Investigation of novel technologies for milk preservation

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Some hide in the shadows, encumbered with many thoughts they may never say.

Alone yet surrounded

By and by, one pulls the blurred lines into sharp focus – becoming a journey reclaimed by dreams.
For above the parapet, there is a Canary birdsong

Abstract

Presently, thermal treatment is widely applied in the dairy industry to preserve and extend milk products' shelf life, including pasteurization and ultra-high temperature (UHT) treatment. Each thermal treatment method is unique, rendering different microbiological grades and storage conditions depending on the time-temperature combination during the treatment. However, the resurgence in demand from consumers for more fresh and improved quality and nutritious food products has prompted researchers to investigate the potential of emerging technologies as an alternative to the current thermal treatments.

Therefore, the main objective of this study is to achieve microbial inactivation at a reduced temperature or shorter treatment time by a combination of effects to attain minimally impacted treated milk products. The first part of this study investigates milk sterilisation using pulsed electric field (PEF) processing in combination with heat with the optimised operating parameters. Results showed 4.02 and 3.37 log reductions in skim milk at 300 Hz, 28 $kVcm^{-1}$ and 100 Hz, 50 $kVcm^{-1}$, respectively, at an operating temperature of 115°C. As the microbial inactivation kinetics is associated with the residence time in a particular system, the residence time distribution (RTD) in the PEF unit was determined. Findings presented that the mean residence times obtained experimentally in all five flowrates tested are slightly more significant than the calculated residence time.

Further, the toxicological aspect of food safety is an essential criterion in human acceptance, prompting an investigation of electrode corrosion occurrence in the PEF unit. Experimental results presented all metal elements studied obtained at least ten times lower than the maximum allowable values for human consumption set by the health standards. In the last chapter, this thesis also focuses on the application of ultra-high-pressure homogenisation (UHPH) processing to study its efficacy in microbial spore inactivation and the shelf-life stability of raw milk in a refrigerated condition. Log reductions of 1.368 and 0.864 were achieved at valve temperatures of 129 °C and 127 °C for both bovine and goat milk, respectively, at 250MPa. Additionally, raw sheep milk reached at least 36 days of total bacterial count under the safe limit in a refrigerated condition after being UHPH-treated at 250MPa and 118 °C. Minimal variation in pH and conductivity of UHPH-treated sheep milk were observed throughout the 36 days period.

The findings from the research are expected to be the primary basis for an in-depth investigation of novel technologies for sterilisation and extended shelf-life application in the dairy industry to complement the current thermal treatment technology.

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Conferences and Presentations

1. Food Industry Enabling Technology (FIET) 2017 Colloquium, 14-15th June 2017, Palmerston North, New Zealand
2. 2nd World Congress on Electroporation & Pulsed Electric Fields in Biology, Medicine, Food and Environmental Technologies, Norfolk, Virginia, USA. 24th-28th September 2017
3. 3rd Annual Chemical and Materials (CHEMMAT) Engineering Ph.D. Research Symposium, 10th of September 2018, Auckland, New Zealand
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1 Introduction

Throughout the years, milk has become a desired product in daily life as it contains essential nutrients such as proteins, fats, carbohydrates, minerals, and water (Balthazar et al., 2017). However, milk as a raw material has a relatively short shelf life. It has also been reported that the consumption of raw milk carries a disproportionately high risk of infection by milk-borne microorganisms (Varnam, 1994). Food products' microbiological safety and stability are essential for their marketability; hence, milk products are required to undergo treatment to ensure they are safe for human consumption (Dutreux et al., 2000).

A broad range of preservation technologies has been applied in the food industry to increase the safety of milk to the consumers by destroying diseases causing microorganisms (pathogens) that may present in milk. Additionally, it is essential to improve the quality of milk products by destroying spoilage microorganisms and enzymes that contribute to milk's reduced quality and shelf life. Currently, thermal treatments such as pasteurisation and ultra-high temperature (UHT) sterilisation are widely used in the industry (Deak, 2014). However, due to their limitation in which high temperature will have a negative impact on milk quality, conventional treatments such as irradiation, ultra-high-pressure (UHPH) processing, pulsed electric field (PEF) processing, pulsed light, as well as a combination of technologies such as PEF with antimicrobials and PEF with ultrasound are explored (Martín-Belloso, Soliva-Fortuny, Elez-Martínez, Robert Marsellés-Fontanet, & Vega-Mercado, 2014).

2 Literature review

2.1 Thermal treatments

In thermal treatment, temperature selection and duration of heating are crucial for the required microbial quality of the milk products. Different thermal processing methods have been commonly applied in the food industry, with well-known effectiveness in microbial inactivation (A. G. Da Cruz et al., 2010; Deak, 2014). These thermal treatment methods result in different microbiological grades and storage durations depending on the processing temperature and time (Rysstad & Kolstad, 2006).

<u>Pasteurisation of liquid foods</u>

Pasteurisation is achieved through introducing mild temperature to food products in which relatively longer shelf life is obtained by inactivating all non-spore-forming pathogenic bacteria and most of the vegetative spoilage microorganisms, as well as inhibiting enzymatic activity. These pasteurised foods

must be stored in a refrigerated condition to maintain the required shelf life. Typically, the refrigerated shelf-life of pasteurised food products could range from several days (milk) to several weeks (juices) until food spoilage occurs (Teixeira, 2014).

Generally, the pasteurisation of milk can be simplified into two main categories: slow pasteurisation and rapid pasteurisation. Slow pasteurisation of milk typically implies the temperature-time combinations of 63-65 °C for 30 minutes. Meanwhile, rapid pasteurisation of milk typically applies the temperature-time combinations of 72 °C (15 s) and 89-100 (1s) (Deak, 2014). Despite having minimal impact on the nutritional values and sensory characteristics (Teixeira, 2014), some spoilage microorganisms, such as heat-resistant bacterial spores, may not be destructed due to the mild severity of heat implied on the food products (Fellows, 2009; van Zuijlen et al., 2010; Wohlgemuth & Kämpfer, 2014). It has also been stated by (Bylund, 2003) that spores have greater resistance than bacteria even to chemical disinfectants, heat, drying, and ultraviolet light.

Nonetheless, despite the efficacy of thermal pasteurisation in inactivating vegetative microorganisms, the relatively short shelf life of pasteurised milk is a drawback when there is a surplus of milk, and longer shelf life is essential to meet transportation and distribution requirements.

Sterilisation of liquid foods

To produce increased shelf life food products, sterilisation such as ultra-high temperature (UHT) treatment is applied by targeting the bacterial spores and thereby achieving the commercial sterility level (Martín-Belloso et al., 2014). Generally, sterilisation is defined as the destruction of all viable microorganisms (Teixeira, 2014). Meanwhile, regarding commercial sterilization, it is determined by the inhibition of microbial activity instead of their presence or absence during the prescribed shelf life (Deak, 2014). UHT treatment involves the application of high temperatures (120-140 °C, 2-4 seconds) (Bendicho, Espachs, Arntegui, & Martn, 2002; Deak, 2014).

UHT treatment requires aseptic processing, in which the UHT-treated product is filled into sterile containers in a sterile environment to minimise post contamination from the microorganisms (Teixeira, 2014). According to (Deak, 2014), UHT treatment consists of three main steps, which are:

1. Heating of food products to the required sterilization temperature
2. Holding the desired sterilization temperature for a period of time
3. Rapid cooling of food products to the desired temperature

Regarding the effectiveness of sterilisation, several authors have reported that *Bacillus spp*. endospores are the most challenging microbial species to be inactivated in the food industry; therefore, the inactivation of this species is worth to be considered as the primary basis of any

technology with the potential for sterilisation from an industrial perspective (Cregenzán-Alberti, Arroyo, Dorozko, Whyte, & Lyng, 2017; Rozali, Milani, Deed, & Silva, 2017). Within the Bacillus genus, a wide range of resistance to heat has been reported, with *Bacillus sporothermodurances*, *Bacillus subtilis*, *Bacillus cereus,* and *Geobacillus stearothermophilus* endospores species being considered the most resistant in the dairy industry (Cregenzán-Alberti et al., 2017).

However, even though UHT treatment is capable of producing extended shelf-life milk products under ambient conditions, some authors have reported noticeable modifications in the nutritional, physicochemical, and sensory properties of milk products as a result of exposure to extreme heat conditions during the treatment (Martín-Belloso et al., 2014). In some areas, UHT milk is less preferable to consumers due to the product's perceived ‘burnt’ flavour (Rysstad & Kolstad, 2006).

Consequently, the requirement to compensate for the short shelf life of HTST pasteurised milk without the noticeable flavour modification commonly associated with UHT milk products has driven the development of a milk product with an acceptable taste profile but a more extended storage ability of the product.

Extended shelf life (ESL) of liquid foods

Extended shelf life (ESL) milk complements the gap between HTST pasteurised milk and UHT milk to benefit from minimal processing while obtaining a considerable shelf life. ESL milk products are being treated beyond standard pasteurisation conditions by targeting the inactivation of psychrotrophic spores with extremely sterile packaging conditions and have a defined extended shelf-life in a refrigerated state. Typically, commercial ESL milk products have a shelf life ranging from 30 to 90 days at four °C storage (Kapadiya, Prajapati, & Pinto, 2015; Rysstad & Kolstad, 2006).

Presently, two main processing methods have been used to produce ESL milk industrially: microfiltration and bactofugation. Microfiltration uses a size-exclusion principle that involves a mechanical separation step using ceramic membranes (0.8-1.4 mm) to remove microbial cells, including spores, from the milk. However, the milk must be centrifugally separated to produce skim milk before microfiltration, as the particle size distribution of the microbial cells is similar to that of fat globules in milk (Fernández García & Riera Rodríguez, 2014; Rysstad & Kolstad, 2006). On the other hand, bactofugation is a method in which microbial cells, including spores, are separated from milk due to their greater density than milk. The separation process is done by using a specially fabricated centrifuge unit. After undergoing microfiltration or bactofugation, these methods are always followed by thermal pasteurisation to eliminate vegetative pathogens in the milk products (Pak, 2016).

On the other hand, several researchers have also explored alternative technologies such as pulsed electric field (PEF) processing in combination with moderate heating in the application of shelf-life extension, which could lead to an improved inactivation of pathogenic bacteria with better quality retention in food products (Walkling-Ribeiro, Noci, Cronin, Lyng, & Morgan, 2009). The outcome shows that applying HTST pasteurisation in combination with PEF at an elevated temperature (70-75 °C) could be a promising alternative to produce foods with greater shelf life.

2.1.1 Structure of spores

Different thermal treatment has various target microorganisms and microbial inactivation levels. HTST pasteurization generally targets vegetative cells, UHT targets thermoduric bacterial spores, and the ESL processing line targets the inactivation of psychrotrophic spores in raw milk (Pak, 2016; Rysstad & Kolstad, 2006).

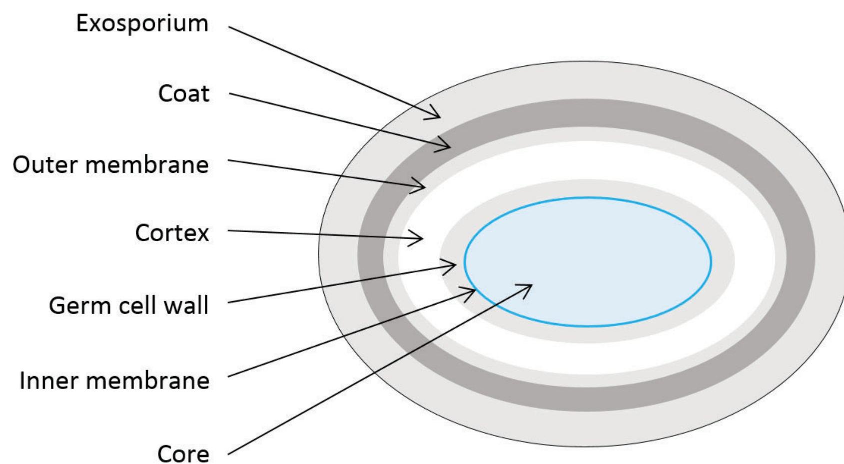

Figure 1: Structure of a typical bacterial spore (not drawn to scale).

Figure 1 demonstrates the structure of a typical bacterial spore. Generally, a spore consists of exosporium, coat layer, outer and inner membrane, cortex, germ cell wall, and the innermost core (Rozali et al., 2017). The innermost core consists of genomes, proteins, and a large amount of Ca^{2+} (Choi et al., 2008). Further, a spore is also distinguished by its pyridine-2,6-dicarboxylic acid (PDC) content in its core, which is not found in vegetative cells. The interior core of the bacterial spores consists of low-level water content (10-25%), which is an essential criterion for determining the resistance of spores to heat and chemicals (Qiu, Lee, & Yung, 2014). The outer and inner membranes are enzymatically active (Choi et al., 2008), where the inner membrane acts as a primary permeability barrier against chemicals.

On the other hand, the cortex layer is composed of a thick, loosely cross-linked peptidoglycan structure that can prevent hydration of the spore core. Meanwhile, the spore is covered in a multi-layered protein shell called the coat, which protects the spore and acts as a permeability barrier. After germination of the endospores, the germ cell wall located outside the inner membrane will become the bacterium's cell wall (Rozali et al., 2017).

2.1.2 Fundamentals of heat destruction of microorganisms

The destruction of the microbial population takes place continuously with time. Practically, only the living cells can be studied and enumerated instead of the dead cells. Therefore, the decrease in a cell population with time can be determined by counting the surviving cells after the treatment. The kinetics of cell death can be described by the first-order reaction by the change in the number of surviving microorganisms over treatment time Figure 2 (Deak, 2014).

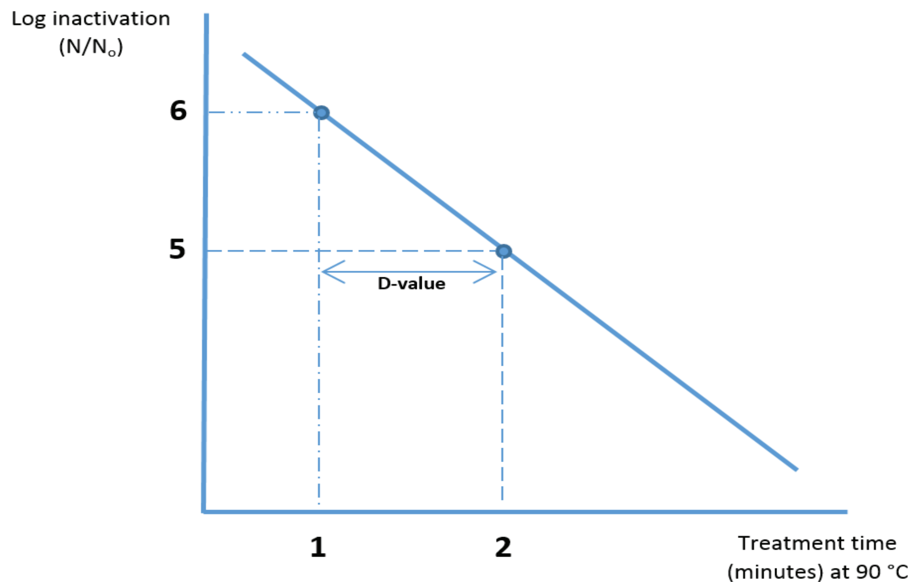

Figure 2: Thermal death curve for a typical bacterial spore

The death kinetics of microbial inactivation can be described using the first-order reaction:

$$\frac{dN}{dt} = -kN \qquad (Equation\ 1)$$

Where *dN* and *dt* represent the change in the number of survivors and the time, respectively. The *k* factor is the death rate coefficient, and the negative sign indicates the decreasing number of microorganisms with time. Equation 1 can be integrated into a logarithmic form, which is called the

equation of survival curve:

$$\ln\left(\frac{N}{N_O}\right) = -kt \qquad (Equation\ 2)$$

Where N is the microbial population at a given time, t and N_o is the initial microbial population. The changes in microbial population as a function of time can be expressed as:

$$\log\left(\frac{N}{N_O}\right) = -\frac{t}{D} \qquad (Equation\ 3)$$

Combining Equations 2 and 3, the relationship between death rate coefficient, *k,* and D-value can be expressed as:

$$k = \frac{2.303}{D} \qquad (Equation\ 4)$$

where the D-value of a microorganism refers to the time needed to result in a 90% reduction (one log reduction) in the initial numbers of pure culture spores or vegetative cells at a specific temperature (Adams, 1995). D-value represents the heat resistance of a microorganism at a particular temperature. The longer the D-value, the greater the resistance of the microorganisms toward the destructive factor. D-value can also be affected by other factors such as types of microorganisms, pH and composition of the medium (Deak, 2014). For instance, the different strains of bacteria have different D-values even in the same food medium and vice versa (Adams, 1995).

2.1.3 Drawbacks of thermal treatment

Thermal treatment is widely used in industry due to its superior effectiveness in microbial inactivation. However, the severity of heat implied during the treatment cause protein denaturation and vitamin loss (J. Li, Wei, Xu, & Wang, 2009), in addition to the adverse effects on the colour, flavour, and taste (Jayaram, 2000; Molinari, Pilosof, & Jagus, 2004). According to (Rosenberg, 2002), a whitening effect and formation of cooked flavour were reported in milk after undergoing UHT treatment. (Shin et al., 2010) also mentioned that even though thermal processing is a widely effective and relatively inexpensive method for the inactivation of bacterial spores, it can reduce nutritional content and alter the sensory attributes of food products.

Therefore, the resurgence in demand from consumers for improved quality or more nutritious food products has prompted researchers to evaluate the potential of emerging technologies as an alternative to the current thermal processing methods (Sharma, Oey, & Everett, 2014). Presently, there are several emerging novel technologies available for food preservation, including ultrasonication (US), high-pressure processing (HPP), and pulsed electric field (PEF) treatment. According to (Craven et al., 2008), among these processes, PEF has a great potential to overcome the

limitations in thermal treatments due to its capability to process liquid foods continuously while providing minimal impact on the nutritional, physicochemical, and sensory properties of milk.

2.2 PEF processing

PEF is an emerging technology that is being researched in a multitude of directions; preservations (Reineke, Schottroff, Meneses, & Knorr, 2015; Toepfl, Heinz, & Knorr, 2007), extraction of valuable compounds (G Pataro et al., 2017; Xue & Farid, 2015), and improvement of functionality in food products (Gholamibozanjani et al., 2021; Ignat, Manzocco, Brunton, Nicoli, & Lyng, 2015; Wu & Zhang, 2014). PEF processing implies a series of high voltage pulses to the food products for a very short duration of 1-100 µs. PEF processing can be used for liquid, semi-liquid, and even solid foods (X. Li & Farid, 2016). It has been reported that PEF treatment has been applied to a wide variety of liquid food products, including milk, fruit juices, and liquid egg (Barbosa-Cánovas & Altunakar, 2006).

