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Moderate intensity Pulsed Electric Fields (PEF) as alternative mild preservation technology for fruit juice

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ABSTRACT

Moderate intensity Pulsed Electric Fields (PEF) was studied for microbial inactivation as an alternative to high intensity PEF or to classical thermal pasteurization. The process is characterized by the application of electric pulses, allowing an increase of the product temperature by the ohmic heat generated by the pulses. A systematic evaluation of the effect of parameters electric field strength (E) and pulse width (τ) on the inactivation of *Escherichia coli*, *Listeria monocytogenes*, *Lactobacillus plantarum*, *Salmonella* Senftenberg and *Saccharomyces cerevisiae* in orange juice was carried out in a continuous flow system. A wide range of conditions was evaluated, and both E and τ were shown to be important in the efficacy to inactivate micro-organisms. Remarkably, PEF conditions at $E = 2.7$ kV/cm and $\tau = 15$–1000 μs showed to be more effective in microbial inactivation than at $E = 10$ kV/cm and $\tau = 2$ μs.

Inactivation kinetics of the tested PEF conditions were compared to an equivalent thermal process to disentangle non-thermal effects (electroporation) from thermal effects responsible for the microbial inactivation. At standard high intensity PEF treatment a non-thermal inactivation at $E = 20$ kV/cm and $\tau = 2$ μs pulses was observed and attributed to electroporation. Non-thermal effects could also be resolved with moderate intensity PEF at $E = 2.7$ kV/cm and pulse width between $\tau = 15$–1000 μs. Microbial inactivation at these moderate intensity PEF conditions was studied in more detail at different pH and medium conductivity for *E. coli* and *L. monocytogenes* in watermelon juice and coconut water. Under moderate intensity PEF conditions the effectiveness of treatment was independent of pH for all evaluated matrices in the pH range of 3.8–6.0, whereas under high intensity PEF conditions the pH of the product is a critical factor for microbial inactivation. This suggests that the inactivation proceeds through a different mechanism at moderate intensity PEF, and speculations for this mechanism are presented. In conclusion, moderate intensity PEF conditions at $E = 2.7$ kV/cm and pulse width of 15–1000 μs has potential for industrial processing for the preservation of fruit juices and pH neutral liquid food products.

1. Introduction

Novel preservation processes with reduced temperature and/or with short holding time have received considerable interest as an alternative to thermal pasteurization and sterilization, because these processes better retain food quality. High intensity pulsed electric fields, often referred to as 'Pulsed Electric Fields' (PEF) (Toepfl et al., 2007; Vega-Mercado et al., 2007) is an example of a continuous flow process for the mild preservation of liquid food products, such as fruit juices. Typical process conditions studied in literature use an electric field strength (E) in the range of 15–40 kV/cm, and total pulse time of 20 μs up to 2000 μs (Álvarez et al., 2003; Toepfl et al., 2007). For industrial scale equipment, the electric field strength is lower than those used in scientific studies, ranging between 10 and 20 kV/cm (Toepfl, 2012), balancing between effectiveness of the treatment and costs (Kempkes, 2017). High intensity PEF induces electroporation of cell membranes leading to inactivation of micro-organisms, which was demonstrated for the first time by Sale and Hamilton (1967). The transmembrane potential generated on a cell membrane by an external applied electric field depends on the intensity of the electric field strength applied and the size and shape of the cell. The external electric field strength required to reach the transmembrane voltage threshold is known as critical electric field

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Table 1
Bacterial strains and yeast strain used in this study.

Species/strains used	Source of isolation	Cell wall structure	Size[a]	Reference
Escherichia coli ATCC 35218	PEF resistant surrogate for *E. coli* O157:H7	Gram-negative	1.1–1.5 μm × 2–6 μm	Gurtler et al. (2011)
Lactobacillus plantarum ATCC 14917	Fermenting fruit juice isolate	Gram-positive	0.9–1.2 μm × 3–8 μm	Campos and Cristianini (2007)
Listeria monocytogenes NV8	Heat resistant variant from a bovine carcass	Gram-positive	0.4–0.5 μm × 0.5–2 μm	Van der Veen et al. (2009)
Saccharomyces cerevisiae CBS 1544	Fermenting fruit juice isolate	–	3–15 μm × 2–8 μm	Put et al. (1976)
Salmonella enterica subsp. *enterica serovar* Senftenberg ATCC 43845	Thermotolerant foodborne pathogen	Gram-negative	0.7–1.5 μm × 2–5 μm	Ng et al. (1969)

ATCC: American Type Culture Collection, USA.
CBS: Centraal Bureau voor Schimmelcultures (Fungal Biodiversity Centre, Utrecht, The Netherlands).
[a] Characteristic dimensions taken from Bergey (1986).

strength (E_c), and when $E > E_c$ electroporation is induced (Álvarez et al., 2006). For microbial cells (1–10 μm) an electric field strength > 10 kV/cm is required to induce electroporation, while for eukaryotic plant cells (40–200 μm) an electric field strength < 5 kV/cm is sufficient (Raso, 2016). Later studies have proposed additional effects of high intensity PEF that occur at the lipid domain (Teissié et al., 2005; Weaver and Chizmadzhev, 1996), on transport channels in the membrane (Tsong, 1990) or cell wall (Pillet et al., 2016).

Moderate intensity PEF conditions, defined in this study as conditions with an electric field strength $E < 5$ kV/cm, have been extensively studied as a pre-step in mass transfer phenomena in food and biotechnological processes, aiming to mildly treat plant cells to reduce the use of chemicals and energy to disintegrate the cells (Donsì et al., 2010). Although the electroporation effect in plant cells was observed at low field strength, this regime of process conditions has received no attention for the application as alternative preservation process to inactivate micro-organisms, presumably due to the small cell size (Raso, 2016). Moderate intensity PEF conditions have been extensively studied for application for gene transfer, and electroporation effects on micro-organisms have been reported with long exposure times (Eynard et al., 1998). In some cases the temperature increase of the sample can be avoided and the application of the pulse treatment can last more than 1 h (El Zakhem et al., 2006; El Zakhem et al., 2007).

Although high intensity PEF treatment aims for minimal thermal effects, the temperature of the treated medium inevitably increases as the result of ohmic heating, also called joule heating. An Ohmic heating process aims at the conversion of electric energy into thermal energy resulting in rapid heating of the food (Jaeger et al., 2016) and usually an electric field strength of less than $E < 0.5$ kV/cm. In practice, the differences between high intensity PEF and ohmic heating are fading, especially since high intensity PEF processing is combined with heat to improve its efficacy (Buckow et al., 2013) and ohmic heating processing are sometimes associated with an additional electroporation effect (Lebovka et al., 2005; Loghavi and Sastry, 2009).

Understanding of the effect of moderate and high intensity pulse conditions on microbial inactivation, requires a systematic evaluation of process parameters, microbial characteristics and culture conditions, and treatment medium characteristics, as these factors all contribute to microbial inactivation (Raso et al., 2014). Improved understanding of the effect of these individual and combined parameters could help to design an optimal pasteurization process, with minimal energy input and maximal microbial inactivation.

The research question of this study was to investigate if moderate intensity pulsed electric fields could be used as alternative PEF condition to inactivate micro-organisms and serve as an alternative, easy to scale mild preservation process for fruit juices, incorporating an increase of the product temperature by ohmic heating that is inevitable generated by the PEF process.

