Current Research in Food Science 5 (2022) 1906–1915

Contents lists available at ScienceDirect

Current Research in Food Science

journal homepage: www.sciencedirect.com/journal/current-research-in-food-science

Microbial inactivation of milk by low intensity direct current electric field: Inactivation kinetics model and milk characterization

Feihong Ji [a], Jing Sun [a], Yiming Sui [a], Xiangming Qi [a,b,*], Xiangzhao Mao [a]

[a] College of Food Science and Engineering, Ocean University of China, Qingdao, 266003, China
[b] Shandong Meijia Group Co. Ltd., Rizhao, 276826, China

ARTICLE INFO

Keywords:
Electric field
Low intensity direct current electric field
Inactivation kinetics
Milk
Microflora
Physicochemical properties

ABSTRACT

Microbial inactivation by pulsed electric field (PEF) has been studied widely although with high operational risk, while few studies on the potential of low intensity electric fields for microbial inactivation have been reported. In this study, the feasibility of inactivating microorganisms in milk by low intensity direct current (DC) electric field was investigated. Then a kinetics model was proposed based on the inactivation curves. Finally, the effect of electric field on the microflora and physicochemical properties of milk was analyzed. Results showed that the bacterial reduction >5 log CFU/mL could be achieved at 50–55°C, 0.3 A–0.6 A, and with 5 min starting intensity of 5 V/cm-9 V/cm. The inactivation kinetics consisted of three stages, therein, the middle stage, main part of the inactivation curve, followed 1st-order reaction kinetics, and the effect of temperature on it was consistent with the Arrhenius Law, which implied that the electric field itself can inactivate bacteria without thermal inactivating effect. The microflora analysis showed that naturally occurring bacteria in the milk contained typical potential pathogenic bacteria (e.g., 56.9% of *Acinetobacter* spp.) and spoilage bacteria (e.g., 27.5% of *Pseudomonas* spp.), and the electric field can inactivate them. Moreover, the inactivation chemically preserved the milk's fresh-like characteristics (according to indexes of whey protein denaturation rate, furosine content), and physical stability (turbidity, zeta potential, particle size, color and so on). Therefore, a promising approach is provided for microbial inactivation in dairy industry.

1. Introduction

Milk is a type of food that provides balanced nutrition for human beings. As is rich in protein, carbohydrate, fats, vitamins and minerals, it is very beneficial to human health (Chen et al., 2014). Thus, there are many dairy products, such as yoghurt (Lang et al., 2022) and, cheese (Arias-Roth et al., 2022) that are favored by consumers. However, the abundant nutrients and high-water activity of milk make it easy to be contaminated by microorganisms during collection, transportation, processing and storage (Tian and Li, 2019; Stratakos et al., 2019).

Safety incidents arising from raw milk consumption occur frequently in Europe and the United States (US). Most of them were caused by harmful microorganisms that contaminated milk (EFSA report, 2015; CDC food safety raw milk outbreak studies, 2017). Consequently, many countries had introduced legislations that do not recommend consumers to drink raw milk directly (Linn, 2019; , 2022Clark and Harte). Nevertheless, because fresh-like milk, many consumers still risk drinking raw milk. So, how to eliminate harmful microorganisms without changing the biological activity and taste of milk has always been the ultimate pursuit of milk sterilization (Liao et al., 2019).

In traditional thermal sterilizations, some techniques try to balance preserved taste and extended shelf life, for instance pasteurization. Others achieve sterilization at the expense of taste and bioactive substances, for instance ultra-high temperature sterilization. Some potential innovative thermal inactivation methods, such as radio frequency (RF) heating (Zhao et al., 2022), may give better results. With strong demand for fresh-like milk, the non-thermal methods, such as high-pressure (Stratakos et al., 2019), plasma (Gurol et al., 2012), ultrasonic (Li et al., 2018) and electric field (Machado et al., 2010; Walter et al., 2016; Boudjema et al., 2019; Al-Hilphy et al., 2020) are increasingly explored.

By now, the most commonly used electric field in microbial inactivation is high-voltage pulse electric field (PEF), which has been proven to have slight impact on physicochemical and nutritional properties (Niu et al., 2020). However, it is difficult to industrialize and risky to operate

Abbreviations: DC, Direct current; PEF, Pulsed electric field; RF, Radio frequency; RMSE, Root mean squared error.

* Corresponding author. College of Food Science and Engineering, Ocean University of China, Qingdao, 266003, China.
E-mail address: qixm@ouc.edu.cn (X. Qi).

https://doi.org/10.1016/j.crfs.2022.10.015
Received 10 July 2022; Received in revised form 6 October 2022; Accepted 10 October 2022
Available online 12 October 2022

since the electric field voltage is up to several kV (Machado et al., 2010). RF heating, as mentioned, has rapid microbial inactivation effect, but it mainly uses electric field to obtain thermal inactivating effect. Rencently, electric field at lower currents or field intensities has attracted more interest in microbial inactivation (Machado et al., 2010; Boudjema et al., 2019; Al-Hilphy et al., 2020). In these studies, the electric field intensity has been gradually reduced from 50 to 280 V/cm (Machado et al., 2010) to 8.33–20.8 V/cm (Al-Hilphy et al., 2020), and the technique has lately been used for milk's microbial inactivation (Al-Hilphy et al., 2020). Here, lower energy consumption and ease of operation were the common attempts.

However, in almost all researches, the temperature has not been monitored or controlled. Whether the inactivation should be attributed partially or completely to thermal effect, in other word, whether the electric field alone inactivates microorganisms, remains unclear. Meanwhile, most of the studies were conducted with broths of specific bacteria (Machado et al., 2010) or simulated food systems inoculated with specific bacteria (Alkhafaji and Farid, 2008), which ignores the complexities of real food systems and naturally occurring bacteria. In addition, the electric field intensity or current intensity of the above studies were still high. It is also worth to explore to what extent the microbial inactivation by this electric field remains at lower field intensity.

So, this work firstly aims to verify the feasibility of inactivating naturally occurring microorganisms in incubated raw milk at low-intensity (less than 9 V/cm) DC electric field. Then, the inactivation kinetics under different conditions were investigated, and to what extent the inactivation was attributed to thermal effect was determined. Meantime, the microflora change of incubated milk was analyzed using high-throughput sequencing technology, so as to detect whether the inactivation was applicable to various naturally occurring bacteria. Finally, in order to figure out if the inactivation can meet the fresh-like milk demand, the physicochemical properties of raw milk were characterized.

2. Materials and methods

2.1. Materials and experimental equipment

2.1.1. Materials

The raw milk used in the experiments was obtained from a local dairy farm in Qingdao (Shandong, China) and stored temporarily (within 12 h) in a refrigerator at 4 °C before use. The total bacterial count of raw milk was tested to be 2–5 log CFU/mL, depending on the seasons. All chemicals used were of analytical grade and obtained from Sigma-Aldrich (St. Louis, MO), unless otherwise specified.

In order to increase natural bacterial counts of the milk samples to 7–8.5 log CFU/mL (be greater than 5 log CFU/mL), the raw milk was placed in an oscillating incubator at 30 °C for 10–12 h after collection. This incubated milk was used as the control sample and experiment material in the investigations of inactivation kinetics (Section 2.2.1) and microflora analysis (Section 2.2.2). While, for physicochemical properties (Section 2.2.3), fresh raw milk was used as control and raw material instead of the incubated milk.

2.1.2. Experimental equipment

An equipment was designed for the inactivation experiments, as shown in Fig. 1. It mainly consists of a DC power supply (MN-1003 A, ZHAOXIN Electronic Instrument Equipment Co., Ltd. Shenzhen, China), a sample treatment chamber, and a magnetic stirring water bath.

The power supply provides 0–100 V in constant electric field intensity mode or 0–3 A in constant current mode. A transparent plastic sample treatment chamber of 10 cm in length, 6 cm in width and 10 cm in height was customized according to the need of sample volume. For a sterile experimental circumstance, the chamber was placed under UV lamp for 30 min before use. A magnetic stirring water bath was applied outside the treatment chamber to control temperature, as shown in Fig. 1.

