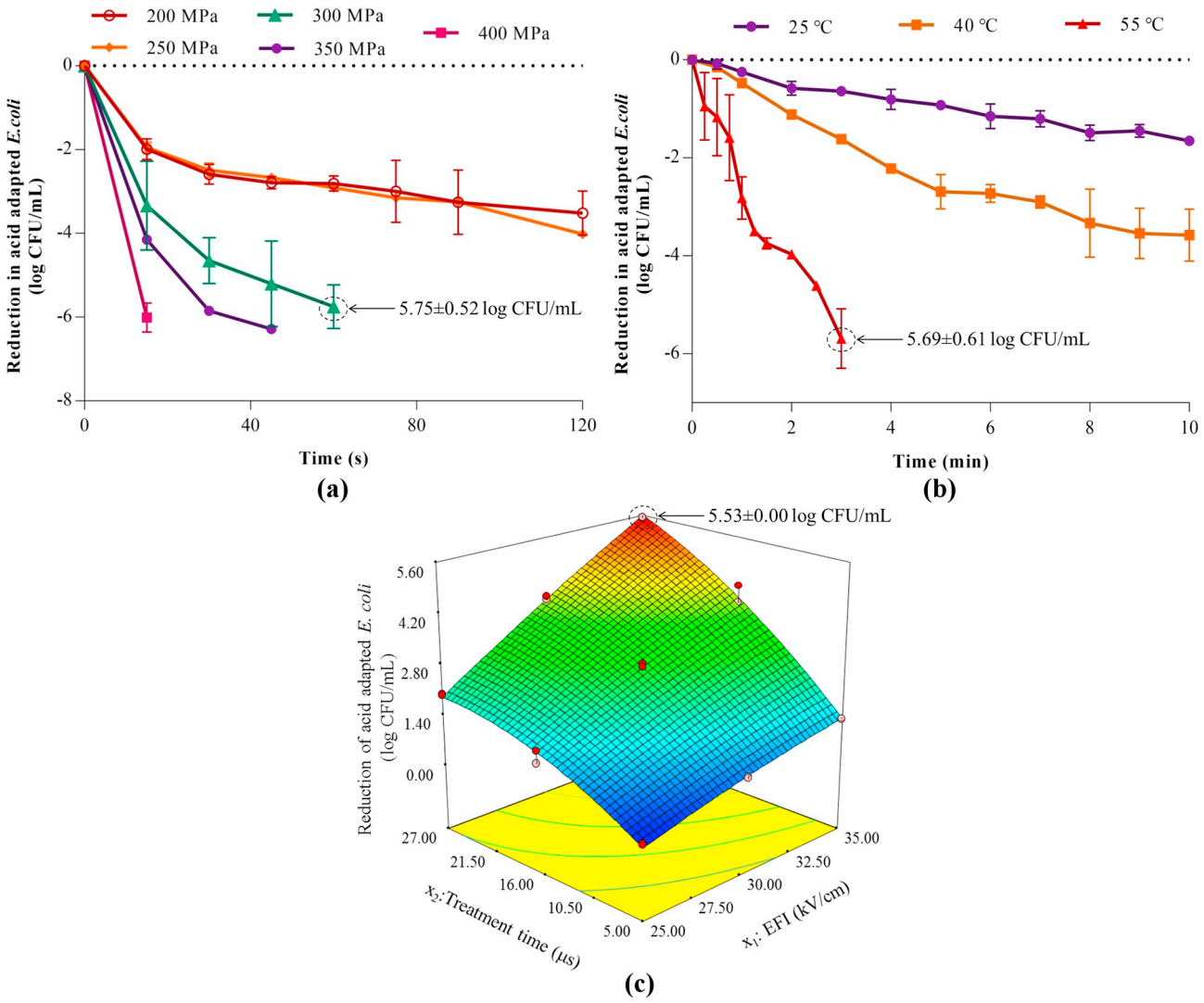

Fig. 3. Inactivation of acid-adapted *E. coli* 11775 in SJ by (a) high pressure processing (HPP), (b) thermosonication, and (c) pulsed electric fields (PEF) to identify processing conditions to reduce the microbial load by at least 5 log CFU/mL.

