UNIVERSITI PUTRA MALAYSIA

EFFECTS OF PULSED ELECTRIC FIELD ON ESCHERICHIA COLI ATCC 8739 INACTIVATION, METAL MIGRATION AND PHYSICOCHEMICAL PROPERTIES OF GOAT MILK

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By

AZIZAH BINTI MOHAMAD

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The increasing demand and interest in goat milk worldwide have evolved many studies on the nutritional and functional properties of goat milk. The typical pasteurization method for goat milk is thermal pasteurization. However, thermal pasteurization can cause many unwanted changes such as browning of milk due to Maillard reaction, increase in titratable acidity, degradation of lactose producing formic and lactic acid, decrease in pH, protein and lactose content and precipitation of calcium. Thus, promising non-thermal processing such as pulsed electric field (PEF) has gained greater attention in the food and drink industry, as a substitute for thermal pasteurization due to the rising demand for high nutritional value and fresh-like characteristic of goat milk. This study aims to evaluate the inactivation of *Escherichia coli* ATCC 8739, physicochemical properties of PEF-processed goat milk and investigate the possibility of the metal migration effect from the electrode material of PEF. In this study, raw goat milk was processed with a laboratory-scale PEF system with continuous mode, monopolar square-wave pulses and fixed pulse width. This model only allows the selection of electric field strength (EFS) and processing time parameters, while the remaining parameters were predetermined by the PEF system, with the maximum EFS and processing time at 40 kV/cm and 13 µs respectively. Therefore, the EFS of 20, 30 and 40 kV/cm for 5 and 10 µs processing time were used to determine the effect of PEF on physicochemical properties of goat milk (pH, titratable acidity, total soluble solids, viscosity, particle size distribution, colour, vitamin A (as β-carotene), B-group vitamin (thiamine (B1), riboflavin (B2) and niacin (B3)), fatty acid profiles and metal migrations). However, it was found that the data were insufficient to be fitted to the inactivation models of *Escherichia coli* (*E. coli*) by PEF. Hence, the additional parameters (EFS: 25 and 35 kV/cm; processing time: 7 and 13 µs) were included in the study. The results indicated that PEF-processed goat milk at 40 kV/cm for 10 µs had the lowest pH (6.53 ± 0.03) and the highest titratable acidity (0.21 ± 0.01) value of the samples due to the decrease in calcium ion (Ca^{2+}), which leads to the protonation of the phosphate. The specific surface area (SSA) value significantly increased ($p < 0.05$) with the increase of PEF processing time. Whereas, PEF processing has shown to reduce 0.03 – 0.06% of the total saturated fatty acids (ΣSFAs) and 8.32 – 51.11% total polyunsaturated fatty acids (ΣPUFAs) of goat milk, while total monounsaturated fatty acids (ΣMUFAs) were increased ranging from 0.02 – 0.05%. Mean concentrations of heavy metals (Fe, Cr, Mn and Ni) in all PEF-processed samples were within the permissible limit of heavy metals according to the USFDA drinking water quality standard. The retention value for vitamin A as β-carotene in PEF-processed samples were between 99.90 – 99.99% with only 0.01 – 0.1% destruction. While the retention value for B-group vitamin were between 95.37 – 98.26% (thiamine), 97.65 – 98.65% (riboflavin) and 98.44 – 99.29% (niacin). The fitting performance for the Weibull and Peleg model was observed to be more accurate and good fits to the observed data of the inactivation kinetics of *E. coli* by PEF processing, as a function of time and EFS respectively. However, the maximum inactivation level of *E. coli* by PEF in this study was 3.87 log^{10} CFU/mL at 40 kV/cm for 13 µs, below the requirements set by the FDA (minimum of 5 log^{10} CFU/mL). Thus, PEF processing would be recommended as a complementary method to conventional thermal pasteurization. By combining PEF with mild thermal pasteurization (< 55 °C) as a hurdle processing technique, the microbial stability and safety of goat milk can be obtained at the minimum intensity of PEF.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN MEDAN ELEKTRIK DENYUTAN TERHADAP PENYAHAKTIFAN *ESCHERICHIA COLI* ATCC 8739, MIGRASI LOGAM DAN CIRI FIZIKOKIMIA DALAM SUSU KAMBING

