A Study of Electrode Material Performance During Food Processing by Pulsed Electric Fields

by

Ahmed Gad

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Abstract

Upon the application of high-field pulses across a liquid food product (such as milk, juices, or beer), electric charge accumulates across the membrane of any microbial cell present in the processing zone between the two electrodes. Within a few hundred nanoseconds, this charge accumulation results in an irreversible membrane breakdown which causes the cell to lose its viability. By this means, an extended shelf-life could be achieved. Thus, the use of pulsed electric fields (PEFs) provides an alternative method to preserve liquid food products instead of the conventional thermal pasteurization. The advantages of using the PEF processing over conventional thermal pasteurization include greater retention of the original nutritional value and fresh-like taste, less usage of processing energy, and lower contamination from the processing equipment.

Physical and electrical contact between the liquid food product and the metallic electrodes during the PEF processing is unavoidable and causes some metallic ions to be released from the electrodes into the processed food. This undesirable release of metallic ions from the electrodes can affect food safety and taste, degrade food compounds/color, and shorten the electrode lifetime over a long period of use. As such, it represents one of the main challenges for the commercialization of such non-thermal preservation technique.

In this study, the concentration of released metallic ions in PEF-processed milk, orange juice, and beer has been determined using the inductively coupled plasma – atomic emission spectroscopy (ICP-AES) under various operating conditions. It has been found that the mitigation of arcing events protects the electrode surface from experiencing “pits”, which is one source of metallic ion release. For electrochemical reactions, satisfying a “zero net charge delivery” reduces the metallic content of PEF-processed foods considerably. Allowing the charging current of the generator’s capacitor to flow through the PEF processing chamber is used here to ensure the zero net charge delivery with mono-

polar pulses. Lower metallic content has been obtained by using pulses of shorter width; this observation agrees with the nature of an electrically-driven electrochemical process. Variation in the voltage peak, within the typical PEF conditions, did not have a significant effect on metal release.

The comparison of the behavior of electrodes coated with chromium, nickel, silver, and titanium has revealed the superior performance of titanium. A solid titanium electrode did not lead to any detectible metal release after PEF processing of milk (neutral) and orange juice (acidic); whereas, the concentration of iron from stainless steel electrodes was detectible in both liquids. The temperature of the PEF processing zone did not influence the metallic content when varied in the range from 1° to 30ºC.

The study also highlights the applicability of the PEF processing on carbonated beer, in particular through a sealed and pressurized processing chamber. Brewery regulations in Canada do not necessitate the thermal pasteurization of carbonated beer; hence, PEF processing finds a potential application in the beer industry. Sensory panels have shown that PEF-processed beer is preferred when compared to thermally pasteurized beer with respect to flavor, aroma, and foam condition. Untrained panelists preferred samples processed with low-dosage of PEF in week 0, but preferred samples with high-dosage in week 12 of the shelf-life. Trained panelists reported a metallic feeling in the PEF-processed beer which could be due to the presence of metallic ions that was detected analytically.

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Chapter 1

Introduction

Food is one of the essential requirements for any living creature. Nutrition, clothing, and housing are defined by economists to be the only “needs” for any human being. Living requirements other than these three are defined as “wants”. Nutrition provides the human body with the required energy for all activities starting from micro-scale cell metabolism to macro-scale body motion. It is well-known that insufficient or unbalanced nutrition reduces immunity of the human body and increases the possibility of diseases.

Historically, man started satisfying his nutrition needs by gathering crops and fruits from plants and trees that grew naturally. With the invention of tools, fishing and hunting of animals became possible and gave humankind an additional source of protein. As climate changed, the most effective way to grow plants and trees was through agriculture. This forced people to settle down into communities and allowed them to raise animals such as cows, goats, sheep ... etc.

Since these old times, the problem of food storage has arisen for any food surplus. Food preservation concepts are all based on killing or at least inhibiting spoilage and pathogenic microorganisms such as yeasts, molds, bacteria, and/or viruses. Successful food preservation techniques included boiling, drying, salting, smoking, pickling, and sugaring. A food such as jam is nothing but a product of fruit preservation through boiling and sugaring.

Later on, Louis Pasteur invented a thermal preservation method in which the food, usually liquid, is heated to a certain temperature for a specific period and then cooled immediately. Typical temperature and time that can be used vary widely from home pasteurization at 63ºC for 30 min to high-temperature short-time (HTST) pasteurization at 72ºC for 15 sec and to ultra-high temperature (UHT) pasteurization at 135ºC for 1 sec. Although this thermal pasteurization technique was originally developed for preserving wine and beer, it is also frequently used for preserving milk and juices. In addition to the thermal pasteurization, some chemical additives may also be used for food preservation.

Nowadays, food processing is one of the largest industries in the world. It includes not only farming and food processing but also distribution, marketing, and even research and development. Customers today demand high quality in food products relating to both fresh-like (non-artificial) flavor and valuable nutrition. Since conventional thermal pasteurization can sometimes adversely affect flavor and nutritional value, non-thermal food preservation techniques started to emerge in the last few decades. These techniques include the use of high hydrostatic pressures, microfiltration, ultrasonic waves, ultraviolet radiation, pulsed electric fields, and hurdle technologies [1-4].

High hydrostatic pressures (HHP) can successfully extend the shelf-life of solid food products such as poultry and red meat, goat milk cheese, and cooked ham as well as liquid food products, leaving many flavor compounds and vitamins intact. Using HHP, food products are essentially processed in batch mode with or without packaging. However, HHP alone do not achieve substantial inactivation of spores and reduction in activity of certain enzymes [1]. The majority of HHP-processed products available in the market are high acid products like fruit juices. Due to low pH, these products are good candidate for HHP processing because they are mainly spoiled with yeast, molds, and lactic bacteria, and do not support the germination of bacterial spores. Costs associated with implementing HHP are expensive and, therefore, its use is limited to high value products [2].