Chanes, 2011; Ramaswamy et al., 2003). Hence, 300 MPa for 1 min was selected as the final HPP conditions for pasteurization of SJ in the current study. The temperature and pressure profiles during HPP at 300 MPa are shown in Fig. 4. The initial temperature of the pressurizing liquid inside the treatment chamber (18.33 ± 1.04 °C) reached to 24.70 ± 1.47 °C after 0.5 min of pressure come-up-time (CUT) due to the compression heating. The temperature was slightly elevated to 26.13 ± 1.10 °C during a holding time of 1 min at 300 MPa and subsequently reduced to its initial level after decompression.

3.3.2. Ultrasonication

Fig. 3b represents the inactivation kinetics of acid-adapted *E. coli* in SJ treated by US. The microbial load was reduced by 1.65 ± 0.07 and 3.58 ± 0.53 log cycles when treated at 25 °C and 40 °C for 10 min, respectively. This finding indicated that sonication at sub-lethal temperatures was not sufficient to fulfill the desired level of inactivation. On the other hand, ultrasound at 55 °C for 3 min resulted in an adequate treatment by reducing the number of acid-adapted *E. coli* by 5.69 ± 0.61 log cycles. Thus, the identified thermosonication conditions rendered a process good enough to reach the targeted level of microbial inactivation. Similarly, Moody et al. (2014) also claimed positive results (> 6 log reduction of *E. coli*) for apple juice sonicated at 120 μm amplitude and 60 °C for 5 min. Ultrasound at higher temperatures has been reported to provide synergism between sonication and heat by increasing the microbial sensitivity to heat and low pH due to the cavitation and changes in the outer membrane of the cell structure (Wordon, Mortimer, & McMaster, 2012). Although sonication is easy to use, one of the challenges during processing is temperature control (Rastogi, 2011). Due to cavitation and energy transmission to the material, the temperature inside the product increases as shown in Fig. 4b. The maximum temperatures reached during sonication at 25, 40, and 55 °C were 34.55, 43, and 56.48 °C, respectively. Energy analysis indicated that SJ required ultrasonic power of 206.8 W and acoustic energy density of 517.1 mW/mL when the sonication was applied at 55 °C for 3 min. Moody et al. (2014) also emphasized the relevance of the applied temperature and recommend, to reach pasteurization, temperatures higher than 50 °C for the reduction of bacterial loads in most juices. Recently, Dundar, Agcam, and Akyildiz (2019) reported the combination of 59 °C and 455 J/g as the optimum thermosonication conditions to minimize the quality degradation of strawberry nectar.

3.3.3. Pulsed electric fields processing

Variance analysis of the response surface quadratic model for the inactivation of acid-adapted *E. coli* in the MS by PEF is given in Table 2. The significance of main (x_1: EFI and x_2: treatment time), interaction