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Peningkatan permintaan dan minat terhadap susu kambing seluruh dunia telah menghasilkan banyak kajian terhadap nutrisi dan fungsi susu kambing. Kaedah pempasteuran yang biasa digunakan untuk susu kambing ialah pempasteuran terma. Walau bagaimanapun, pempasteuran terma boleh menyebabkan banyak perubahan yang tidak diingini seperti keperangan susu disebabkan oleh reaksi Maillard, peningkatan nilai keasidan, kemerosotan laktosa kepada asid formik dan laktik, penurunan nilai pH, kandungan protein dan laktosa dan pemendapan kalsium. Jadi, pemprosesan tanpa terma seperti medan elektrik terdenyut (PEF) telah menjadi perhatian industri makanan dan minuman, sebagai pengganti kepada pempasteuran terma kerana terdapat peningkatan permintaan terhadap susu kambing yang mempunyai nilai nutrisi yang tinggi dan mempunyai ciri seakan susu kambing segar. Oleh itu, kajian ini bertujuan untuk menilai penyahaktifan *Escherichia coli* ATCC 8739, ciri fizikokimia susu kambing yang diproses menggunakan PEF dan mengkaji kebarangkalian kesan migrasi logam daripada bahan elektrod PEF. Dalam kajian ini, susu kambing mentah diproses dengan sistem PEF berskala makmal dengan mod berterusan, denyutan gelombang persegi monopolar dan lebar denyutan yang tetap. Model ini hanya membolehkan pemilihan kekuatan medan elektrik (EFS) dan parameter masa pemprosesan, manakala parameter yang selebihnya telah ditetapkan oleh sistem PEF tersebut dengan EFS maksimum dan masa pemprosesan masing-masing pada 40 kV/cm dan 13 μs. Oleh itu, EFS 20, 30 dan 40 kV/cm untuk 5 dan 10 μs masa pemprosesan digunakan untuk menentukan kesan PEF terhadap sifat fizikokimia susu kambing (pH, keasidan boleh dititrasi, jumlah pepejal terlarut, kelikatan, taburan saiz zarah, warna, vitamin A (seperti β-karotena), vitamin B-group (thiamine (B1), riboflavin (B2) dan niacin (B3)), profil asid lemak dan migrasi logam). Walau bagaimanapun, didapati bahawa data itu tidak mencukupi untuk dipadankan pada model penyahaktifan *Escherichia coli* (*E. coli*) oleh PEF. Oleh itu, parameter tambahan (EFS: 25 dan 35 kV/cm; serta masa pemprosesan: 7 dan 13 μs) telah dimasukkan ke dalam kajian ini. Hasil kajian menunjukkan bahawa susu kambing yang diproses dengan PEF pada 40 kV / cm dan 10 μs mempunyai sampel dengan pH yang paling rendah dan nilai keasidan yang paling tinggi (0.21 ± 0.01) kerana terdapat penurunan ion kalsium (Ca^{2+}), yang menyebabkan protonasi ion fosfat. Nilai luas permukaan zarah (SSA) meningkat dengan ketara ($p < 0.05$) dengan peningkatan masa pemprosesan PEF. Manakala, pemprosesan PEF telah menunjukkan penurunan 0.03 – 0.06% dari jumlah asid lemak tepu (ΣSFA) dan 8.32 – 51.11% dari jumlah asid lemak politaktepu (ΣPUFA) susu kambing, sementara jumlah asid lemak monotaktepu (ΣMUFAs) meningkat sekitar 0.02 – 0.05%. Purata kepekatan logam berat (Fe, Cr, Mn dan Ni) dalam semua sampel yang diproses oleh PEF berada dalam had logam berat yang dibenarkan menurut piawaian kualiti air minuman USFDA. Nilai pengekalan untuk vitamin A sebagai β-karotin dalam sampel yang diproses menggunakan PEF adalah di antara 99.90-99.99% dengan nilai kemerosotan sebanyak 0.01 – 0.1% sahaja. Manakala, nilai pengekalan untuk vitamin kumpulan B adalah di antara 95.37 - 98.26% (tiamin), 97.65 - 98.65% (riboflavin), dan 98.44 – 99.29% (niasin). Perilaku penyesuaian untuk model Weibull dan Peleg adalah lebih tepat dan sesuai dengan data yang diperhatikan dari kinetik penyahaktifan *E. coli* oleh proses PEF, dengan fungsi masa dan EFS. Namun, tahap penyahaktifan maksimum *E. coli* oleh PEF dalam kajian ini adalah 3.87 log^{10} CFU / mL pada 40 kV/cm selama 13 μs, rendah dari keperluan yang ditetapkan oleh FDA (minimum 5 log^{10} CFU/mL). Oleh itu, proses PEF dicadangkan untuk menjadi kaedah pelengkap kepada pempasteuran terma konvensional. Dengan menggabungkan PEF dengan pempasteuran terma sederhana sebagai teknik pemprosesan rintangan, kestabilan mikrob dan keselamatan susu kambing dapat diperoleh pada intensiti minimum pemprosesan PEF.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science The members of the Supervisory Committee were as follows:

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AC	Alternating Current
Af	Accuracy Factor
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ATCC	American Type Culture Collection
B1	Thiamine
B2	Riboflavin
B3	Niacin
B6	Pyridoxine
B9	Folic Acid
B12	Cyanocobalamin
Bf	Bias Factor
Ca	Calcium
CAGR	Compound Annual Growth Rate
Ca^{2+}	Calcium Ion
CFU	Colony Forming Unit
CLA	Conjugated Linoleic Acid
CM	Casein Micelles
Cr	Chromium
DVS	Department of Veterinary Services
Ec	Critical Field Strength
EFS	Electric Field Strength
E. coli	Escherichia coli
ECU	European Currency Unit
FAME	Fatty Acid Methyl Ester

FG	Fat Globule
GCMS	Gas Chromatography-Mass Spectrometry
GRASS	Generally Recognized As Safe
HMF	Hydroxymethylfurfural
HPLC	High-Performance Liquid Chromatography
HTST	High-Temperature Short Time
HUS	Haemolytic-Uraemic Syndrome
ICPMS	Inductively Coupled Plasma Mass Spectrometry
IVT	Induced Transmembrane Voltage
Fe	Iron
FID	Flame Ionization Detector
LCFA	Long-Chain Fatty Acids
LDPE	Low Density Polyethylene
LV	Low Viscosity
LTLT	Low Temperature Low Time
Min	Minute
Mg	Magnesium
Mn	Manganese
MCFA	Medium-Chain Fatty Acids
MFGM	Milk Fat Globule Membrane
MSE	Mean Square Error
NaOH	Sodium Hydroxide
Ni	Nickel
NB	Nutrient Broth
OD	Optical Density
PEF	Pulsed Electric Field

PMO	Pasteurized Milk Ordinance
RTV	Resting Transmembrane Voltage
S	Second
SCFA	Short-Chain Fatty Acids
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
SSA	Specific Surface Area
STEC	Shiga Toxin-Producing E. coli
TA	Titratable Acidity
ΣMUFA	Total Monounsaturated Fatty Acids
ΣPUFA	Total Polyunsaturated Fatty Acids
ΣSFA	Total Saturated Fatty Acids
TSS	Total Soluble Solids
UHT	Ultra-High Temperature
UPM	Universiti Putra Malaysia
USFDA	United States Food and Drug Administration
UV	Ultraviolet
Wpulse	Pulse Energy
Wspec	Specific Energy Inputs
Zn	Zinc
α-la	α-lactalbumin
β-lg	β-lactoglobulin
^{o}C	degree celsius

CHAPTER 1

INTRODUCTION

1.1 Research Background

Milk is an essential food for a human being. It has many beneficial nutrients and biological values. There are a niche market and great potential for goat milk due to its functional properties and low allergenic effect. Goat milk is reported to contain vitamins, minerals, trace elements, electrolytes, enzymes, proteins and fatty acids that are readily incorporated by the body (Abbas et al., 2014). Moreover, goat milk is beneficial for people suffering from health problems such as eczema, asthma, migraine, colitis, stomach ulcer, digestive disorder, liver and gallbladder diseases (Ravula & Ramachandra, 2016). Softer curd formation, smaller fat globules and a greater amount of short and medium-chain fatty acids in goat milk are beneficial for better digestibility, inhibits cholesterol deposition and aid lipid metabolism (Park et al., 2007). However, it has been reported that the constituents of goat milk have differed with species, breeds, diet, stage of lactation, body size, season and other factors (Yurchenko et al., 2018; Stergiadis et al., 2019). The functional properties and nutritional benefits of goat milk also contribute to the expansion of the global goat milk market. Nowadays, most infant milk formula manufacturers employ goat milk powder for infant formula in the market due to its low allergenic effect.

The majority of global production of goat milk is reported in Asia (58.4%), Africa (24.1%) and Europe (14.2%) (Skapetas & Bampidis, 2016). Goat milk is considered a major contributor to non-bovine milk production which accounts for 13.5% out of 17% non-bovine milk production globally (Ranadheera et al., 2019). Turkmen et al. (2019) reported that global goat milk production is anticipated to increase due to a large number of non-commercial local goat milk production. In Malaysia, there is no conclusive data on goat milk production at the moment of writing.