The bactericidal effect of electric current or pulses had already been investigated in the nineteenth century (Krüger, 1893; Prochownick & Spaeth, 1890). (Sale & Hamilton, 1967) was among the first to report a comprehensive investigation on microbial inactivation using electric current or pulses. PEF treatment application has become more desirable in processing food and agricultural products since the 1990s (Sitzmann, Vorobiev, & Lebovka, 2016). This emerging technology is considered for commercialization, and the food industry and regulatory bodies have shown reasonable interest in PEF (Buckow, Ng, & Toepfl, 2013; X. Li & Farid, 2016). However, several authors have mentioned the challenges in scaling up the process (Góngora-Nieto, Sepúlveda, Pedrow, Barbosa-Cánovas, & Swanson, 2002; Van den Bosch, 2007) and the high capital cost and operation costs (Góngora-Nieto et al., 2002; X. Li & Farid, 2016).

The effectiveness of PEF processing is primarily attributed to several factors, which are the process parameters (electric field strength, frequency, inlet temperature, pulse width, and treatment time), product parameters (conductivity and composition of food), and microbial characteristics (type and size of microorganism, growth phase and initial microbial concentration) (Sharma, Oey, et al., 2014).

Electric field strength

The introduction of electric field intensity (E) is defined as the electric potential difference (V) for two given points in space separated by the distance (d) between the two electrodes:

$$E = \frac{V}{d} \qquad (Equation\ 5)$$

Treatment time

The treatment time, (t), is the time that the food sample is subjected to the electric field in the treatment chamber. The treatment time can be described using the following relationship:

$$t = n \times \tau \qquad (Equation\ 6)$$

Where n is the pulse number (frequency), and τ is the pulse width.

2.2.1 Mechanism of PEF

It is widely acknowledged that microbial inactivation of PEF primarily depends on the electroporation phenomenon, which increases the permeabilization of the cell membrane. (Raso, Condón, & Álvarez, 2014) and (Jaeger, Meneses, & Knorr, 2014) suggested that as the intensity of the electric field across the membrane exceeds the threshold, the permeabilization of microbial cells will be irreversible, resulting in a leakage of intercellular compounds and cell lyses.

It is generally accepted that, according to (Saulis, 2010), the whole process of cell electroporation in food treatment by PEF can be simplified into four main stages (Figure 3) as follow:

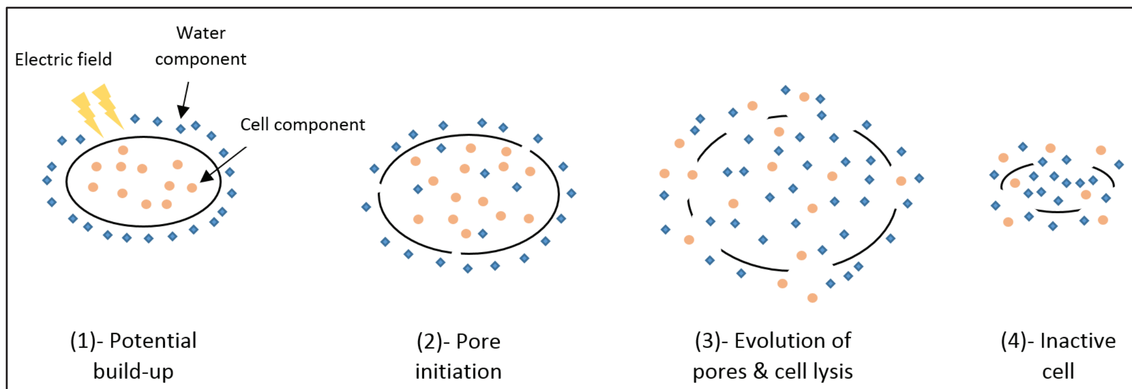

Figure 3: Mechanism of electroporation in a microbial cell

1. Transmembrane potential builds up – As the electric field intensity increase, the potential across the cell membrane increases.
2. Pore initiation – the creation of tiny metastable hydrophilic pores.
3. The evolution of the pore population is the number of pores or their sizes changes.
4. Post-treatment – After the treatment, leakage of intracellular compounds, the entrance of extracellular substances, pore resealing, and other processes occur.

Regarding bacterial spores, the information on the exact mechanism of spore inactivation using PEF treatment is still limited. In a study by (Choi et al., 2008), they observed dark colour in the cortex region and deep dark spots in the core through the transmission electron microscope (TEM). These findings are related to the irreversible loss of the inner membrane's function. Further, according to (Qiu et al., 2014), the mechanism of bacterial spores inactivation by PEF can be attributed to protein denaturation and enzyme inactivation. In another study by (Yonemoto et al., 1993), cracks were found on the spores' surfaces through electron microscopic observation after electric pulse treatment. In addition, a few particles in the spores' black granules were also crushed after the exposure to electric pulses. However, several authors have suggested that the mechanism of spore inactivation is likely to be similar to the inactivation of vegetative cells (X. Li & Farid, 2016).

2.2.2 Application of PEF in microbial inactivation

One of the essential criteria for applying PEF processing in milk is the effectiveness of the microbial inactivation (Deeth & Datta, 2011). There is numerous research that has been done on the application of PEF to inactivate bacteria such as *Escherichia coli* (S. R. Alkhafaji & Farid, 2007; Evrendilek & Zhang, 2005), *Staphylococcus aureus* (Sobrino-López & Martín-Belloso, 2008), *Listeria innocua* (Sharma, Bremer, Oey, & Everett, 2014). However, a limited number of publications explain the inactivation of bacterial spores by applying PEF-only treatment. According to (Deeth & Datta, 2011), unlike most vegetative cells, bacterial spores are more challenging to be inactivated by PEF treatment. (Narsetti, Curry, McDonald, Clevenger, & Nichols, 2006) found that *Bacillus subtilis* spores are difficult to inactivate when compared to *Escherichia coli*. (Sale & Hamilton, 1967) also reported that *Bacillus cereus* and *Bacillus subtilis* spores were resistant to PEF up to 30 kV/cm. The findings from these authors are in agreement with (X. Li & Farid, 2016), who described the sensitivity to PEF treatment in the order of gram-negative bacteria > gram-positive bacteria and yeasts > spores. The greater sensitivity of gram-negative bacteria to PEF can be explained due to its thin cell wall as compared to the gram-positive bacteria which has thicker cell wall (Bylund, 2003).

Further several authors have reported that inactivation of spores was not observed in cranberry juice (Devlieghere, Vermeiren, & Debevere, 2004) in simulated milk ultra-filtrate after PEF-only treatment (Pagan, Esplugas, Gongora-Nieto, Barbosa-Canovas, & Swanson, 1998). On the contrary, (Marquez, Mittal, & Griffiths, 1997) showed at least 3.4 log inactivation of *B. subtilis* with 30 pulses in 0.15% NaCl solution and 5 log inactivation for *B. cereus* with 50 pulses in 0.10% NaCl solution, both after PEF treatment at 50 $kVcm^{-1}$. In another study conducted by (Yonemoto et al., 1993), they found that yeast spores showed greater sensitivity towards PEF processing as compared to bacterial spores, which is likely attributed to the larger size of yeast spores. The findings from these authors suggest that the

type of microorganisms or size is an important criterion in evaluating the lethal effect of PEF treatment.

These findings show that heat-resistant bacterial spores are recalcitrant to be inactivated, making sterilisation of food challenging to attain with the application of non-thermal PEF-only treatment (Condón, Mañas, & Cebrián, 2010; Reineke et al., 2015). Therefore, this situation has driven researchers to explore the potential of combining technologies such as heat, antimicrobial agents, and high pressure with PEF processing (Álvarez & Heinz, 2007). Such combinations could potentially provide the required lethality with improved retention of quality and sensory characteristics compared to thermal-only processing (Sharma, Oey, et al., 2014). One such technology is the combination of PEF with heat allowing one to achieve the desired degree of microbial inactivation with less severe treatments.

2.2.3 Synergistic application of PEF

The potential of PEF processing in combination with heat as a sterilisation technology has been shown by many researchers (X. Li & Farid, 2016). The combination of PEF and heat is typically defined as the effect of the inlet temperature of the product to be treated on the microbial inactivation by PEF (Álvarez & Heinz, 2007). According to (Reineke et al., 2015), pre-heating and maximum treatment temperatures in PEF processing are the main criteria to be considered to determine PEF lethality. Generally, it is widely known that increasing the treatment temperature results in an increase in microbial lethality of PEF synergistically with the electric field (Floury, Grosset, Lesne, & Jeantet, 2006; Narsetti et al., 2006; Reineke et al., 2015).

It has been elucidated that the increase in the lethality rate with temperature is attributed to the temperature-related phase transition of the phospholipids layer from gel to liquid crystalline. As the temperature increases, cell membranes will lose their elasticity, making it easier to be disrupted from the exposure of electric field (Álvarez & Heinz, 2007). (Amiali, Ngadi, Smith, & Raghavan, 2007) also mentioned that the impact of processing temperature in PEF treatment is attributed to its influence to change the cell membrane fluidity, where the membranes become more fluid and their mechanical resistance decreases with an increase in temperature. Meanwhile, according to (Choi et al., 2008), they proposed a notion, in which PEF processing with heat is assisted by the thermal shock that induces the structural fatigue of spores. Several authors have also explained that an increase in temperature causes an increase in the conductivity of the media, hence the charging time of the membrane decreases (Floury, Grosset, Lesne, et al., 2006).

The combination of thermal treatments with PEF has also been proposed as an approach to reduce costs and to increase the effectiveness due to the high processing costs from PEF-only treatment (Guerrero-Beltrán, Sepulveda, Góngora-Nieto, Swanson, & Barbosa-Cánovas, 2010). Several researchers have published their work on the application of PEF processing with heat to inactivate microbial spores and to extend shelf life of milk products. It has been reported by (D R Sepulveda, Góngora-Nieto, Guerrero, & Barbosa-Cánovas, 2005) that the shelf-life of milk was increased up to 60 days after thermal pasteurisation at 72 °C for 15 s directly before PEF treatment. They also managed to obtain an extended shelf life of milk up to 78 days after thermal pasteurisation at 72 °C for 15 s followed by PEF after 8 days of storage in 4 °C condition. Both PEF treatments were conducted at 35 kVcm^{-1}, pulse width of 2.3 µs, inlet temperature of 50 °C and outlet temperature of 65 °C for less than 10 seconds. Further, other researchers have also investigated the inactivation of spores in liquids by thermal assisted PEF processing Table 1.

The findings from these authors suggested that the application of PEF in combination with heat shows a promising potential towards achieving microbial spore inactivation. It can be seen that the inactivation of microbial spores is dependent on numerous factors, in addition to the diversity of PEF equipment making it challenging to compare the results obtained by the different researchers. However, it has been reported that the effectiveness of PEF treatment mainly depends on the intensity of electric field applied and the treatment time (Condón et al., 2010). Therefore, it is essential to establish the most suitable combinations of PEF parameters (field strength, energy input, initial and final temperature) that can provide acceptable levels of microbial inactivation in food products and suitable for industrial application (Álvarez & Heinz, 2007)

Table 1: Inactivation of spores using PEF with heat obtained from different studies

Microorganisms	Medium	Electric Field Strength (kV/cm)	Conductivity (mS/cm)	Pulse width (µs)	Frequency (Hz)	T_{inlet} (°C)	T_{outlet} (°C)	Log reduction	References
Geobacillus stearothermophilus ATCC 7953	Skim milk (0.3% fat)	17.1	5.3	4	75	95	132.7	3.1	(Reineke et al., 2015)
	Saline water	10.26	5.3	6	200	95	131.7	3.2	
	Carrot juice	13.68	7.73	4	125	95	129.5	3.5	
Bacillus subtilis PS832	Skim milk	17.1	5.3	8	100	80	111.0	3.0	
	Saline water	17.1	5.3	8	100	80	115.9	3.5	
Bacillus subtilis PS832	Ringer solution – pH 7	9	4.0	20	NA	80	133	4.4	(Siemer, Toepfl, & Heinz, 2014)
Geobacillus stearothermophilus ATCC 10149	Simulated milk ultra-filtrate 50% (wt/wt)	45	2.47	1.7	1000	70	115	4.1	(S. R. Alkhafaji & Farid, 2012)
Bacillus cereus ATCC 7004	Skim milk (0.3% fat)	40	5.12	2.5	10	65	NA	2.5	(Bermúdez-Aguirre, Dunne, & Barbosa-Cánovas, 2012)

*NA = information was not reported

2.2.4 Application of PEF in shelf-life extension of milk products

Several studies have been reported regarding the shelf-life extension of milk using PEF only or PEF with mild thermal treatment as presented in Table 2.

The combination of PEF and thermal treatment can result in synergistic effects on reducing the growth of bacteria in refrigerated milk. (Fernandez-Molina, Barbosa-Canovas, & Swanson, 2005) reported that ESL of skim milk was more than 22 days (73 °C for 6s and 50 kV/cm) and 30 days (80 °C for 6s and 30 kV/cm). The synergistic effect of temperature and PEF processing has also been applied to study the shelf-life extension of fruit juices. According to (Evrendilek et al., 2000), the fresh apple cider was found to have over 68 days of shelf life after applying thermal treatment (60 °C for 30 s) followed by PEF processing (35 kV/cm, pulse width of 1.92 μs, treatment temperature of 26-27 °C). Moreover, (D R Sepulveda et al., 2005) showed that raw milk had an ESL of 60 days when PEF was applied immediately after HTST pasteurisation and an ESL of 78 days when PEF was applied 8 days after HTST pasteurisation. The results from these authors show that a combination of technologies consisting of thermal pasteurisation (HTST) prior to PEF processing with an elevated inlet temperature of 70-75 °C can potentially be a sound strategy and more effective approach to extend the shelf life of milk than PEF alone.

Table 2: PEF processing application in shelf-life extension study of milk products

Medium	Conditions	Analysis	Results	References
Raw whole milk	PEF followed by self-generated electric heat. **PEF**: Inlet flow rate of 20 mL/s. 5 exponentially decaying pulse, 35 kV/cm, pulse width 2.3 µs, T_{outlet} = 65 °C, holding tube after PEF for <10 s, cylindrical concentric electrodes chamber. **Heating**: 65 °C for <10 s.	Mesophilic and enteric bacteria counts (every other day), psychrotrophic counts (once a week). Titratable acidity (TA) (every other day). Visual and olfactory testing (coagulation, thickening, curdling, colour change, sedimentation, off-odour, and/or bubbling) every other day.	Shelf life up to 24 days[a]. TA remained below the minimum sensory detection level (0.2%). No abnormalities were detected in the visual and olfactory inspection up to the end of the storage period.	(David R Sepulveda, Góngora-Nieto, Guerrero, & Barbosa-Cánovas, 2009)
Raw skim milk (0.2% fat content)	PEF followed by thermal treatment. **PEF**: Inlet flow rate of 8.33 mL/s. 28 kV/cm, 32 kV/cm, 36 kV/cm, total treatment time 84 µs, exponentially decaying wave, pulse width of 2.8 µs, 3 Hz, continuous coaxial electrodes chamber, T_{outlet} = <32 °C **Heating**: Using coil in glycol bath. Skim milk was heated at 60 °C and 65 °C for 21 s.	Total growth of aerobic bacteria counts in 7-day intervals. The growth was monitored for a 1-month period.	At T_{outlet} = 60 °C, total aerobic bacteria count increased from 2.4 to 6.8 log cfu/mL (28 kV/cm) and 6.6 log cfu/ml (32 kV/cm and 36 kV/cm) after 30 days. At T_{outlet} = 65 °C, total aerobic bacteria count increased from 2.4 to 6.2 log cfu/mL (28 kV/cm), to 5.9 log cfu/mL (32 kV/cm), to 5.3 log cfu/ml after 30 days. TA analysis shows that the acidity changes after PEFH affected the least, followed by PEF alone and conventional heating alone.	(Fernández-Molina et al., 2005)
Raw skim milk (0.2% fat content)	Thermal treatment followed by PEF. **Heating:** Using tubular heat exchanger in glycol bath. 73 °C or 80 °C for 6 s. Inlet flow rate of 8.33 mL/s. Cooled to 4 °C before PEF. **PEF:** T_{inlet} = 40 °C, 50 or 30 kV/cm, 30 exponentially decay pulses of 2 µs pulse duration each at 4 or 3 Hz, continuous coaxial electrodes chamber.	Total aerobic and coliform bacteria count were performed at 5-7 days interval. Count total aerobic and coliform bacteria. A 6-7 log cfu/mL microbial population was established as the standard for acceptable quality and safety of PEF processed skim milk during shelf-life studies.	The combination of thermal/PEF skim milk achieved 4.1 and 4.9 log cfu/mL at 22 days and 30 days respectively at 4 °C. The thermal (73 or 80 °C for 6s)/PEF (50 or 30 kV/cm, 30 pulses) treated skim milks resulted in equivalent TA after 14 days at 4°C reaching a value of 0.2%. It maintained a constant TA at 0.23% LA from day 22 to day 30 at 4°C.	(Fernandez-Molina et al., 2005)
Raw milk	Strategy 1: HTST pasteurisation followed by 4 °C storage for 8 days, and then PEF processing with a mild thermal treatment.	Mesophilic and enteric bacteria (every other day), psychrotrophic (once a week). Daily analysis of titratable	PEF immediately after HTST pasteurization has an ESL of 60 days[a] meanwhile PEF after 8 days of HTST pasteurisation has an ESL of 78 days[a].	(D R Sepulveda et al., 2005)

	Strategy 2: HTST pasteurisation immediately followed by PEF at mild temperature. **Heating:** Using plate heat exchanger. High temperature short time (HTST) pasteurization at 72 °C for 15 s. **PEF:** Inlet flow rate of 20 mL/s, 5 exponentially decay pulses, 35 kV/cm, 2.3 µs pulse width, T_{inlet} = 50°C, T_{outlet} = 65 °C for less than 10 s holding tube, exponentially decay pulse wave, cylindrical concentric electrodes chamber.	acidity, visual (coagulation, thickening, curdling, changes of color, sedimentation, and bubbling) and olfactory (sour or putrid-like odours).	No apparent changes in the olfactory or visual characteristics of the treated samples. Sweet curdling was present in some of the studied samples. The TA of all treated samples remained below the sensory detection level (0.2% of lactic acid).	
Raw whole milk	Thermal treatment followed by PEF. **Heating:** Using coil submerged in hot water bath. T_{inlet} = 25 °C, T_{outlet} = 30 °C, 40 °C, 55 °C. Residence time in coil of 60 s. The milk was then cooled to 15 °C of 60 s. **PEF:** 0.263 mL/s of milk, residence time 3.3 s in PEF chamber, 40 kV/cm, 60 µs, monopolar square wave, 1 µs pulse width, 15 Hz, T_{inlet} = 20 °C, T_{outlet} = 55 °C, cooled to 10 °C in 120s. Native microbiota (approx. 10^4 cfu/mL) was grown (incubation at 37 °C for 24 h) producing a diversified microbial population of approx. 10^{10} cfu/mL.	Microbiological analyses on the total bacterial count (TBC), *lactobacilli*, pseudomonas, lactose fermenting coliforms and non-lactose fermenters, and *staphylococcus aureus* at day 0, 1, 4, 7, 14, and 21.	ESL of 21 days[a] at 4 °C Pseudomonas, coliform, and non-lactose fermenting bacteria did not show a quantifiable growth throughout 21 days. *Staph. aureus* and *Lactobacilli* were less than the detectable numbers at 21 days.	(Walkling-Ribeiro et al., 2009)

[a]Limit of 2.0 x 10^4 cfu/mL (4.3 log units) indicates the end of microbiological shelf life of milk

2.2.5 Impact of PEF on quality of liquid foods

Despite having the ability to inactivate microorganisms, the application of PEF also induces modifications on the physicochemical and nutritional characteristics of milk products. Several authors have reported the impact of PEF application on the quality of foods. However, the information in regard to the influence of PEF sterilisation in combination with heat on the modifications of food quality is relatively limited. Table 3 shows a summary of the literature regarding the effect of PEF treatment on the modification of quality in milk products.