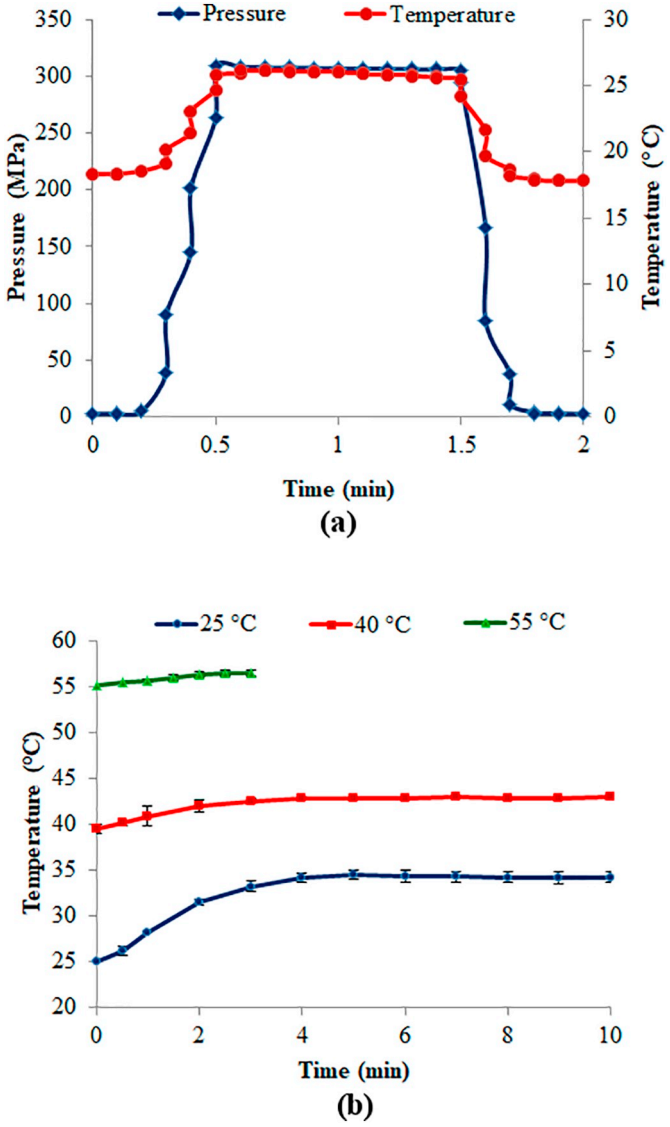

Fig. 4. Changes in strawberry juice temperature during processing by (a) high pressure processing (HPP) and (b) thermosonication (US).

($x_1.x_2$) and quadratic (x_1^2 and x_2^2) effects on the inactivation of acid-adapted *E. coli* in MS by PEF was evaluated. EFI, treatment time (t), interaction of EFI and t, and t^2 were found to be significant due to small *p*-values ($p < 0.05$). However, the EFI quadratic effect (x_1^2) was non-significant with a *p*-value of 0.1825 and thus, x_1^2 was removed from the model. The quadratic model was significant with a nonsignificant lack-of-fit value. The R^2 and the adjusted R^2 were in good correlation with values of 0.9942 and 0.9920, respectively. Hence, the reduction of acid-adapted *E. coli* in MS subjected to PEF was estimated by the following equation in coded terms:

$$\text{Reduction}\left(\log\frac{\text{CFU}}{\text{mL}}\right) = 2.77 + 1.23 \times x_1 + 1.53 \times x_2 + 0.60 \times x_1x_2 - 0.43 \times x_2^2 \quad (5)$$

Fig. 3c shows the response surface plot of logarithmic reduction of *E. coli* in MS exposed to PEF treatment considering different EFI (25, 30, and 35 kV/cm) and treatment time (5, 16, and 27 μs). PEF at 35 kV/cm for 27 μs reduced the acid-adapted *E. coli* by 5.53 log cycles. Mosqueda-Melgar, Elez-Martinez, Raybaudi-Massilia, and Martin-Belloso (2008) stated that the applied EFI ranges from 20 to 80 kV/cm depending on the media characteristics, operating parameters and target micro-organism. PEF treatment time could range from 12 to 400 μs for inactivation of *E. coli* in different types of liquid foods (Mosqueda-Melgar et al., 2008). Additionally, some other studies have applied PEF for > 400 μs. For example, Plaza et al. (2006) treated orange juice with an electric field of 35 kV/cm for 750 μs. In another study, orange juice was processed by PEF at 17 kV/cm of EFI for a treatment time of 1034 μs (Agcam, Akyildiz, & Evrendilek, 2016). SJ was also processed by PEF at 35 kV/cm of EFI for 1700 μs (Aguilo-Aguayo, Oms-Oliu, Soliva-Fortuny, & Martin-Belloso, 2009). The variations in treatment times among previously published studies could depend on the conductivity of the juice, pulse polarity, pulse width, *etc.* (Wouters, Alvarez, & Raso, 2001).