Microfiltration is characterized by introducing minimal changes to the processed food. The price of microfilters and the relatively high pressure difference required across them can significantly increase the preservation costs. Other non-thermal methods that have potential for food processing are ultrasonic waves and ultraviolet radiation. Although ultrasonic waves have the ability to travel through gas, liquid, and solid materials, they have not been adopted in food processing probably due to the long processing needed for substantial microbial inactivation [3]. Ultraviolet (UV) radiation is an effective method of killing bacteria, viruses, yeast, molds (and their spores). It has been traditionally, mainly due to its poor penetration level, used to sterilize or reduce microbial population on food surfaces, packaging material, or processing plants. The exposure to UV light causes damage to the organoleptic qualities of the food products. For that reason, UV is nowadays extensively used for disinfection of water or air. Finally, hurdle technologies employ several methods, some of which may include mild heat, in synergy to preserve food products [4].

This thesis is concerned with the use of pulsed electric fields (PEFs) for liquid food preservation. Applying high-field short-duration pulses to liquid food leads to an extension to its shelf-life. These high-field pulses induce a potential difference across the cell membranes of microorganisms that may exist in the liquid food. If this induced potential exceeds a threshold value for a sufficient period of time, irreversible pore formation occurs in the cell membrane. As a result, the membrane loses its semi permeable characteristic and causes the cell to lose its viability. Experiments have already verified the applicability of this approach in extending the shelf-life of milk products, beer, fruit juices, and liquid egg [5-10]. The advantages of using PEFs over conventional thermal pasteurization include greater retention of the original nutritional value and fresh-like taste, less usage of processing energy, and lower contamination from the processing equipment.

Figure 1-1 summarizes the effects associated with the application of electric pulses. Other than microbial inactivation, and perhaps some enhancement in the inactivation caused by the ohmic heating, these effects are considered unavoidable and have been impeded the commercialization of such preservation techniques. Therefore, research is currently being carried out either to assess whether these side effects are within the acceptable limits or to provide feasible methodologies to eliminate/reduce them.

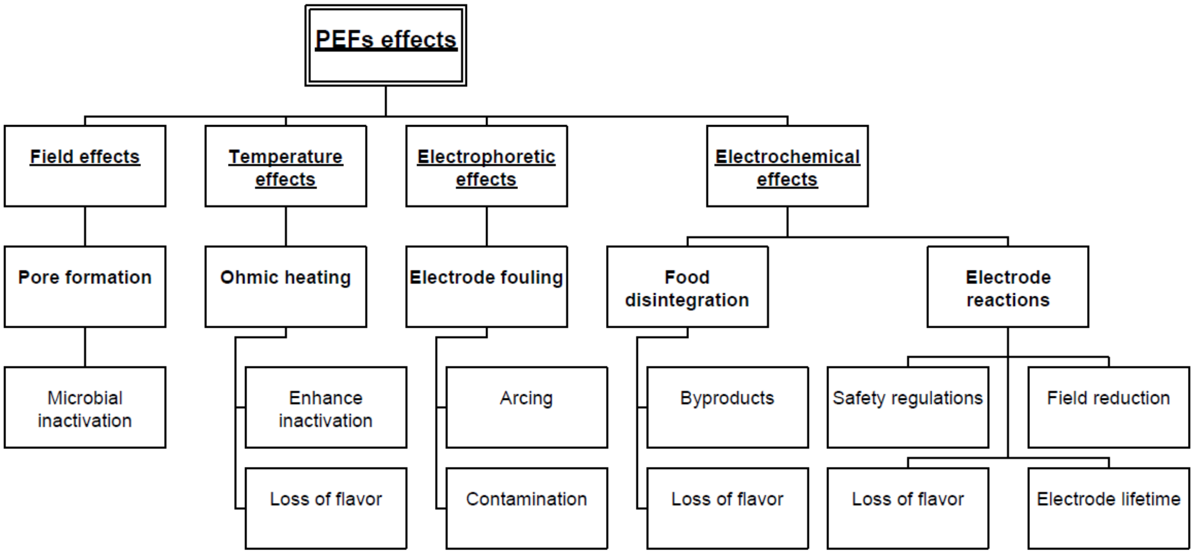


Figure 1-1: Effects associated with food preservation by PEFs

1.1 Literature review

In this section, a literature review pertaining to the PEF technique is conducted. First, the general application of PEFs in food preservation is presented. The reported electrode problems during PEF processing are then discussed in detail. Finally, some solutions for electrode reactions adopted with similar electrical processes (like ohmic heating) are presented.

1.1.1 Food preservation by pulsed electric fields (PEFs)

The use of PEFs as a non-thermal microbial inactivation technique was first reported by Sale and Hamilton [11-13]. The first part [11] of their study aimed to evaluate the lethal effect of PEFs on microorganisms. They found that the distribution of killed microorganisms suspended within a gel sample was spatially uniform and that the killing rate was independent of the current density. These results indicated that the lethal effects are not caused by electrolysis products. Further, the authors showed that raising the starting temperature from 20ºC to 40ºC did not affect the killing rate during PEF processing, which refuted claims that thermal inactivation was the main mechanism. Experiments carried out on various species of vegetative bacteria and yeasts revealed that survival rates exhibited similar characteristics as a function of the applied electric field but with different sensitivities. In the second part [12] of their study, changes in the structure and the nature of the cell-membrane explained the mechanism of microbial inactivation during the PEF processing. The application of electric pulses caused an irreversible loss of the membrane effectiveness as a semi-permeable barrier; as a result, the cell lost its ability to multiply even without a catastrophic destruction of its membrane. In the third part [13], a potential difference of about 1 V developed across the cell-membrane was experimentally shown to cause the membrane to lose its permeability as a result of an electrical breakdown.