Although PEF processing affects some changes towards the quality of milk, most of the authors reported that the impact of PEF treatment on the quality was less severe as compared to that in thermal processing (Shamsi, 2008; Sui, Roginski, Williams, Versteeg, & Wan, 2011; Zhang et al., 2011). In regards to PEF processing in combination with heat, (Sui, Roginski, Williams, Versteeg, & Wan, 2010) reported that at higher treatment temperature (60-70 °C), the concentration of unmodified native lactoferrin (N-LF), iron-depleted lactoferrin (Apo-LF) and iron-saturated lactoferrin (Holo-LF) decreased. Apart from that, they also observed large protein aggregates at the top of the gel matrix in the simulated milk ultrafiltrate (SMUF) solution after PEF treatment at temperature of 70 °C. These findings suggest that the potential PEF to impact the physicochemical and nutritional qualities in milk products, which therefore highlights the necessity for in-depth studies to elucidate the extent of modification induced by PEF with heat in comparison to the current thermal treatment.

Table 3: Impact of PEF processing on quality of milk products from different studies

Medium	Parameters	Functional properties	References
Raw skim milk (2% fat)	EFS of 40 kV/cm, 20 pulses, treatment time of 2 µs, T_i <12 °C, Tp <55°C, continuous mode	No change in whey protein, taste and flavour.	(Qin et al., 1995)
Raw milk	EFS of 21.5 kV/cm, 1-20 exponentially decaying pulses, T_p of 45-50 °C, batch mode	No significant change in whey protein and taste. About 90% reduction of vitamin C observed at high energy input (>200 kJ/L).	(Grahl & Märkl, 1996)
Raw milk	EFS of 20-80 kV/cm, pulse width of 2 µs, treatment time of 1-10 µs, T_p of 55 °C, continuous mode	No change in fat and protein integrity, no detrimental flavour degradation.	(Dunn, 1996)
Raw milk	EFS of 35 kV/cm, 30 exponentially decaying pulses, 3.3 Hz, flow rate of 600 ml/min, T_o <30 °C, continuous mode	No change in the adhesiveness and cohesiveness of cheese produced from PEF-treated milk. Increase in hardness and springiness.	(Sepúlveda-Ahumada, Ortega-Rivas, & Barbosa-Cánovas, 2000)
Skim milk and simulated milk ultra-filtrate (SMUF)	EFS of 18.3-27.1 kV/cm, exponentially decaying pulses, treatment time of 400 µs, T_p of 20-25 °C & 50-55 °C, continuous mode	No change in fat-soluble and water-soluble vitamins. Reduction of ascorbic acid with greater retention in skim milk.	(Bendicho et al., 2002)
Raw skim milk	EFS of 34.7 kV/cm, 64 bipolar square wave pulses, treatment time of 188 µs, flow rate of 60 ml/min, T_i at 22 °C, T_o at 52 °C, continuous mode	No substantial change in pH, conductivity, colour, protein, total solids, viscosity, particle size and density.	(Michalac, Alvarez, Ji, & Zhang, 2003)
Raw skim milk	EFS of 45-55 kV/cm, square wave monopolar pulses of 500 & 250 ns, 40-120 Hz, flow rate of 83.3 ml/min, T_o of <50 °C, continuous mode	Casein micelle size, viscosity and clotting time decreased substantially, with no change in pH and no modification in the micellar mineral particle.	(Floury, Grosset, Leconte, et al., 2006)
Raw whole milk (3.6% fat)	EFS of 35.5 kV/cm, bipolar pulses of 7 µs, 111 Hz, treatment time of 300-1000 µs, flow rate of 60 ml/min, T_o of <40 °C, continuous mode	pH decreased slightly during storage, free fatty acids increased, whey protein denaturation of α-lactalbumin (40%) > bovine serum albumin (24.5%) > β-lactoglobulin (20.1%).	(Odriozola-Serrano, Bendicho-Porta, & Martín-Belloso, 2006)
Raw, skim, and whole milk	EFS of 35 & 38 kV/cm, monopolar square wave pulses of 2 µs, 200 Hz, treatment time 19.2 µs, flow rat eof 60 ml/min, T_i of 15 & 45 °C, corresponding T_o of 30 & 60 °C, continuous mode	No effect on casein micelle and milk fat globule. Rheological and coagulation properties changed much less compared to heat treatments (LTLT, HTST, 97 °C for 10 minutes).	(Shamsi, 2008)

Whole milk	EFS of 36 & 42 kV/cm with 24-64 & 8-24 exponentially decaying pulses, respectively, treatment time of 2.6 µs, flow rate of 383.3 ml/min, T_o of <25 °C, continuous mode	Changes in milk fat globule in PEF were similar to LTLT treatment	(Garcia-Amezquita, Primo-Mora, Barbosa-Cánovas, & Sepulveda, 2009)
Raw milk (3.8% fat)	EFS of 15-35 kV/cm, treatment times of 12.5-75 µs, T_p of 30 °C, continuous mode	No significant change in vitamins content	(Riener, Noci, Cronin, Morgan, & Lyng, 2009)
Skim milk	EFS of 15-20 kV/cm, 20-60 exponentially decaying pulses of 0.5 Hz, T_o of <35 °C, batch mode	Shear thinning behaviour was observed, consistency index increased	(Xiang, Simpson, Ngadi, & Simpson, 2011)
Raw milk (3.4%)	EFS of 15-30 kV/cm, bipolar square wave pulses of 2 µs, 200 Hz, t_T of 800 µs, T_o of <40 °C, continuous mode	Aldehydes increased and methyl ketones were lower than pasteurised milk treated at 75 °C for 15 seconds. 2(5H)-furanone was only detected in PEF-treated milk	(Zhang et al., 2011)
Raw milk	EFS of 5-40 kV/cm, pulse width of 5-35 µs, square wave pulses, 50-1000 Hz, flow rate of 500 ml/min, T_i of 20-45 °C, T_o of 39-72 °C, continuous mode	No change in colour, drop in conductivity, maximum of 70% reduction in the native form of milk proteins. Reduction of lactoferrin with increasing specific energy.	(Mathys et al., 2013)
Whey proteins (β-lactoglobulin, α-lactalbumin, IgG and lactoferrin)	EFS of 37.6 kV/cm, 50, 100, 200 exponentially decaying pulses of 2 µs at 1 Hz, T_o at <35 °C, batch mode	No considerable changes of IgG and lactoferrin were observed after PEF processing.	(De Luis et al., 2009)
Lactoferrin (native: 98.3% protein; iron depleted: 95.7% protein; iron saturated: 89.5% protein) with different iron saturation levels 24.5, 6, & 78.7%, respectively	EFS of 35 kV/cm, 200 monopolar and 100 bipolar square wave pulses of 2 µs, treatment time of 19.2 µs, flow rate of 60 ml/min, T_o at 30-70 °C	No substantial changes in physicochemical properties of bovine lactoferrin after PEF treatment, however at higher treatment temperature (60-70 °C) of PEF treatment, native folded lactoferrin concentration dropped, protein aggregation was observed and hydrophobicity increased.	(Sui et al., 2010)

*EFS = Electric field strength

2.2.6 Residence time distribution analysis in PEF unit

According to Equation 3 in Section 2.1.2, the total treatment time, t is one of the essential factors to be considered in order to determine D-value of the microorganism. In PEF, the total treatment time typically refers to the summation of the treatment time (pulse width × number of pulses) that takes place in the treatment chamber and the holding time in the holding tube. Therefore, in order to obtain an accurate D-value for microbial analysis, it is essential to determine the accurate residence time in a particular system.

Residence time of an element of fluid is described as the time spent in a system from its entry until it reaches the exit (Torres, Oliveira, & Fortuna, 1998). The residence time distribution is one essential criteria for designing a reactor and performance evaluation (Sievers, Kuhn, Stickel, Tucker, & Wolfrum, 2016) as well as to determine the extent of reaction (R. E. Hayes, 2001). It also can be applied as an evaluation tool to determine the performance of an aseptic system (Ramaswamy, Abdelrahim, Simpson, & Smith, 1995). Currently, residence time was determined by using the theoretical equation as follow:

$$Residence\ time\ (s) = \frac{Volume\ of\ pipe\ (cm^3)}{Volumetric\ flow\ rate\ (cm^3 s^{-1})} \qquad (Equation\ 7)$$

Residence time distribution is related to various interrelated or interdependent factors which include the systems configuration such as tube diameter and length, the rheological properties, flow rate, temperature and the density of the fluid, as well as the shape, density and concentration of the solid particles. Therefore, for complicated and fabricated systems, it is unsuitable to be in an agreement with the assumptions of either a plug or laminar flow regimes, or that the residence times can be determined from the volume of the system and the flow rate of the fluid (Ramaswamy et al., 1995).

Further, as mentioned by (Torres & Oliveira, 1998) flow behaviour in continuous systems are typically complex to be measured experimentally or evaluated theoretically from Navier-Stokes equation. This is because real flow systems such as in PEF unit usually deviate from the ideal flow patterns of plug flow, continuous stirred tank or even flow patterns predicted from mechanistic models. The deviation from the ideal flow patterns is attributed by the formation of stagnant points or by channelling of fluid in the reactor (Levenspiel, 1999). Therefore, the understanding of the residence time distribution in PEF system is critical for a more precise estimation of the extent of microbial inactivation.

Typically, residence time distribution measurements can be obtained from tracer experiments. An inert tracer which is non-reactive, non-adsorbing and has close properties resemblance to the desired

fluid is usually selected. Tracer experiment consists of a pulse response method whereby the injection of tracer is rapidly introduced at the inlet of the reactor and the concentration of the tracer in the effluent is then recorded as a function of time. The time at which the tracer exits the system may vary depending on the flow pattern inside the system (R. E. Hayes, 2001).

Once the graph of tracer concentration against time is obtained, the area under the curve is then normalized by dividing the concentration values with the total area under the curve. This normalised form known as the residence time distribution (RTD) of an element in the system defined as *E(t)* and is described by the following equation (Mohammadi & Boodhoo, 2012):

$$E(t) = \frac{C(t)}{\int_0^\infty C(t)dt} \qquad (Equation\ 8)$$

As the tracer must enter and eventually leave the vessel and due to the normalisation, the area under the curve is equal to unity and is expressed as (Heppell, 1985):

$$\int_0^\infty E(t)dt = 1 \qquad (Equation\ 9)$$

2.2.7 Electrode corrosion during PEF processing

During PEF treatment, the electrodes will be in direct contact with the liquid food product. Therefore it is of a concern that there is a possibility for contamination from the PEF treatment systems to occur. (Morren, Roodenburg, & de Haan, 2003) mentioned that electrochemical reactions can occur at the electrode-liquid interface due to the large currents that pass through the interface in the PEF treatment chamber. The undesirable reaction may result in electrode fouling, electrode corrosion and the modification in food quality. According to (Matser, Schuten, Mastwijk, & Lommen, 2007), degradation of some parts of the electrodes may cause the metallic elements to migrate from the electrodes into the food which could affect the taste and safety of the treated food as well as shortening the lifetime of the electrode (Gad, Jayaram, & Pritzker, 2014). However, due to the limited literature data on the occurrence of electrode corrosion in PEF as well as the wide range of parameters dependant on the electrochemical reactions during PEF treatment, in addition to the variety in the PEF equipment, there is no clear relationship between the metallic ions transfer in the electrodes and the operating conditions that has been explained up to now (Gianpiero Pataro, Falcone, Donsì, & Ferrari, 2014). Therefore, in addition to understanding PEF lethality on microorganisms, the acceptability of PEF processing as an alternative method for liquid food preservation requires attention in regard to the possible chemical and toxicological safety issues (Gad & Jayaram, 2014).

There are several aspects that contribute to the extent of these undesired effects related to the electrode, which include the chamber design and electrode material, process parameters such as pulse shape, peak voltage, polarity and pulse duration as well as the physicochemical properties of the treated food (Guerrero-Beltrán et al., 2010; Gianpiero Pataro, Barca, et al., 2014).

As reported by (Matser et al., 2007) electrode corrosion takes place because of the direct current (DC) leakage into the system and low frequency alternating current (AC) voltages with the following presence of metallic ions of iron, nickel, chromium and manganese in the food. (Wang & Farid, 2015) investigated the effect of frequency on the extent of corrosion in several types of stainless-steel electrodes from ohmic heating (Wang & Farid, 2015) (Table 4) where corrosion was found in almost all types of electrodes studied at low frequency (50 Hz) with alternating current. Similar findings were reported in which the severity of pitting corrosion from ohmic cooking decreases with higher frequencies (Gin & Farid, 2007) and the migration of metallic ions decreases at higher frequency in ohmic heating (Gianpiero Pataro, Barca, et al., 2014).

Table 4: Elemental composition of stainless-steel electrodes

Elemental composition (wt %)	Fe	C	Si	Mn	P	S	Cr	Ni	Mo	Nb	Cu	Co	N
304	70.7	0.03	0.4	1.3	0.04	0.03	18.5	9	-	-	-	-	-
316L	61.05	0.03	0.75	2	0.045	0.03	18	14	4	-	-	-	0.1
904L	46.989	0.12	0.29	1.52	0.22	0.001	20.10	24.16	4.35	0.17	1.34	0.21	0.53

On the other hand, (Jaeger et al., 2014) reported that the undesirable electrochemical reactions can be minimised by applying appropriate parameters such as pulse shape and width in addition to selecting suitable materials for the electrodes. Similarly, (Góngora-Nieto et al., 2002) also suggested on installing suitable electrode materials such as stainless steel, gold, platinum and metal oxides (iridium/ruthenium) as one of the approaches to avoid electrode corrosion. Further, according to (Griffiths & Walkling-Ribeiro, 2014) stainless steel, titanium coated stainless steel or solid titanium as well as silver and germanium (alloy) have been selected as the most appropriate materials for electrodes.

2.2.8 Mechanism of corrosion

According to (Roodenburg, Morren, Berg, & de Haan, 2005a), PEF treatment chamber can be considered as a system with two electrodes in an electrolyte (fluid). Generally, the electrolyte contains dissolved ions. In the electrolyte, the current is carried by ions rather than by free electrons. In the small layer between the stainless-steel electrode and the electrolyte, electron transfer reactions take

place. This layer is named as double layer, which acts like an electrical capacitor. When the applied voltage across the double layer increases and exceeds threshold voltage, two independent electrochemical half-reactions (oxidation and reduction) will take place at the two electrodes (Morren et al., 2003).

The electrode corrosion problem is related to the dissolution of the anode material due to the oxidation of the metal of the electrode at the anode-solution interface and to the deposition of part of the dissolved metals on the surface of the cathode due to the reduction reactions (Gianpiero Pataro, Falcone, et al., 2014).

2.3 UHPH processing

Milk is an important source of nutrient in human dietary consumption. It is perishable and made up of high percentage of water and vulnerable to microorganisms and act as a harbour for their growth. Conventionally, milk is commonly treated using pasteurisation at low temperature long time and high temperature short time or ultra-high temperature (UHT) processing in order to extend the shelf life. However, pasteurisation has a limitation of the shelf life of not high while UHT processing is associated to having negative effects in milk quality including the sensory and flavour profile due to the extremely high temperature. Consequently, the increasing demand for a fresh taste with an extended shelf-life milk product has stimulated an exploration for a technology that can complement the gap in pasteurisation and UHT processing. Currently, there has been several proposed technologies as an alternative including high hydrostatic pressure, pulsed electric field, ultrasound, and ultra-high-pressure homogenization. UHPH has shown potential in several applications including extended shelf life of milk (Addo & Ferragut, 2015; J Pereda, Ferragut, Quevedo, Guamis, & Trujillo, 2007; Fábio Henrique Poliseli-Scopel, Hernández-Herrero, Guamis, & Ferragut, 2013), extended shelf life juices (Guan et al., 2016), inactivation of spores in milk products (E Georget et al., 2014) and juices (Roig-Sagués, Asto, Engers, & Hernández-Herrero, 2015), and extraction of valuable compounds by cell disruption (Praveenkumar et al., 2020). The high pressure homogenization (HPH) has been proposed also for the bacterial spore inactivation, generally in combination with other physico-chemical methods for spore surviving and or gemination due to the ability of bacterial spores to survive to the most prohibitive conditions (Patrignani & Lanciotti, 2016).

UHPH is also used in combination with heat at an elevated inlet temperature which has a potential to germinate and inactivate spore. This has initiated the exploration to apply the concept to produce ESL milk with an extended shelf-life stability than pasteurisation and a better-quality retention than UHT.

The word “homogenization” is referred to the ability to produce a homogeneous size distribution of particles suspended in a liquid, by forcing the liquid under the effect of pressure through a specifically designed homogenization valve. Homogenizer able to process fluid matrices at pressure ranging between 20–100 MPa are nowadays employed in the dairy beverage, pharmaceutical, and cosmetic industries mainly to reduce particle size and consequently increase stability of emulsions in order to avoid creaming and coalescence phenomena. In UHT processing, milk undergoes homogenization to reduce the effect of fat separation during storage. Initially high-pressure homogenization is used in a pre-process until the parameter improved and expand its application. Modern high-pressure homogenizer enables pressures 10-15 times higher than traditional ones and covers pressure ranges between 300-400 MPa. These ranges have been referred to as UHPH. The progression toward UHPH has also opened the view to new sterilisation opportunities, including the inactivation of spores by HPH. The inlet temperature of pumpable products and the level of pressure, which both determine the temperature reached during the UHPH treatment, have been considered as the main factors of the microbial inactivation (Patrignani & Lanciotti, 2016).