To answer this question, high intensity PEF conditions used in industrial applications ($E = 10$ and 20 kV/cm at a typical pulse width of $\tau = 2$ μs) were compared to moderate intensity PEF conditions ($E = 0.9$ and 2.7 kV/cm and pulse widths in the range of $\tau = 15$–1000 μs) in orange juice using three pathogenic (*Escherichia coli*, *Salmonella* Senftenberg and *Listeria monocytogenes*) and two spoilage micro-organisms (*Lactobacillus plantarum* and *Saccharomyces cerevisiae*). The inactivation kinetics for the tested conditions were modelled and compared to the inactivation kinetics of an equivalent thermal process to retrieve non-thermal effects. The most promising moderate intensity PEF conditions that were found, were evaluated for other fruit juices to evaluate the potential of this alternative for heat pasteurization.

2. Material and methods

2.1. Micro-organisms, culture and recovery conditions

Pathogenic and spoilage micro-organisms relevant for fruit juices

Table 2
Pulse conditions and characterizations for the different PEF configurations I-III used in this study.

Configuration	Pulse shape	Number of treatment chambers	Pulse width (τ) (μs)	Electric field strength (E) (kV/cm)	Dimensions of treatment chamber (diameter × length) (mm)	Residence time (ms)	Matrix	Frequency (Hz)	Number of pulses
I	Monopolar square wave	2	2	10	2.8 × 4.0 (u, l)	227	Orange juice	0–964	0–220
				15	1.5 × 3.0 (u, l)	49	Orange juice	0–964	0–204
				20	1.0 × 2.0 (u, l)	14	Orange juice	0–964	0–14
II	Bipolar square wave	2	1000	0.9	4.0 × 6.0 (u, l)	696	Orange juice	0–55	0–77
			1000	2.7	1.0 × 2.0 (u, l)	14	Orange juice	0–255	0–7.4
							Coconut water	0–100	0–2.9
							Watermelon juice	0–238	0–6.8
			100	2.7	1.0 × 2.0 (u, l)	14	Orange juice	0–1040	0–30
III	Bipolar square wave	3	100	2.7	1.0 × 2.0 (u, l) 2.0 × 4.0 (m)	72	Orange juice	0–270	0–39
			15	2.7	1.0 × 2.0 (u, l) 2.0 × 4.0 (m)	72	Orange juice	0–1700	0–247

u = upper treatment chamber.
m = middle treatment chamber.
l = lower treatment chamber.

were selected based on differences in morphology, PEF- or heat-resistance (Table 1). Selected micro-organisms were stored at −80 °C in stock cultures containing 15% (vol/vol) glycerol (Fluka). Fresh cultures were prepared by plating −80 °C stock cultures on suitable agar plates that were incubated overnight at 20 °C. For *Escherichia coli* and *Salmonella* Senftenberg, 30 g Tryptic Soy Broth (TSB, Oxoid) per 1 L distilled water was used as medium, for *Lactobacillus plantarum* 52.2 g De Man, Rogosa and Sharpe medium (MRS, Merck) per 1 L distilled water, for *Listeria monocytogenes* 30 g Brain Heart Infusion (BHI, Oxoid) per 1 L distilled water and for *Saccharomyces cerevisia* 40 g glucose (Sigma-Aldrich), 5 g peptone (Fluka) and 5 g yeast extract (Oxoid) (GPY) per 1 L distilled water was used as growth medium. For preparation of solid media for plate counting, above mentioned media were supplemented with 15 g/L agar (Oxoid). A single colony of the overnight incubated plates was used to inoculate 10 mL of suitable broth (TSB, MRS, BHI or GPY) in a 100 mL Erlenmeyer flask and cultivated for 24 h at 20 °C in an Innova shaking incubator (180 rpm). From the resulting culture, 1 mL was used to inoculate 95 mL fresh broth supplemented with 1% glucose (Sigma-Aldrich) in 500 mL Erlenmeyer flask, and incubated for 24 h at 20 °C at 180 rpm as described previously (Timmermans et al., 2017) to mimic the sugar content that is present in fruit juice. The resulting cultures were pelleted by centrifugation (4000 rpm, 5 min) at 20 °C. Pellet was resuspended in 20 mL sterile peptone physiological salt diluent (Tritium) and washed twice. The resulting pellet was dissolved in 2 L pasteurized juice, resulting in a cell density of 10^6–10^7 cfu/mL (yeast) and 10^7–10^8 cfu/mL (bacteria). Expected cell numbers were verified by plate counting untreated samples. The number of viable cells was determined by plating 100 μL of a serially diluted sample in sterile peptone physiological salt diluent on suitable agar plates in duplicate.

Medium was supplemented with 15 g/L agar (Oxoid) and 0.1% sodium pyruvate (Sigma Aldrich) to enhance outgrowth of sub-lethally damaged cells (Timmermans et al., 2017). Surviving cells were enumerated after 3–5 days incubation at either 25 °C (*S. cerevisiae*), 30 °C (*L. monocytogenes*, *L. plantarum*) or 37 °C (*S.* Senftenberg, *E. coli*), and only plates with 10 to 300 colony forming units (cfu) were included for calculations. Plates with < 10 colonies were considered below detection limit.

2.2. Product matrices

Three different fruit juices varying in pH and conductivity were selected for PEF treatment experiments: orange juice (Minute Maid, original) and coconut water (HealthyPeople) are commercially available, the watermelon juice, was manually extracted according to the method described in Timmermans et al. (2014), and stored at −20 °C. Juices were sieved (pore size 0.225 mm) to remove large fibres and particles, and pasteurized (30 min holding time at 98 °C) prior to inoculation. Electric conductivity and pH of the juices was measured before and after inoculation with target micro-organisms using a conductivity meter (Greisinger GMH 3430) and pH meter (Metrohm 744), and no change was detected in conductivity and pH before and after inoculation.

2.3. Pulsed electric field processing

2.3.1. Processing parameters and equipment

Moderate intensity PEF conditions (bipolar square wave pulses of E = 0.9 and 2.7 kV/cm) were compared to high intensity PEF conditions used in industrial applications (monopolar square wave pulses of E = 10 or 20 kV/cm and pulse width τ = 2 μs). The pulse width of the moderate intensity conditions was varied (τ = 15, 100 or 1000 μs) to evaluate if this parameter is critical to the effectivity of the PEF process. Bipolar pulses were used to minimize the occurrence of electrochemical reactions that are known to occur under monopolar conditions or long pulse duration (Roodenburg, 2007). All PEF processing conditions were studied in a continuous-flow system using different configuration of electrode design. The configurations that were used are given below and in Table 2, according to guidelines established by the precursor of the International Society for Electroporation-Based Technologies and Treatments (Raso et al., 2016). Different dimensions of the treatment chambers were used for the different configurations to vary electric field strength. Insulators were made of polyetherimide (PEI, Ultem™ resin), and electrodes consisted of stainless steel (SS-316) for configuration I and titanium for configurations II and III.

The detailed account of the treatment chamber and electrode design is described by Mastwijk et al. (2007), and all treatment chambers were scaled to this design. As a result of the variations in length and diameter, the residence times of a fluid element within the treatment chambers were different for every tested configuration (Table 2). The average number of pulses (n) that is applied to a fluid element was calculated according to Eq. (1), where V is the volume of the high electric field region approximated by the volume defined by the distance of the electrodes and diameter of the gap (Mastwijk et al., 2007) (L), f is the frequency (Hz) and ϕ the flow rate (m^3/s).

$$n = \frac{V \bullet f}{\phi} \quad (1)$$

For calculations with bipolar pulses this number was multiplied by 2, as both a positive and negative pulse are given within one cycle.

Table 2 shows the range of frequencies and number of pulses applied for each setting.

The liquid handling for the experiments was identical for the three configurations, with sterilization, start-up and cleaning procedures as described previously in Timmermans et al. (2014). A detailed description and overview of the set-up is shown in the Appendix.