Hulsheger et al. [14] studied the role of different experimental parameters such as the type and concentration of electrolyte, pulse width, field strength, pH, and temperature on the lethal action of PEFs. Solutions containing chloride compounds led to undesirable electrolytic production of chlorine that rendered the processed solutions toxic even after PEF processing had finished. No significant difference appeared between the results obtained depending on the presence of the two monovalent cations Na^+ and K^+. Bivalent cations, Mg^{+2} and Ca^{+2}, induced a protective mechanism against PEF processing. The logarithm of the survival rate was found to vary linearly with the processing time, whereas the field strength had a linear effect on the logarithm of the survival rate. Neither parameter had a significant lethal effect below critical threshold values. Increasing the starting temperature of suspensions led to higher killing rates. No effect of varying the pH was observed.

Applying a given field intensity peak and amount of electric energy input, Qin et al. [15] have studied the inactivation of microorganisms with different voltage waveshapes including exponential decay, oscillatory decay, square waves, and bi-polar pulses. An average increase in the level of inactivation by 60% was obtained when comparing square to exponential decay waveforms. In two other comparisons, the authors showed that exponential decay waveforms were preferable over oscillatory decay waveforms, and bi-polar over mono-polar exponential decay waveforms. They noticed a layer of small bubbles at the surface of the electrodes during microbial tests with mono-polar pulses; however, such a layer was not observed during tests with bi-polar pulses. The use of bi-polar pulses also reduced the amount of solid deposits on electrode surfaces by 80%. In conclusion, bi-polar square wave pulses were found to be the most efficient in terms of microbial inactivation for commercial PEF processing.

Raso et al. [16] investigated the kinetics of microbial inactivation during the PEF processing by suspending the bacterial species under study in a McIlvaine buffer. For the same total processing time (number of pulses × pulse width), inactivation was independent of both the pulse width and pulse repetition rate. Attempts to fit the obtained results (under various electric field strengths) preferred a log-logistic model for the survival percentage as a function of the total processing time over both the first-order kinetics model and the log-log model. It confirmed the hypothesis of a distribution of resistances towards PEF inactivation among the cells under processing. This distribution was expected to arise from the inherent variation in the population or from an adaptive response of cells during the processing. Based on the fitted model, it was concluded that after a certain electric field strength, a further increase in the field would not lead to a faster inactivation.

Alvarez et al. [17] examined the influence of growth phase, initial cell concentration, and processing medium parameters (pH and electrical conductivity) on the inactivation of a microbial species during PEF processing. The initial concentration and medium conductivity did not significantly affect the inactivation results. In contrast, microbial cells were observed to be more resistant in their stationary phase as well as when suspended in acidic medium.

The presence of ions of different molecules, polarity, and valency has been suggested by Jayaram [18] to influence the destruction of a microbial cell due to the following causes: making the cells more susceptible to the high fields at potentials lower than the critical potential, and/or forming toxic compounds due to undesirable electrochemical processes in the presence of the high fields (e.g., hydrochloric acid and hypochlorous acid formation as a secondary reaction of chlorine with water).

The effectiveness of PEF processing in the inactivation of pectin methyl esterase (PME), an active enzyme that can be present in orange juice, has been evaluated by Yoem et al. [19]. PME causes cloud loss of orange juice, which plays a role in its turbidity, flavor, aroma, and characteristic color. PME is known to be more heat resistant than the spoilage microorganisms of orange juice. Shorter processing time (39 μs) with PEFs did not cause any inactivation of PME, whereas about 90% reduction in PME activity with longer processing time (59 μs) was observed.

In a study with four microbial species, Aronsson et al. [20] examined the change in microbial inactivation when varying the pulse parameters namely electric field strength, pulse width, and number of pulses. The effect of PEF processing is found to be largely species-dependent; yeasts were more sensitive to electric field than bacteria and gram-negative bacteria were more sensitive than gram-positive ones. Within each species, an analysis of the variance revealed a statistical significance for all three pulse parameters as well as their interaction. The authors concluded that an increase in one parameter could intensify the effect of another one.

Heinz et al. [9] carried out a detailed review on liquid food preservation by PEF processing in order to formulate the basic concepts for process design. If the membrane potential difference required for irreversible electroporation of the cell membrane is assumed the same for a number of microorganisms, the critical electric field strength will depend on the cell shape, size, and orientation. It was concluded that a field strength of 30 kV/cm is sufficiently high to trigger electroporation even in small bacteria.

However, due to the random cell orientation relative to the external electric field, a fraction (which progressively decreases with increasing cell diameters) of the microbial population will remain unaffected. To overcome this orientation effect, the suggestion has been made to apply multiple pulses instead of only one and promote rotation of the cells during the break times between pulses. Above a threshold energy level, these authors indicated that the efficiency of PEF processing strongly depends on the field strength applied, i.e. the higher the field strength at constant energy levels, the higher the lethality of the processing.

Bendicho et al. [5] evaluated the effect of PEF processing in comparison with thermal pasteurization on water-soluble and fat-soluble vitamins in skim milk. Ascorbic acid was the only vitamin that suffered some destruction; however, milk retained more ascorbic acid after PEF processing than after low (63ºC for 30 min) or high (75ºC for 15 sec) heat pasteurization. No significant differences were found in the results obtained when applying PEF at room or moderate temperature.

Heinz et al. [10] showed that the synergistic effects of elevated temperature (35–65°C) of PEF processing on the microbial inactivation reduce the energy consumption considerably. Temperature has a significant influence on membrane properties due to a temperature-related phase-transition of phospholipids from gel to liquid-crystalline structure, which affects the stability of the cell membrane. As the unprocessed liquid has to be preheated, these authors suggested recovering the dissipated electric energy through a heat exchanger. Since the adaptation of microbial cells to a specific type of stress may cross-protect them against other types of stresses, Evrendilek and Zhang [21] concluded that long preheating durations are not preferable. Amiali et al. [22] showed that inactivation of one microbial species was enhanced after increasing the field strength at elevated temperature, whereas another species behaved oppositely. In the range 15–50°C, the synergistic effect of processing temperature was reported by Saldana et al. [23] to take place in acidic, but not in neutral, media.