As shown in Fig. 3c, the higher EFI values the more reductions of acid-adapted *E. coli* were achieved in the PEF treated MS. This is actually attributed to the better disintegration of cell matrices due to increased EFI (Töpfl, 2006). Table 3 shows the PEF processing parameters used for MS based on CCD where corresponding frequencies for the applied t_{dose} of 5, 16, and 27 μs were 29, 92, and 155 Hz, respectively. Accordingly, the total number of pulses applied to the four treatment chambers ranged from 3 to 14 pulses, depending on the treatment time (t_{dose}). The MS initial temperature was approximately 22.7 ± 0.07 °C. Even though the MS temperature at the outlet of the chamber increased up to 46 °C due to energy dissipation, it was still below the lethal temperature. Thus, the term "nonthermal" would still be valid and justified since the lethal effect of this treatment could be originated from the electric field itself. The experimental and predicted logarithmic reduction data obtained from the quadratic model are shown in Table 4. Consequently, 5.53 log reduction of *E. coli* was achieved in MS by PEF at EFI of 35 kV/cm, t_{dose} of 27 μs, frequency of 155 Hz, pulse width of 2 μs, flow rate of 350 mL/min, and electrical conductivity of 3.9 mS/cm.

3.3.4. Thermal processing

Complete inactivation of acid-adapted *E. coli* cells was achieved in SJ by heat treatment at 72 °C for 15 s, and thereby the FDA's criterion of 5 log reduction in the pertinent microorganism was satisfied. Kaya, Yildiz, and Unluturk (2015) used *E. coli* K-12 as a surrogate for the determination of thermal pasteurization conditions for a lemon–melon juice blend. The authors achieved approximately 6 log reduction of *E. coli* K − 12 in the juice blend subjected to thermal pasteurization at 72 °C for 71 s. More severe conditions (90 °C for 60 s) were also applied

Table 2
Variance analysis of central composite design for a model solution (MS) treated by pulsed electric fields (PEF).

Source	Sum of squares	df	Mean square	F value	p-Value	
Model (R^2:0.9942)	50.18	5	10.04	447.66	< 0.0001	Significant
EFI (x_1)	18.24	1	18.24	813.73	< 0.0001	
Treatment time (x_2)	28.19	1	28.19	1257.43	< 0.0001	
$x_1.x_2$	2.85	1	2.85	127.25	< 0.0001	
x_1^2	0.04	1	0.04	1.98	0.1825	
x_2^2	0.81	1	0.81	35.93	< 0.0001	
Residual	0.29	13	0.02			
Lack of fit	0.11	3	0.04	1.92	0.1896	Not significant
Pure error	0.18	10	0.02			
Cor total	50.47	18				

Table 3
Change in temperature of model solution (MS) during PEF trials.

EFI (kV/cm)	t (μs)	Frequency (Hz)	n	T_{in} (°C)	T_{out} (°C)	ΔT (°C)
25	5	29	3	22.7	23.9	1.2
35	5	29	3	22.6	25.9	3.3
25	27	155	14	22.8	31.9	9.1
35	**27**	**155**	**14**	**22.7**	**46.0**	**23.3**
25	16	92	8	22.8	27.6	4.8
35	16	92	8	22.6	34.6	12
30	5	29	3	22.7	24.8	2.1
30	27	155	14	22.6	37.7	15.1
30	16	92	8	22.7	30.8	8.1

Electrical conductivity (3.9 mS/cm), flow rate (350 mL/min), and pulse width (2 μs) were kept constant. The row with bold numbers indicates the selected processing conditions for PEF treatment of strawberry juice.
EFI: Electrical field intensity.
t: treatment time.
n: number of pulses.
T_{in}: MS temperature at the inlet point.
T_{out}: MS temperature at the outlet point.
$\Delta T = T_{out} - T_{in}$.

Table 4
Inactivation of acid-adapted *E. coli* 11775 in a model solution (MS) by PEF based on central composite design.