2.3.1 Mechanism of UHPH

Mechanical homogenization has been defined as the ability to generate a distribution of particles of homogeneous size, in a liquid, by forcing the liquid under high pressure through a disruption valve (Donsì, Ferrari, & Maresca, 2009). A homogenizer principally consists of a pump and homogenizing valve. The pump is used to force the fluid into the valve where the homogenization occurs. In the homogenizing valve the fluid is forced under pressure through a constricted orifice between the valve and the valve seat. The operating pressure is controlled by adjusting the distance between the valve and the seat (Patrignani & Lanciotti, 2016).

High pressure homogenization (HPH), also known as dynamic high-pressure homogenization, utilizes pressures 10 to 15 times higher than those of conventional homogenizers. It covers the full range of 100 to 400 MPa and the range 300–400 MPa is generally referred to as ultra-high pressure homogenization (E Georget et al., 2014). Ultra-high-pressure homogenization technology is a novel process for the treatment of liquid food products. Based on traditional homogenization, the process features improvements to both the valve material and valve geometry, which helps to reach pressures up to 400 MPa. The most important processing parameters are operating pressure, inlet temperature (T_i), and the number of passes (Amador-Espejo, Hernández-Herrero, Juan, & Trujillo, 2014). The main mechanisms involved in the microbial inactivation by UHPH treatment are mostly mechanical such as shear stress, high speed collisions, impingement, cavitation and changes in internal energy (Datta, Hayes, Deeth, & Kelly, 2005; Donsì, Ferrari, Lenza, & Maresca, 2009; Michiels, 2006; Paquin, 1999).

2.3.2 Application of UHPH in inactivation of spores

Ultra-high pressure homogenization technology was first used in the pharmaceutical, chemical and biochemical industries. Several applications of UHPH for the food industry have been reported. Possible uses of this technology for the dairy industry include reduction of fat globule size, inactivation of enzymes, and destruction of bacteria (J Pereda et al., 2007). UHPH extension of the homogenization pressure range offers a new area to investigate bacterial spore inactivation and to attempt to achieve commercial sterility through a single, continuous step combining dynamic high pressure and other stress factors, such as temperature, shear and cavitation (E Georget et al., 2014). With regard to microbiology, both HPH and UHPH have been commonly used for cell disruption of dense microbial cultures (Saboya, Maillard, & Lortal, 2003), and to cause a reduction of the microbial population, improving the microbial safety of dairy products (Amador-Espejo et al., 2014; E Georget et al., 2014; J Pereda et al., 2007) and fruit juices (Á Suárez-Jacobo, Gervilla, Guamis, Roig-Sagués, & Saldo, 2009). High microbial reductions in pathogenic microorganism such as *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli* have been achieved through UHPH technology (Briñez, Roig-Sagués, Herrero, & López, 2007; Diels, Callewaert, Wuytack, Masschalck, & Michiels, 2004; Donsì, Ferrari, Lenza, et al., 2009).

Of all microorganisms, bacterial spores are resistant to most conventional treatments and are the main reason for the use of high thermal intensity treatments to sterilise food (Erika Georget et al., 2010). Existing studies showing successful inactivation of bacterial spores through UHPH were reviewed (Erika, Brittany, Michael, Volker, & Alexander, 2014), and it was concluded that the best inactivation could be achieved by combining high homogenization pressures and high inlet and, consequently, valve temperatures (Amador-Espejo et al., 2014; N. Cruz et al., 2007; Poliseli Scopel, 2012; Valencia-Flores, Hernández-Herrero, Guamis, & Ferragut, 2013). Most studies in food matrices focused on *Bacillus cereus* spores or endogenous mesophilic spore strains and claimed sterility based on the full inactivation of these strains. A study conducted with *Geobacillus stearothermophilus* spores, a particularly thermostable strain, showed that high pressure of 300 MPa and 84 °C at the UHPH valve were not sufficient to inactivate this strain, but unfortunately did not assess higher valve temperatures (Pinho, Franchi, Tribst, & Cristianinia, 2011). Further, work on the exploration of UHPH processing to inactivate thermophilic spore inoculated in UHT whole milk had shown that with an inlet temperature of 85 °C, *Geobacillus stearothermophilus* (CECT 47) spores could be inactivated (Amador-Espejo et al., 2014). Summary of other published work in the application of UHPH in spore inactivation is also summarised in Table 5.

Table 5: Application of UHPH to inactivate spores from different studies

Medium	Spores	Parameters	Log reduction	References
PBS buffer 0.01 M	*Bacillus subtilis* PS832,	Pressure: >300 MPa Valve temperature: 145 °C Inlet temperature: 80 °C Residence time: 0.24 s	5	(E Georget et al., 2014)
	Geobacillus stearothermophilus ATCC 7953		2	
UHT whole milk	*Bacillus cereus* (CECT 5144)	Pressure: 300 MPa Valve temperature: 139 °C Inlet temperature: 85 °C Residence time: <0.5 s	6.47	(Amador-Espejo et al., 2014)
	Bacillus licheniformis (DSMZ 13)		6.33	
	Bacillus sporothermodurans (SMZ 10599)		6.91	
	Bacillus coagulans (DSMZ 2356)		6.57	
	Bacillus subtilis (CECT 4002)		5.22	
	Geobacillus stearothermophilus (CECT 47)		5.26	
UHT Skim milk	*Bacillus stearothermophilus* ATCC 7953	Pre-treatment: Heat shock 100 °C/15min Pressure: 300 MPa Inlet temperature: 45 °C Valve temperature: 84°C Passes: 16	0.67 (<1)	(Pinho et al., 2011)
	Clostridium sporogenes PA3679			
Soy milk	Aerobic spores (natural)	Pressure: 300 MPa Inlet Temperature: 80°C Valve Temperature: 144°C	<2.18	(F H Poliseli-Scopel, Hernández-Herrero, Guamis, & Ferragut, 2014)
Soy milk	Aerobic spores (natural)	Pressure: 300 MPa Inlet Temperature: 40°C Valve Temperature: 108°C	>2	(N. Cruz et al., 2007)
Milk (3.5% fat)	Aerobic spores (natural)	Pressure: 300 MPa	>1	(J Pereda et al., 2007)

		Inlet Temperature: 40°C Valve Temperature: 103°C		
Almond beverages (with and without lecithin)	Aerobic Spores (Natural)	Pressure: 300 MPa Inlet Temperature: 75°C Valve Temperature: 129°C	4	(Valencia-Flores et al., 2013)
Soymilk and Almond milk	Aerobic spores (Natural)	Pressure: 300 MPa Inlet Temperature: 75°C Valve Temperature: 135°C	>4	(Ferragut, Hernández-Herrero, Poliseli, Valencia, & Guamis, 2011)
Malt extract broth	*Alicyclobacillus acidoterrestris* (DSMZ 2498, r4 and c8)	Pressure: 170 MPa Valve Temperature: 55°C	DSMZ 2498: 1-2 log r4: 0.5-1 log c8: 0.25 log	(A Bevilacqua, Cibelli, Corbo, & Sinigaglia, 2007)
Laboratory medium apple juice	Alicyclobacillus acidoterrestris (DSMZ 2498 & γ4)	Pressure: 140 MPa Exit temperature: 40°C	γ4: 0.82 log DSMZ 2498: 0.67 log	(Antonio Bevilacqua, Corbo, & Sinigaglia, 2012)
Orange Juice	Alicyclobacillus *acidoterrestris* spores (CECT 7094)	Pressure: 300 MPa Inlet Temperature: 80°C Valve Temperature: 130°C	>5.5	(Roig-Sagués et al., 2015)
	Alicyclobacillus hesperidum spores (CECT 5324)		5.0	
Ice cream mix	*Bacillus licheniformis* ATCC 14580	Pressure: 200 MPa Inlet Temperature: 50°C Valve Temperature: 88°C	0.75 log	(Feijoo, Hayes, Watson, & Martin, 1997)

2.3.3 Application of UHPH in shelf-life extension of liquid foods

On the other hand, the potential of UHPH technology to produce extended shelf life (ESL) liquid food products have also been explored various authors shown in Table 6. Different authors (M. G. Hayes, Fox, & Kelly, 2005; Julieta Pereda, Ferragut, Guamis, & Trujillo, 2006; Vachon, Kheadr, Giasson, Paquin, & Fliss, 2002) have suggested the possible potential of UHPH as a combined pasteurization and homogenization step to obtain commercial milk with a shelf life similar to conventional market milk. However, literature related to the effects of UHPH on milk shelf life is scarce. According to (Smiddy, Martin, Huppertz, & Kelly, 2007), a shelf life between 4 and 7 days was obtained for UHPH-treated raw whole bovine milk at 200 or 250 MPa with inlet temperatures of 55 or 70 °C. To date, physicochemical and microbiological changes of UHPH-treated milks at different temperatures and pressure have been studied immediately after treatment but not at different storage conditions (M. G. Hayes et al., 2005; Julieta Pereda et al., 2006; Thiebaud, Dumay, Picart, Guiraud, & Cheftel, 2003).

Table 6: Application of UHPH in shelf-life extension of liquid foods

Medium	Parameters	Results	References
Apple juice	Pressure: 300 MPa Inlet temperature: 4 °C	• 60 days (4 °C and 30 °C)	(Ángela Suárez-Jacobo et al., 2012)
Raw bovine milk	Set a) Parameters: 100, 200, 300 MPa Inlet temperature: 40 °C Set b) Parameters: 200 and 300 MPa Inlet temperature: 30 °C Outlet temperature: 80 °C Residence time: 0.7 s	• 21 days at 4 °C (200 MPa, 30 °C) • 14-18 days at 4 °C (other parameters) • Significant decrease in psychrotrophic counts at d=1 at 200 and 300 MPa at both inlet temperatures, reaching reductions of about 3.5 log cfu/mL • Coliforms, lactobacilli, and enterococci, were completely inactivated except at 100 MPa • Spore reduction of 0.8-1.1 log reductions, 0.4 log reduction at 100 MPa • No Pseudomonas was detected at 200 and 300 MPa at d=1	(J Pereda et al., 2007)
Raw bovine milk	Pressure: 200 MPa Number of cycles: 5 Inlet temperature: 25 °C	• 2.5 log reductions of total bacterial count	(Sharabi, Okun, & Shpigelman, 2018)

2.3.4 Impact of UHPH of milk quality

Due to the increase demand for fresher and more nutritious food products from the consumer, it is also vital to investigate the impact of UHPH on the treated food. Despite the potential ability of UHPH to improve shelf life, several researchers have investigated the effect in quality components of UHPH-treated food products Table 7. According to (M. G. Hayes & Kelly, 2003a), they observed inactivation of native milk enzymes such as lactoperoxidase, plasmin and alkaline phosphatase in UHPH treated raw whole bovine milk. High pressure homogenization treatment is also reported to act on food constituents, especially proteins and enzymes, modifying their functional properties and activities (M. G. Hayes & Kelly, 2003b; Kheadr, Vachon, Paquin, & Fliss, 2002; Patrignani & Lanciotti, 2016; Vannini, Lanciotti, Baldi, & Guerzoni, 2004).

Table 7: Impact of UHPH processing on the quality of liquid foods

Medium	Parameters	Effects on quality	References
Raw bovine milk	Pressure: 300 MPa	• Reduction of L* value and reduce viscosity at 200 MPa • Great reduction of particle size at 200 MPa, no further reduction could be obtained at 300 MPa • Milks at 300 MPa showed a significantly lower D50 value compared with pasteurised milk but slightly higher compared with milks treated at 200 MPa • Fat aggregation at 300 MPa due to an increase in $D_{4,3}$ but no creaming observed • Complete inactivation of lactoperoxidase and alkaline phosphatase after UHPH at both pressure and inlet temperatures • Differences in instrumental colour and viscosity measurements between UHPH and pasteurised milks were not visually or sensorially obvious.	(J Pereda et al., 2007)
Raw bovine milk	Pressure: 200 MPa Inlet temperature: 25 °C	• Vitamin C, riboflavin, antioxidant less affected compared to pasteurised milk • Decrease in particle size	(Sharabi et al., 2018)
Almond milk	Pressure: 350 MPa Inlet temperature: 85°C	• Significant effect was observed on particle size • Almond proteins antigens were not detected in UHPH treated product. • Vitamin B1 and Vitamin B2 were similar to raw milk • 50% reduction in free sulfhydryl groups were observed	(Briviba, Gräf, Walz, Guamis, & Butz, 2016)
Soy milk	Pressure: 200 MPa, 300 MPa	• Intense particle size reduction was observed, although aggregates were formed at 300 MPa. • UHPH products were more stable (less particle settling) than UHT milk. • Decrease in L values were observed with UHPH treated milk	(N. Cruz et al., 2007)
Soy milk Almond milk	Pressure: 200 MPa, 300 MPa Inlet temperature: 55°C, 65°C, 75°C	• Finer emulsion was observed. Although aggregation was observed. • Increased oxidation was observed in almond milk.	(Ferragut et al., 2011)
Soy milk	Pressure: 300 MPa Inlet temperature: 80°C	• UHPH treated milk produce high colloidal stability during storage.	(F H Poliseli-Scopel et al., 2014)

	Valve temperature: 144°C	• No difference in sensory were detected by panels members between UHT and UHPH treated milk.	
Soy milk	Pressure: 200 MPa, 300 MPa Inlet temperature: 55°C, 65°C, 75°C	• Particle size was reduced • Sedimentation was reduced • Lower hydro peroxide formation was observed in comparison to UHT milk	(Fábio H Poliseli-Scopel, Hernández-Herrero, Guamis, & Ferragut, 2012)
Raw bovine milk	Pressure: 300 MPa Inlet temperature: 40 °C Valve temperature: 101.5 °C	• Showed highest values for 2-pentanone, 2-heptanone, 2-nonanone for UHPH milk and high-pasteurized milk • Hexanal content remained almost the same for all samples • No significant differences in dimethyl sulphide were detected between raw and treated milk samples • Hexanoic acid was detected • No lipolysis in 4 °C storage after 300 MPa	(J Pereda et al., 2008)

3 Objectives and scopes

In this thesis, the main aim is to perform microbial inactivation at a reduced temperature or shorter treatment time using novel technologies such as pulsed electric field (PEF) and ultra-high-pressure homogenisation (UHPH) processing in combination with heat. Experimental procedures were undertaken within the following scopes:

- Microbial studies with PEF and UHPH processing

Firstly, thermal treatment using capillaries method were carried out using UHT skim bovine milk, reconstituted UHT sheep milk, and water. Secondly, *Bacillus subtilis* spores were treated in UHT skim milk using PEF processing in combination with heat. Thirdly, same spore species in UHT whole bovine milk and goat milk were treated using UHPH processing at an elevated inlet temperature. A shelf-life study was also conducted with UHPH processing using raw sheep milk in a refrigerated condition. The total bacterial count and physical properties were monitored through the storage period.

- Hydrodynamic testing in the PEF unit

This goal was executed by monitoring the residence time distribution (RTD) in PEF unit whereby the determined residence time will be applied in the microbial analysis of PEF.

- Migration of metallic ions in PEF chamber

This experiment was executed by investigating the rate of metallic ions migration from the electrodes in the PEF chamber into the liquid foods as a step to justify the safety of the PEF unit in terms of ions transfer.

4 Methodology

4.1 Pulsed Electric Field (PEF) unit

A continuous PEF system that was previously fabricated at the workshop of the Faculty of Engineering, University of Auckland, New Zealand was used in this study. The system shown in Figure 4 is composed of a high voltage (AC) pulse generator, treatment chamber, cooling system, degassing unit, and monitoring devices to measure and record the current, voltage and temperature during the application.

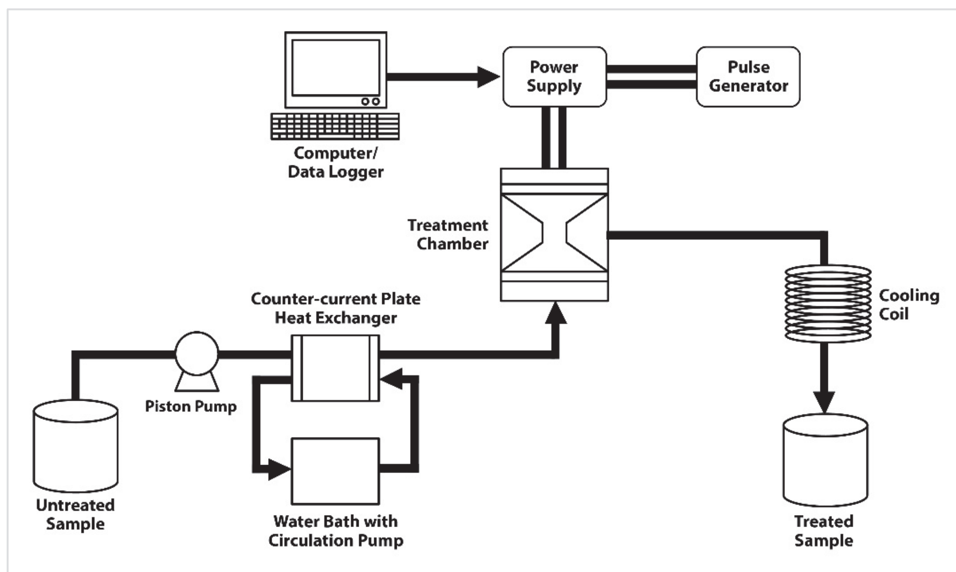

Figure 4: A schematic experimental set-up of pulsed electric field (PEF) processing

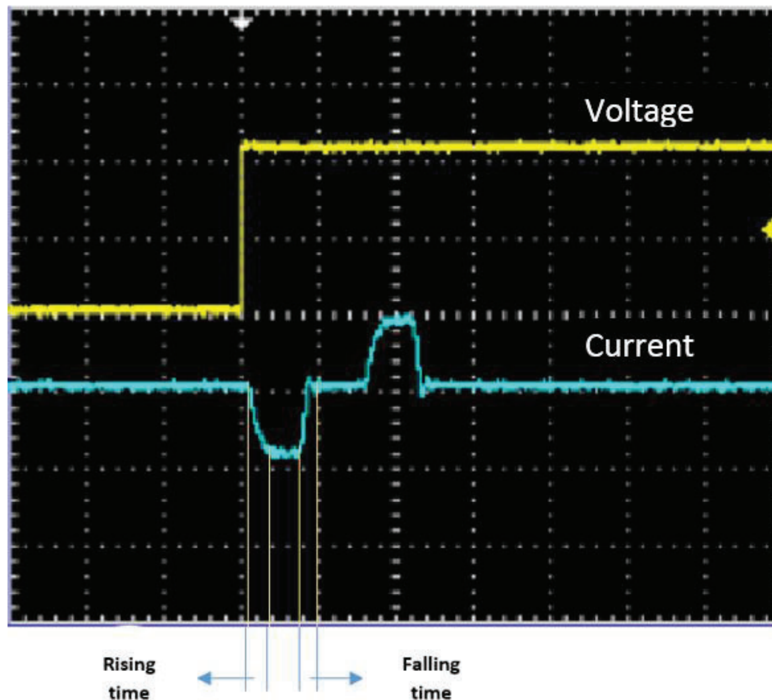

Figure 5: The square bipolar pulse generated by the PEF unit

The high voltage pulse generator produces bipolar square wave pulses Figure 5 with a pulse width of 1.7 µs, pause duration of 2.5 µs and short pulse rising and falling times of less than 0.5 µs with a frequency of up to 1000 Hz. The generator peak power is 4.5 MW. An input transformer and a rectifier designed to increase the voltage to 1 kV and another transformer to raise the output voltage to 30 kV was included in the generator so that the nominal output impedance can be changed by changing the transformer turns ration without having to alter the switching circuits. The cell can be configured to operate symmetrically around ground potential, i.e., one terminal of the cells does not have to be grounded. This method reduces unnecessary ground currents through the processed liquid. Safety of the system was also improved as the maximum voltage to ground is now equal to only half the maximum voltage between the cell electrodes. The use square wave in this experiment is selected because it has been reported to be superior to exponentially decaying pulses as it gives the treatment a sustained and constant intensity for the total duration of the pulse (Wan, Coventry, Swiergon, Sanguansri, & Versteeg, 2009). The application of bipolar square wave in this work was selected as it results in extra stress in the microbial cell membrane with better energy utilization, reduced the deposition of solids on the electrode surface and reduce the occurrence of electrode corrosion. Additionally, as mentioned by (Álvarez, Pagán, Condón, & Raso, 2003), short rising time increases the efficiency of PEF treatment as it is slightly dependant on the pulse rising time. At a very short rising time, all the power delivered is used for microbial inactivation with minimum heating.