The applicability and effectiveness of PEF processing on high viscosity yogurt-based products has been verified by Yeom et al. [24]. A combination of mild heat and PEF processing was carried out by heating the yogurt to 60ºC for 30 s, cooling it down to 30ºC, and then applying electric pulses. The results obtained by applying the mild heating alone without applying any electric pulses confirmed that non-thermal inactivation of microorganisms by PEF processing occurred. Sensory evaluation by untrained students and technical panelists as well as physical measurements of color, ºBrix, and pH indicated no significant difference between the control and PEF-processed products.

Using mono-polar and bi-polar square pulses for PEF processing, Beveridge et al. [25] studied the influence of the pulse width on the microbial inactivation while rigorously controlling temperature. They concluded that shorter pulses provide a more energy-efficient inactivation. The superiority of mono-polar over bi-polar pulses was only observed with pulse widths less than 3µs since the bi-polar pulses undermine charge accumulation. Reorientation torque, induced by charge accumulation, was suggested as an explanation for bi-polar pulses becoming superior with longer pulse widths.

The physical–chemical characteristics of blended orange and carrot juice after PEF processing has been investigated by Rivas et al. [26] and compared to that after HTST thermal pasteurization (98ºC for 21 sec). The intensity of thermal pasteurization chosen was suggested by juice manufacturers, whereas the conditions for PEF processing were chosen so as to minimize the energy consumption while achieving a reasonable shelf-life of 4 weeks at 2ºC. Thermal pasteurization was more efficient in inactivating microbial flora and in extending the shelf-life; however, sensory analysis of odor and taste favored PEF processing. No significant difference was detected when comparing the effect of both preservation techniques on pH, ºBrix, and color.

Microbial growth can also play a role in the efficiency of PEF processing as indicated by El-Hag et al. [7]. Freshly-squeezed orange juice was stored at room temperature for eight days to allow the growth of natural contaminants before being subjected to PEF processing. For the sake of comparison, the naturally-grown microorganisms were collected from the stored juice and then inoculated in pasteurized orange juice. Results revealed that a greater kill could be achieved in the inoculated juice compared to the stored one.

Jayaram and Kameswara [27] presented a new design for the PEF processing chamber. It contained two electrodes of opposing convex electrode surface sections defining a biconcave processing zone between them. This electrode assembly has several advantages. From an electrostatics viewpoint, it generates the most intense electric field inside the processing zone and is continuous without edges to avoid arcing. From a fluid dynamics viewpoint, steady non-turbulent flow avoids eddy currents, bubble formation, and mixing between processing and unprocessed liquid food. From a microbiological viewpoint, the average microbial decay was superior than that achieved in the conventional parallel plate electrode assembly. The addition of a pre-processing stage involving temperature adjustment (40 to 60ºC) enhanced microbial inactivation relative to that obtained using PEF alone or temperature adjustment alone.

Walkling-Ribeiro et al. [28] introduced a hurdle treatment for reconstituted orange juice by combining thermosonication (TS) with PEF processing and compared it to HTST thermal pasteurization (94ºC for 26 sec) through a microbial shelf-life study and a sensory evaluation. Although the microbial counts in TS/PEF-processed juice were higher than in thermally pasteurized juice, overall counts in both treatments remained within safe levels (<1000 CFU/mL) over a storage period of 168 days. All sensory attributes were rated equivalent for both preservation techniques apart from the detection of a blander flavor in thermally pasteurized juice and a metallic flavor in TS/PFE-treated juice by a few panelists. It was suggested that the combination of TS and PFE hurdles could explain the lower impact on the juice color observed experimentally.

When a combination of moderate heating (55ºC for 60 sec) and PEF processing was applied by Walkling-Ribeiro [29] for the preservation of a fruit smoothie, the processed smoothie exhibited a longer microbiological shelf-life than that obtained after mild thermal pasteurization (72ºC for 15 sec). The accompanying sensory analysis showed no significant difference in the overall acceptance among unprocessed, heated/PEF-processed, and mildly pasteurized fruit smoothies. The authors suggested that sensitive fruits such as pineapple and banana are one of the possible reasons that the overall sensory quality of the three products was the same.

After consulting brewing specialists, Walkling-Ribeiro et al. [30] defined the minimum requirements for microbiologically safe 'cold' PEF processing to be a microbial reduction of 4.0 $\log_{10}$ CFU/mL at temperatures below 42ºC. Out of the four typical beer microorganisms used in this study, aforementioned requirements were met with three of them, which required the input of at least 819 J/mL of energy. Results did not show a strong influence of the carbonation and alcohol levels on the microbial inactivation.

Table 1-1 lists some typical conditions for PEF processing used for liquid food preservation. It can be seen peak fields ranging from 25–40 kV/cm and pulse widths from 1–4 μs commonly applied. These ranges account for the various microorganisms, which differ in their sensitivity to the PEF processing, as well as various media. Since the pulse energy could vary over a wide range, the energy density is considered for any comparison in this thesis. For the experimental work, the nominal operating region of energy density is defined to be 500–750 J/mL.

The typical specific energy reported for modern milk pasteurization plants ranges from 972 to 1584 J/mL [31]. Thus, thermal pasteurization requires almost double the energy required in the case of PEF processing; i.e., the use of PEF requires a lower processing energy.