The inoculated suspension was pumped at a flow rate of 13 ± 1 mL/min, and preheated prior to the PEF treatment from room temperature (20 °C) to 36 °C in 48 s in a heating spiral (SS-316) that was immersed in a water bath (40 °C), as cells were more susceptible towards electroporation at this temperature as shown previously (Timmermans et al., 2014). Next, the suspension entered either two or three vertical positioned co-linear treatment chambers for electrical treatment, and different PEF conditions were applied, varying in electric field strength, pulse duration and number of pulses. Due to these variable PEF conditions, a different amount of electrical energy was delivered, resulting in different maximum temperatures. More heat was generated in the upper treatment chamber, due to the temperature-dependent increase of the electrical conductivity. As no cooling between the treatment chambers was carried out, the maximum temperature obtained can be correlated to a given energy input (see Section 2.3.2).

No (explicit) temperature holding section was included: directly after leaving the last treatment chamber, the juice entered (after 2.3 s) a cooling spiral (SS-316) that was immersed in an ice-water bath. At the exit, samples were collected. An example of a reconstructed temperature-time profile of the PEF treatment using two treatment chambers is shown in Fig. 1 and a photo for each section is shown in the Appendix, Figs. S1 and S2.

At the length scale flow conditions are laminar, leading to a parabolic velocity profile and corresponding residence time distribution in the treatment chamber. This has an effect on the homogeneity of the temperature distribution and hence on the electric field strength. Yet, the measurement of the actual average outlet temperature is still the most suitable method for an in-line process characterization (Jaeger et al., 2009). Therefore, temperature before and directly after the treatment chambers was measured using HYP-O T-type thermocouples (Omega). Additionally, the maximum temperature was measured indirectly at the exit of the last treatment chamber using a calibrated NTC-resistor. Pulse shape, voltage and current realized in the treatment chamber were recorded with a digital oscilloscope (Rigol DS1102E), and an example of come up time and fall time of the pulses is given in the Appendix (Fig. S6). Fall time of the square wave pulses, known as the decay time of the pulse height, was calculated as time to reduce the intensity to 37% of the peak value, similar to what was defined for exponential decay pulses (Raso et al., 2016), and was 60 µs in configuration II, and 1.5 µs in configuration III.

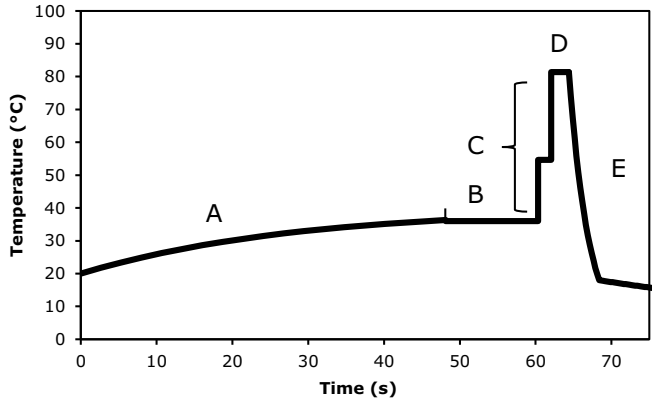

Fig. 1. Example of a temperature-time profile during PEF treatment, using two treatment chambers and heating to a maximum temperature of 81 °C. Inoculated juice is preheated to 36 °C (A), transferred from preheating section to treatment chambers (B), heated in the treatment chambers by applying pulses, i.e. to T_{max} 81 °C (C), transferred to the cooling section in 2.3 s (D) and cooled down for 122 s (E, partly displayed). Fig. S2 in the Appendix indicates each section on a photo.

2.3.2. Energy balance

The electrical energy input was calculated by numerical integration of the actual voltage and current traces (Eq. (2)) which was balanced by the caloric power measured in the bulk (Eq. (3)) within the experimental error (< 5%), with absolute deviations of 0.5–3.0 °C for the maximum outlet temperature. Specific energy, w (J/kg), is calculated with $P_{electric}$ as the electrical power (W), ρ is the density of the juice, 1020 kg/m^3, U is the voltage signal (V), I is the current signal (A), T_{out} as the outlet temperature (°C), T_{in} is the inlet temperature (°C) and c_p is the specific heat capacity, which is 3.8 kJ/kg·K for fruit juice and 4.1 kJ/kg·K for coconut water (Fontan et al., 2009).

$$w = \frac{P_{electric}}{\phi \cdot \rho} = \frac{f \cdot \int U(t) I(t) \mathrm{d}t}{\phi \cdot \rho} \quad (2)$$

$$w = (T_{out} - T_{in}) \cdot c_p \quad (3)$$

2.3.3. Inactivation studies

Directly after inoculation of selected juice, the inactivation experiment was started and samples were collected at each setting, and kept on ice until plating. At finalising a kinetic series, the pulse generator was turned off and a control sample (zero pulses) was taken at the exit of the equipment. Experiments were carried out on two different days to include biological variability for the different cultures. For each experiment, electric field strength and pulse duration were pre-set, and a kinetic series was obtained by variation of the pulse frequency. This adjustable frequency could also compensate for the different energy densities per pulse found for the specific juices and their corresponding conductivity. Note that for PEF treatment the total exposure time to pulses leads to a proportional change in the outlet temperature (i.e. a kinetic series for inactivation is similar to a temperature series for inactivation).

2.4. Thermal reference samples

Thermal treatment of the selected micro-organisms in orange was carried out in capillary glass tubes that were immersed in a water bath for specific temperature – time combinations as described in previous research (Timmermans et al., 2017), and are referred to as configuration 0.

2.5. Modelling PEF inactivation

Microbial inactivation is expressed as $\log_{10}(N/N_0)$, where N is the number of surviving micro-organisms at a specific PEF-condition in cfu/mL, and N_0 is the average number of surviving micro-organisms at start and control, in cfu/mL.

The Gauss-Eyring model for inactivation provides a combined kinetic (inactivation versus time) and thermal model (inactivation versus temperature) with temperature and exposure time as independent variables (Mastwijk et al., 2017) and was used to fit to the survival data $\log_{10}(N/N_0)$ as a function of maximum temperature (T, °C) according to Eq. (4), with $Tc(t)$ describing the critical temperature when 50% of the population is inactivated (°C) and σ is the width of the temperature distribution (°C), interpreted as measure of the heterogeneity in sensitivity towards temperature resistance (Mastwijk et al., 2017). Note that $Tc(t)$ is defined for a specific process time, which is calculated for t = 2.3 s corresponding to the transit time for leaving the exit treatment chamber and entering the cooling section (section D in Fig. 1).