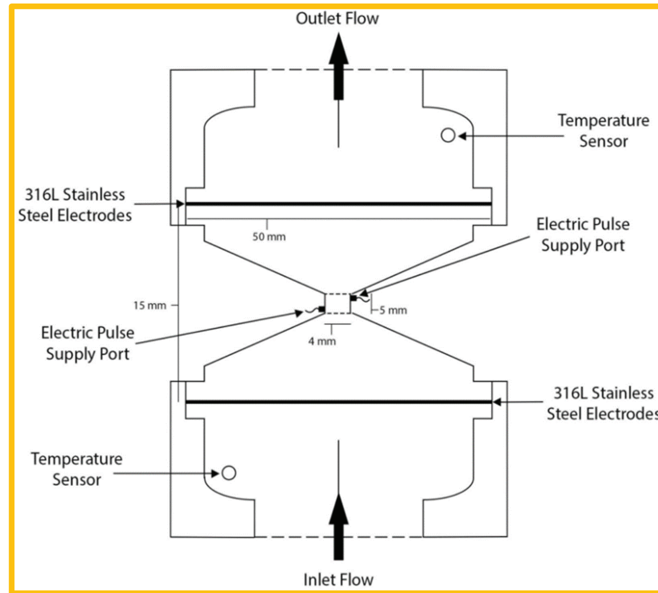

Figure 6: Design of the PEF unit treatment chamber

The treatment chamber shown in Figure 6 designed by (S. R. Alkhafaji & Farid, 2012) consists of two parallel electrodes which is made of the food-grade 316L stainless steel with the composition of iron (62-69%), chromium (16-18), nickel (10-14%) and manganese (2%). The distance between the electrodes is 15 mm and the volume of the treatment chamber is 0.063 cm^3. The chamber was designed in such a way that it could provide uniform high intensity electric field strength in the treatment region and minimize the occurrence of electrode corrosion (S. Alkhafaji, 2006).

4.2 Ultra-high-pressure homogenisation (UHPH) unit

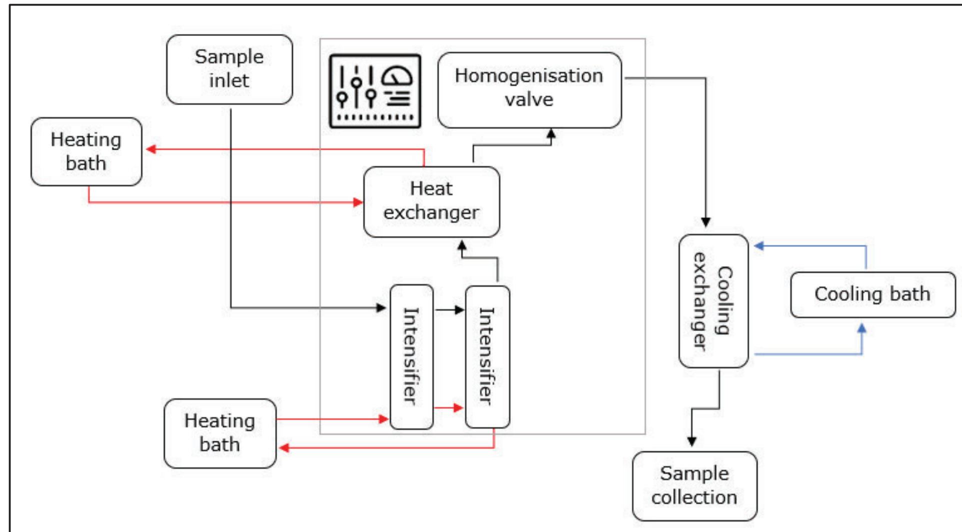

Figure 7: Flow diagram of UHPH unit

In this study, UHPH treatments were carried out using a continuous flow system pilot scale unit (FPG7575:300, Stansted Power Fluid Ltd., UK) shown in Figure 7. The equipment comprised of two intensifiers driven by a hydraulic pump and high-pressure ceramic valves where the primary valve can sustain up to 400 MPa. In order to achieve different inlet and outlet temperatures, the intensifiers were jacketed and could be maintained to required temperatures by circulating hot/cold water using water bath. There is a spiral type of heat exchanger located between intensifiers and ceramic valves to preheat the sample before treatment. For cooling after homogenization treatment, another spiral type of heat exchanger is connected to a chiller operating at -4 °C. The processing parameters were indicated by processing valve pressure (P), feed temperature (Tf), inlet temperature correspond to temperature before homogenization valve (Ti), homogenization valve temperature (Tv) and outlet temperature that corresponds to temperature after cooling (To), the time of which treated samples were collected aseptically into sterile containers for later microbial analysis.

4.3 Microbial studies

4.3.1 Production and enumeration of spores

Agar medium preparation and inoculation

Nutrient agar (NA) plates were prepared by adding 1mg $MnSO_4$, 0.5 G $CaCl_2$ to 23 g NA powder and making it to 1L with distilled water. Then, the bottles containing the agar solutions were autoclaved for 15 minutes at 121 °C. Once autoclaved, the agar was poured into sterilised petri plates. After it is cooled to room temperature and solidified, the plates were kept in the refrigerator in sealed bags for future use.

The freeze-dried culture of *Bacillus subtilis* ATCC 6633 was obtained from Fort Richard Lab Ltd., New Zealand. The culture was transferred into cooked meat medium and later placed in 35 °C incubator for seven days. Using a sterile wire loop, a sample was taken out from the mother suspension and then streaked onto a NA plate. The plate was then incubated at 30 °C for 24 hours. After that, approximately 1 mL of sterilised water was poured onto the plate and the spores were scraped out. Next, 0.1 mL (100 µL) of the scraped spores was pipetted out into a test tube containing 9.9 mL of nutrient broth (make a total of 10 mL). Then, the broth was incubated at 30 °C for 24 hours.

Spore sporulation and harvesting

For spore sporulation, 0.1 mL (100 µL) of the inoculated nutrient broth was pipetted out and streaked onto NA plates and incubated at 30 °C for 14 days. During the sporulation period, the growth proportion of spores was monitored by phase contrast microscopy (Motic microscope BA 410 Series, Canada) as reported by (Ansari, Ismail, & Farid, 2017). Then, spores were harvested by gently scraping the colonies with a sterile loop spreader while flooding the plates with sterilised water. After scraping, the spore suspension was carefully transferred into sterile tubes and centrifuged 3 times at 4816 g for 15 minutes at 4 °C to separate the vegetative cells from the spores. After each centrifugation cycle, the supernatant was discarded and the pellet was washed with sterile distilled water for 3 times. At the end, the pellet was re-suspended in 20 mL of sterile distilled water. The suspension was heated at 80 °C for 10 minutes in order to inactivate the vegetative cells. Finally, the spore concentration was determined by following a serial dilution technique. The spore suspension was spread onto plate count agar (PCA) plates with appropriate dilutions and then the plates were incubated at 30 °C for 24-36 hours. After incubating, the concentration of the colonies was determined and expressed in terms of colony forming units per ml (cfu/mL). The suspension was kept in the fridge at 4 °C for later use.

Spore enumeration

Plate count agar (PCA) plates were prepared similar to NA plates by adding 23.5 g of PCA powder into 1L of distilled water. The spore concentration was determined by following a serial dilution technique. The spore suspension was spread onto PCA plates with appropriate dilutions and then the plates were incubated at 30 °C for 24-36 hours. After incubating, the concentration of the colonies was determined and expressed in terms of colony forming units per mL (cfu/mL). The suspension was kept in refrigerated conditions at 4 °C for later use. A similar technique of spore enumeration was used to calculate spore count in milk samples in PEF and UHPH processing studies.

4.3.2 Thermal inactivation

The capillary tube method technique was conducted to investigate the thermal inactivation for *Bacillus subtilis* spores in milk with a method described by (Ansari et al., 2017). The glass capillary tube has 3 mm outside diameter and 1.2 mm inside diameter. All the capillary tubes were first autoclaved before use. The spore suspension was pasteurised at 80 ℃ for 15 minutes to eliminate all vegetative cells. Then 100 µL of the spore suspension with concentration of 10^9 spores/ml was inoculated in 100 ml milk. 65 µL of the inoculated milk was injected into each of the glass capillary tubes using pipettes with sterile tips. Then both the ends of the glass capillary tubes were sealed by flame. The glass capillary tubes were then immersed in an oil bath at preselected measurement temperatures of 100 °C and 110 °C. The time gaps for measurement temperature at 100 °C and 110 ℃ were 100 s and 14 s, respectively. Then the capillary tubes were cooled immediately in an ice water bath. They were washed with 70% alcohol and both ends were clipped under sterile conditions. In order to check the number of survivors, 50 µL of the sample was diluted and inoculated on PCA plates. All enumeration studies were performed in duplicates.

4.3.3 PEF treatment

All UHT milk used in this study was purchased from Countdown supermarket in New Zealand. Prior to the operation of PEF with inoculated milk, all processing devices including pipes of the PEF unit were sterilised using 3L of 3% w/v Oxonia Active sanitizer and then rinsed with 3-L sterilised water thoroughly. A suspension of *Bacillus subtilis* spores (10^9 cfu/mL) maintained at the microbiology laboratory of University of Auckland was used in this study. For each run, 3L of skim milk was inoculated with 3 mL of Bacillus subtilis ATCC 6633 spores (10^9 cfu/mL) producing a solution with an initial concentration of spores of approximately 10^6 cfu/mL.

Then the inoculated milk was heated to 80 °C with hot water through a counter-current plate heat exchanger. Once the system has reached steady state, a sample of inoculated milk was taken out for the accurate measurement of the initial microbial load. The milk was then circulated using a piston pump at a flow rate of 4.3 cm^3s^{-1}. Subsequently, the pulse generator was turned on, and the high voltage pulses were applied to the milk flowing through the treatment chamber. Since the treatment was conducted over 100 ℃, the treatment chamber was pressurised up to 6 bar to avoid boiling in the chamber.

The temperatures of the treated milk were measured at two positions using thermocouples located at the outlet point of treatment chamber and also at the end of the holding tube. The milk was then immediately cooled by the chiller that consists of two cooling coils immersed in cold glycol. The treated samples were collected in aseptic sample tubes and refrigerated at 4 ℃ before enumerating the number of survivors as described in Section 4.3.1.

PEF experiments were conducted at 28 $kVcm^{-1}$ with 300 Hz and 50 $kVcm^{-1}$ with 100 Hz with a pulse width of 1.7 µs. The total treatment time was determined from the addition of the treatment time in the treatment chamber and the time spent in the holding tube (L = 73 cm, ID = 0.4 cm). The treatment time in the treatment chamber was determined using Equation 6 mentioned in Section 2.2 whereas the residence time in the holding tube can be calculated using Equation 7.

After each run, the system is cleaned immediately. The system was first rinsed with continuous flow of deionised water, followed by 3% of NaOH solution. Then, it was rinsed with continuous flow of deionised water followed by 3% Oxonia Active sanitizer solution for about 20 minutes and at last the entire system was washed with 3L of sterilised water. The feed tank was filled with 3L of 3% of Oxonia Active solution and kept overnight or until the next use to keep it sterilised.

4.3.4 UHPH treatment

4.3.4.1 Inactivation of microbial spores

Prior to treatment, 5-L batch of store-bought UHT bovine and goat milk was inoculated respectively, with B. subtilis spore suspension to obtain ~10^6 cfu/ml as the final concentration of spores inside the pre-treated milk. Then it was pre-heated to 55 °C using a water bath. This pre-heating was necessary to achieve a high inlet temperature and subsequently high valve temperature. Meanwhile UHPH pressure was adjusted up to ~250 MPa. The inoculated batch of milk was then circulated through the UHPH unit at a flow rate of 40 L/h, and valve pressure was adjusted up to about 250 MPa using the

first pressure valve knob. Measurements of processing valve pressure, valve temperature and outlet temperature were recorded once the system reached a steady state.

The total treatment time was determined from the addition of the treatment time inside the pipe right from the first processing valve until the entry of the cooling chamber (L = 106.5 cm, ID = 0.4 cm), which is 1.2 seconds.

For UHPH cleaning purposes, 1% Trigene solution was passed through the unit for 10 minutes followed by sterilised water to flush the system. Subsequent to any experiment, the microbial count of rinsed water was taken to ensure proper sterility of the system.

4.3.4.2 Extended shelf-life (ESL) study

Raw sheep milk obtained from Fernglen Farm in New Zealand was used in this study. Raw milk samples were ordered freshly from the farm and once it has arrived the university in a refrigerated container, experiment using the UHPH was performed right away. UHPH unit was run at a similar pressure setting up to about 250MPa and the processing valve temperature and outlet temperature were monitored and noted once the system has reached steady state. Then, treated raw sheep milk was collected aseptically into sterile bottles for later microbial analysis.

The total plate count (TPC) before and after UHPH processing will be assayed via the standard plate count method. The microbiological shelf life of processed milk will be considered at an end when the limit of 2.0×10^4 cfumL^{-1} is exceeded according to the milk ordinance for Grade A milk and milk products (Walkling-Ribeiro et al., 2009). Samples of treated raw sheep milk will be collected after treatment to evaluate the total bacterial count at 4 °C storage. Plate Count Agar (PCA) was used in the preparation of agar plates. The milk samples were diluted using distilled water and spread on PCA plates. Then the plates were placed in a 30 °C incubator for 24-36 hours. Thereafter, the number of colonies were counted in terms of colony forming units per ml (cfu/mL).

4.3.4.3 Physical properties analysis

Prior to pH measurements, pH meter (HANNA Edge pH Meter Instrument) was calibrated using standard solutions of pH 4.0, 7.0 and 10. Then, milk samples were transferred into a 100 mL beaker at room temperature and the electrode probe was immersed until a constant reading was reached.

For conductivity measurement, the conductivity (mS/cm) of milk samples was measured using a conductivity meter (Mettler Toledo AG, Seven Compact Conductivity Meter S230, Switzerland) by

immersing the electrode probe (inLAB 710 probe) in milk samples until a constant reading was achieved.

4.4 Residence time distribution (RTD) study of PEF system

In this study, RTD in the PEF unit was investigated by injecting a tracer amount of salt solution of a known conductivity at the inlet of PEF chamber and the change of tracer voltage with time at the effluent stream was monitored. The apparatus set up is shown in Figure 8.