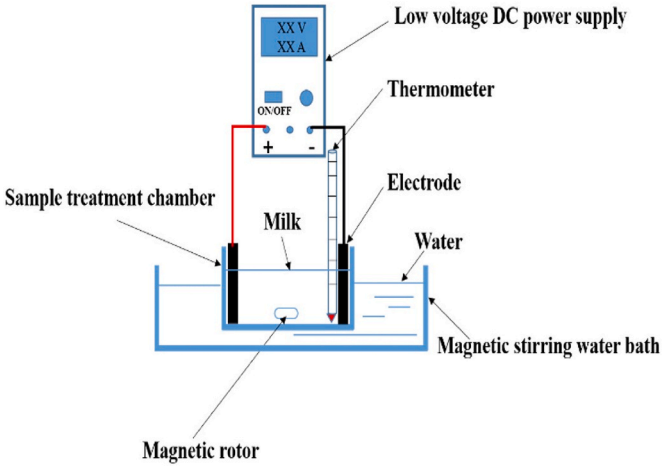

Fig. 1. Experimental device diagram.

2.2. Experimental methods

2.2.1. Inactivation of microorganisms in milk by low intensity DC electric field

A volume of 200 mL incubated milk was added to the sample chamber. Effects of temperature, current and starting electric field intensity on the microbial inactivation were investigated. The incubated milk was taken as control sample, and then the first sample was taken after the first 5 min treatment. In the following process, samples were taken every 30 min and their bacterial counts were tested. Meanwhile, the current, field intensity, and temperature were recorded every 5 min during the inactivation, respectively.

The effect of temperature (35 °C, 40 °C, 45 °C, 50 °C, 55 °C) was investigated with a constant current of 0.5 A and 5 min starting electric field intensity of 9 V/cm. Compared to the inactivation in electric field, a control experiment was executed in 50 °C water bath without electric field. The effect of current intensity (0.2 A, 0.3 A, 0.4 A, 0.5 A, 0.6 A) was investigated at 50 °C and with 5 min starting electric field intensity of 9 V/cm. The effect of starting electric field intensity (5 V/cm, 6 V/cm, 7 V/cm, 8 V/cm, 9 V/cm) was investigated at 0.5 A and 50 °C, where the starting electric field intensity acted in the first 5 min. The kinetics analysis was conducted by the results of the above-mentioned experiments.

2.2.2. Microflora change in milk after inactivation with electric field

The inactivation at 0.5 A and 50 °C with 5 min starting electric field intensity of 7 V/cm for 270 min was taken for high throughput sequencing analysis (marked as DC.0 d). The incubated milk (marked as CON.0 d) and heat-sterilized milk (heated at 72 °C for 15 min, marked as HT.0 d) were investigated as well. In addition, the remaining samples were stored at 4 °C for 7 days and labeled as CON.7 d, DC.7 d, HT.7 d. Finally, DNA of the above 6 samples were extracted, amplified and sequenced.

2.2.3. Evaluation of the physicochemical properties of milk after inactivation

Here, the real raw milk was treated at 0.5 A and 50 °C with 5 min starting electric field intensity of 7 V/cm. The samples were taken every 30 min. Their pH value, furosine content, whey protein denaturation rate, turbidity, zeta potential, particle size, lipid droplet size, color and viscosity were then measured.

2.3. Testing methods

2.3.1. Microbial enumeration

The total bacterial count (TBC) was measured by the spread plate method. 100 μL sample was taken and serially diluted 10 times with 0.85% sterile saline. Then the 100 μL dilutions were spread on Plate Count Agar (PCA) and incubated at 37 °C for 48 h. After 48 h, the number of colony-forming units (CFU) were counted and the results were expressed as log CFU/mL.

For the microbial inactivation, the inactivation effect was expressed by $\log_{10}(\frac{N_t}{N_0})$, where N_t and N_0 represent the bacteria count at time t and before the electric field treatment, respectively.

2.3.2. DNA extraction, amplification and sequencing

Genomic DNA of the 6 samples for microflora analysis (Section 2.2.2) were extracted by cetyltrimethylammonium bromide (CTAB) method. Briefly, 1000 μL of CTAB lysis solution and proper amount of lysozyme and sample were mixed into a 2.0 mL EP tube, then incubated in a 65 °C water bath to allow the sample to fully lysis. Then the mixture was centrifuged (12,000 rpm, 10 min) to obtain the supernatant. Phenol (pH = 8.0): chloroform: isoamyl alcohol (25:24:1) was added and mixed well. The mixture was centrifuged again and chloroform: isoamyl alcohol (24:1) was added to the centrifuged supernatant. After centrifugation again, the supernatant was sucked into a new EP tube, isopropanol was added and, mixed then placed at −20 °C for precipitation. The samples were centrifuged again, and the precipitates were collected and washed twice with 1 mL of 75% ethanol. The precipitates were collected by centrifugation and dried naturally. Then, ddH_2O was added to dissolve the DNA sample, and 1 μL of RNase A was added to digest the RNA to obtain pure DNA. 16 S V3–V4 region of rRNA genes were amplified using the specific primer 341 F (CCTAYGGGRBGCASCAG) and 806 R (GGACTACNNGGGTATCTAAT) with the barcodes. Polymerase chain reaction (PCR) was carried out following the protocol described by Caporaso et al. (2011). The tag-encoded high throughput sequencing was carried out by Illumina HiSeq platform (Novogene Bio-information Science and Technology Co., Ltd., Beijing, China).

2.3.3. Chemical stability characterization

Whey protein denaturation rate, pH value and furosine content of the samples from Section 2.2.3 were characterized. The pH meter (PHS–3C, INESA SCIENTIFIC INSTRUMENT Co., Ltd, Shanghai, China) was calibrated with pH standard buffers at 25 °C, and the pH values of samples were measured using the calibrated pH meter.

Whey protein denaturation rate was measured according to the methods described by Li et al. (2018) with some modifications. The samples were centrifuged at 4000 rpm, 4 °C to remove fat. The pH was then adjusted to 4.6 with 1 mol/L HCl solution and the samples were centrifuged to remove the precipitate. Un-denatured whey protein content in the supernatant was measured by Bradford protein assay kit (Beyotime Biotechnology, Shanghai, China). Then the whey protein (WP) denaturation rate was calculated by Eq. (1):

$$\text{WPDR}\ (\%) = \frac{\text{electrically untreated protein content} - \text{electrically treated protein content}}{\text{electrically untreated protein}} \quad (1)$$

Furosine content was measured according to the method described by Li et al. (2021).

2.3.4. Physical stability characterization

Turbidity, zeta potential, particle size, lipid droplet size, color and viscosity of the samples from Section 2.2.3 were characterized. The turbidity was measured using a UV–Vis spectrophotometer (UV-5200, Metash Instruments Co., Ltd, Shanghai, China) (Shanmugam et al., 2012). The zeta potential and average particle size were measured using Malvern Zetasizer Nano ZS90 (Malvern Instruments, Ltd., Malvern, UK) (Shanmugam et al., 2012).

The lipid droplet size was observed in optical microscope (using 100 x oil microscope and 10 x eyepiece) by taking 1 mL milk and 1% Sudan Red III dye for mixing (Scudino et al., 2020). The results were listed in Fig. S4.

The color parameters, L^* (lightness), a^* (redness) and b^* (yellowness), were detected using a Konica Minolta Spectrophotometer (CR 400, Konica Minolta Investment Ltd., Japan) based on the CIELAB color space (Yang et al., 2020). The total color difference (ΔE) was calculated by Eq. (2):

$$\Delta E = \sqrt[2]{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2} \quad (2)$$

where L_0, a_0, b_0 represents the average color parameters of the milk untreated, and L, a, b represents the average color parameters of the milk electrically treated, respectively.

The viscosity of milk samples was measured by rheometer (MCR 301, Anton Paar GmbH, Graz, Austria) at 25 °C. Viscosity data were obtained by varying the stress at a shear rate range of 20–100 S^{-1}.

2.4. Analytical methods

2.4.1. Kinetics model fitting analysis

The inactivation mentioned in Section 2.2.1 can be divided into three stages. Here the middle stage takes the majority of the process, so kinetics analysis mainly focused on this stage. From the experimental results, first-order reaction kinetics model (Eq. (3)) was the most suitable for fitting the middle stage.

$$\log_{10}\frac{N_t}{N_0} = -\frac{1}{D}t \quad (3)$$

Where N_t and N_0 represent the bacteria count at time t and before treatment, respectively, D is the time taken to kill 90% of bacteria in the sample, and t is treatment time.

The goodness of the model fitting was tested by R-square (R^2) and root mean squared error (RMSE) values using software Origin (V 2018) (Chen et al., 2019; Zhao et al., 2019).