EFI (kV/cm) (x_1)		t (μs) (x_2)		Reduction in *E. coli* cells (log CFU/mL)	
Actual	Coded	Actual	Coded	Experimental	Predicted
25	−1	5	−1	0.18	0.17
25	−1	5	−1	0.14	0.17
35	+1	5	−1	1.29	1.44
35	+1	5	−1	1.33	1.44
25	−1	27	+1	2.02	2.04
25	−1	27	+1	1.97	2.04
35	**+1**	**27**	**+1**	**5.53**	**5.70**
35	**+1**	**27**	**+1**	**5.53**	**5.70**
25	−1	16	0	1.12	1.53
25	−1	16	0	1.45	1.53
35	+1	16	0	3.75	4.00
35	+1	16	0	4.23	4.00
30	0	5	−1	0.76	0.80
30	0	5	−1	0.72	0.80
30	0	27	+1	3.83	3.87
30	0	27	+1	3.93	3.87
30	0	16	0	2.87	2.77
30	0	16	0	2.77	2.77
30	0	16	0	2.77	2.77

EFI and t refer to electrical field intensity and treatment time, respectively. The rows with bold numbers indicate the selected processing conditions for PEF treatment of strawberry juice.

by Odriozola-Serrano, Puigpinos, Oliu, Herrero, and Martin-Belloso (2016) for heat treatment of SJ. However, thermal processing of SJ at 72 °C for 15 s was enough to facilitate the conventional pasteurization in our case.

3.4. Validation of processing conditions

Fig. 5 shows the reduction of TMAC and YM count of SJ treated with HPP, US at 55 °C, PEF, and heat. The initial levels of TMAC and YM count were 3.68 ± 0.30 and 5.25 ± 0.20 log cycles, respectively. Regarding HPP, 300 MPa for 1 min reduced the TMAC and YM count by 1.81 ± 0.15 and 4.07 ± 0.58 log cycles, respectively. These findings are in agreement with Fonberg-Broczek et al. (2005) that claimed prokaryotes were more pressure-resistant than YM. Similarly, HPP treated strawberry puree was reported to contain the total aerobic bacteria in the range of 2.2–2.7 log CFU/g while no yeast and mold were detected (Aaby, Grimsbo, Hovda, & Rode, 2018). However, HPP

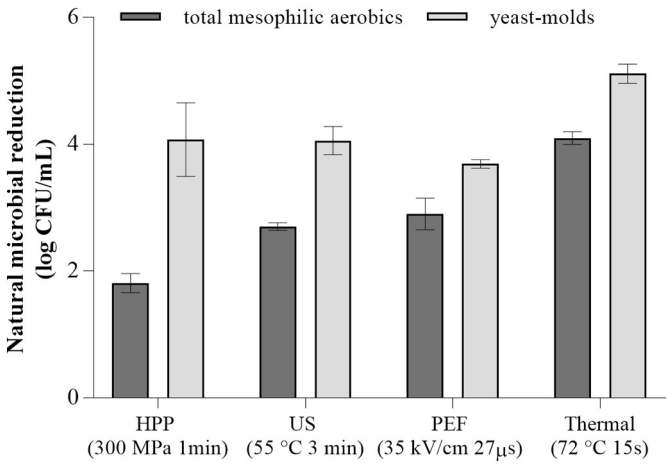

Fig. 5. Strawberry juice natural microbiota after treatments with selected equivalent processes using high pressure processing (HPP), thermosonication (US), pulsed electric fields (PEF), and heat.

under the same conditions achieved a higher inactivation level for acid-adapted *E. coli* in SJ (as previously mentioned, 5.75 ± 0.52 log cycles) compared to natural flora. This could be associated with the increment in the sensitivity of *E. coli* O157:H7 to the acidic conditions due to high pressure (Bull et al., 2004). Thermosonication conditions (55 °C, 120 μm, 24 kHz, 3 min) that resulted in 5.69 ± 0.61 log reduction of acid-adapted *E. coli* in SJ was able to reduce TMAC and YM count by 2.72 ± 0.06 and 4.06 ± 0.22 log cycles, respectively. Gabriel (2012) studied both pathogenic bacteria and spoilage yeasts in cloudy apple juice subjected to sonication; and concluded that yeasts showed more resistance to sonication compared to acid-adapted or non-adapted pathogenic bacteria such as *E. coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes*. This study is in agreement with the findings of our study where SJ natural microbial flora showed more resistance to thermosonication in comparison to the acid-adapted *E. coli*.