Table 1-1: Typical conditions for PEF processing used for liquid food preservation

Food data			Process data		Voltage pulse data								
Name	Cond mS/cm	pH	Flow mL/min	Temp ºC	Shape	Polarity	Peak kV/cm	Width µs	Rate Hz	Energy J	Pulse density mL^{-1}	Energy density J/mL	Ref
Yogurt	5.6	4.2	1666.7	30	square	mono	30	1.4	500	(2.24)	(72)	(161)	[24]
Orange – carrot	4.5	3.8	60	-	square	bi	25	2.5	767	(0.086)	(9204)	(788)	[26]
Fruit smoothie	4.62	4.3	-	15	square	(bi)	34	1	<25	(9.48)	(68.6)	650	[29]
Orange	4.17	3.8	(15.75)	55	square	mono	40	1	15	(11.68)	(57.1)	(667)	[28]
Beer	1.58	-	60	4	square	bi	41	4	(300)	(0.164)	(3600)	(589)	[8]
Orange	3	3.8	6000	40	square	bi	30	1	(335)	(7.46)	6.7	50	[32]
Nutritive Treatment Medium	4	5	41	30	square	bi	30	4	250	(0.126)	(4390)	(553)	[20]
							30	4	500	(0.126)	(8780)	(1105)	
							35	4	250	(0.171)	(4390)	(752)	
							35	4	500	(0.171)	(8780)	(1505)	

Values within brackets are not reported in the references, rather they are the calculated values by the author from the available information in the published papers

1.1.2 Electrode material performance during PEF processing

In order to prevent electrode material from being released in the liquid food upon the application of PEFs, Dunn and Pearlman [6] introduced an ion permeable polymer membrane functioning as a physical barrier between the electrodes and the liquid food as shown in Figure 1-2. Cation and anion selective membranes (208, 210) prevent the liquid food (202) from being in direct contact with the metallic conductive electrodes (204, 206). Electrical conduction between each membrane and the adjacent electrode is provided through an ionically conductive electrolyte (212, 214). Such electrolytic solutions should be frequently replaced with fresh ones to avoid undesired degree of concentration or depletion of ionic components due to the ionic transport to/from the electrolyte zones across the membranes. Both the ion permeable membranes and the electrolytic solutions should exhibit relatively high electrical conductivity and be suitable for food processing applications. This arrangement guarantees a physical separation between the electrodes and the liquid food, and hence the isolation of any electrode reaction products. The disadvantages of using this arrangement are as follows: only mono-polar pulses are permitted due to the selective nature of membranes, additional cost of membrane material and high sensitivity to puncture by any electrical discharges, frequently required maintenance, and complicated arrangement for industrial implementation. Due to these complexities, the reduction of electrode products rather than their complete prevention in the absence of a membrane might be preferred as long as metal concentrations from the electrodes remain within limits.

Electrophoresis accompanying the flow of electric current during PEF processing is discussed by Bushnell et al. [33] to account for the motion (without electrochemical reactions) of charged particles suspended in the liquid food. Problematically, in rich protein foods such as milk, a film of material can collect or agglomerate on at least one electrode. The formation of such a film on the electrode(s) during extended processing periods can cause electrical breakdown in the cell, fouling or contamination of the system, and can even cause the flow of fluid to stop. The rate of agglomeration on the electrode(s) is suggested to be proportional to the net electric charge delivered to the processing cell. Based on this reasoning, a claim has been made that “zero net charge delivery” substantially prevents fouling of electrode(s). Although the claim could be reasonable, neither analytical proofs nor quantitative experimental results have been presented for its justification.

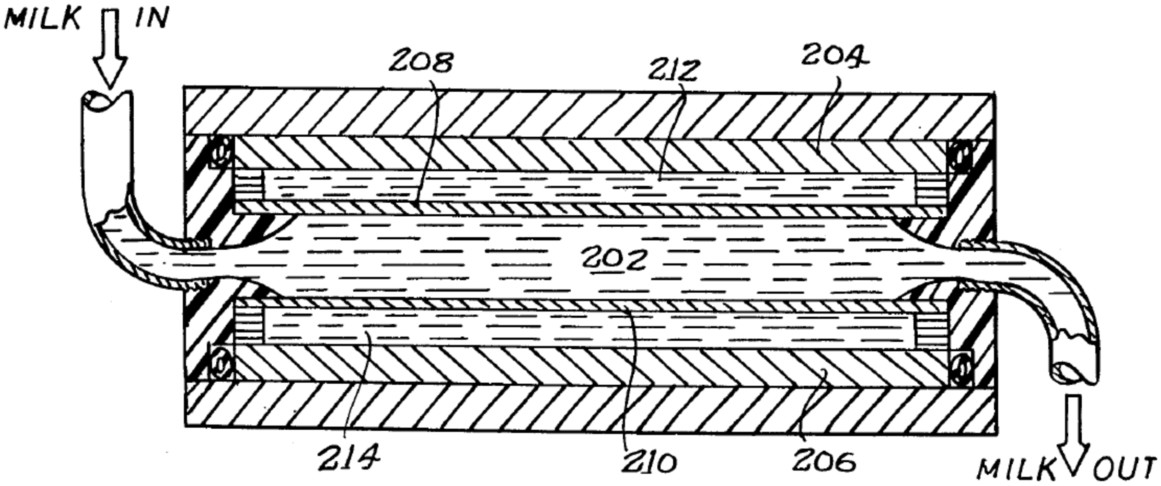


Figure 1-2: Continuous-flow PEF processing chamber with ion permeable membranes [6]. Parts (202-214) are explained in detail through the text

In a later stage, Bushnell et al. [34] added electrochemical reactions into the picture as a continuation of their previous study [33]. The same “zero net charge delivery” concept, which could be used to reduce fouling of electrodes due to electrophoresis, functions to prevent or reduce electrochemical reactions, in general, during the PEF processing. It is emphasized that only Faradaic current will contribute to electrochemical reactions according to Faraday’s law of electrolysis. Faradaic current is defined as the current flowing through the processing cell after charging the electric double-layer (or double-layer capacitor) at the boundary of the electrodes by a non-Faradaic capacitive current up to the reaction potential of the reacting species. The time required for this charging process is given by the following equation:

$$t_{ch} = \frac{C_d V_R}{\sigma E}$$

where C_d= specific capacitance (capacitance per unit area) of the two electrode/electrolyte interfaces in series, V_R= reaction potential of the reacting species at both electrodes ($V_R = V_{anode} - V_{cathode}$), σ= electrical conductivity of fluid, and E= electric field intensity. As long as no cumulative build-up of charge occurs, no electrochemical reactions will take place for any pulse of width shorter than the charging time ($t_{pulse} < t_{ch}$). The charge build-up can be avoided by the same concept of zero net charge delivery where the double-layer capacitors are forced to discharge any residual charges after each high voltage pulse. Attempts to prevent the electrochemical reactions can involve applying multiple pulses of lower electric field and shorter pulse width and/or using electrode material having a high specific double-layer capacitance. This claim is theoretically reasonable; however, no quantitative experimental results have been presented for justification.