2.4.2. Bioinformatics analysis

The genomic sequences were submitted to the NCBI under the BioProject accession number PRJNA871761. It was assembled using the software FLASH (V 1.2.7, http://ccb.jhu.edu/software/FLASH/), and the quality controlled was assessed using the software QIIME (V 1.9.1, http://qiime.org/scripts/split_libraries_fastq.html) to obtain effective data. The effective data of all samples were clustered with 97% uniformity using the software Uparse (V 7.0.1001, http://drive5.com/uparse/) to obtain OUTs. Species annotation analysis was performed using the Mothur method with the Silva Database (http://www.arb-silva.de/) to obtain taxonomic information. In order to study phylogenetic relationship of different OTUs, and the difference of the dominant species in

different samples, multiple sequence alignment was conducted using the software MUSCLE (V 3.8.31, http://www.drive5.com/muscle/).

2.4.3. Statistics analysis

All experiments were repeated three times. Data analysis was conducted using the software SPSS (V 22). Data were expressed as mean ± standard deviation (SD), and means of values were performed by one-way ANOVA ($p < 0.05$ was regarded significant). Pictures were drawn by using R and software Origin (V 2018).

3. Results and discussion

3.1. Feasibility of inactivating microorganisms in milk by low intensity DC electric field

In order to evaluate the potential of this new technique to inactivate all microorganisms in milk, here the milk with natural microflora was applied rather than inoculated samples with specific bacteria. Moreover, the naturally-occurring bacterial counts of the milk samples were incubated to 7–8.5 log CFU/mL. Compared to raw milk, no evident differences in the physicochemical properties (e.g., fluidity and odor) of incubated milk was found.

Fig. 2a proved that, at temperatures below 55 °C, microorganisms in the milk could be thoroughly inactivated using low intensity DC electric field (the intensity of these experiments was around 5 V/cm, as shown in Fig. S1). Compared to the work of Boudjema et al. (2019), which achieved sterilization in DC electric field, in this study, the DC electric field of lower current intensities (below 0.5 A in this experiment) also inactivated microorganisms in liquid food material, milk (as shown in Fig. 2b). Moreover, here it is proven that all the naturally occurring microorganisms in the milk can be inactivated. In a previous study (Boudjema et al., 2019), only *Escherichia coli* was inactivated in aqueous solution under 3 A DC electric field.

In the current of 0.5 A, microorganisms of 7.5 log CFU/mL in the incubated milk were completely inactivated, which met the target of milk pasteurization (greater than 5 log CFU/mL) (Cole et al., 2022). Also, complete inactivation of 4.8 log CFU/mL microorganisms in fresh raw milk could be achieved in the low intensity DC electric field.

As for temperature, more than 60 °C is often required in conventional thermal sterilization (Bezie, 2019). In this study, all the experiments were carried out below 55 °C. A control experiment showed that inactivation of 50 °C (Fig. 2a) without the electric field only achieved 2.5 log CFU/mL reduction, while bacteria at 7.5 log CFU/mL could be thoroughly inactivated in the electric field after 270 min. In the reported work (Boudjema et al., 2019), the uncontrolled temperature reached 80 °C eventually, therefore, it was impossible to distinguish whether the inactivation therein was mainly attributed to temperature or electric field. The results in this study demonstrated that the low intensity DC electric field itself, not thermal effects, dominated the inactivation.

Additionally, compared with the other two researches on microbial inactivation in moderate electric field (Machado et al., 2010; Al-Hilphy et al., 2020), these experiments challenge the known perception. It demonstrated the potential of low-intensity DC fields to sterilize food materials, even in the presence of matrix effects, since milk exhibited strong matrix effects in various sterilization techniques (Osaili et al., 2009; Bai et al., 2022; Ruiz-De Anda et al., 2022). Consequently, it technically confirmed that, in such low-intensity steady DC electric fields (at currents below 0.5 A or at field intensities below 5 V/cm, as shown in Fig. S1), without thermal inactivating effect, inactivating microorganisms in milk was feasible.

3.2. Kinetics model of microbial inactivation in milk by low intensity DC electric field

As a novel microbial inactivation technique, the process is worthy of further exploration. Hence, the kinetics were analyzed, and the effects of

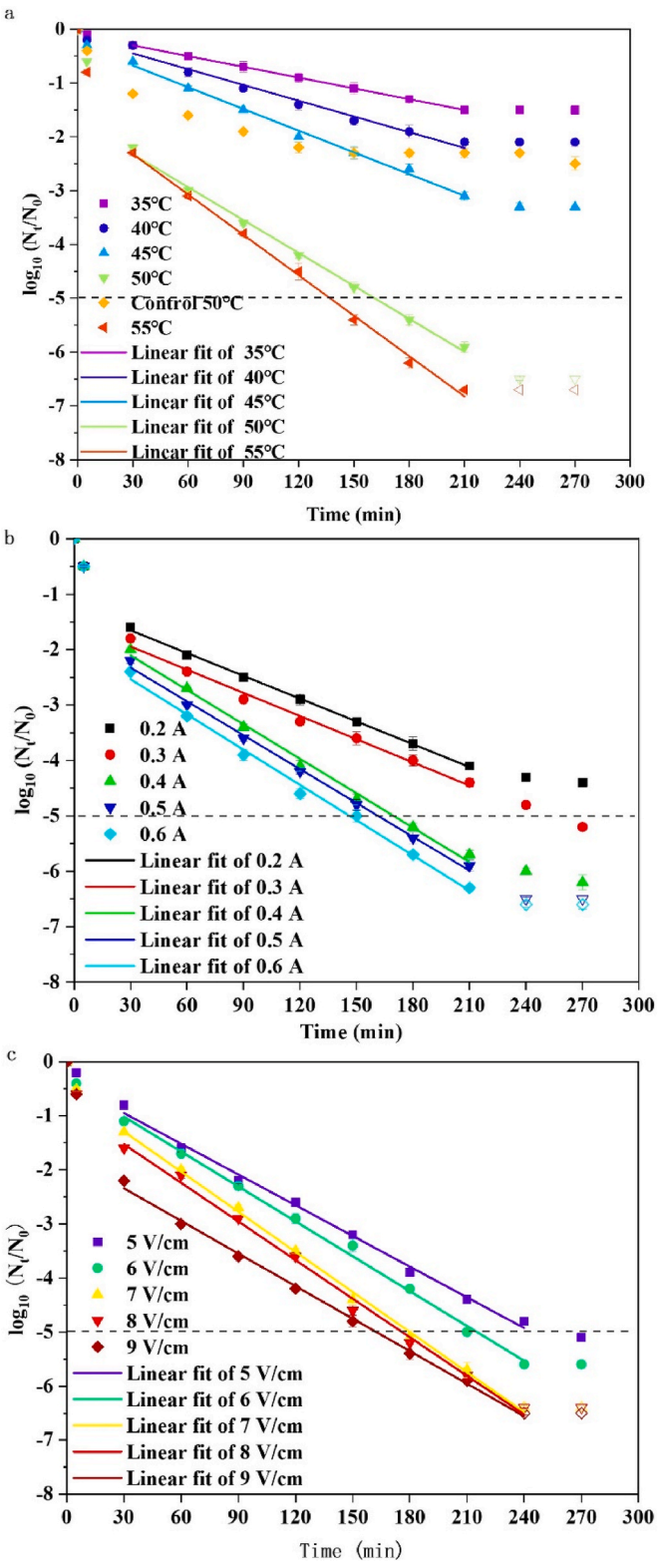

Fig. 2. Inactivation kinetics of low intensity DC electric field on the total number of bacteria in milk. The inactivation process consisted of three stages, the middle stage followed first-order reaction kinetics. The hollow points in the figures mean that all bacterial were killed. Note: (a) Different temperatures (35°C–55 °C), (b) Different currents (0.2 A–0.6 A) and (c) Different starting electric field intensities (5 V/cm-9V/cm). Table 1 lists the model fitting and evaluation parameters.

temperature, current and starting electric field intensity on it were investigated (as shown in Fig. 2a, b, 2c). It is also expected that this kinetics investigation will further lead to some clues on the inactivation mechanism.

It can be seen that, regardless of changing temperature, current or starting electric field intensity, the process always can be clearly divided into three stages: a rapid decrease in the first stage (about 30 min), a linear decrease in the middle stage and a slowdown decrease in the last stage. It is a new form of inactivation kinetics curve. So, a new three-stage kinetics inactivation model is proposed here.

The linear middle stage takes the majority of the process, from 30 min to 210 min. The middle stages were fitted using Eq. (3) (as shown in Fig. 2 and Table 1). From Table 1, it was seen that almost all results of R^2 were above 0.99, and average RMSE value was 0.071, 0.088, and 0.104 respectively when temperature, current, and staring intensity were changed. RMSE is generally considered as the most informative goodness index of non-linear and linear fittings, and lower RMSE values indicate better fitness of the model (Chen et al., 2022). Both the results strongly suggested that the middle stage was a first-order reaction kinetics process.