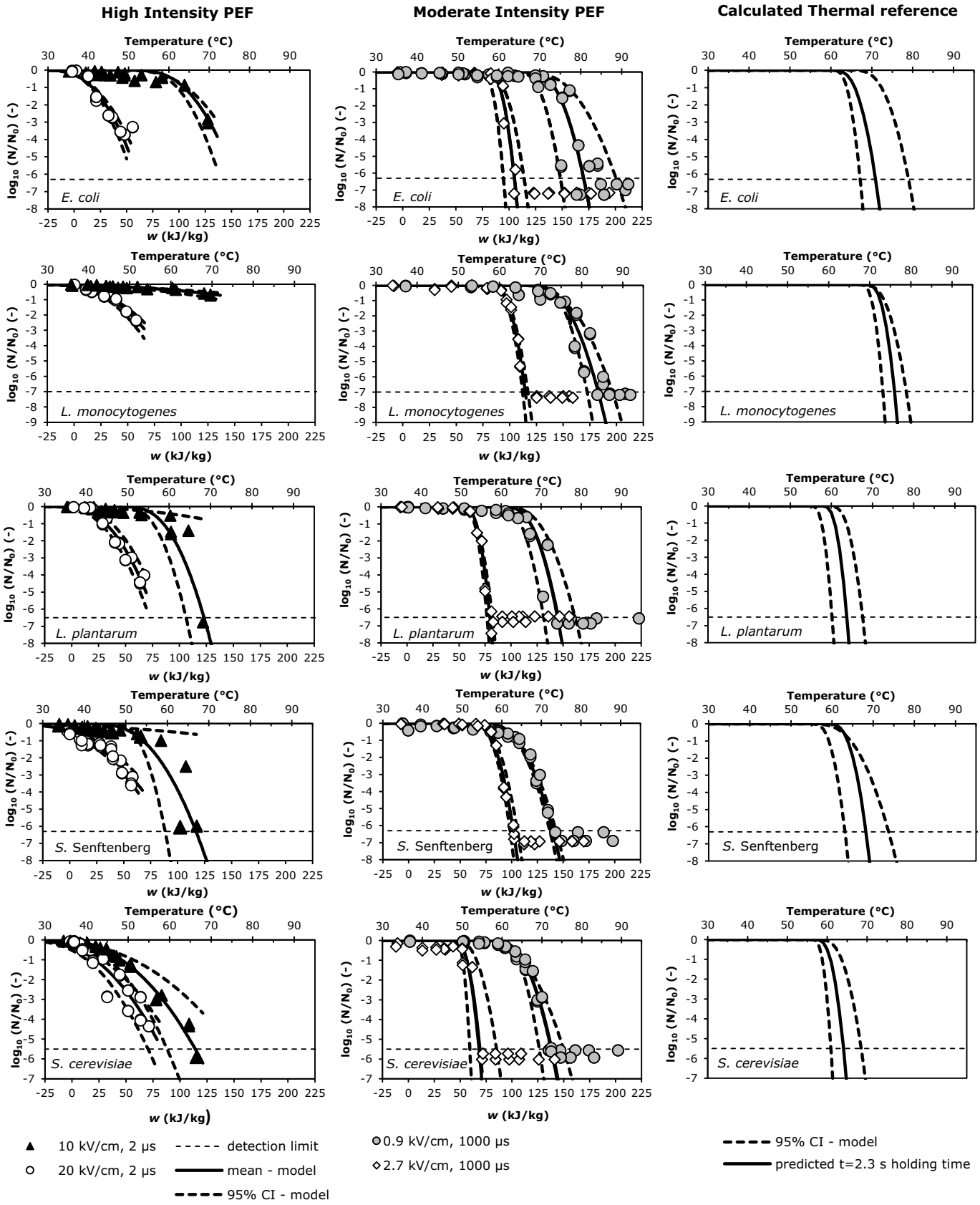

Fig. 2. Inactivation of *E. coli*, *L. monocytogenes*, *L. plantarum*, *S.* Senftenberg and *S. cerevisiae* in orange juice after PEF (left and middle panel) or thermal treatment (right panel). Inactivation, $\log_{10}(N/N_0)$, after PEF treatment is expressed as a function of maximum temperature (°C) or electrical energy applied (kJ/kg). Symbols represent measured data, lines represent the mean value and confidence interval estimated with the Gauss-Eyring model.
Part of the data at $E = 20$ kV/cm was published in Timmermans et al. (2014). Predictions were based on thermal reference data from Timmermans et al. (2017), and calculated according to Eqs. (4) and (5).

Table 3
Parameter estimates for microbial inactivation using the Gauss-Eyring model for PEF treatment (Eq. (4)) and thermal treatment (configuration 0) at $t = 2.3$ s (Eqs. (5) and (6)). Predictions to calculate the temperature required to have a 5 $\log_{10}$ reduction are presented, except for *L. monocytogenes* at $E = 10$ kV/cm, as virtually no inactivation was observed. Values between brackets show the 95% confidence interval.

Micro-organism	Electric field strength (kV/cm)	Pulse width (μs)	Medium	Data shown in figure	Configuration	n (exp.)	n (data points)	σ (°C)	Tc ($t = 2.3$ s) (°C)[a]	Predicted Tmax (°C) necessary for 5 $\log_{10}$ reduction
E. coli	20	2	Orange juice	Fig. 2	I	2	20	3.2 [2.9–3.5]	36.9 [36.5–37.3]	50.6 [49.3–51.8]
E. coli	10	2	Orange juice	Fig. 2	I	1	16	3.5 [2.9–4.4]	59.2 [57.3–61.2]	74.0
E. coli	2.7	1000	Orange juice	Figs. 2, 3, 4	II	2	24	1.0 [0.7–1.2]	59.1 [56.7–61.5]	63.1 [61.5–64.7]
E. coli	2.7	1000	Coconut water	Fig. 4	II	2	30	0.8 [0.7–1.0]	63.4 [62.1–64.6]	66.7 [64.0–69.4]
E. coli	2.7	1000	Watermelon juice	Fig. 4	II	2	34	1.0 [0.9–1.1]	62.9 [59.3–66.5]	67.1 [61.6–72.6]
E. coli	2.7	100	Orange juice	Fig. 3	III	2	32	2.3 [0.7–3.9]	61.9 [59.0–64.1]	72.0 [65.3–78.8]
E. coli	2.7	15	Orange juice	Fig. 3	III	2	32	1.0 [0.4–1.7]	62.3 [61.7–62.9]	66.4 [63.0–69.8]
E. coli	0.9	1000	Orange juice	Fig. 2	II	3	50	2.1 [1.0–3.1]	71.4 [67.4–75.3]	80.0 [72.0–88.1]
E. coli	0 (thermal)	0	Orange juice	Fig. 2	0	8	304	1.4 [0.7–2.2]	64.0 [60.5–67.7]	69.5 [64.7–74.3]
L. monocytogenes	20	2	Orange juice	Fig. 2	I	1	14	4.5 [4.1–5.1]	39.8 [38.7–40.8]	59.1
L. monocytogenes	10	2	Orange juice	Fig. 2	I	2	34	17.3 [13.3–21.3]	56.9 [52.4–61.4]	X
L. monocytogenes	2.7	1000	Orange juice	Figs. 2, 3, 4	II	2	30	1.1 [1.0–1.2]	60.4 [60.1–60.7]	65.2 [64.1–66.3]
L. monocytogenes	2.7	1000	Coconut water	Fig. 4	II	1	18	1.4 [1.3–1.4]	60.4 [60.1–60.7]	66.2
L. monocytogenes	2.7	1000	Watermelon juice	Fig. 4	II	2	32	1.6 [1.4–1.8]	58.2 [55.2–61.2]	64.7 [59.2–70.1]
L. monocytogenes	2.7	100	Orange juice	Fig. 3	III	2	36	1.4 [0.8–2.0]	66.1 [64.8–67.4]	72.1 [70.0–74.2]
L. monocytogenes	2.7	15	Orange juice	Fig. 3	III	2	40	1.6 [0.9–2.3]	65.1 [63.8–66.4]	72.1 [70.0–74.2]
L. monocytogenes	0.9	1000	Orange juice	Fig. 2	II	2	34	2.3 [1.7–2.9]	72.1 [70.4–73.8]	81.9 [76.1–87.6]
L. monocytogenes	0 (thermal)	0	Orange juice	Fig. 2	0	4	147	0.9 [0.6–1.2]	71.4 [68.8–74.2]	72.0 [61.0–83.0]
S. cerevisiae	20	2	Orange juice	Fig. 2	I	2	28	5.0 [4.3–5.7]	35.6 [32.5–38.6]	56.9 [53.6–60.2]
S. cerevisiae	10	2	Orange juice	Fig. 2	I	2	34	5.5 [4.0–7.0]	41.3 [39.3–43.3]	64.6 [58.3–70.9]
S. cerevisiae	2.7	1000	Orange juice	Figs. 2, 3	II	2	22	1.0 [0.5–1.5]	49.9 [47.0–52.8]	54.1 [53.0–55.3]
S. cerevisiae	2.7	100	Orange juice	Fig. 3	III	2	43	1.7 [1.6–1.8]	55.9 [55.8–56.0]	63.1 [62.5–63.6]
S. cerevisiae	2.7	15	Orange juice	Fig. 3	III	2	40	1.7 [0.9–2.7]	54.6 [52.2–56.9]	62.3 [60.4–64.3]
S. cerevisiae	0.9	1000	Orange juice	Fig. 2	II	2	34	2.2 [1.6–2.8]	62.5 [59.8–65.3]	72.0 [71.9–72.1]
S. cerevisiae	0 (thermal)	0	Orange juice	Fig. 2	0	9	272	1.0 [0.6–1.5]	59.5 [56.0–63.1]	63.8 [61.6–66.0]
S. Senftenberg	20	2	Orange juice	Fig. 2	I	2	28	5.5 [5.2–5.7]	34.0 [32.8–35.2]	57.3 [53.9–60.7]
S. Senftenberg	10	2	Orange juice	Fig. 2	I	2	36	3.5 [0.7–7.9]	49.6 [46.8–52.3]	68.3 [50.2–86.4]
S. Senftenberg	2.7	1000	Orange juice	Fig. 2	II	2	30	1.5 [1.3–1.6]	55.7 [55.2–56.2]	62.0 [61.8–62.1]
S. Senftenberg	0.9	1000	Orange juice	Fig. 2	II	2	36	2.5 [2.3–2.6]	60.7 [60.3–61.1]	71.4 [69.9–72.8]
S. Senftenberg	0 (thermal)	0	Orange juice	Fig. 2	0	9	263	1.2 [0.6–1.9]	62.2 [58.0–66.5]	67.3 [62.8–71.8]
L. plantarum	20	2	Orange juice	Fig. 2	I	2	28	3.3 [3.1–3.4]	40.5 [39.4–41.6]	54.4 [51.9–57.0]
L. plantarum	10	2	Orange juice	Fig. 2	I	2	36	3.7 [1.5–10.8]	55.2 [51.7–58.7]	75.8 [47.2–104.4]
L. plantarum	2.7	1000	Orange juice	Fig. 2	II	2	23	0.9 [0.8–1.0]	52.6 [52.1–53.1]	56.6 [55.2–58.0]
L. plantarum	0.9	1000	Orange juice	Fig. 2	II	2	32	1.9 [1.3–2.4]	65.0 [62.7–67.3]	72.7 [66.2–79.1]
L. plantarum	0 (thermal)	0	Orange juice	Fig. 2	0	10	237	0.8 [0.3–1.3]	59.8 [56.2–63.5]	63.4 [59.6–67.2]