With respect to PEF, processing conditions that resulted in 5.53 log reduction of *E. coli* for the MS were firstly confirmed by applying the same conditions to SJ. PEF treatment (35 kV/cm, 27 μs, 155 Hz, 350 mL/min, 2 μs pulse width in monopolar mode) reduced the *E. coli* counts in SJ by 5.16 ± 0.15 log cycles which was similar to the acid-adapted *E. coli* reduction in MS ($p > 0.05$). These results were in line with a study conducted for apple cider where 4.5 log reduction of *E. coli* O157:H7 (35150) was achieved at EFI of 34 kV/cm, 160 μs, pulse duration time of 4 μs, and frequency of 800 pps (Evrendilek et al., 2000). In the present study, the reductions of TMAC and YM counts under the optimum PEF processing conditions were 2.90 ± 0.25 and 3.69 ± 0.07 log cycles, respectively. It is quite apparent the reduction of natural flora was lower than that of acid-adapted *E. coli* cells. This result may be attributed to the type of species in the mixed population where the microbial load was intentionally increased by keeping the juice at refrigerated conditions for five days before processing. Moreover, formation of any particles and metabolic products during SJ incubation (*i.e.,* fermentation) can change the homogeneity of the electric fields when PEF is applied. In addition, total mesophilic aerobic bacteria showed more resistance to PEF treatment compared to yeast-mold count. This could be explained by the differences in morphological characteristics since larger cells could be permeabilized in an easier manner compared to smaller cells (Wouters et al., 2001). Since the FDA's requirement of 5 log reduction in the population of the target microorganism was satisfied and a considerable reduction was achieved for TMAC and YM counts, these conditions were chosen as PEF processing parameters for SJ pasteurization. In summary, it is relevant to mention that SJ natural flora was reduced below 2 log CFU/mL by HPP, US, and PEF at the selected equivalent conditions.

Before thermal pasteurization, the initial TMAC and YM count in SJ

were 4.82 ± 0.61 and 5.86 ± 0.50 log cycles, respectively. Thermal pasteurization at 72 °C for 15 s resulted in 4.1 ± 0.10 and 5.11 ± 0.15 log reductions of TMAC and YM count, respectively. Hence, thermal pasteurization that yielded at least 5 log reduction of *E. coli* in SJ also significantly reduced the natural SJ microbial flora.

4. Conclusions

This study identified the processing conditions for pasteurization of strawberry juice based on equivalent level of inactivation of acid-adapted *E. coli* considering three nonthermal technologies including high pressure, pulsed electric fields and ultrasound. The main outcomes of this work were:

- The identified processing conditions can be used for pasteurization of strawberry juice since at least 5 log CFU/mL reduction was achieved for the acid-adapted *E.coli*.
- Microbiological quality of strawberry juice was improved under the identified equivalent conditions due to the reduction of the natural microbiota (total mesophilic aerobic and yeast-mold counts) below 2 log CFU/mL regardless of treatment type. It can be strongly suggested that the natural microbiota should also be taken into consideration while establishing the pasteurization conditions due to their high survival characteristics in the fruit juices.
- The use of a model system simulating strawberry juice was very successful for the selection of proper PEF conditions since it prevented wasting both time and large amount of juice.
- Further studies should be focused on the evaluation of physicochemical, phytochemical and sensorial properties of strawberry juice subjecting to the processing conditions identified in the present study.

Declaration of Competing Interest

We have no conflict of interest to declare.

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