As to the other two stages, the reason for the rapid inactivation in the first stage and the slower inactivation in the last stage may be attributed to the resistance difference of various microorganisms to electric fields. Similar phenomena and attributions have been reported in other sterilization techniques (El-Hag et al., 2011; Huang et al., 2014).

3.2.1. Effect of different temperatures on inactivation kinetics

Temperature had a positive effect on the inactivation in this experiment (Fig. 2a). Although similar law has been found in other sterilizations (Amiali et al., 2007; Saldaña et al., 2010), the predominant role of the electric field in this process has been clearly fixed in the foregoing discussion (See section 3.1). However, how the combined effect of temperature and electric field is in the inactivation, in other words, whether temperature herein also contributed to some chemical reactions that lead to the inactivation of microorganisms, remains unclear.

Taking the microbial inactivation as a chemical reaction, the $1/D$ in Eq. (3) is directly proportional to the reaction rate constant k (The deduction was shown in S1). According to Arrhenius' law, the relationship between $\ln k$ and $\frac{1}{T}$ is shown in Fig. 3 (The deduction was shown in S2). As can be seen, the change of $\ln k$ value from 35 to 50 °C was consistent with Arrhenius' law, while the $\ln k$ value at the 55 °C point decreased slightly, which indicated that, in this process, at temperatures

Table 1
Model fitting and evaluation parameters under different conditions.

Condition		Model fitting parameter		Evaluation parameter		
		D	log N_0 CFU/mL equivalent	R^2	RMSE	Average RMSE
Temperature (°C)	35	150	7.2	1	0	0.071
	40	102	7.2	0.974	0.103	
	45	74.6	6.9	0.993	0.075	
	50	49	5.3	0.996	0.082	
	55	40	5.4	0.997	0.094	
Current (A)	0.2	73	5.9	0.999	0.033	0.088
	0.3	72	5.7	0.988	0.101	
	0.4	48.3	5.5	0.993	0.111	
	0.5	49	5.3	0.996	0.082	
	0.6	47.2	5.2	0.993	0.111	
Starting intensity (V/cm)	5	52.9	6.7	0.992	0.113	0.104
	6	46.5	6.4	0.988	0.107	
	7	40.5	6	0.992	0.087	
	8	42	5.8	0.99	0.133	
	9	49.8	5.2	0.984	0.081	

Note: For the regression, the point of the first 30-min is taken as t_0 (the first point of the middle stage), and the resulting log N_0 equivalents (the log CFU/mL of 30 min) are shown in Table 1.

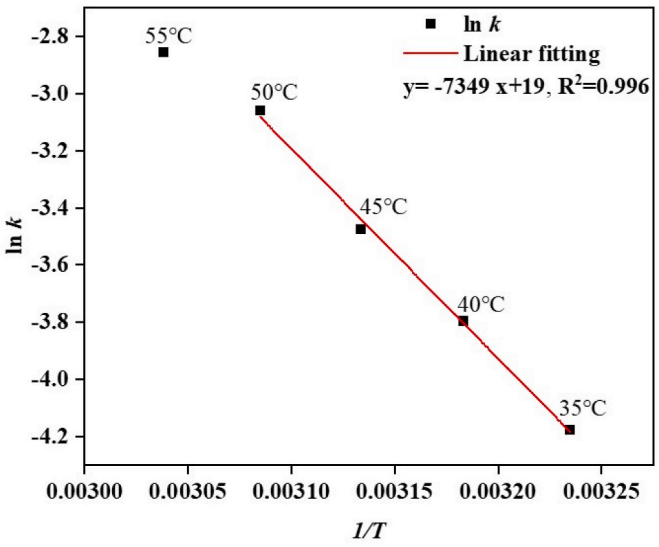

Fig. 3. Arrhenius plot (relationship between $\ln k$ and $\frac{1}{T}$) of low intensity DC electric field inactivation of bacteria.

below 50 °C, temperature only exerted a physical influence and did not cause chemical changes (Cheng et al., 2013). It is suggested that the chemical changes leading to inactivation during this process were only caused by electric fields at temperatures below 50 °C.

As for 55 °C, the deviation from Arrhenius' law means that, temperature here might have caused some kind of chemical change that impacted the inactivation effect (Huang, 2019). Interestingly, the chemical effect of temperature on the inactivation seemed to be negative, the reason for which needs further research. As a result, subsequent experiments were carried out at 50 °C.

3.2.2. Effect of different currents on inactivation kinetics

Experiments have shown (Fig. 2b) that the current also had a significant positive effect on the inactivation. Intuitively, the increase of current might have a positive effect on both the first and middle inactivation stage. But here, in the first stage the increase of current resulted in better inactivation, while in the middle stage, the inactivation effect of current appeared to differ between the two investigated ranges: below 0.3 A and above 0.4 A. As shown in Table 1, the D value below 0.3 A seemed to be a constant while above 0.4 A it seemed to be another. In other word, in the range below 0.3 A or above 0.4 A, the increase of current seemed to only slightly affect the inactivation of middle stage.

The possible reason for this phenomenon is that the protective effect of milk components makes the microorganisms in milk insensitive to the electric field below 0.4 A (Kim and Kang, 2015; Kim et al., 2017). From 0.3 A to 0.4 A, the effect of current on the middle stage also rose abruptly.

When the current was raised from 0.4 A to 0.6 A, although a slightly better inactivation effect was obtained, the margin of this increase was rather limited. Further considering that at this point the heat production of the electric field gradually increased, and therefore the temperature control became more difficult, the other experiments were conducted at 0.5 A.

3.2.3. Effect of different starting electric field intensity on inactivation kinetics

For better inactivation effect and shorter treatment time, the effect of different starting electric field intensity at 0.5 A and 50 °C was tested (Fig. 2c). It was seen that the increase of starting electric field intensity also affected the inactivation. Compared to current, higher starting electric field intensity was more beneficial to the first stage inactivation. However, interestingly, starting electric field intensity also had effects

on the middle stage inactivation, which was positive before 7 V/cm and then negative with starting electric field intensity increasing, although it lasted as short as 5 min at the beginning.

Correspondingly the *D* values showed an increasing trend before 7 V/cm and then decreasing (Table 1). It was speculated that the short duration of higher electric field intensities has caused microorganisms to undergo stress physiological changes. Similar phenomena have been reported in other inactivation techniques (Lou and Yousef, 1996; Liao et al., 2018). Hence, the starting electric field intensity increase needs to be limited to a relatively effective range.

3.3. Milk microflora change after inactivation with electric field

To reveal inactivation selectivity to bacteria in the low intensity DC electric field, microflora of the incubated milk samples before and after inactivation was investigated using high throughput sequencing techniques (Fig. 4a, b, 4c).

From Fig. 4a, it can be seen that the community of control sample (CON.0 d) consisted mainly of *Pseudomonas*, *Acinetobacter*, *Pantoea, Lactococcus*, and, *Serratia.* Among them, there were both potential pathogenic bacteria and spoilage bacteria. *Pseudomonas* spp. and *Acinetobacter* spp. were the dominant genera, accounting for 27.5%, and 56.9%, respectively. As reported previously (Zhang et al., 2019; Stratakos et al., 2019), both of them were the dominant genera of raw milk in low temperature storage. It indicated that the microorganisms in the incubated milk sample were appropriate for the inactivation investigation. As to species level (Fig. 4b), the four most abundant species, *Acinetobacter johnsonii*, *Pseudomonas fragi*, *Pseudomonas tolaasii*, and *Acinetobacter guillouiae*, belong to the two dominant genera shown in Fig. 4a, *Acinetobacter* spp. and *Pseudomonas* spp. respectively.

3.3.1. Relative abundance of milk microflora

At both the genus level and species level (Fig. 4a and b), the electric field treatment (DC.0 d) changed the community composition slightly, mainly in abundance, which was similar to the heat treatment (HT.0 d). Similarly, selectivity also has been reported in high-pressure sterilization of oysters (Rong et al., 2018). The results indicated that, as well as heat treatment and other sterilization technologies, inactivating effect of the electric field was also slightly selective to various bacteria.

In addition, it was found that after 7 days of storage (at 4 °C), compared to the control sample, the microbial growth of the electric field treated samples was significantly lower ($p < 0.05$) (Fig. 4d). It suggested that the technology may have an advantage over traditional thermal sterilization for extending shelf life.