[a] Based on parameter estimates given in Timmermans et al. (2017).

$$\log_{10}\left(\frac{N(t,T \mid E)}{N_0}\right) = \log_{10}\left(\frac{1}{2}\left[\operatorname{erfc}\left(\frac{T - Tc\,(t)}{\sigma\sqrt{2}}\right)\right]\right) \quad (4)$$

Parameter estimation was done via nonlinear least-squares regression using Athena Visual Studio, version 14.2 (www.athenavisual.com).

The Gauss-Eyring model was fitted to single temperature series of collected inactivation data. Mean and standard deviation of the parameter estimates of the duplicate experiments was calculated. To compensate for variation in the number of data points used in each individual experiment, weighted means and weighted standard deviations were calculated.

2.6. Comparison between PEF and thermal treatment

The thermal reference data was analysed by fitting the Gauss-Eyring model to thermal inactivation data. The parameters and confidence interval estimates allow for quantitative statistical comparison to the PEF data. Due to the fast heating and cooling times, this static model is an accurate tool to predict and compare inactivation, both for thermal inactivation as well as for a PEF process.

The temperature-time profile of the PEF process included an exposure of $t = 2.3$ s to a selected maximum temperature (see Fig. 1, section D). Consequently, the thermal reference process should have the same exposure-time of $t = 2.3$ s to this temperature. This short exposure time is below the range of exposure times that could be experimentally determined by the capillary method. Therefore inactivation was extrapolated to $t = 2.3$ s by predictive modelling, based on thermal inactivation of the same strains in orange juice for temperatures and holding times acquired in the range of 46–73 °C and 4–3004 s, respectively (Timmermans et al., 2017).

The model parameters in the Gauss-Eyring model (Mastwijk et al., 2017) are the reference temperature Tr (°C) and Z-value (°C) and are related to the exposure time (t) (Eq. (5)), where $Tc(t)$ is the characteristic temperature where the onset of the inactivation is observed and $\tau_r = 1$ s the chosen unit of time.

$$Tc\,(t) = Tr - Z{\cdot}\log_{10}\,(t/\tau_r) \quad (5)$$

The Z-value expresses the temperature increase/decrease required to reduce/raise the holding time by a factor of ten, and is identical to the expression used in log-linear inactivation kinetics. Tc was recalculated to correspond to a reference exposure time of $t = 2.3$ s to coincide with the holding time in the experiment, using Eq. (5), as

$$Tc\,(2.3) = Tr - Z{\cdot}\log_{10}\,(2.3) \quad (6)$$

Using Eq. (6), the (extrapolated) critical temperatures in a thermal series at the specific chosen holding time of $t = 2.3$ s can in this way be compared to the actual observed critical temperatures for PEF treatment determined at the actual 2.3 s holding time.

Monte Carlo simulations were made ($n = 1000$ simulations, Excel) based on parameter estimates to determine the 95% Confidence Interval (95% CI) of the inactivation curves after PEF or thermal treatment. The 5% and 95% quantiles of the observed data in the simulation define the parameter interval around the Maximum Likelihood. This method accounts for possible unsymmetrical confidence intervals occurring from nonlinear regression, in contrast to linear approximation methods used in most software packages (Poschet et al., 2003; Van Boekel, 2009).

For each single experiment carried out, the temperature that is required to obtain a 5 $\log_{10}$ reduction of the target micro-organism for $t = 2.3$ s holding time was predicted, based on the parameter estimates of the Gauss-Eyring model at $t = 2.3$ s. Data of the single experiments was combined per treatment condition to calculate the mean value, and an independent sample t-test was conducted (Excel) to compare the mean values between two treatments at 5 $\log_{10}$ reduction.

3. Results and discussion

3.1. Influence of electric field strength

The influence of electric field strength on inactivation of *E. coli*, *L. monocytogenes*, *L. plantarum*, *S.* Senftenberg and *S. cerevisiae* in orange juice was determined for $E = 10$ and 20 kV/cm using monopolar pulses of 2 µs (Fig. 2 left panel). Inactivation kinetics of the individual experiments were modelled using the Gauss-Eyring model (Eq. (4)) and model fit and parameter estimates for the different treatments are shown in Fig. 2 and Table 3, respectively. Other data in Fig. 2 will be discussed later. A decrease in the intensity of the electric field strength from 20 kV/cm to 10 kV/cm led to a reduced degree of inactivation for all tested micro-organisms, meaning that a higher electrical energy input (kJ/kg) and corresponding maximum temperature is required to obtain the same level of inactivation.

The value of parameter $Tc(2.3)$ increased at lower electric field strength, indicating that a higher temperature is required to inactivate 50% of the population (Table 3). This observation is in agreement with results of Grahl and Märkl (1996), Heinz et al. (2002), Hülsheger et al. (1983), and Sale and Hamilton (1967), who described increased inactivation at elevated electric field strength. Interestingly, parameter estimates for $Tc(2.3)$ and σ (the temperature width of the distribution) of the yeast *S. cerevisiae* showed smaller differences between conditions at $E = 10$ kV/cm and $E = 20$ kV/cm than was observed for the bacteria, indicating that an elevation of the electric field strength from $E = 10$ kV/cm to $E = 20$ kV/cm has less effect on the degree of inactivation for this species.