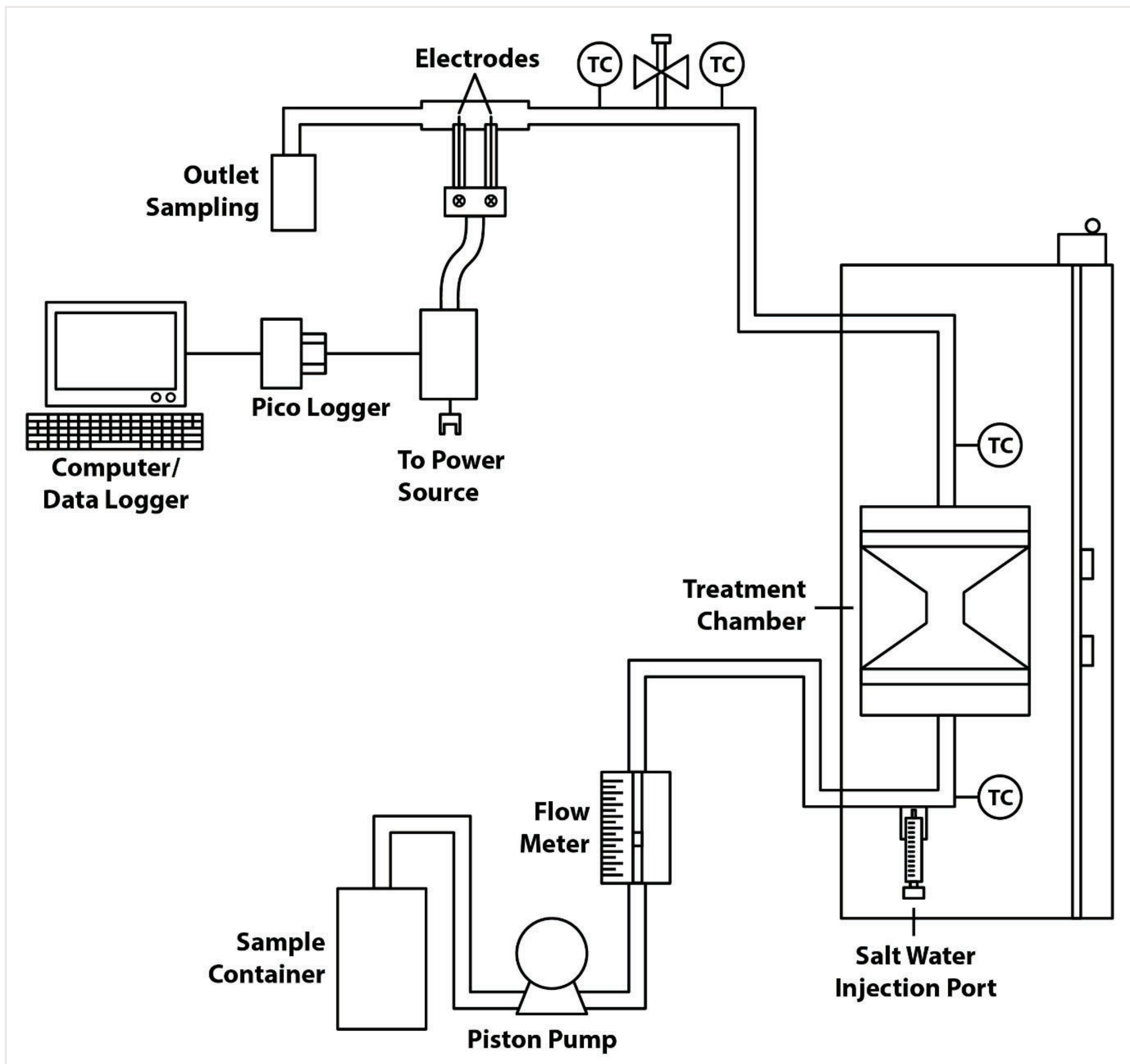

Figure 8: A schematic experimental set-up for RTD study

At the outset, the pump was switched on with a continuous flow of deionized water at five different flowrates, respectively. After it reached steady state, 0.5 mL NaCl solution with a known conductivity of 10 $mScm^{-1}$ was injected through a one-way syringe fitted onto the pipe section at the inlet of the treatment chamber. The time of injection was kept constant in all experiments. A fabricated electrical instrumentation that consists of measuring probe was attached at the outlet pipe right after the holding tube section. Change in signal at the effluent stream was monitored using the Pico-log data logger and the data was used for later RTD determination.

In order to determine the mean residence time, t_{MRTD} from the experimental data, a weighted average over all residence time was considered. The mean residence time can be obtained by multiplying the individual residence time for each element by the fraction of the salt solution, summed over all elements. The equation can be expressed as follow:

$$t_{MRTD} = \frac{\sum t_i C_i \Delta t_i}{\sum C_i \Delta t_i} \qquad (Equation\ 10)$$

where C_i is the measured tracer concentration (voltage) at time t_i, and Δt_i is the interval between two measurements, which is constant. Therefore, the expression can be further simplified into:

$$t_{MRTD}(s) = \frac{\sum t_i C_i}{\sum C_i} \qquad (Equation\ 11)$$

On the other hand, the residence time can also be estimated offline from the volumetric throughput, Q, passing through a fixed volume of equipment, υ, and is expressed as follow:

$$t_{MRTD} = \frac{\upsilon}{Q} \qquad (Equation\ 12)$$

If the two mean residence times do not coincide, then there is an evidence that there is channelling or bypassing in parts of the equipment (Heppell, 1985).

4.5 Corrosion study in PEF system

3 L of saline water with 5.3 $mScm^{-1}$ conductivity (to emulate a similar conductivity with the store-bought UHT skim milk used in PEF microbial study) was put into a stainless-steel container and then heated to an inlet temperature of 80 °C in an oil bath. The inlet flow rate was set at 4.3 cm^3s^{-1} in all experiments. Once the solution reached 80 °C, the pump was switched on to ensure the entire system was at steady temperature of 80 °C then untreated samples were collected using the sampling tubes. Next, experiments were conducted at both 50 $kVcm^{-1}$ and 42 $kVcm^{-1}$, at 100 Hz, 75 Hz, and 50 Hz, respectively. Treated samples were collected when the outlet temperature has reached steady state.

After sampling, the pulse generator was switched off. Then, the entire system was rinsed with de-ionised water before starting the next run.

Each sample was taken out in three replicates with 10 mL each. The analysis of metallic elements was performed by using the inductively coupled plasma mass spectrometry (ICP-MS) instrument. Evaluation of the performance of the electrode materials was done by monitoring the change in metal concentrations of iron (Fe), chromium (Cr), nickel (Ni) and manganese (Mn). The concentrations obtained were compared to the maximum allowable values in standards.

5 Results

This section consists of three parts: microbial analysis using PEF and UHPH processing, electrode corrosion studies and residence time distribution studies.

5.1 Microbiological analysis

5.1.1 Thermal treatment

An investigation on thermal treatment was conducted by using the capillaries method mentioned in Section 4.3.2. D-values of *Bacillus subtilis* spores from the thermal treatment were determined by using Equation 3 mentioned in Section 2.1.2.

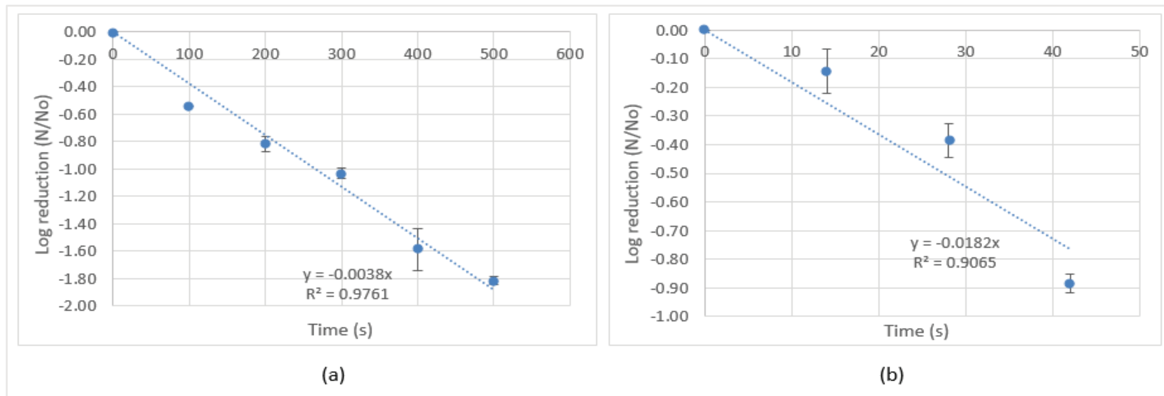

Figure 9: Thermal inactivation of Bacillus subtilis spores in sheep milk at (a) 100°C and (b) 110°C

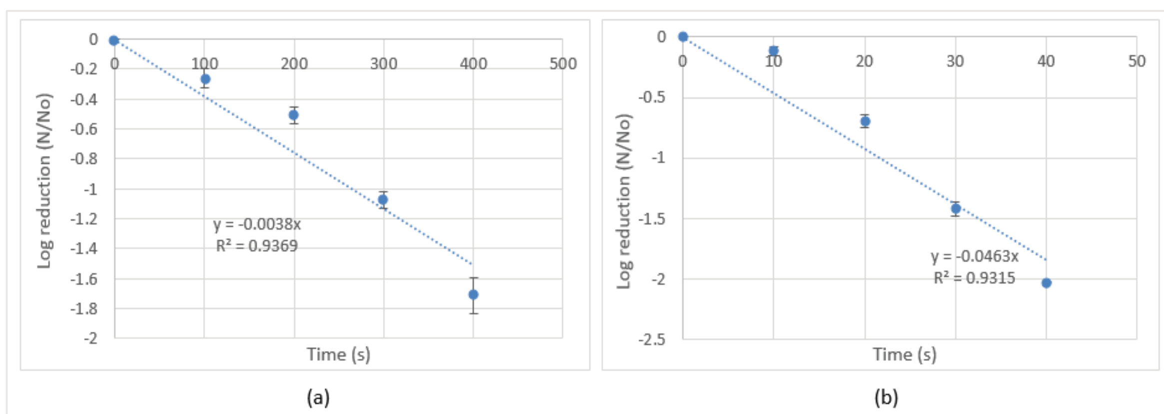

Figure 10: Thermal inactivation of Bacillus subtilis spores in skim milk at (a) 100°C and (b) 110°C

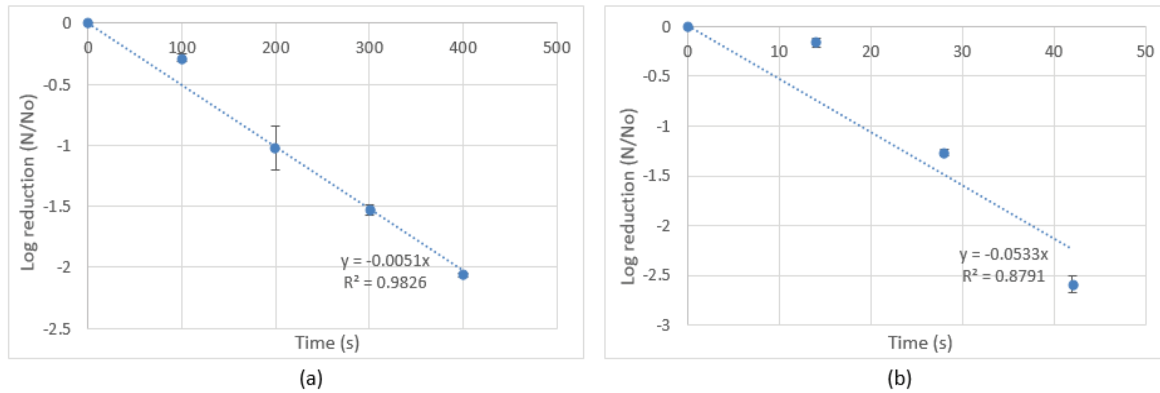

Figure 11: Thermal inactivation of Bacillus subtilis spores in water at (a) 100°C and (b) 110°C

Table 8: Comparison of D-values of thermal treatment obtained from the study

Microorganism	Medium	D-values (minutes)	
		100 °C	110 °C
Bacillus subtilis spores ATCC 6633	Sheep Milk	4.45 ± 0.25	0.92 ± 0.01
	Skim milk	4.39 ± 0.20	0.37 ± 0.03
	Water	3.3 ± 0.14	0.31 ± 0.01

Table 8 shows that the D-values for sheep milk are higher than that in skim milk and water at both temperatures tested. Additionally, the D-values of sheep milk obtained from this study are greater that obtained by (Reineke et al., 2015) in bovine skim milk. They obtained $D_{100\ °C}$ and $D_{110\ °C}$ of 2.47 minutes and 0.18 minutes, respectively after thermal treatment of skim milk inoculated with *Bacillus subtilis* PS 832. This difference can be explained due to the high content of total solids in sheep milk than in bovine milk (Ramos & Juarez, 2011) forming the protective effect on the spores resulting in lower inactivation (Reineke et al., 2015). Table 9 (Park, Juárez, Ramos, & Haenlein, 2007) below also shows the differences in basic components in several milk types such as goat, sheep, and bovine, which may have contributed to the difference in the effect of heat treatment in milk products.

Table 9: Average composition of essential nutrients in several milk types

Composition	Goat	Sheep	Cow	Human
Fat (%)	3.8	7.9	3.6	4.0
Solids-non-fat (%)	8.9	12.0	9.0	8.9
Lactose (%)	4.1	4.9	4.7	6.9
Protein (%)	3.4	6.2	3.2	1.2
Casein (%)	2.4	4.2	2.6	0.4
Albumin, globulin (%)	0.6	1.0	0.6	0.7
Non-protein N (%)	0.4	0.8	0.2	0.5
Ash (%)	0.8	0.9	0.7	0.3
Calories/100 ml	70	105	69	68

Note for the thermal treatment, the time required for the content in the capillaries to reach the treatment temperature is very small. (S. R. Alkhafaji & Farid, 2012) reported that the temperature increased rapidly when 1.2mm diameter of heat capillary is applied in the thermal treatment. In addition, according to (Ansari, Ismail, & Farid, 2019; M Lund, C Baird-Parker, & Warwick Gould, 2000) the lagging time in the application of heat capillary is negligible. Therefore, the lagging time in this experiment was not considered.

5.1.2 PEF treatment

An investigation on microbial spore inactivation was performed by applying PEF in combination with heat as described in Section 4.3.3. Log reductions and D-values were calculated as described in Section 2.1.2. The values are then compared to that obtained from thermal treatment only. Pulse width and flow rate were set at constant values, which are 1.7 µs and 4.3 cm^3s^{-1}, respectively. The outlet temperature of 115 °C was taken from the average value of the temperature reading at the outlet of chamber and the temperature reading at the end of the holding tube at a steady state.

Table 10: Log reductions of Bacillus subtilis spores in skim milk

Parameters	Inlet temperature, (°C)	Outlet temperature, (°C)	Treatment time, (μs)	Holding time, (s)	Energy density, (kJ/kg)	Log reduction, (N/N_o)
300 Hz, 28 $kVcm^{-1}$	80	115	7.47	5.22	99.63	4.02
100 Hz, 50 $kVcm^{-1}$	80	115	2.49	5.22	110.2	3.37

Table 10 above shows the findings from the application of PEF at an inlet temperature of 80 °C in skim milk. The results presented 4.02 log reduction at 300 Hz, 28 $kVcm^{-1}$ and 3.37 log reduction at 100 Hz, 50 $kVcm^{-1}$, respectively. According to other published work on the application of PEF, (Reineke et al., 2015) reported a log reduction of 3.1 for Geobacillus stearothermophilus ATCC 7953 spores at 17 $kVcm^{-1}$, 75 Hz and a log reduction of 3.0 for Bacillus subtilis PS832 spores at 17 $kVcm^{-1}$, 100 Hz, both in skim milk as the medium suspension. Further, (Bermúdez-Aguirre et al., 2012) also reported a total log reduction of 2.55 in skim milk for Bacillus cereus ATCC 7004 after PEF treatment at 40 $kVcm^{-1}$ and 10 Hz.

Table 11: Comparison of D-values at 115°C for PEF and thermal treatment of Bacillus subtilis spores in skim milk

$D_{115\,°C}$ (min)		
PEF treatment		Thermal treatment
300 Hz, 28 $kVcm^{-1}$	0.022 (1.29s)	0.56 (33.6s)
100 Hz, 50 $kVcm^{-1}$	0.026 (1.55s)	

From Table 11, the D-values for Bacillus subtilis spores ATCC 6633 obtained from PEF treatment are lower than that obtained from thermal treatment in skim milk sample. The relatively lower D-values achieved from PEF treatment can be explained due to its lower treatment time as compared to that in thermal treatment.

5.1.3 UHPH treatment

5.1.3.1 Inactivation of microbial spores

Table 12: Log inactivation of Bacillus subtilis spores using UHPH processing

Medium	**T fresh feed, T_f (°C)**	**T inlet, T_i (°C)**	**T valve, T_v (°C)**	**T outlet, T_o (°C)**	**Pressure (MPa)**	**Log inactivation (B. *subtilis* spores)**	**D-values (min)**
Whole bovine	45	71	106	28.5	200 ± 1.25	0.363	0.055 (3.306s)
	55	84	129	25.8	250 ± 2.07	1.368	0.015 (0.877s)
Whole goat	55	84	127	27.7	250 ± 3.87	0.864	0.023 (1.389s)

Store-bought UHT whole bovine and goat milk were used in this study, and milk samples were inoculated with Bacillus subtilis spores. Bovine milk samples were pre-treated to an initial temperature of 55 °C and treated at 200 MPa and 250 MPa. Results in Table 12 show log reductions of 0.363 and 1.368, respectively. Meanwhile, a log reduction of 0.864 was achieved in goat milk at a similar initial temperature and 250 MPa. In comparison to log reductions achieved by (Amador-Espejo et al., 2014) which were 5.22 for *Bacillus subtilis* CECT 4002 and 5.26 for *Geobacillus stearothermophilus* CECT47, log reductions achieved in this study were relatively lower probably due to the use of different strains. Additionally, they have operated at 300 MPa with a valve temperature of 139 °C, which correspondingly resulted in a higher log reduction of spores.

Further, in a study conducted by (E Georget et al., 2014), they achieved a total log reduction of 5 and 2 for *Bacillus subtilis* PS832 and *Geobacillus stearothermophilus* ATCC7953, respectively, after the introduction of UHPH treatment at 300 MPa and valve temperature of 145 °C in phosphate-buffered saline (PBS) buffer 0.01M. Another work (Pinho et al., 2011) with UHT skim milk presented <1 log reduction of both *Bacillus stearothermophilus* AT7953 and *Clostridium sporogenes* PA3679 strains after 100 °C/15min heat-shock pre-treatment followed by UHPH pressure at 300 MPa and valve temperature of 84 °C.

5.1.3.2 Pressure-temperature variation during UHPH treatment

The initial feed temperatures were set at 45 °C and 55 °C, and UHPH was operated at two different pressures; 200 MPa and 250 MPa. A rise in temperature of 17.5 °C per 100 MPa was achieved with feed temperature of 45 °C and inlet temperature before the homogenization valve of 71 °C and 200 MPa. Meanwhile, an increase in temperature of 18 °C per 100 MPa was achieved with bovine milk at 55 °C of feed temperature, 84 °C of the inlet temperature before the homogenization valve, and 250 MPa. As for goat milk, an increase in temperature of 17.2 °C per 100 MPa was obtained with 55 °C as the feed temperature, 84 °C as the inlet temperature before the homogenisation valve, and 250 MPa.

The findings from this study show that the temperature increases are relatively close to the values reported by (Amador-Espejo et al., 2014), which obtained 17.83 °C per 100 MPa at 320 MPa. Further, in work published by (M. G. Hayes & Kelly, 2003b), an increase in temperature of 17.6 °C per 100 MPa was achieved at a pressure of 50-200 MPa. It is worth reporting that according to several studies, the rise in temperature during the UHPH treatment is contributed by the combination of the effect of shear stress and the occurrence of cavitation. Additionally, the adiabatic heating generated in the unit with the change in kinetic energy to pressure as well as the turbulence inside the system may have also contributed to the increase in temperature during the treatment (Amador-Espejo et al., 2014; M. G. Hayes & Kelly, 2003b; J Pereda et al., 2007; Thiebaud et al., 2003).

Subsequently, after the UHPH treatment, the treated milk was rapidly cooled down to 20-30 °C, followed by the collection of samples. The total treatment of the milk is from the region of the first homogenisation valve, where the pressure was introduced up until the entry of the cooler region. The total time was approximately 1.2 seconds, which was relatively lower than that used in UHT treatment. Therefore, it is hypothesised that the short treatment time obtained during UHPH operation could produce better quality retention in milk products (Bendicho et al., 2002; Deak, 2014).