Pulsed electric field (PEF) is a promising non-thermal processing technique in the food and drink industry due to low processing cost, energy-efficient with minimal energy loss, flexible, instant, non-thermal and environment-friendly (Pereira et al., 2016; Pal, 2017). PEF has gained recognition in food processing through halting an adverse issue of thermal processing including the destruction of spoilage microorganisms, inactivation of enzymes as well as better retention of organoleptic and nutritional characteristics of fruit juices (Roobab et al., 2018); bovine milk (Ahmad et al., 2019); milk and fruit juices (Nowosad et al., 2020). Besides food processing, the feasibility of PEF can be used in diverse applications and fields including biotechnology, bioprocess engineering, medicine, as well as in drug and gene delivery (Kanduser et al., 2017). In September 1996, the U.S. Food and Drug Administration (FDA) released a letter of no objection for the use of PEF to treat liquid eggs (Vega-Mercado et al., 2007;

Kanduser et al., 2017). This leads to the conclusion that the use of PEF in food processing is safe. The results of PEF processing are shown to be encouraging in food and drinks manufacturing due to its low processing cost, minor energy loss and environmental friendliness (Aadil et al., 2015b; Arshad et al., 2020). There are several published reports on milk processed with PEF including skim milk and whole milk (Walkling-Ribeiro et al., 2011) where it was observed that there was no significant difference between the pH of PEF-processed and thermally processed or unprocessed raw bovine skimmed milk (0.08% fat) when processed at a field strength of 16 – 42 kV/cm and processing times of 612 – 2105 µs. Lee et al. (2015) also demonstrated that PEF processing at 10 kV/cm for 30 µs did not change the physical properties of commercial low-fat bovine milk including pH, colour and particle size distribution. Sharma et al. (2018) also reported that the pH of PEF-processed raw bovine whole milk did not change significantly ($p > 0.05$) after processing at 25.7 kV/cm for 34 µs.

PEF is operated at ambient temperature (20 – 25 °C) with short processing time (from several nanoseconds to several milliseconds) and pulsed electric field strength from 100 – 300 V/cm to 20 – 80 kV/cm (Barba et al., 2015; Sack et al., 2017; Silva et al., 2018; Alirezalu et al., 2019). In the PEF process, the sample is positioned in the middle of two or more electrodes before exposing the sample to high voltage electric field pulses (typically 10-80 kV/cm) (Ahmad et al., 2019; Nowosad et al., 2020). To the best of our knowledge, PEF-processed goat milk has not yet been applied at the industrial level or on a large scale. Therefore, it is important to study the effects and efficiency of PEF processing on goat milk at a lab scale to ensure the process can be implemented on an industrial scale. Moreover, PEF has a great potential to be commercialized as it can reduce the production cost due to its lower processing temperature, shorter processing time and energy-efficient. PEF also provides better retention of nutritional and functional properties of goat milk, therefore milk manufacturers do not have to spend more on fortifying goat milk with vitamins and other nutrients.

1.2 Problem Statements

The safety and quality of milk and dairy products are essential issues to deal with due to their perishable nature. The most common method used to maintain food safety and shelf-life stability for liquid food and beverages is pasteurization. Pasteurization is crucial to assure that the microbial load of liquid food is significantly reduced leading to prolongation of its shelf-life. The typical pasteurization method for goat milk is thermal pasteurization at 72 – 76 ˚C for 15 – 25 s (National Dairy Development Board, 2018). However, thermal pasteurization can negatively affect the organoleptic properties and nutritional quality of milk. Thermal pasteurization can also cause many unwanted changes such as browning of milk due to Maillard reaction (Stojanovska et al. 2017), degradation of lactose to formic and lactic acid by 10 – 80% (Sakkas et al., 2014; Melini et al., 2017; Najib et al., 2020), reduction of pH value in the range of 3.14 – 10.06% (Ma & Barbano, 2003; Haq et al., 2013), protein denaturation from 15 – 33% (Lewis & Deeth, 2009; Sakkas et al., 2014) and hydrolysis of milk lipid up to 72% (Sharma et al., 2016). The decrease in milk pH is attributable to the production of lactic acid, which could have a detrimental effect on the overall sensory quality of milk (McAuley et al., 2016). According to Lee et al. (2015), thermal processing results in cooked flavour due to the destruction of sulphydryl groups from sulphur-containing amino acids. Thus, non-thermal processing such as pulsed electric field (PEF) has been introduced as a substitute for thermal pasteurization due to the rising demand for high nutritional value and fresh-like characteristic of processed foods (Aadil et al., 2015a, 2018; Ahmad et al., 2019; Pankiewicz et al., 2019; Zhang et al., 2019).