3.3.2. Species clustering heat map of milk microflora

As can be seen from the heat map (Fig. 4c), both the electrically treated sample (DC.0 d) and the heat-treated sample (HT.0 d) showed a little difference in Z-value from the control sample (CON.0 d). After storing the samples at 4 °C for 7 days, the Z-value of *Enhydrobacter* spp.,

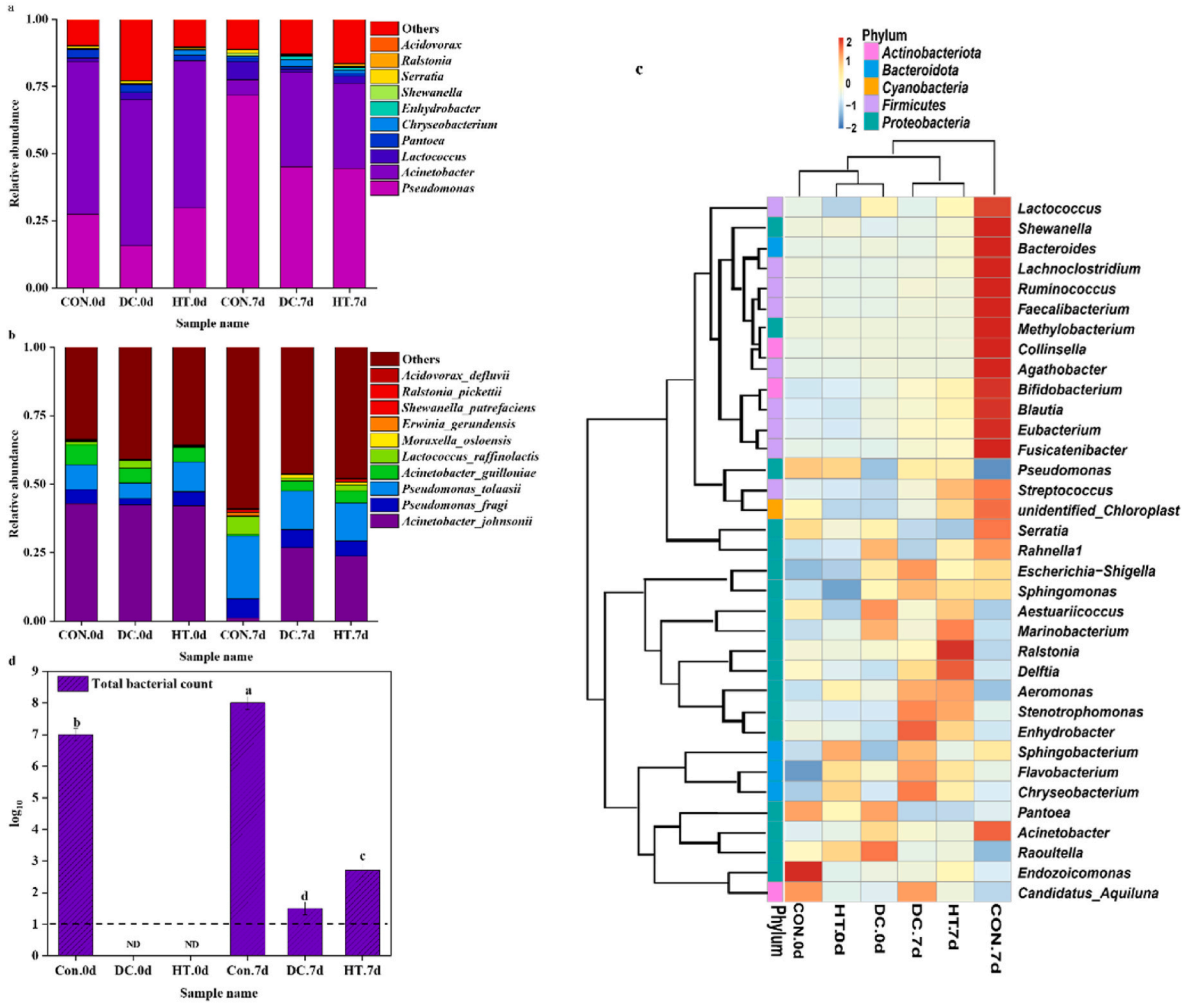

Fig. 4. Microflora change in milk after inactivation with electric field. CON.0 d, DC.0 d, HT.0 d represent the control sample, electric field treated sample and heat-treated sample, respectively. After stored at 4 °C for 7 days, three samples, CON.7 d, DC.7 d, HT.7 d were obtained. Note: Relative abundance of microorganisms at (a) genus, (b)species levels of samples; (c) Heat map of genus abundance (d) Total bacterial count determined by plate counting method.

Chryseobacterium spp. and *Stenotrophomonas* spp. were higher in the electrically treated sample (DC.7 d), while *Ralstonia* spp., *Delftia* spp. and *Marinobacterium* spp. were higher in the heat-treated sample (HT.7 d). Nevertheless, samples DC.0 d and HT.0 d were clustered into the same group, and so were samples DC.7 d and HT.7 d. It again indicated that the microbial inactivation in electric field had similar selectivity as the thermal sterilization.

3.4. Physicochemical-properties evaluation of milk after inactivation

To evaluate whether the quality of milk after microbial inactivation met the fresh-like milk demand, pH, furosine content, whey protein denaturation rate, turbidity, zeta-potential, particle size, size of lipid droplets, color and viscosity physicochemical parameters were determined. Preliminary experiments revealed that 4.8 log CFU/mL of bacteria count in raw milk would be completely inactivated after 150 min treatment in the electric field. So, the samples used here were raw milk with <5 log CFU/mL of bacterial count.

3.4.1. Chemical stability characterization

As can be seen from Fig. 5a, there was no significant difference ($p > 0.05$) among pH within the first 60 min, then pH decreased slightly from 6.71 to 6.33 with time lasting. The pH decreasing by similar extent was also found in pasteurization (Pegu and Arya, 2021) and other non-thermal sterilization technology (Li et al., 2018). Here the pH decrease may be attributed to a slight isoelectric focusing phenomenon in milk proteins (Sui et al., 2021).

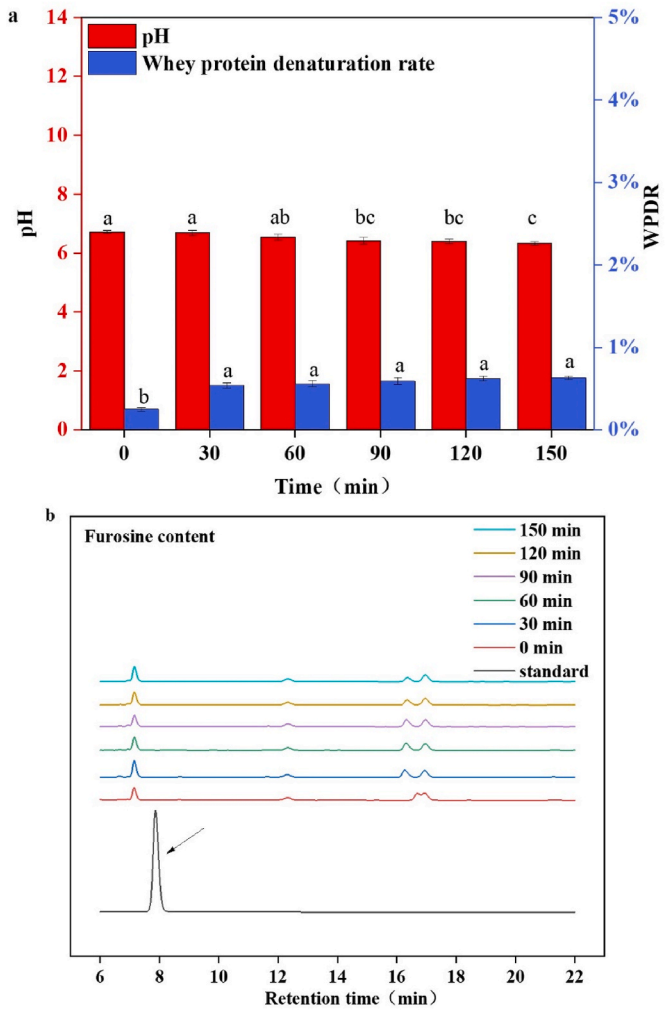

Fig. 5. Characterization of chemical stability of raw milk after inactivation. Different letters above bars indicate significant differences ($p < 0.05$). Note: (a) Whey protein denaturation rate and pH (b) Furosine content.