5.1.3.3 Shelf-life study of raw sheep milk

Table 13: UHPH parameters in ESL study

Milk	T fresh feed (°C)	T inlet (°C)	T valve (°C)	T outlet (°C)	Pressure (MPa)
Raw sheep milk	55	84	118	19.2	250.29 ± 2.98

Parameters shown in Table 13 were achieved, and the treated milk was analysed by monitoring the total place count over a period time. Total bacterial count was analysed using PCA plates on Day 0 (the day of the experiment), Day 1, Day 5, Day 8, Day 12, Day 15, Day 19, Day 22, Day 26, Day 29, Day 33 and Day 36. In general, according to (Silcock et al., 2014), milk product is deemed to reach the end of shelf life when the microbial count reaches 10^6 – 10^7 cfu/mL which would result in unacceptable sensory changes in milk. Results showed that the concentration of the total bacterial count in the UHPH-treated milk remains less than the threshold concentration of 2.0×10^4 cfu/mL set by the ordinance for Grade A pasteurised milk (Walkling-Ribeiro et al., 2009) after 36 days of storage in a 4°C. Untreated raw sheep milk obtained from the farm showed a concentration of 5.08×10^6 cfu/mL on Day 5, which has exceeded the threshold of microbiological count. Note that temperature increase in ESL study with UHPH at this parameter was 13.6 °C per 100 MPa.

Published work on the application of UHPH to study the shelf life of raw sheep milk is relatively scarce. A study was conducted using raw bovine milk by (J Pereda et al., 2007) reported that the UHPH treated milk at 200 MPa and valve temperature of 80 °C had total psychrotrophic count below the limit at 21 days of 4 °C storage. Further, (Smiddy et al., 2007) also reported a study using raw bovine milk with UHPH treatment at 200-250 MPa and inlet temperature of 55-70 °C resulted to total bacterial counts (TBC) of ~10^8 cfu/mL after 14 days of storage at 5 °C, which exceeded the safe limit for human consumption.

5.1.3.4 Physical properties of UHPH-treated milk

The physical properties of the UHPH-treated raw sheep milk such as pH and conductivity were monitored throughout the period of study. Measurements were taken when milk products reached room temperature at 20.4 °C and presented in Table 14. Milk conductivity is an essential parameter that is linked to bacterial growth. Lactic acid that is correlated to bacteria is used to study the changes in conductivity, whereby as the acidity increases, the conductivity of milk products will increase too (Ansari, Ismail, & Farid, 2020).

Table 14: pH and conductivity measurements of raw sheep milk during the ESL study period

Day	pH	Conductivity (mS/cm)
Fresh untreated raw milk upon arrival	6.60	3.71
0	6.54	3.68
1	6.53	3.71
5	6.52	3.72
8	6.51	3.70
12	6.52	3.65
15	6.52	3.70
19	6.52	3.69
22	6.52	3.71
26	6.54	3.71
29	6.51	3.68
33	6.54	3.69
36	6.52	3.69

The results show that the pH of the raw sheep milk treated at 118 °C and 250 MPa did not have significant variation throughout 36 days of storage at 4 °C. Similar pattern was observed in the conductivity of the milk throughout the storage period.

The observed patterns can be attributed to the UHPH treatment at the temperature and pressure that resulted in a considerable microorganism inactivation, which consequently resulted in less changes in pH and conductivity.

5.2 Residence time distribution analysis in PEF system

This section elaborates the results obtained from the residence time distribution analysis in PEF unit. Experiments were performed to study the effect of different flowrates on the tracer voltage response with four replicates for each setting. The change in voltage reading for all three settings are exhibited as follows.

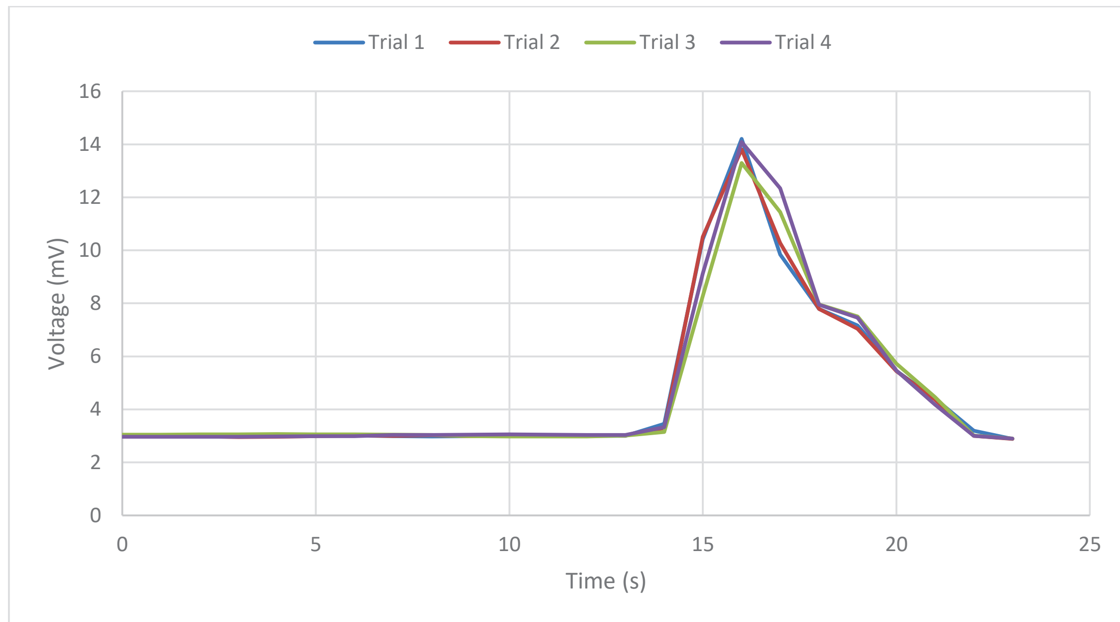

Figure 12: Voltage response against time at 5.23 ml/s (65%)

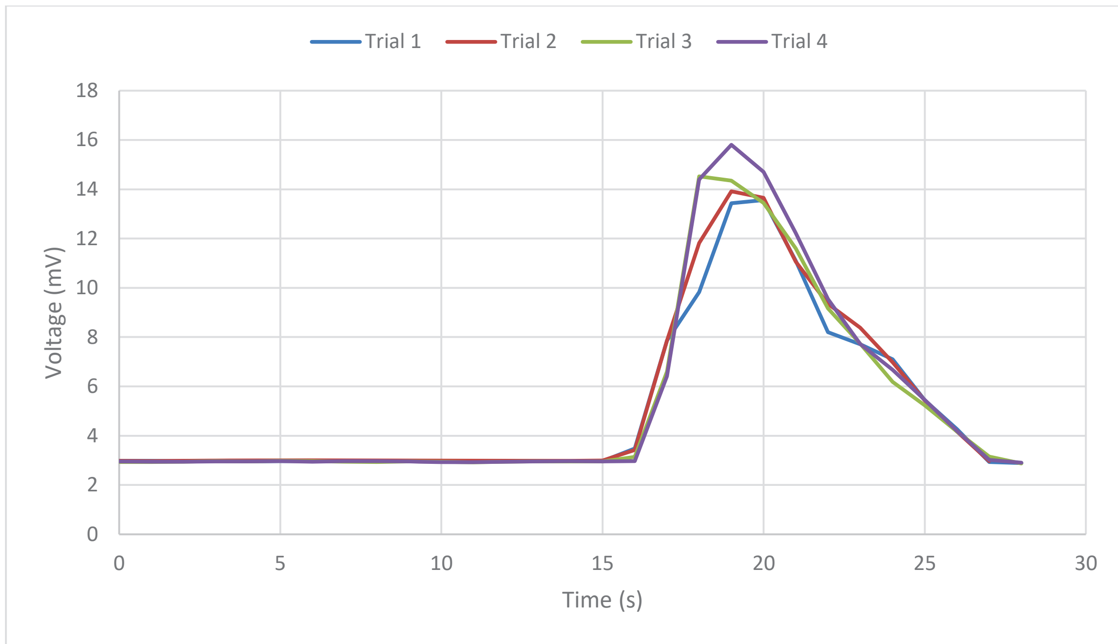

Figure 13: Voltage response against time at 4.72 ml/s (55%)

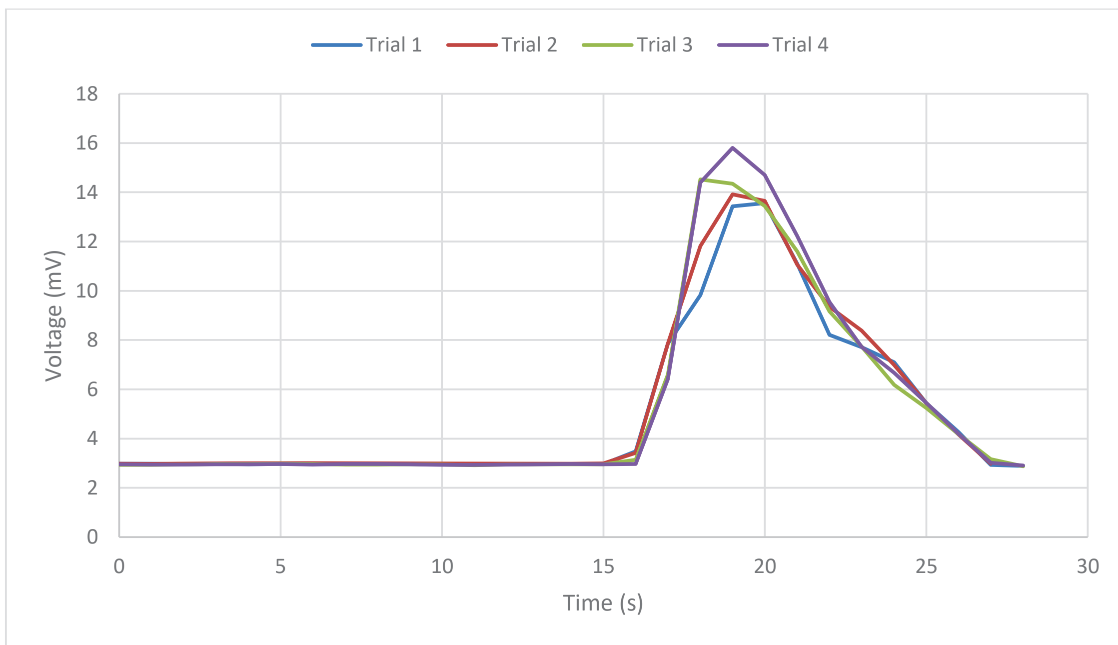

Figure 14: Voltage response against time at 3.74 ml/s (45%)

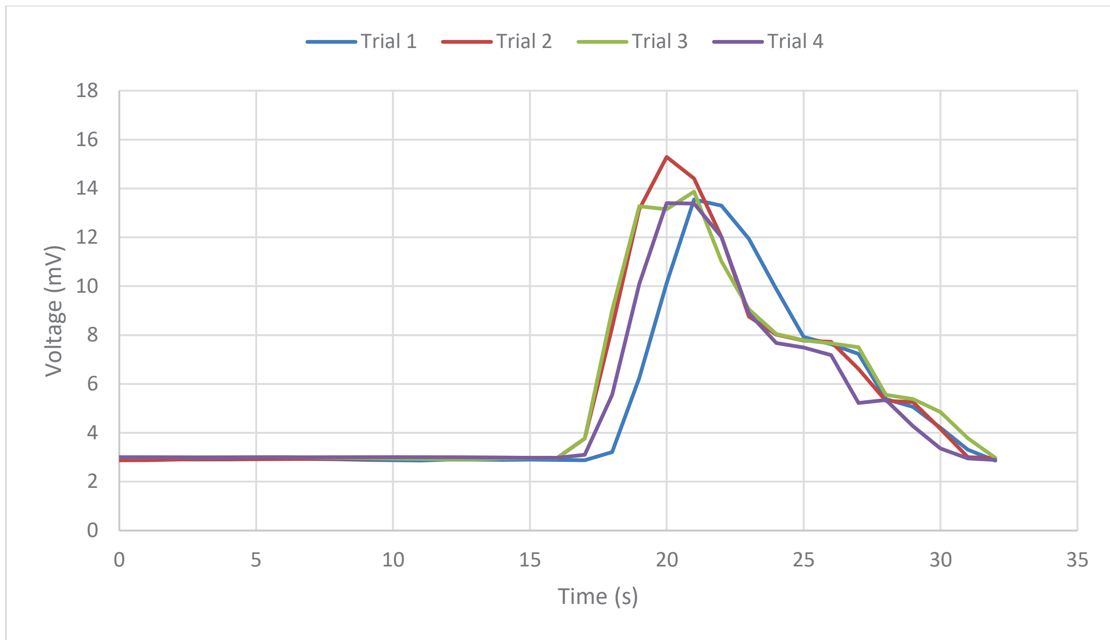

Figure 15: Voltage response against time at 2.96 ml/s (35%)

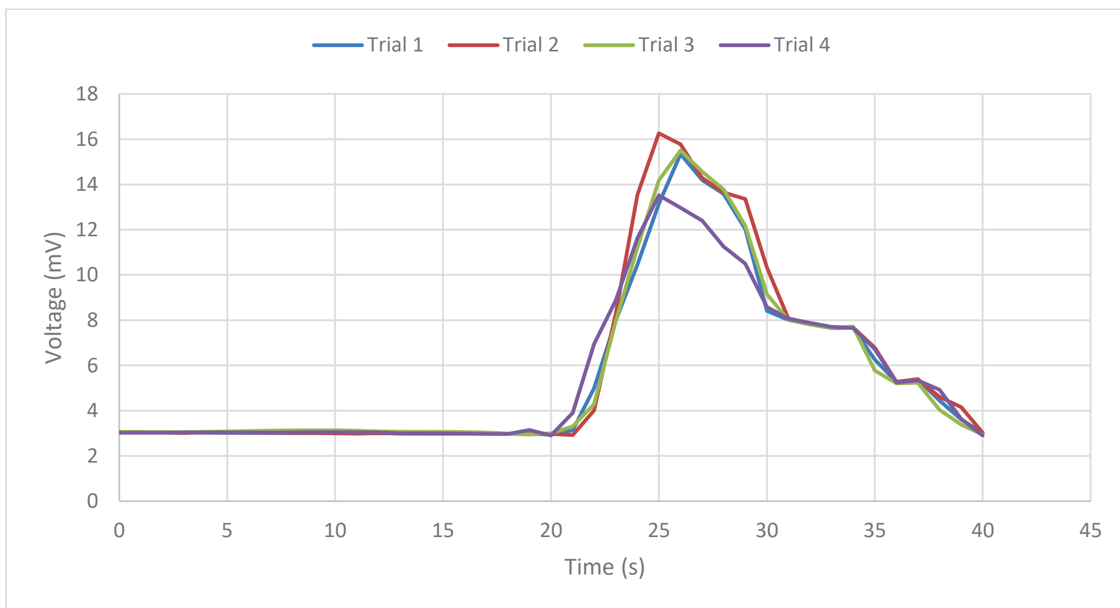

Figure 16: Voltage response against time at 2.03 ml/s (25%)

Based on graphs plotted, the mean residence time for each flowrate setting was determined by applying Equation 11 and the values are then compared to the calculated values which were obtained by using Equation 12 (Table 15). In order to determine the average residence time using Equation 12, the total volume of the treatment chamber and pipe as well as the flowrate of deionised water must be known.

The total volume of treatment chamber is based on a combination of cylindrical and frustum shapes:

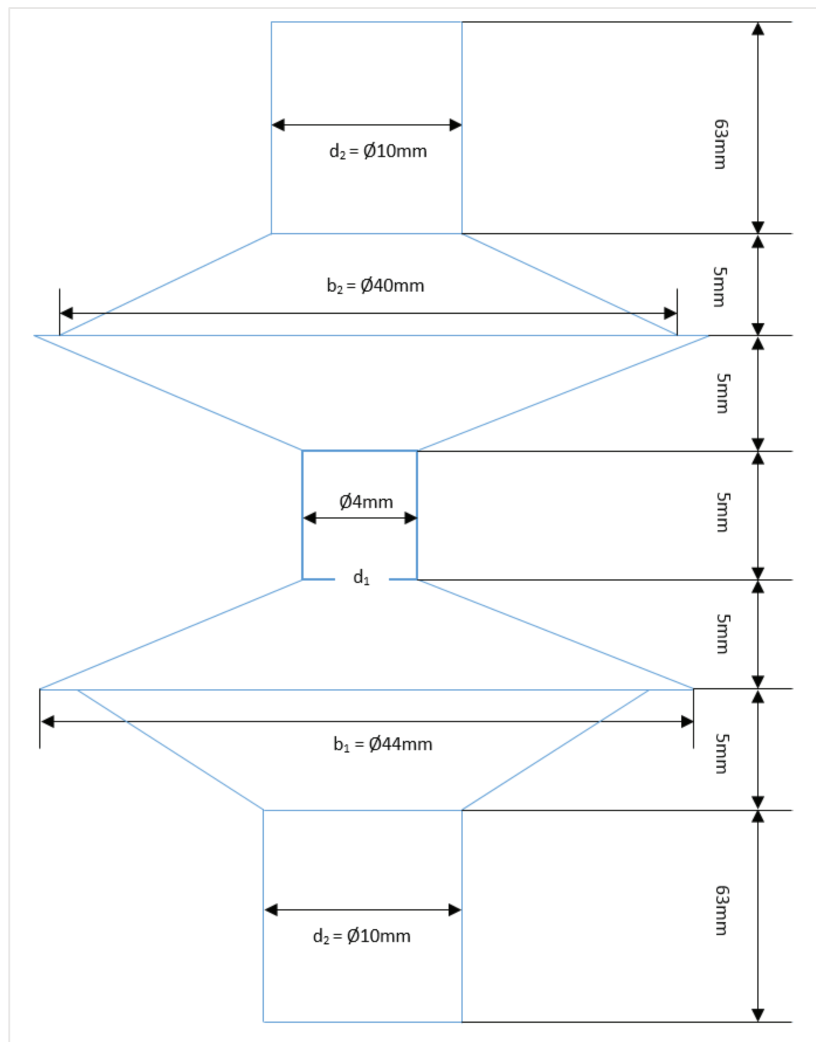

Figure 17: Total volume distribution of the 4mm treatment chamber

Therefore, the total volume, v_T is:

$$Total\ volume, v_T = volume\ of\ pipe + total\ volume\ of\ treatment\ chamber$$

$$Total\ volume, v_T = 11.37\ cm^3 + 21.04\ cm^3 = 32.41\ cm^3$$

Once the total volume is obtained, the volumetric flowrate is substituted in the following Equation 13;

$$t_{RTD}(s) = \frac{v_T\ (cm^3)}{Q\ (cm^3 s^{-1})}$$

$$t_{RTD}(s) = \frac{32.41\ cm^3}{5.23\ cm^3 s^{-1}} = 3.43\ s$$

Table 15: Mean residence time distribution at five different flow rates

Pump setting (%)	Flowrate (ml/s)	Residence time, t_{RTD} (s)					
		Method 1 (Experimental)					Method 2 (Calculated)
		1	2	3	4	Average	
65	5.28	6.96	6.92	7.18	6.92	6.99 ± 0.12	6.14
55	4.56	8.22	8.15	8.68	8.14	8.30 ± 0.26	7.11
45	3.70	10.52	10.41	10.30	10.39	10.40 ± 0.09	8.76
35	2.82	13.17	12.09	12.52	12.30	12.52 ± 0.47	11.49
25	2.00	18.67	18.56	18.31	18.39	18.48 ± 0.16	16.21

In all five flowrates tested, the mean residence times obtained experimentally are slightly greater than the calculated residence times which indicates that there is a possibility for the formation of stagnant points or channelling of fluid to take place in the PEF system. This outcome highlights the importance of online experimentally obtained RTD.