PEF is regarded as an excellent technology compared to conventional thermal processing techniques because it minimizes adverse effects in physical, functional and sensorial attributes as well as extends the shelf-life of treated food (Syed et al., 2017; Pal, 2017; Mohamed & Eissa, 2011). PEF is promising to commercialisation and effectively enhance energy usage, by offering microbiologically safe and nominally processed food (Syed et al., 2017; Nowosad et al., 2021; Mohamed & Eissa, 2011). Many studies reported that PEF allows a reduction in enzymatic activity and inactivation of vegetative cells of bacteria and yeasts in various food. In addition to that, thermally susceptible food such as fruit juices and milk are best suited to process with PEF because it hinders the depletion of vitamins and nutrients (Syed et al., 2017; Pal, 2017). Therefore, the physicochemical analysis of PEF-processed goat milk at different electric field strengths and PEF processing time were critical to be determined in this study.

In order for PEF processing to be accepted and commercialized as one of the non-thermal processing methods for milk and liquid foods, the occurrence of electrochemical reactions must be minimized as it can cause safety issues to the food products. The electrochemical reaction occurs when the voltage across the electrode-electrolyte (sample) interface surpassed the threshold voltage, which results in metal oxidation (loss) of the electrode and/or reduction (gain) of the dissolved metals on the electrode (Pataro et al., 2014). From the angles of food safety and quality, fouling and electrode lifetime, electrode corrosion is objectionable and must be reduced (Roodenburg et al., 2005; Pataro et al., 2014). Evrendilek et al. (2004) reported a significant release of Fe, Cr, Zn and Mn in the PEF-processed beer at 22 kV/cm for 154 µs. Apart from this study, very limited data on the effects of metal migration on PEF-processed milk is available in the literature. Hence, the study aims to investigate the possibility of the metal migration effect from the electrode material of PEF in the goat milk sample.

Most of the studies on milk pasteurization by PEF have been conducted on bovine milk with microbial and enzymatic inactivation being the main focus area. Studies on PEF-processed goat milk are very limited because bovine milk is the most consumed product compared to products from other ruminants. Until the moment of writing, an article by Hariono et al. (2020) has been published on the effects of PEF processing on goat milk. The authors studied the combined effect of high-PEF (HPEF) with ultraviolet (UV) processing on microbial inactivation and physicochemical properties of goat milk. A total of 89.49% microbial reduction was reported with no significant effects ($p > 0.05$) on the physicochemical properties (pH, viscosity, density, conductivity, freezing point and specific heat, lipid content, protein content, lactose content and peroxide value) of HPEF and UV processed goat milk at 10, 15, 20 Hz and 0.62 Gy.kg/cm^2 respectively. Despite the positive synergistic effects of HIPEF and UV, further study is needed to understand the fundamental effect of PEF on goat milk before it can be commercially applied in goat milk processing.

Goat milk is an ideal medium for various microorganisms and is easily spoilt due to its ample amount of nutrients. Hence, there is a dire need for further advancement of goat milk processing for the local goat milk industry with the ultimate aim to produce shelf-stable goat milk with minimal effects to its nutritive quality perusing alternative pasteurizing technology. To date, no studies on the use of PEF processing to inactivate *E. coli* in goat milk have been reported, because most studies were focused on the effect of PEF processing on microbial inactivation in the bovine milk sample. For instance, Guerrero-Beltran et al. (2010) reported a 4.3 $\log_{10}$ reduction of *Listeria innocua* by hurdle processing of PEF at 30 and 40 kV/cm for 10 µs, with thermal processing at 72 °C in bovine whole milk. The authors suggested that the synergistic effect between PEF and mild-thermal processing could increase the bacterial inactivation rates. Bermudez-Aguirre et al. (2011) studied the inactivation of mesophilic and psychrophilic bacteria in skim milk and whole milk. The authors observed 3.6 and 0.4 $\log_{10}$ reductions of mesophilic, and 3.0 and 0.8 $\log_{10}$ reductions of psychrophilic bacteria in skim and whole milk respectively. The study also found a lower inactivation rate in whole milk compared to skim milk, due to the fat and protein content that acts as an insulator and safeguarded the cells from the electric field (Bermudez-Aguirre et al., 2011). Sharma et al. (2014) obtained 5 – 6 $\log_{10}$ reductions of *Pseudomonas aeruginosa* in PEF-processed bovine whole milk at 22 – 28 kV/cm for 17 – 101 µs. The same authors also found that the reduction of *E. coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Listeria innocua* (ATCC 33090) were below the detection limit (< 10^2 CFU/mL). Based on the literature search, it was found that no study has been conducted on the effect of PEF processing on the inactivation level of *E. coli* ATCC 8739 in goat milk. Therefore, the present study could fill the gap with the new findings and demonstrate the efficiency of PEF in goat milk, before it can be recommended to be commercialized at the industrial level. Moreover, the works in the present study are lab-based and it has not yet been implemented in industry or on a large scale. Perhaps, the results from this research could provide useful information to the dairy industry especially for those who are interested to adopt PEF pasteurizing method for goat milk processing.