With treatment time lasting, the denaturation rate of whey protein increased by 0.29% at the first 30 min, and there was no significant difference ($p > 0.05$) over the rest time (Fig. 5a). As the second most abundant protein in milk, whey protein has many bioactivities (Zhang et al., 2021a). The denaturation rate in this study was much lower than that (43%) of pasteurized heat-treated milk whey proteins (Giroux et al., 2020). It indicated that the inactivation preserved the biological activity of fresh milk better (Troise et al., 2014), thus, meeting the fresh-like demand for fresh-like milk.

Fig. 5b shows the furosine content changing during the inactivation in low intensity DC field. The results of the external standard method suggested that no furosine formed during the process. Since furosine is usually found in heat-treated milk (Li et al., 2021), the furosine content is also an important index to determine whether the milk is fresh-like (Zhang et al., 2021b). Moreover, it was found to have adverse effects on human health recently (Boitz et al., 2015). So, the results again implied that, after the inactivation in low intensity DC field, fresh-like milk can be obtained.

3.4.2. Physical stability characterization

Turbidity, zeta-potential and particle size characterize the stability of milk and are often used to evaluate the stability of the milk colloidal system (Shanmugam et al., 2012; Scudino et al., 2020). Fig. 6a–b shows their values during the inactivation.

The turbidity of milk increased a little with the time lasting. It was reported that the turbidity of milk was positively correlated with the size of protein particles and lipid droplets (Ragab et al., 2019), which was also verified by the slightly-increased particle size results in this experiment (Fig. 6b and Fig. S4). At the same time, the absolute value of the zeta-potential was slightly reduced from 28.7 mV to 25.5 mV, which may imply a slight reduction in the stability of the treated milk (Pegu and Arya, 2021). This may also be responsible for the particle size increase. These changes may be a direct result of the electric field treatment (Costa et al., 2018), or derived from pH changes.

As can be seen from Table 2, with the time lasting, the L, a and b values were also changed slightly. The ΔE was 0.96 at 150 min. This minor difference was generally considered to be unnoticeable (Cserhalmi et al., 2006). This was further supported by image of the electrically treated samples (Fig. 6d).

Fig. 6c shows that the viscosity of milk decreased with the increasing time. A slight fat separation was observed after inactivation. This was inferred to be the result of increased particle size. Similar decrease of viscosity was also reported in the study on ultrasonic sterilization of milk (Shanmugam et al., 2012).

In a word, the slight changes of the physical indexes are similar with the result reported in previous milk sterilization studies. It indicates that after microbial inactivation the milk was physically stable.

4. Conclusions

In this study, the feasibility of low intensity steady DC electric field for milk's microbial inactivation was successfully verified. At 50–55 °C, current of 0.3–0.6 A, and starting electric field intensity of 5 V/cm-9 V/cm conditions, the inactivation effect can meet the target of milk pasteurization. The inactivating effect of the electric field itself was also preliminary demonstrated here.

According to the inactivation curves, a new kinetics model was proposed. The inactivation is divided into three stages: a rapid decrease in the first stage, a linear decrease in the middle stage and a slowdown decrease in the last stage. The middle stage, which took up about 2/3 of the whole process, followed first-order reaction kinetics model. A more in-depth analysis of the kinetics showed that, below 50 °C, the effect of temperature on the middle stage was consistent with the Arrhenius Law. It again supported that the electric field itself can inactivate bacteria in

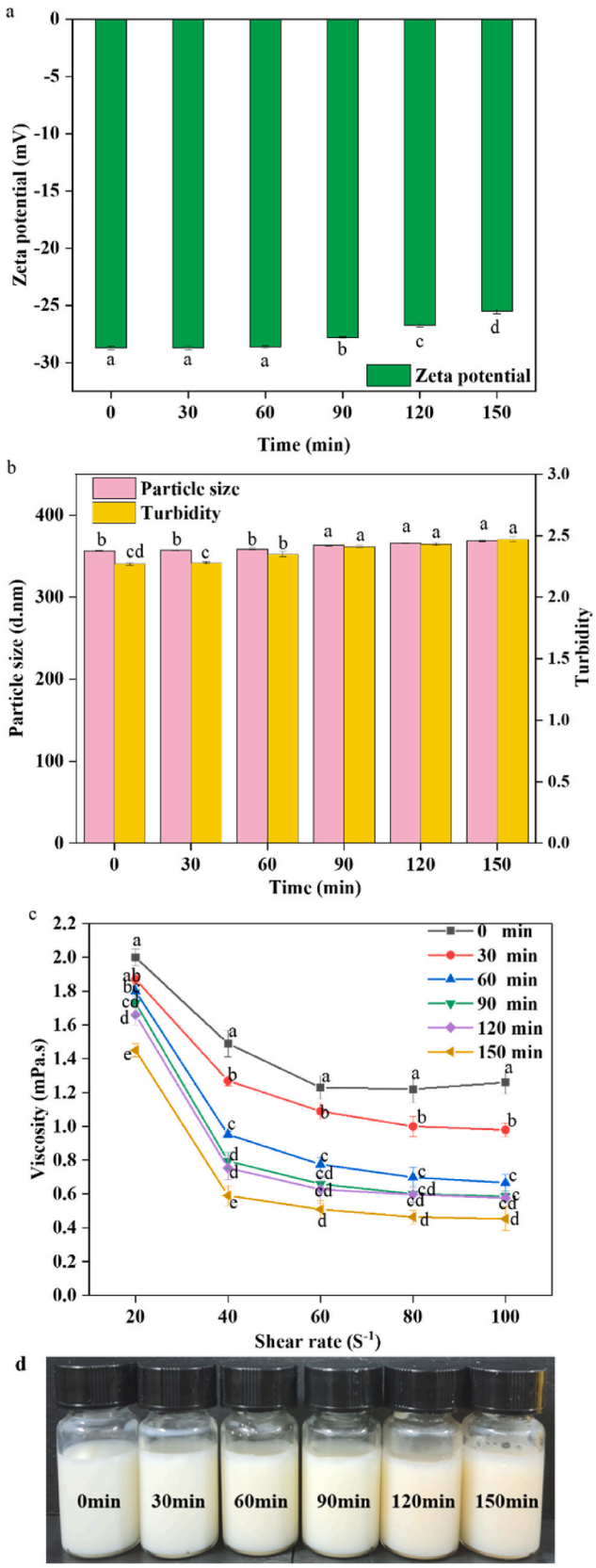

Fig. 6. Characterization of physical stability of raw milk after inactivation. Different letters above bars indicate significant differences ($p < 0.05$). Note: (a) Zeta potential, (b) Turbidity, particle size (c) Viscosity and (d) Visual display color of milk. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Effect of low intensity DC field treatment on milk color.

Time (min)	L^*	a^*	b^*	ΔE
0	78.67 ± 0.04^{a}	-0.99 ± 0.01^{b}	3.83 ± 0.01^{d}	–
30	78.64 ± 0.05^{a}	-0.98 ± 0.01^{b}	3.83 ± 0.02^{d}	0.03 ± 0.01^{e}
60	78.61 ± 0.02^{a}	-0.95 ± 0.02^{ab}	3.95 ± 0.03^{cd}	0.13 ± 0.03^{d}
90	78.52 ± 0.02^{a}	-0.95 ± 0.03^{ab}	4.01 ± 0.05^{c}	0.24 ± 0.01^{c}
120	78.12 ± 0.09^{b}	-0.92 ± 0.02^{a}	4.20 ± 0.03^{b}	0.66 ± 0.02^{b}
150	77.88 ± 0.07^{c}	-0.91 ± 0.02^{a}	4.38 ± 0.04^{a}	0.96 ± 0.03^{a}

Note: The same lowercase letter in one column means that the difference is not significant at 0.05 level.

milk without thermal effect.

Meanwhile, the microflora analysis indicated that *Pseudomonas* spp. and *Acinetobacter* spp. were identified as the naturally occurring dominant genera in the incubated milk, and the low intensity DC electric field can inactivate the naturally occurring microorganisms in milk with slight selectivity, similar to thermal and other sterilization techniques which is acceptable. Physicochemical-properties evaluation showed that, the inactivation chemically preserved the milk's fresh-like characteristics, and maintained the milk physically stable to an acceptable extent. It is proven that the low intensity DC electric field can be a promising liquid food sterilization device.