Next, it can be seen that the response to the PEF treatment is species dependent, showing highest values for the parameter estimates σ and Tc (2.3) for *L. monocytogenes*, indicating a higher resistance to PEF for this species compared to any other species tested. Especially at $E = 10$ kV/cm, a significantly larger σ value was observed for *L. monocytogenes* compared to the other micro-organisms. To facilitate the comparison among different species and treatment conditions, the temperature required to obtain a 5 $\log_{10}$ reduction was calculated, based on parameter estimates $Tc(2.3)$ and σ given in Table 3. Most values had to be extrapolated from the measured data, because observed microbial inactivation did not exceed 4 $\log_{10}$ reduction. Conditions at $E = 10$ kV/cm showed a higher variance between the duplicate experiments than conditions at $E = 20$ kV/cm, resulting in a larger confidence interval (Table 3). The calculation of *L. monocytogenes* at $E = 10$ kV/cm was not included, as virtually no inactivation was observed within the process conditions tested and parameter estimates could not make a sound prediction.

PEF resistance of the species followed the order *L. monocytogenes* > *E. coli* > *L. plantarum/S.* Senftenberg/*S. cerevisiae*. Differences between the latter three species were small. High PEF resistance of *L. monocytogenes* was also observed by Hülsheger et al. (1983), Grahl and Märkl (1996), and Toepfl et al. (2007), who concluded that smaller sized cells and Gram-positive bacteria require a higher energy input for inactivation compared to larger sized and Gram-negative micro-organisms. Theoretical predictions of the electric field strength required to induce electroporation have been calculated for different sized micro-organisms, and showed that larger sized cells are more susceptible to electric field strengths than smaller sized cells (Heinz et al., 2002; Toepfl et al., 2007). Difference in cell wall composition might also contribute to the PEF resistance. It has been suggested that the thicker peptidoglycan layer of the Gram-positive bacterial cell envelope is responsible for a higher resistance towards PEF, compared to a thinner peptidoglycan layer of Gram-negative bacteria (Hülsheger et al., 1983). This may also explain the differences observed in this study (based on characteristics of the species presented in Table 1), albeit that other intrinsic properties of the micro-organisms as for example stress resistance properties also play a role (Cebrián et al., 2009; Gurtler et al., 2010; Lado and Yousef, 2003).

Moderate intensity electric field strength of 0.9 or 2.7 kV/cm using bipolar pulses of $\tau = 1000$ µs was tested in orange juice for the same species (Fig. 2, middle panel). Inactivation could be modelled using the Gauss-Eyring model, and model fits (Fig. 2) and parameter estimates (Table 3) are provided. Also for this configuration, a higher electric field strength resulted in more inactivation at constant energy input or maximum temperature. The resistance of the species at moderate intensity ($E = 0.9$ and 2.7 kV/cm) and long pulse duration ($\tau = 1000$ µs) followed the same order as at high intensity pulses ($E = 10$–20 kV/cm) of short duration ($\tau = 2$ µs): *L. monocytogenes* > *E. coli* > *L. plantarum*/*S.* Senftenberg/*S. cerevisiae*.

Comparison of the inactivation kinetics for each individual strain showed that conditions at $E = 20$ kV/cm were most effective to reach a 5 log reduction, except for *S. cerevisiae* (Table 3). Interestingly, comparison of the inactivation kinetics of the moderate and high intensity PEF conditions at the same energy input/maximum temperature showed for all species more inactivation at $E = 2.7$ kV/cm using bipolar pulses with a pulse width of 1000 µs compared to monopolar pulses of $E = 10$ kV/cm and pulse width of 2 µs. In addition, a significant difference between the maximum temperature required to reach a 5 $\log_{10}$ reduction was observed for these two PEF conditions, for all species (Table 3). This suggests that not only the electric field strength, but also the duration of a single pulse (pulse width) plays a role in the effectivity of the PEF process.

Although a relationship between electric field strength and minimal total treatment time was noticed before and empirically determined for *E. coli* by Hülsheger et al. (1981), the effect of short and long pulse width, as shown in Fig. 2, has not been described before, and will be discussed in Section 3.2.

3.2. Influence of pulse width

To study the effect of single pulse duration at $E = 2.7$ kV/cm, the PEF equipment was adapted, to reduce the fall time of the pulse from 60 µs to 1.5 µs (Appendix Fig. S6, configuration II and III). As a consequence of this adaption, the number of treatment chambers was increased from 2 to 3. This change in lay-out and fall time did not influence the degree of inactivation, as verified in an inactivation experiment with *E. coli* exposed to bipolar pulses of $\tau = 100$ µs at $E = 2.7$ kV/cm conducted at both configurations II and III (Fig. S7 and Table S1 in Appendix).

The effect of pulse width at identical electric field strength of 2.7 kV/cm was studied for *E. coli*, *L. monocytogenes* and *S. cerevisiae* in orange juice. A clear distinction could be observed when the inactivation kinetics (curves) of the long pulse width of 1000 µs was compared to the inactivation kinetics at shorter pulse width of 100 and 15 µs (Fig. 3, and Table 3). A significant lower temperature was required to obtain a 5 $\log_{10}$ reduction of *L. monocytogenes* and *S. cerevisiae* when pulse width was increased from 100 µs to 1000 µs. No significant differences in temperature requirements for a 5 $\log_{10}$ reduction were observed when the pulse width was increased from 15 µs to 100 µs, for none of the three species tested. Similar effects were reported by Fox et al. (2008), who varied pulse width between 100 ns and 100 µs at an electric field strength of 47 kV/cm and observed no effect on *L. plantarum* inactivation. Also Moonesan and Jayaram (2013) observed no effect when varying the pulse width between 1 µs–7 µs at 40 kV/cm for *E. coli*.

Based on these results, it can be concluded that at constant electric field strength of 2.7 kV/cm longer pulse width ($\tau = 1000$ µs) was more effective than a short pulse width ($\tau = 100$ or 15 µs).

3.3. Comparison of PEF process with an equivalent thermal process

To compare the PEF process described above to a thermal process, a thermal reference process was developed. To this end, thermal inactivation was calculated at an equivalent exposure time of $t = 2.3$ s