In future, a systematic study can be performed in order to investigate the influence of other factors affecting the residence time distribution of an element. One such factor is the type of media in which real food products such as milk will be used to replace deionized water to study effect of viscosity on the residence time distribution behaviour. Additionally, different treatment chambers can also be incorporated in further studies in order to investigate the influence of different chamber's diameter on the residence time distribution.

5.3 Analysis of metal concentration in PEF unit

This section discusses the results obtained from the investigation of ions migration from the electrodes during PEF processing. Experiments were conducted to investigate the influence of electric field strength and frequency on the concentration of metallic ions in liquid samples. Saline water with a conductivity of 5.3 $mScm^{-1}$ was used in all runs in order to imitate a similar conductivity of UHT skim milk. The conditions of PEF treatment during the processing are shown in Table 16 where flow rate and pulse width were set constant in all experiments at 4.3 cm^3s^{-1} and 1.7 µs, respectively.

Table 16: PEF conditions at electric field strength of 42 kVcm^{-1} and 50 kVcm^{-1}

At 42 kVcm^{-1}				
Frequency (Hz)	Current (A)	Pulse Width (µs)	Flowrate (cm^{3}s^{-1})	Outlet temperature (°C)
100	86	1.7	4.3	97
75	81	1.7	4.3	90
50	77	1.7	4.3	83.5
At 50 kVcm^{-1}				
Frequency (Hz)	Current (A)	Pulse width (µs)	Flowrate (cm^{3}s^{-1})	Outlet temperature (°C)
100	105	1.7	4.3	127
75	96	1.7	4.3	97
50	96	1.7	4.3	90.2

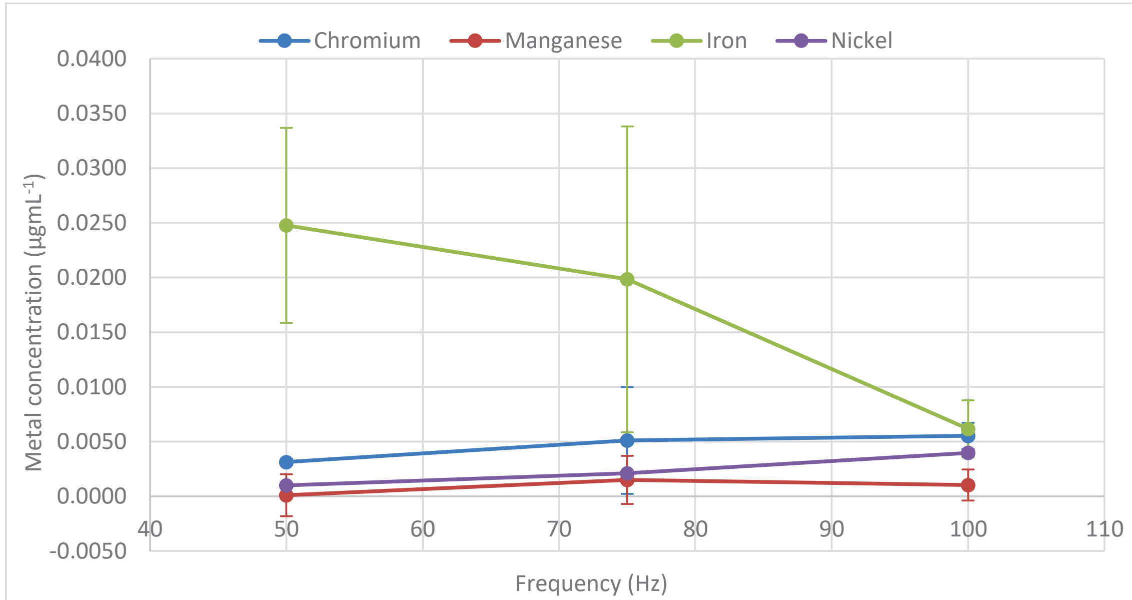

Figure 18: Change in metal concentration at 50 kVcm^{-1} at 100 Hz, 75 Hz, and 50 Hz

Figure 18 above presents no significant relationship that can be drawn with respect to the influence of electric field strength and frequency on the change of all four metallic elements concentrations. Additionally, it can be seen that iron has the highest change in concentrations in relative to other metallic elements tested. This is because iron has the highest percentage in the 316L stainless steel electrodes.

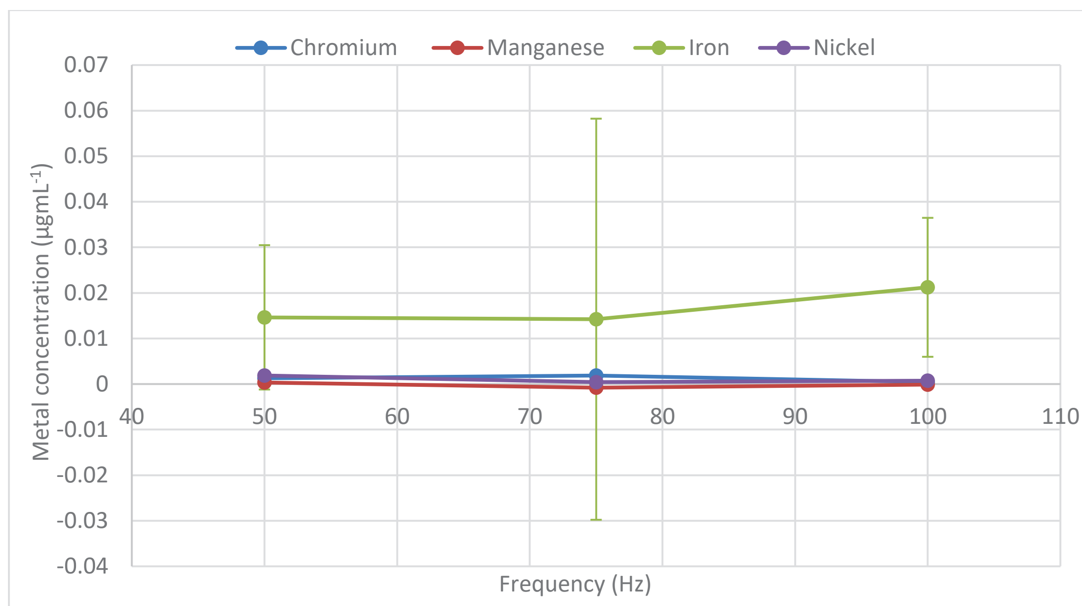

Figure 19: Change in metal concentration at 42 kVcm^{-1} at 100 Hz, 75 Hz, and 50 Hz

PEF treatment at 42 kVcm^{-1} as shown in Figure 19 also shows a similar outcome as at 50 kVcm^{-1} cm, whereby no clear relationship can be drawn on the influence of the electric field strength and frequency on the change in metal concentrations, respectively.

The lowest operable frequency of 50 Hz was tested for the experiments since several authors that have reported to observe high concentrations of electrode elements in the liquid sample at lower frequencies as compared to high frequencies in an alternating current system (Matser et al., 2007; Morren et al., 2003; Wang & Farid, 2015). Further, the inconclusive trends can be explained probably due to the small range of frequencies tested thus no significant pattern can be drawn from the results. In future, as the PEF unit is operable up to 1000 Hz, a larger range of frequency could be selected to further clarify the relationship between electric field strength and frequency with the extent of the migration of metallic elements.

In regards to error analysis, the variances in both, change in metal concentrations samples and inlet metal concentrations samples were determined.

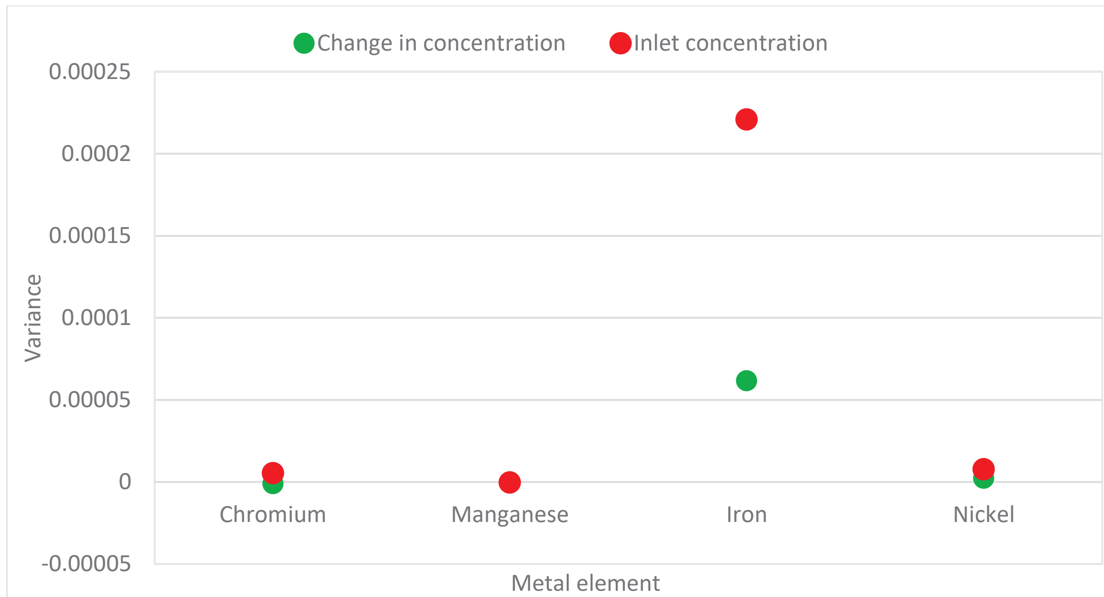

Figure 20: Variance obtained at 50 kVcm^{-1} with respect to the change in concentration and inlet concentration of metallic elements

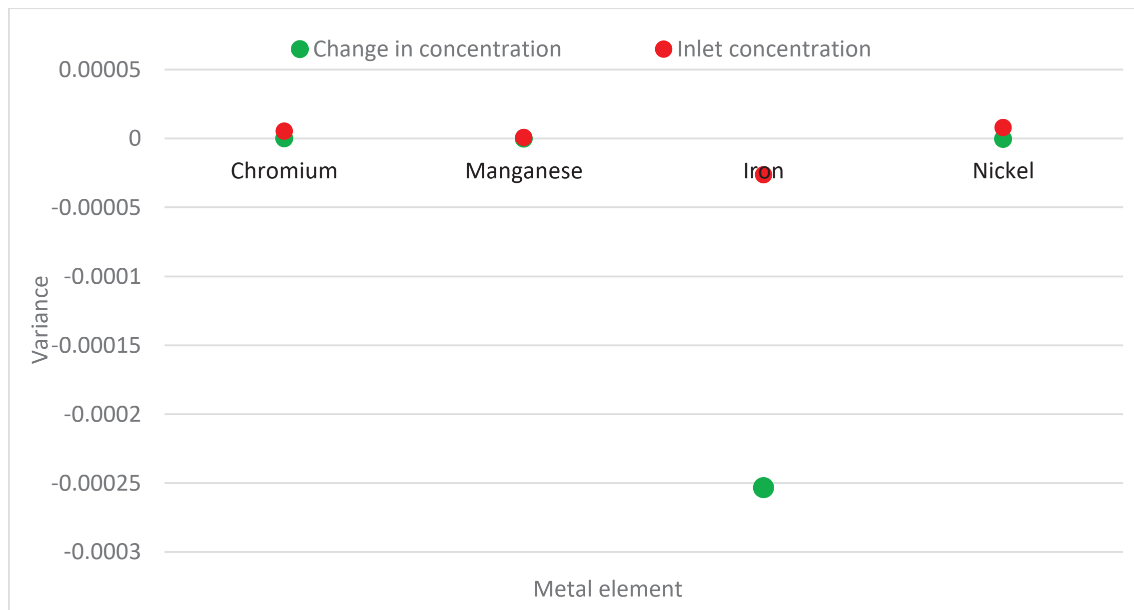

Figure 21: Variance obtained at 42 kVcm^{-1} with respect to the change in concentration and inlet concentration of metallic elements

Based on Figure 20 and Figure 21, the variance obtained for untreated samples are higher than the variance obtained from the change in concentrations in all four elements studied, at both 50 kVcm^{-1} and 42kVcm^{-1}, respectively. These results suggest that the variation in the concentration is probably caused by the external factor such as the source of water used rather than due to the deposition of metal in the PEF system. Therefore, it is recommended that Milli-Q water to be used in the future due to its consistency in water content.

Apart from only having replicates for samples from PEF experiment itself, replicates should also be made during the dilution step prior to ICP-MS testing in order to further clarify the source of errors in this study.

Table 17: Comparison of change in metal concentrations with the standards

Electric Field Strength ($kVcm^{-1}$)	Frequency (Hz)	Iron (Fe) (μgmL^{-1})	Chromium (Cr) (μgmL^{-1})	Manganese (Mn) (μgmL^{-1})	Nickel (Ni) (μgmL^{-1})	Maximum Permissible Concentration for Drinking Water (μgmL^{-1})	
						EU Legislation	NZ Legislation
50	100	0.0062	0.0055	0.0010	0.0040	Iron = 0.2, Chromium = 0.05 Nickel = 0.02, Manganese = 0.05	Iron = 0.2, Chromium = 0.05, Nickel = 0.08, Manganese = 0.4
	75	0.0198	0.0051	0.0015	0.0021		
	50	0.0248	0.0031	0.0001	0.0010		
42	100	0.0212	0.0004	-0.0001	0.0008		
	75	0.0142	0.0019	0.0008	0.0004		
	50	0.0146	0.0012	0.0003	0.0019		

The change in metal concentrations obtained for all four elements were also compared with the legislations. Table 17 demonstrates that all metal elements studied obtained are at least 10 times lower than the maximum allowable values for health significance provided by the European Union Legislation (Roodenburg, Morren, Berg, & de Haan, 2005b) and New Zealand Legislation (*Drinking-water standards for New Zealand*, 2008) for drinking water. The outcome of this study supports the selection of design for the PEF treatment chamber whereby the occurrence of electrode corrosion is minimized and shows promising potential in the application of PEF in food preservation.

6 Conclusions and Future Work

Milk is presently processed with ultra-high temperature or pasteurisation treatment to increase its shelf life. Due to the exposure of extreme heat to the milk product, it has negative impact on the milk quality. Therefore, the basis of this research is to explore novel technologies that could operate at high temperature with less processing time to reduce the negative impact on the quality of milk products. In this study, the application of PEF and UHPH processing were investigated using milk products.

Firstly, the application of PEF in combination with heat resulted in 4.02 and 3.37 log reductions of *Bacillus subtilis* spores in skim milk at 300 Hz with 28 $kVcm^{-1}$ and 100 Hz with 50 $kVcm^{-1}$, respectively. The D-values at 115°C for *Bacillus subtilis* in skim milk obtained from PEF with heat are 0.77 s and 0.65 s at 300 Hz with 28 $kVcm^{-1}$ and 100 Hz with 50 $kVcm^{-1}$, respectively while longer D-value of 33.6 s was obtained using thermal treatment. The application of PEF with heat in this study shows its potential to inactivate microbial spores, and therefore further explorations are necessary. On the other hand, since the residence time is an essential factor to determine the D-values and its exposure to the treatment, the residence time inside the PEF was also investigated. The outcome reveals that the experimentally obtained mean residence times using dye tracing method are greater than that obtained from the offline theoretical method, which suggests the formation of stagnant points or channelling of fluid in the PEF system. The results reflect the necessity to explore further into the factors contributing to the residence time behaviour, such as designs of the treatment chamber, or the viscosity of the liquid foods used in the system. Regarding the safety of the PEF unit, corrosion study that was carried out shows that the PEF unit is safe to use as the change in metal concentrations obtained are at least 10 times lower than the maximum allowable values provided by both New Zealand and European Union standards. The outcome supports the safety of PEF processing as an alternative technology for microbial spore inactivation in milk products.

Secondly, UHPH processing has been selected in this study to investigate its efficacy using milk products. With store-bought UHT whole bovine and goat milk, UHPH processing operated at 250MPa and 125 °C valve temperature resulted in 1.368 and 0.864 log reductions of *Bacillus subtilis* spores, respectively. Further, to explore the efficacy of UHPH processing for shelf-life extension, a shelf-life study was conducted with raw sheep milk at 250MPa and 118 °C valve temperature, which produced a total bacterial count (TBC) below the limit for Grade A ordinance for at least 36 days in 4 °C refrigerated condition. Additionally, the pH and conductivity of the UHPH-treated raw sheep milk show minimal variation throughout the 36 days period of storage at 4 °C. The results reflect that UHPH

processing has the potential to be used as an alternative technology to produce extended shelf-life (ESL) milk products in the markets.

In future, more in-depth investigations into the use of PEF and UHPH processing are required to further elucidate their efficacy to produce safe and quality milk products. It is essential to perform an extended shelf-life (ESL) studies with raw milk using PEF with heat and UHPH processing at a longer period, in both refrigerated and room temperature storage conditions. The outcome can be a basis of comparison with other UHT or pasteurised, or extended shelf-life milk products.

Further quality studies are also important to support the efficacy of the novel technology besides its ability in microbial aspect. The impact of PEF with heat and UHPH processing on milk nutritional and enzymatic properties can also be explored thoroughly. A comprehensive investigation on the heat-related sensory quality such as hydroxymethyl-furfural (HMF) and dimethyl sulphide (DMS) components are also other aspects that could be studied to support the efficacy of PEF and UHPH processing as alternative technologies to the current thermal treatment that is widely used in the industry.

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