CRediT authorship contribution statement

Feihong Ji: Methodology, Investigation, Writing – original draft, Validation. **Jing Sun:** Software, Data curation, Formal analysis. **Yiming Sui:** Design, maintenance of dedicated power supply and supporting equipment. **Xiangming Qi:** Conceptualization, Writing – review & editing, Funding acquisition, Resources. **Xiangzhao Mao:** Project administration, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by the Key Technology Research and Development Project of Shandong Province, China (No. 2019JZZY010814).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2022.10.015.

References

Al-Hilphy, A.R., Abdulstar, A.R., Gavahian, M., 2020. Moderate electric field pasteurization of milk in a continuous flow unit: effects of process parameters, energy consumption, and shelf-life determination. Innovat. Food Sci. Emerg. Technol. 67 https://doi.org/10.1016/j.ifset.2020.102568.

Alkhafaji, S., Farid, M., 2008. Modelling the inactivation of *Escherichia coli* ATCC 25922 using pulsed electric field. Innovat. Food Sci. Emerg. Technol. 9, 448–454. https://doi.org/10.1016/J.IFSET.2008.02.003.

Amiali, M., Ngadi, M.O., Smith, J.P., Raghavan, G.S.V., 2007. Synergistic effect of temperature and pulsed electric field on inactivation of *Escherichia coli* O157:H7 and *Salmonella enteritidis* in liquid egg yolk. J. Food Eng. 79, 689–694. https://doi.org/10.1016/j.jfoodeng.2006.02.029.

E. Arias-Roth, H. P. Bachmann, M. T. Fröhlich-Wyder, R. S. Schmidt, D.Wechsler, E. Beuvier, S. Buchin, C. Delbès.Raw milk cheeses. In: P. L. H. McSweeney, J. P. McNamara (Ed.), Encyclopedia of Dairy Sciences. Oxford: Academic Press, pp. 299-308.

Bai, F., Guo, D., Wang, Y., Zhang, S., Li, J., Zhi, K., Shi, C., Xia, X., 2022. The combined bactericidal effect of nisin and thymoquinone against *Listeria monocytogenes* in Tryptone Soy Broth and sterilized milk. Food Control 135. https://doi.org/10.1016/j.foodcont.2021.108771.

Bezie, A., 2019. The effect of different heat treatment on the nutritional value of milk and milk products and shelf-life of milk products. A Review. J. Vet. Sci. 11 https://doi.org/10.19080/JDVS.2019.11.555822.

Boitz, L.I., Mayer, H.K., Evaluation of furosine, 2015. Lactulose and acid-soluble β-lactoglobulin as time temperature integrators for whipping cream samples at retail in Austria. Int. Dairy J. 50, 24–31. https://doi.org/10.1016/j.idairyj.2015.06.002.

Boudjema, N., Kherat, M., Drouiche, N., Mameri, N., 2019. Investigation of the mechanisms of *Escherichia coli* cells sterilization by the application of an electric field. Int J Environ Sci Te 16, 6259–6266. https://doi.org/10.1007/s13762-019-02218-5.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. USA 108, 4516–4522. https://doi.org/10.1073/pnas.1000080107.

CDC food safety raw milk outbreak studies, 2017. https://www.cdc.gov/foodsafety/rawmilk/rawmilk-outbreaks.html.

Chen, C., Mi, X., Yuan, Y., Chen, G., Ren, L., Wang, K., Zhu, D., Qian, Y., 2014. A preliminary risk assessment of potential exposure to naturally occurring estrogens from Beijing (China) market milk products. Food Chem. Toxicol. 71, 74–80. https://doi.org/10.1016/j.fct.2014.05.028.

Chen, L., Zhang, H., Liu, Q., Pang, X., Zhao, X., Yang, H., 2019. Sanitising efficacy of lactic acid combined with low-concentration sodium hypochlorite on *Listeria innocua* in organic broccoli sprouts. Int. J. Food Microbiol. 295, 41–48. https://doi.org/10.1016/j.ijfoodmicro.2019.02.014.

Chen, L., Liu, Q., Zhao, X., Zhang, H., Pang, X., Yang, H., 2022. Inactivation efficacies of lactic acid and mild heat treatments against *Escherichia coli* strains in organic broccoli sprouts. Food Control 133. https://doi.org/10.1016/J.FOODCONT.2021.108577.

Cheng, X.F., Zhang, M., Adhikari, B., 2013. The inactivation kinetics of polyphenol oxidase in mushroom (*Agaricus bisporus*) during thermal and thermosonic treatments. Ultrason. Sonochem. 20, 674–679. https://doi.org/10.1016/j.ultsonch.2012.09.012.

S. Clark, F. Harte. Raw milk: nature's most perfect food? In: P. L. H. McSweeney, J. P. McNamara (Ed.), Encyclopedia of Dairy Sciences. Oxford: Academic Press, pp. 451-462.

S. Cole, A.Goetze, L. Meunier-Goddik.Pasteurized milk. In: P. L. H. McSweeney, J. P. McNamara (Ed.), Encyclopedia of Dairy Sciences. Oxford: Academic Press, pp. 444-450.

Costa, N.R., Cappato, L.P., Ferreira, M.V.S., Pires, R.P.S., Moraes, J., Esmerino, E.A., Silva, R., Neto, R.P.C., Tavares, M.I.B., Freitas, M.Q., Silveira Junior, R.N., Rodrigues, F.N., Bisaggio, R.C., Cavalcanti, R.N., Raices, R.S.L., Silva, M.C., Cruz, A. G., Heating, Ohmic, 2018. A potential technology for sweet whey processing. Food Res. Int. 106, 771–779. https://doi.org/10.1016/j.foodres.2018.01.046.

Cserhalmi, Z., Sass-Kiss, Á., Tóth-Markus, M., Lechner, N., 2006. Study of pulsed electric field treated citrus juices. Innovat. Food Sci. Emerg. Technol. 7, 49–54. https://doi.org/10.1016/j.ifset.2005.07.001.

EFSA report, 2015. EFSA BIOHAZ panel (EFSA panel on biological Hazards). Scientific opinion on the public health risks related to the consumption of raw drinking milk, europen food safety authority. EFSA J. 13 (2015) https://doi.org/10.2903/j.efsa.2015.3940.

El-Hag, A.H., Jayaram, S.H., Gonzalez, O.R., Griffiths, M.W., 2011. The influence of size and shape of microorganism on pulsed electric field inactivation. Ieee T Nanobiosci 10, 133–138. https://doi.org/10.1109/TNB.2011.2163078.

Giroux, H.J., Dupont, F., Villeneuve, G., Britten, M., 2020. Effect of heating milk on whey protein denaturation and cheese-making properties. Int. Dairy J. 111 https://doi.org/10.1016/j.idairyj.2020.104831.

Gurol, C.F.Y., Ekinci, N., Aslan, Korachi, M., 2012. Low temperature plasma for decontamination of E. coli in milk. Int. J. Food Microbiol. 157, 1–5. https://doi.org/10.1016/j.ijfoodmicro.2012.02.016.

Huang, L., 2019. Reconciliation of the D/z model and the Arrhenius model: the effect of temperature on inactivation rates of chemical compounds and microorganisms. Food Chem. 295, 499–504. https://doi.org/10.1016/j.foodchem.2019.05.150.

Huang, K., Yu, L., Wang, W., Gai, L., Wang, J., 2014. Comparing the pulsed electric field resistance of the microorganisms in grape juice: application of the Weibull model. Food Control 35, 241–251. https://doi.org/10.1016/j.foodcont.2013.07.011.

Kim, S.S., Kang, D.H., 2015. Effect of milk fat content on the performance of ohmic heating for inactivation of *Escherichia coli O157:H7, Salmonella enterica Serovar Typhimurium* and *Listeria monocytogenes*. J. Appl. Microbiol. 119, 475–486. https://doi.org/10.1111/jam.12867.

Kim, S.-S., Jo, Y., Kang, D.-H., 2017. Combined inhibitory effect of milk fat and lactose for inactivation of foodborne pathogens by ohmic heating. LWT–Food Sci. Technol. 86, 159–165. https://doi.org/10.1016/J.LWT.2017.07.043.

Lang, F., Wen, J., Wu, Z., Pan, D., Wang, L., 2022. Evaluation of probiotic yoghurt by the mixed culture with *Lactobacillus plantarum* A3. FSHW 11, 323–331. https://doi.org/10.1016/j.fshw.2021.11.006.

Li, J., Wang, J., Zhao, X., Wang, W., Liu, D., Chen, S., Ye, X., Ding, T., 2018. Inactivation of *Staphylococcus aureus* and *Escherichia coli* in milk by different processing sequences of ultrasound and heat. J. Food Saf. 39. https://doi.org/10.1111/jfs.12614.