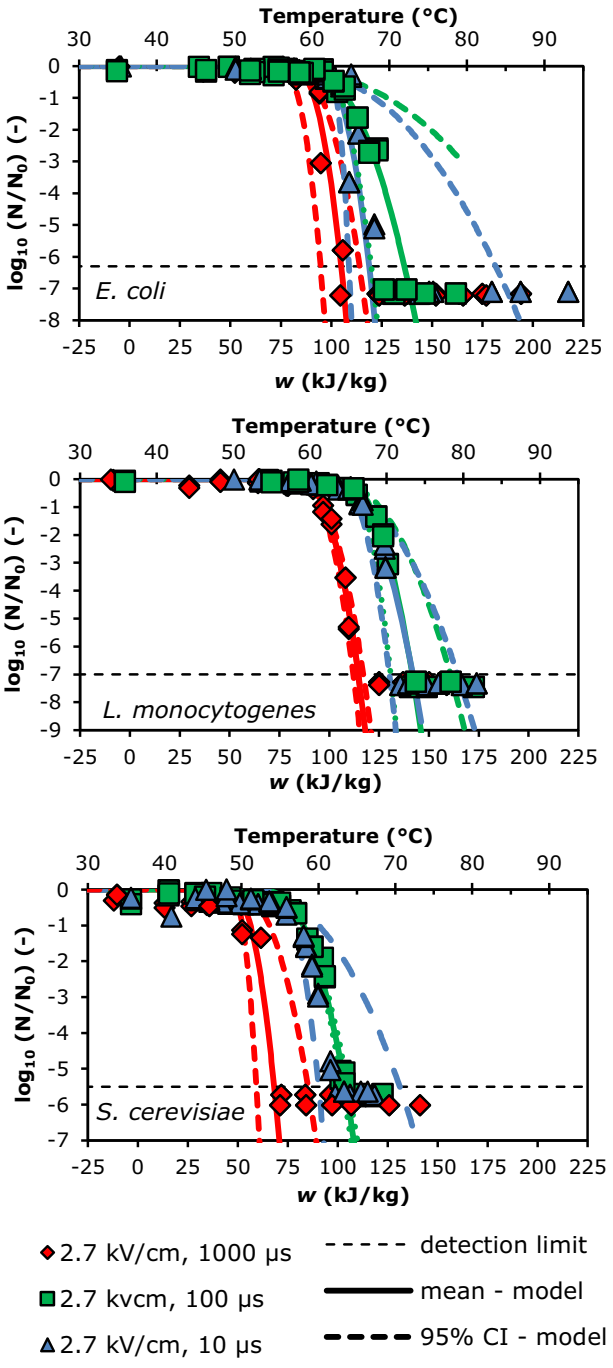

Fig. 3. Inactivation of *E. coli*, *L. monocytogenes* and *S. cerevisiae* in orange juice after PEF treatment, using bipolar pulses at $E = 2.7$ kV/cm with variable pulse duration. Inactivation, $\log_{10}(N/N_0)$, is expressed as a function of maximum temperature (°C) or electrical energy applied (kJ/kg). Symbols represent measured data, lines represent the mean value and confidence interval estimated with the Gauss-Eyring model (Eqs. (4) and (5)).

(Fig. 2, right panel) and parameter estimates were calculated (Table 3, $E = 0$ kV/cm). Since the PEF inactivation and thermal treatment were predicted with the same model, model parameters can be compared.

Comparison of the moderate intensity PEF treatment condition at $E = 0.9$ kV/cm and $\tau = 1000$ µs (Fig. 2, middle panel) to the thermal data (Fig. 2, right panel) showed that for all tested micro-organisms the inactivation kinetics and temperature required to obtain a 5 $\log_{10}$ reduction was significantly lower for the thermal process than for the PEF process. This difference was unexpected, but an underestimation of the thermal equivalent process might be explained by the different

methodologies used; comparing uniform heating in continuous flow PEF to batch-wise conductive heating in capillaries. Berendsen et al. (2015) showed that inactivation kinetics of *Bacillus subtilis* spores in a batch system generally led to a significantly lower *Z*-value than in a continuous flow heating system. Incorporating this effect of a lower *Z*-value in the calculation for a thermal reference, this would lead to a shift of the inactivation curve to the right as presented in Fig. 2 (right panel), requiring a higher maximum temperature to have a 5 $\log_{10}$ reduction than currently presented. The inactivation kinetics at moderate intensity PEF conditions at $E = 0.9$ kV/cm was therefore used as an equivalent continuous thermal process with only 'ohmic heating' and no additional pulse effects. Comparison of the effect of PEF conditions with different pulse width – electric field strength combinations was performed relative to the process conditions at $E = 0.9$ kV/cm. The processing conditions at continuous flow, including preheating, transit time from PEF to the cooler and the cooling profile were identical. Hence, the highest level of discrimination can be expected for latent non-thermal effects from thermal effects.

Comparison of the moderate intensity PEF conditions at $E = 2.7$ kV/cm and $\tau = 1000$ µs to the thermal inactivation data at $E = 0.9$ kV/cm (Fig. 2, middle panel) showed a significant shift of the 2.7 kV/cm inactivation curve to the left, resulting in lower temperature requirement when the electric field strength was increased. A significant lower temperature was required to obtain 5 $\log_{10}$ reduction for $E = 2.7$ kV/cm compared to $E = 0.9$ kV/cm, suggesting that non-thermal effects at $E = 2.7$ kV/cm and $\tau = 1000$ µs enhance inactivation.

The same effect was observed for conditions at $E = 2.7$ kV/cm and $\tau = 100$ and 15 µs (Fig. 3).

Comparison of the high intensity PEF treated data at $E = 10$ kV/cm and pulse width of 2 µs (Fig. 2, left panel) to the thermal data at $E = 0.9$ kV/cm (Fig. 2, middle panel), showed that the $E = 10$ kV/cm inactivation curve completely overlapped with the thermal inactivation curve (*E. coli*, *L. plantarum*, *S.* Senftenberg). The temperature required to obtain 5 $\log_{10}$ reduction, showed no significant difference between $E = 10$ kV/cm and pulse width of 2 µs and the thermal data at $E = 0.9$ kV/cm, for *E. coli*, *L. plantarum*, *S.* Senftenberg and *S. cerevisiae*. Data of *L. monocytogenes* was not compared as no inactivation was measured at $E = 10$ kV/cm up to maximum temperature of 70 °C, the starting point for thermal inactivation of *L. monocytogenes*.

PEF inactivation data at $E = 20$ kV/cm clearly showed a more efficient inactivation curve for all tested micro-organisms when compared to the thermal inactivation curve at $E = 0.9$ kV/cm, conceivably caused by an electroporation effect at the high *E* conditions.

3.4. Effect of different juice matrices

Physical and chemical characteristics of the product matrix can strongly influence the effectivity of PEF inactivation on micro-organisms (Raso et al., 2014). To evaluate if the efficiency of the condition at electric field strength of 2.7 kV/cm and pulse width of 1000 µs would also be affected by the product matrix, inactivation experiments were performed with orange juice (pH = 3.8), coconut water (pH = 5.0) and watermelon juice (pH = 6.0). Temperature dependency of the electrical conductivity was determined and showed the following relation, with electrical conductivity, κ (S/m) as function of temperature (T) to be $\kappa = 0.0019\,T + 0.39$ (coconut water), $\kappa = 0.012\,T + 0.16$ (orange juice) and $\kappa = 0.006\,T + 0.15$ (watermelon juice), meaning that matrices had an electrical conductivity at 36 °C of 0.59 S/m for orange juice, 1.07 S/m for coconut water and 0.37 S/m for watermelon juice. Inactivation data of *E. coli* and *L. monocytogenes* (Fig. 4, Table 3) showed no significant difference in degree of inactivation between the matrices when bipolar pulses of $\tau = 1000$ µs at $E = 2.7$ kV/cm were used, implying that neither the pH of the medium or the conductivity effect the degree of inactivation when moderate intensity PEF was applied.

Contradicting results regarding the effect of pH and conductivity on microbial inactivation using high intensity PEF have been reported in scientific literature, with some studies stating that pH effects inactivation (Saldaña et al., 2010; Wouters et al., 1999) whereas others report no pH influence (Hülsheger et al., 1981; Sale and Hamilton, 1967). Also for conductivity some studies describe effects (Jayaram et al., 1993; Sensoy et al., 1997; Vega-Mercado et al., 1996; Wouters et al., 1999) while others report absence of effects (Álvarez et al., 2003). The different observations and conclusions might be related to the set-up and equipment used, possibly leading to indirect effects, making it difficult to compare data. To overcome these differences, the results in Fig. 4 were compared with our previous study using the same set-up (Timmermans et al., 2014). This study showed, that the efficiency of monopolar pulses of $\tau = 2$ µs at an electric field strength of 20 kV/cm was clearly influenced by the pH of the product matrix, with more inactivation in high acid compared to low acid fruit juices. Therefore, the moderate PEF conditions at $E = 2.7$ kV/cm and $\tau = 1000$ µs, may be applicable to a large group of liquid food products with neutral pH.