1.3 Objectives

The general aim of this study is to pasteurize while minimizing the nutritional and physicochemical qualities of goat milk using a pulsed electric field (PEF) as a non-thermal pasteurization technique. The specific objectives of this study are:

i. To determine the effect of PEF processing at 20, 30 and 40 kV/cm for 5 and 10 µs on physicochemical (pH, titratable acidity, total soluble solids, viscosity, particle size distribution and colour) and nutritional properties (vitamin A (β-carotene) and B-group vitamins (thiamine (B1), riboflavin (B2) and niacin (B3)) of goat milk.

ii. To determine metal migration namely iron (Fe), chromium (Cr), nickel (Ni) and manganese (Mn), and fatty acid profiling of PEF-processed goat milk.

iii. To determine the maximum inactivation level and the best kinetic modelling (Weibull, Bigelow, Peleg and Hulsheger model) for inactivation of *Escherichia coli* ATCC 8739 in PEF-processed goat milk at 20, 25, 30, 35 and 40 kV/cm for 5, 7, 10 and 13 µs.

1.4 Scope of the Thesis

A continuous laboratory-scale of PEF system, PowerMod^{TM} 25 kW by Diversified Technologies, Inc. (Bedford, Mass., U.S.A) was used in this study. This PEF system only allows the selection of electrical field strength (EFS) and processing time parameters. The rest of the parameters were predetermined by the PEF system. The maximum EFS and processing time achieved in this study were 40 kV/cm and 13 µs, respectively. Single circulation (one cycle) of the sample was used in the study, aiming to avoid cross-contamination during sample flowing from inlet to outlet pipeline.

The goat milk samples were purchased from the local dairy farm in Sepang, Selangor, within 2 h after milking, transported directly (4 ± 2 °C) to the Food Processing and Engineering Laboratory, Agro-Biotechnology Institute, Serdang, Selangor, prior to PEF processing. The physicochemical (pH, titratable acidity, total soluble solids, viscosity, particle size distributions, colour), nutritional properties (vitamin A (β-carotene) and B-group vitamin (thiamine (B1), riboflavin (B2) and niacin (B3)), metal migration (iron (Fe), chromium (Cr), nickel (Ni) and manganese (Mn)) and fatty acid profiles of the samples were analysed before and after PEF processing at 20, 30 and 40 kV/cm for 5 and 10 µs.

Escherichia coli (*E. coli*) ATCC 8739 is a gram-negative and rod-shaped bacteria from the family *Enterobacteriaceae*. This strain was used to determine the inactivation kinetics by PEF at 20, 25, 30, 35 and 40 kV/cm for 5, 7, 10 and 13 µs. The advantage of *E. coli* ATCC 8739 was due to its modest biology structure and short generation time. According to our preliminary study, *E. coli* ATCC 8739 only took 4 – 6 h to achieve the exponential or log phase and was ready to harvest towards the end of the log phase in less than 7 h, as depicted in Appendix 5.

The inactivation kinetics of *E. coli* ATCC 8739 by PEF processing were fitted with four different models namely Weibull, Bigelow, Peleg and Hulsheger. These models were chosen due to their robustness, flexible approach and ability to fit the inactivation rate below 4 $\log_{10}$ cycle. The fitting performance of each model was evaluated through predicted parameters including mean square error (MSE), accuracy factor (*Af*) and bias factor (*Bf*).

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