Li, Y., Wu, Y., Quan, W., Jia, X., He, Z., Wang, Z., Adhikari, B., Chen, J., Zeng, M., 2021. Quantitation of furosine, furfurals, and advanced glycation end products in milk treated with pasteurization and sterilization methods applicable in China. Food Res. Int. 140 https://doi.org/10.1016/j.foodres.2020.110088.

Liao, X., Li, J., Muhammad, A.I., Suo, Y., Ahn, J., Liu, D., Chen, S., Hu, Y., Ye, X., Ding, T., 2018. Preceding treatment of non-thermal plasma (NTP) assisted the bactericidal effect of ultrasound on. Staphylococcus aureus Food Control 90, 241–248. https://doi.org/10.1016/j.foodcont.2018.03.008.

Liao, H., Zhong, K., Hu, X., Liao, X., 2019. Effect of high pressure carbon dioxide on alkaline phosphatase activity and quality characteristics of raw bovine milk. Innovat. Food Sci. Emerg. Technol. 52, 457–462. https://doi.org/10.1016/j.ifset.2019.02.005.

Linn, 2019. 2019 A. Linn. Raw milk is always risky: stabilising the danger of raw milk in Australian food safety regulation. Health Risk Soc. 21, 304–317. https://doi.org/10.1080/13698575.2019.1612859.

Lou, Y., Yousef, A.E., 1996. Resistance of *Listeria monocytogenes* to heat after adaptation to environmental stresses. J. Food Protect. 59, 465–471. https://doi.org/10.4315/0362-028X-59.5.465.

Machado, L.F., Pereira, R.N., Martins, R.C., Teixeira, J.A., Vicente, A.A., 2010. Moderate electric fields can inactivate *Escherichia coli* at room temperature. J. Food Eng. 96, 520–527. https://doi.org/10.1016/j.jfoodeng.2009.08.035.

Niu, D., Zeng, X.A., Ren, E.F., Xu, F.Y., Li, J., Wang, M.S., Wang, R., 2020. Review of the application of pulsed electric fields (PEF) technology for food processing in China. Food Res. Int. 137 https://doi.org/10.1016/j.foodres.2020.109715.

Osaili, T.M., Shaker, R.R., Al-Haddaq, M.S., Al-Nabulsi, A.A., Holley, R.A., 2009. Heat resistance of *Cronobacter* species (*Enterobacter sakazakii*) in milk and special feeding formula. J. Appl. Microbiol. 107, 928–935. https://doi.org/10.1111/j.1365-2672.2009.04271.x.

Pegu, K., Arya, S.S., 2021. Comparative assessment of HTST, hydrodynamic cavitation and ultrasonication on physico-chemical properties, microstructure, microbial and enzyme inactivation of raw milk. Innovat. Food Sci. Emerg. Technol. 69 https://doi.org/10.1016/j.ifset.2021.102640.

Ragab, E.S., Lu, J., Pang, X.Y., Nassar, K.S., Yang, B.Y., Zhang, S.W., Lv, J.P., 2019. Effect of thermosonication process on physicochemical properties and microbial load of goat's milk. J Food Sci Tech Mys 56, 5309–5316. https://doi.org/10.1007/s13197-019-04001-3.

Rong, C., Ling, Z., Huihui, S., Qi, L., 2018. Characterization of microbial community in high-pressure treated oysters by high-throughput sequencing technology. Innovat. Food Sci. Emerg. Technol. 45, 241–248. https://doi.org/10.1016/J.IFSET.2017.11.001.

Ruiz-De Anda, D., Casados-Vázquez, L.E., Ozuna, C., 2022. The synergistic effect of thurincin H and power ultrasound: an alternative for the inactivation of *Listeria innocua* ATCC 33090 and *Escherichia coli* K-12 in liquid food matrices. Food Control 135. https://doi.org/10.1016/j.foodcont.2021.108778.

Saldaña, G., Puértolas, E., Álvarez, I., Meneses, N., Knorr, D., Raso, J., 2010. Evaluation of a static treatment chamber to investigate kinetics of microbial inactivation by pulsed electric fields at different temperatures at quasi-isothermal conditions. J. Food Eng. 100, 349–356. https://doi.org/10.1016/j.jfoodeng.2010.04.021.

Scudino, H., Silva, E.K., Gomes, A., Guimaraes, J.T., Cunha, R.L., Sant'Ana, A.S., Meireles, M.A.A., Cruz, A.G., 2020. Ultrasound stabilization of raw milk: microbial and enzymatic inactivation, physicochemical properties and kinetic stability. Ultrason. Sonochem. 67 https://doi.org/10.1016/j.ultsonch.2020.105185.

Shanmugam, A., Chandrapala, J., Ashokkumar, M., 2012. The effect of ultrasound on the physical and functional properties of skim milk. Innovat. Food Sci. Emerg. Technol. 16, 251–258. https://doi.org/10.1016/j.ifset.2012.06.005.

Stratakos, A.C., Inguglia, E.S., Linton, M., Tollerton, J., Murphy, L., Corcionivoschi, N., Koidis, A., Tiwari, B.K., 2019. Effect of high pressure processing on the safety, shelf life and quality of raw milk. Innovat. Food Sci. Emerg. Technol. 52, 325–333. https://doi.org/10.1016/j.ifset.2019.01.009.

Sui, Y., Huang, W.-C., Wu, Y., Qi, X., Mao, X., 2021. Lipid extraction from Greenland halibut (*Reinhardtius hippoglossoides*) by-product in low-voltage DC electric field and its mechanism. J. Clean. Prod. 283 https://doi.org/10.1016/j.jclepro.2020.124673.

Tian, D., Li, C., 2019. Risk assessment of raw milk quality and safety index system based on primary component analysis. Sustain Comput-Infor. 21, 47–55. https://doi.org/10.1016/j.suscom.2018.11.006.

Troise, A.D., Fiore, A., Colantuono, A., Kokkinidou, S., Peterson, D.G., Fogliano, V., 2014. Effect of olive mill wastewater phenol compounds on reactive carbonyl species and maillard reaction end-products in ultrahigh-temperature-treated milk. J. Agric. Food Chem. 62, 10092–10100. https://doi.org/10.1021/jf503329d.

Walter, L., Knight, G., Ng, S.Y., Buckow, R., 2016. Kinetic models for pulsed electric field and thermal inactivation of *Escherichia coli* and *Pseudomonas fluorescens* in whole milk. Int. Dairy J. 57, 7–14. https://doi.org/10.1016/j.idairyj.2016.01.027.

Yang, S., Liu, G., Munk, D.M.E., Qin, Z., Apetersen, M., Cardoso, D.R., Otte, J., Ahrné, L., 2020. Cycled high hydrostatic pressure processing of whole and skimmed milk: effects on physicochemical properties. Innovat. Food Sci. Emerg. Technol. 63 https://doi.org/10.1016/j.ifset.2020.102378.

Zhang, D., Palmer, J., Teh, K.H., Biggs, P., Flint, S., 2019. 16 S rDNA high-throughput sequencing and MALDI-TOF MS are complementary when studying psychrotrophic bacterial diversity of raw cows' milk. Int. Dairy J. 97, 86–91. https://doi.org/10.1016/j.idairyj.2019.06.001.

Zhang, Y., Yi, S., Lu, J., Pang, X., Xu, X., Lv, J., Zhang, S., 2021a. Effect of different heat treatments on the Maillard reaction products, volatile compounds and glycation level of milk. Int. Dairy J. 123 https://doi.org/10.1016/J.IDAIRYJ.2021.105182.

Zhao, L., Zhao, M.Y., Phey, C.P., Yang, H., 2019. Efficacy of low concentration acidic electrolysed water and levulinic acid combination on fresh organic lettuce (*Lactuca sativa Var. Crispa L.*) and its antimicrobial mechanism. Food Control 101, 241–250. https://doi.org/10.1016/j.foodcont.2019.02.039.

Zhao, Y., Yu, X., Zhao, W., Li, G., Liu, G., Ma, Y., Chu, L., Ma, Y., Zhang, Y., Lu, Y., He, F., Liu, X., 2022. Inactivation of endogenous pectin methylesterases by radio frequency heating during the fermentation of fruit wines. Fermentation 8, 265. https://doi.org/10.3390/fermentation8060265.

Zhang, L., Zhou, R., Zhang, Zhou, P., 2021b. Heat-induced denaturation and bioactivity changes of whey proteins. Int. Dairy J. 123 https://doi.org/10.1016/j.idairyj.2021.105175.