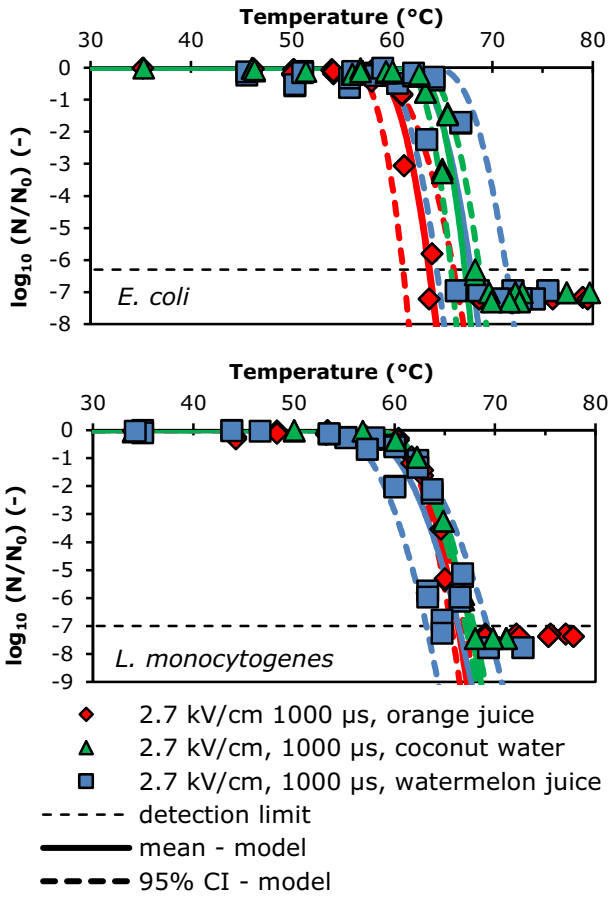

Fig. 4. Inactivation of *E. coli* and *L. monocytogenes* after PEF treatment using bipolar pulses at $E = 2.7$ kV/cm with a duration of $\tau = 1000$ µs, suspended in different fruit juices. Symbols represent measured data, lines represent the mean value and confidence interval estimated with the Gauss-Eyring model (Eqs. (4) and (5)).

3.5. Suggested mechanism for non-thermal effects

The underlying mechanism for the enhanced inactivation at moderate intensity field strength of 2.7 kV/cm and pulse width of $\tau = 15$–1000 µs remains to be determined, but several findings reported previously triggered us to speculate about it.

Often mentioned is the possibility of generating undesired electrochemical species that lead to inactivation. Electrochemical reactions take place at the interface between the electrode and the liquid. The specific electrochemical reactions that are triggered are dependent on

the electrolyte, the electrode material and the pulse conditions used. If electrochemical reactions would occur at an electric field strength of $E = 2.7$ kV/cm using $\tau = 15$–1000 µs pulses, this might contribute to the microbial inactivation. Electrochemical reactions were largely avoided by using bipolar pulses (Roodenburg, 2007). In addition, titanium electrodes were used to minimize potential formation of toxic species by electrode degradation since this material has a low electrochemical degradation rate when in contact with a water based electrolyte (Kempkes, 2010). If reactions between the electrode material with the food products takes place, titanium dioxide is the most likely product to be formed. As titanium dioxide has been reported to have antimicrobial properties (Kubacka et al., 2014), a new study should be performed to exclude a role of titanium dioxide. The combination of bipolar pulsed ohmic heating was demonstrated to minimize electrochemical yield of reactions and the formation of gas bubbles compared to ohmic heating (Samaranayake et al., 2005). Samaranayake and coworkers concluded that pulsed ohmic heating at lower frequencies and longer pulse widths up towards $\tau = 100$ µs are more effective in suppressing the electrochemical reactions at the surface of titanium electrodes. A minimum delay time of $\tau = 10$–15 µs between the positive and negative pulses was found to be a critical factor to avoid hydrogen generation (Samaranayake et al., 2005).

Different pathways for the electroporation of biological cell membranes have been proposed. The most widely accepted theory involves the induction of an (external) electric field applied on the lipid membrane, leading to local instabilities and finally resulting in formation of pores in the membrane (electroporation) (Teissié et al., 2005; Weaver and Chizmadzhev, 1996). Due to the electroporation effect, the permeability of the cell membrane increases (electropermeabilization) and, depending on the applied electric field strength, leads to either cell death (irreversible, complete inactivation) or resealing of the cell membrane and recovery (reversible, sub-lethal or partial inactivation) (Saulis, 2010). It is assumed that at least an electric field strength of 5–10 kV/cm is required to cause irreversible electroporation of the microbial cell membrane (Jaeger et al., 2016; Raso, 2016). The observation in the present study, that no additional non-thermal pulse effect was found at $E = 10$ kV/cm next to the thermal element responsible for microbial inactivation is in line with this. Application of pulses with an intensity of $E = 20$ kV/cm and a duration of $\tau = 2$ µs are likely responsible for pore formation in the lipid domain, as this is proven for red blood cells (Zimmerman et al., 1974), model membranes (Neumann and Rosenheck, 1972) and bacteria (Hamilton and Sale, 1967). As pore formation is dependent on both the intensity of the pulses and the duration of a single pulse (Neumann et al., 1992; Tsong, 1990) it might be possible that the application of $E = 2.7$ kV/cm and $\tau = 15$–1000 µs in this study also acts on the lipid domain, but that different sized pores are formed than when high intensity pulses of short duration were applied.

A study by Tsong (1990) points to an alternative mechanism targeting the protein channels, transport systems or transport pumps. The cell membrane contains many types of membrane transport systems, like pumps and specific channels for cations and anions, imbedded in the lipid bilayer, and several of these channels or pumps are voltage sensitive. The gating voltage to open a protein channel in *E. coli* is typically below 100 mV, which is considerably lower than the 1000 mV required for pore formation in the lipid double layer of the membrane (Schoenbach et al., 1997). Once a channel is forced to open by an excessive potential induced by the applied external field, it will experience an enormous local heating due to the high current density passing through the channel and as a consequence it may be thermally denatured (Tsong, 1990). In this scenario, the longer pulses of $\tau = 15$–1000 µs at an electric field strength of $E = 2.7$ kV/cm, may denature the transport proteins. The observation in the present study showed additional non-thermal effects at longer pulse width of 15–1000 µs, while no effects were observed at pulse width of 2 µs.

3.6. Conclusion and industrial relevance

In this study we show that moderate intensity pulsed electric fields (at $E = 2.7$ kV/cm and $\tau = 15$–1000 µs) is a very efficient, and easy to scale, process to pasteurize fruit juices that could be used as alternative to higher electric field PEF processes at $E = 20$ kV/cm and $\tau = 2$ µs.

A clear advantage of the moderate field PEF at $E = 2.7$ kV/cm and $\tau = 1000$ µs is that it is applicable to both high-acid and low-acid products, in contrast to conditions at $E = 20$ kV/cm and $\tau = 2$ µs that are only suitable for high-acid products. Furthermore, the moderate intensity conditions at $E = 2.7$ kV/cm and $\tau = 1000$ µs showed minor differences in degree of inactivation between the different microbial species tested, while high intensity conditions at $E = 20$ kV/cm showed larger differences between the microbial species. In addition, it is expected that industrial application and scaling up of the moderate intensity electric field strength is easier compared to application of higher electric field strength.

Taken together, the inactivation data indicates that conditions of $E = 2.7$ kV/cm and $\tau = 1000$ µs can be used as a PEF process for the preservation of liquid food products. The lower maximum temperature required and shorter holding time when compared to a conventional thermal pasteurization process are expected to result in a better product quality, which is the subject of investigation in a forthcoming publication.

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Declaration of interest

Conditions described in this work are subject of a pending patent application, WO2017086784.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2019.02.015.

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