One attempt to avoid the direct contact between the liquid food and the electrodes was made by Mazurek et al. [35] through an electrode arrangement comprising cylindrical electrodes with a glass tube immersed (Figure 1-3) with the condition that the inter-electrode space is filled with an insulating liquid (distilled water) with a permittivity equal to or larger than that of the sample being processed. Inactivation curves of four microbial species proved the effectiveness of such an arrangement. Further investigations on the same arrangement have been carried out by Lubicki and Jayaram [36] using various filling media, including transformer and silicon oil. However, Jayaram [18] reporte that the energy transfer efficiency of this electrode arrangement from the point of application to the point of utilization for inactivation is very low since the potential drop near the electrodes is high and, consequently, the field across the suspension may not be sufficient.

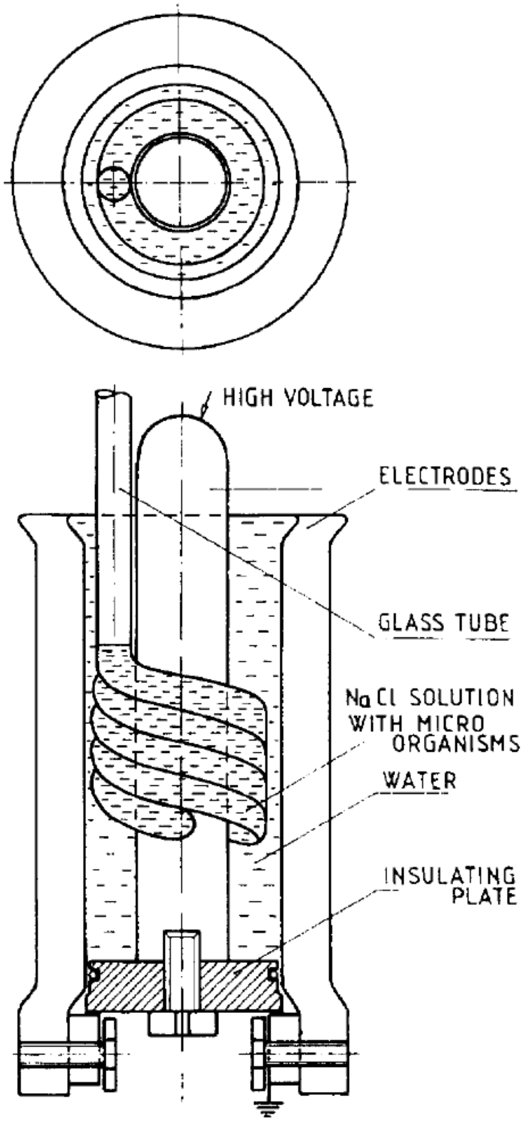


Figure 1-3: Electrode arrangement comprising cylindrical electrodes with a glass tube immersed where the inter-electrode space is filled with distilled water [35]

Morren et al. [37] developed electrical equivalent circuit models to describe the electrode/electrolyte interface under various conditions as shown in Figure 1-4. These circuits involved element for the charging process of the double-layer capacitor by a non-Faradaic current, resistors for the Faradaic processes and electrolyte transport, and Warburg impedance for diffusional limitations whenever applicable. Theoretical formulation resulted in the same threshold pulse width, identified earlier in [34], beyond which electrochemical reactions take place as long as the build-up of charge is avoided. Sinusoidal waveforms at various frequencies were applied to an aqueous solution of sodium chloride where high and low frequencies simulated PEF processing with bi-polar pulses having short and long pulse widths, respectively. Their experimental results verified that, beyond a critical width, the rate of electrochemical reactions is proportional to the width of applied pulses. It should be noted that sinusoidal waveforms with only 15V and 150V peak-to-peak voltages were applied resulting in a maximum electric field of 0.15 kV/cm and a maximum current of 480 mA, which are far below typical PEF conditions. The authors suggested coating electrodes with conductive polymers or using electrode material featuring high double-layer capacitance to reduce the electrochemical reactions.

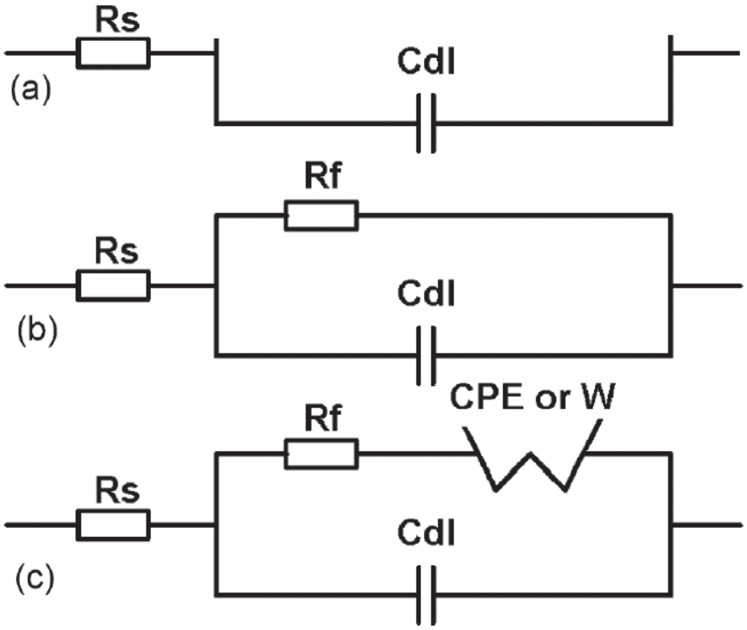


Figure 1-4: Electrical equivalent circuit of the electrode/electrolyte interface for (a) no electrochemical reactions, (b) electrochemical reactions with charge transfer limitations, and (c) electrochemical reactions with diffusion limitations where C_{dl} is the double-layer capacitance, R_f is the polarization resistance of Faradaic process, W is the Warburg impedance due to diffusional limitations, and R_s is the bulk resistance of the solution [37]

To investigate the effectiveness of PEF processing on beer, Evrendilek et al. [8] performed a comprehensive study that included microbial, sensory, and quality analyses. By comparing the concentration of 10 elements in beer samples before and after the PEF processing, a significant increase was observed in the concentrations of iron, chromium, manganese, and zinc in the beer. This increase caused an unfavorable degradation in flavor/aroma and mouth feeling assessed by the sensory panel. In addition, some of the panelists indicated a metallic mouth feeling in the samples. Although stainless steel did not contain zinc, authors did not comment on its presence after the PEF processing. The authors recommended the use of more stable electrode materials under such severe electrochemical conditions.

Roodenburg et al. [32, 38] presented a detailed study of metal release in a PEF processing system with stainless steel electrodes. First, a comparison was made of three mono-polar pulse shapes frequently used in PEF processing based on the metal released from the electrodes when each was applied using a continuous PEF system to a neutral aqueous sodium chloride solution [38]. The applied voltage and corresponding current waveforms for these three pulse shapes shown in Figure 1-5 were generated. For shapes (a) and (b), the concentrations of metals dissolved were almost equal to each other and significantly lower than those produced when shape (c) was applied. This difference was attributed to the fact that waveforms (a) and (b) have negative components, which may reverse some of the electrochemical reactions. In order to verify that metals are released due to electric pulses, an experiment was done by just circulating the aqueous solution through the tubing system and the processing chamber without applying any electric pulses. The resulting metal concentration was found to be below the detection level of the measuring apparatus. It was suggested that the total transferred charge per pulse could be divided into three portions: charge and discharge of the double layer capacitance, release of metallic components, and other electrochemical reactions. As a result, an expression was derived for estimating an upper limit of the amount of metal that might be released per pulse in any specific PEF system. Accumulation of rust on the electrodes indicated that the dissolved metal concentration in the processed liquid might not reflect the full extent of the the electrochemical reactions and accurately predict the electrode. To reduce metal release, polishing the stainless steel electrode surface was suggested since this might expose chromium-oxide, which is more chemically resistant than ordinary iron-oxide.

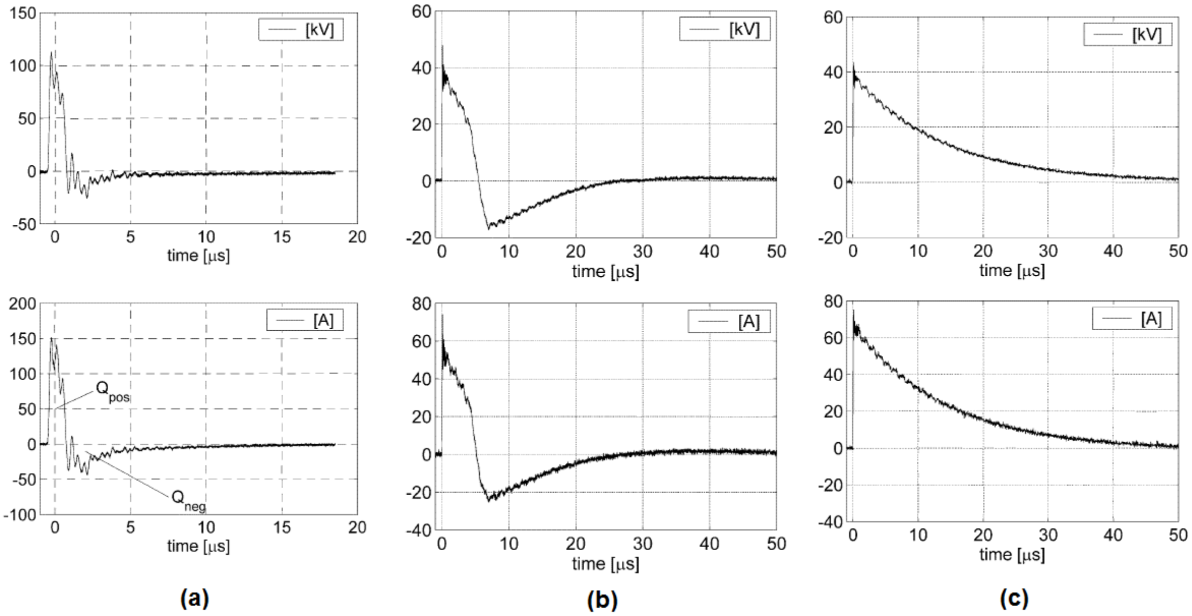


Figure 1-5: Voltage and current waveforms for three pulse shapes: (a) matched load with an output transformer, (b) oscillatory decay with a parallel inductor, and (c) exponential decay [38]

Roodenburg et al. [32] also investigated the effect of orange juice composition and its pH on the released metals by using one of the three pulse shapes presented in [38]. Experiments revealed that only the concentration of iron rose above the detection limit of the measuring apparatus when a single real PEF processing was applied with the conditions listed in the corresponding row in Table 1-1. To gain an estimate of other metals that could be released from the stainless steel electrodes, the juice was circulated 36 times through the unit and the amounts of released chromium and nickel per cycle were determined. The estimate of released iron matches the results obtained from the single PEF experiment, which indicates a linear relationship between the released metals and the number of circulations. In a separate experiment of, orange juice was circulated through the tubing system 36 times without applying any pulses. As a result, the metals released during a single PEF processing could be divided into contributions from electrically-driven electrochemical reactions and from contact with the metallic tubing system in absence of a pulse. When the authors compared the experimental results to the ones presented in [38], the levels obtained in orange juice were 36-44 times those obtained in the aqueous solution. This suggests that real food products should be tested rather than aqueous solutions. Based on levels mandated by European legislation for fruit juices and drinking water, the authors found that the measured values of dissolved metals were far below the regulated limits. One could explain this conclusion by observing that the corresponding energy density is much lower than the average of typical PEF conditions (Table 1-1). The authors also expected that metal release would change the geometry of the processing chamber and consequently reduce the applied electric field below the requirements. On this basis, they estimated that each chamber could process only 276,000 liters of orange juice before being discarded. In order to compensate for these geometry changes and maintain the applied electric field as per requirements, a gradual increase of the applied voltage waveform was suggested but without indicating any details about the corresponding control methodology.

In addition to their study [39] of the crucial parameters in PEF processing, Toepfl et al. tested stainless steel and graphite electrodes to investigate their suitability as electrode materials. Graphite was chosen because it has a relatively higher double-layer capacitance, which plays a role in reducing the electrochemical reactions. The authors did not evaluate the electrochemical reactions quantitatively, but instead observed high microbial inactivation with the graphite electrode. It was concluded that the presence of gas bubbles, formed by electrolysis of water, might have led to perturbations in the electric field distribution in the case of stainless steel electrode. After modeling the electric field, low field strength was obtained at the boundary of a bubble present inside a liquid, possibly leading to under-processing, in particular between several bubbles. Arcing, often observed in the presence of large air bubbles, was not observed with either electrode material.

Kitajima et al. [40] introduced a textile electrode for PEF processing as shown in Figure 1-6. The electrode was woven with polyester fiber as warp yarn and metal wire as weft yarn. The use of both tungsten and titanium for the metal wire was found to lead to similar amount of same microbial inactivation. A white material was observed in scanning electron microscope image on the high voltage electrode only when processing low-conductivity solutions. The authors expected this sediment to contain inactivated microbial cells, although it could be produced by electrochemical reactions.

Due to the lethal effect of silver, Yoshino et al. [41] utilized the released ions from silver electrodes during the PEF processing to enhance the microbial inactivation. When a silver electrode was connected to the high voltage source and a stainless steel electrode was grounded, the elution of silver was significant (1.6–9.4 ppm) resulting in instantaneous sterilization. However, when reversing the connections of the electrodes, a lower and constant (0.01–0.02 ppm) silver concentration was obtained, but the inactivating effect was still greater than when stainless steel was used for both electrodes. In a separate experiment, it was concluded that the eluted silver from the anode had a greater inactivating effect than silver added directly in a nitrate solution.

Altuntas et al. [42] measured the concentration of metallic ions in the course of their quality assessment of PEF-processed sour cherry juice (juice was obtained from concentrates). Although the electrodes were made of stainless steel, no remarkable increase was detected in the concentration of chromium, iron, or nickel. The authors did not comment on the concentration of iron being 4.83 ppm before the PEF processing; it may have originated from the cherry fruit itself or the concentration process.

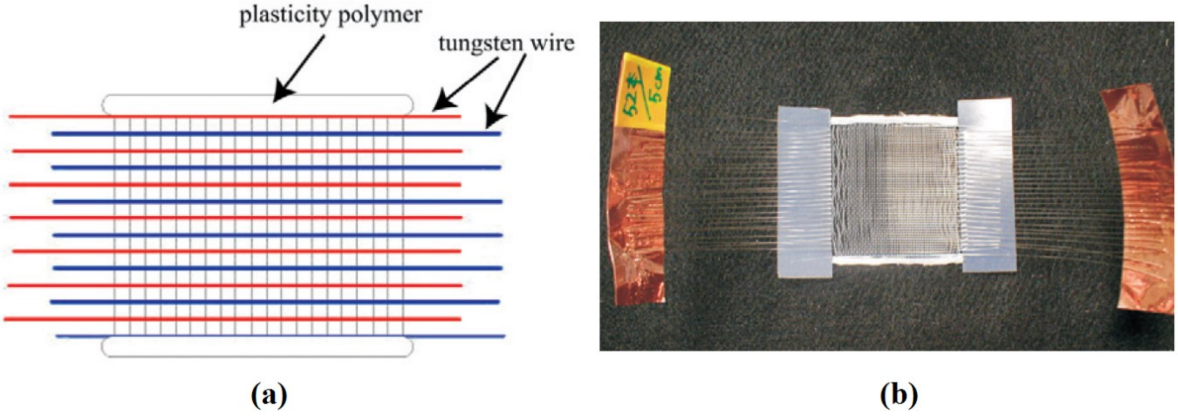


Figure 1-6: (a) Illustration of the textile electrode with red and blue wires connected to the high voltage and ground terminals, respectively, (b) photo of the textile electrode [40]

In another attempt to avoid physical contact between the liquid food and the metallic electrodes, Roodenburg et al. [43] proposed the use of thin conductive plastic films to cover the metallic electrodes as shown in Figure 1-7. A Carbostat® film of 80 µm thickness was used for the electrical and bacterial validation experiments. It consisted of an ethylene vinyl acetate copolymer matrix made conductive by the addition of 30% w/w (i.e. ~8.6% v/v) carbon black particles ranging between 50 and 200 nm. Its electrical conductivity in the direction normal to the surface is ~7.5 mS/cm which allows most of the applied voltage to fall across the liquid being processed. As a proof of concept, reduction levels of 0.8 – 2.1 $\log_{10}$ CFU/mL were obtained in a batch-type processing chamber with both electrodes covered by thin films. The authors computed the time constant of the transition from a capacitive to the resistive field distribution to be in the nanosecond range so that the resistive electric field distribution should be obtained under typical PEF conditions. This conclusion implicitly means that conductive current will flow across the film/liquid interface with the possible occurrence of electrochemical reactions. The authors also measured the average conductive area fraction in the film surface to be ~2.3%, which may result in a very low double layer capacitance on the interface.

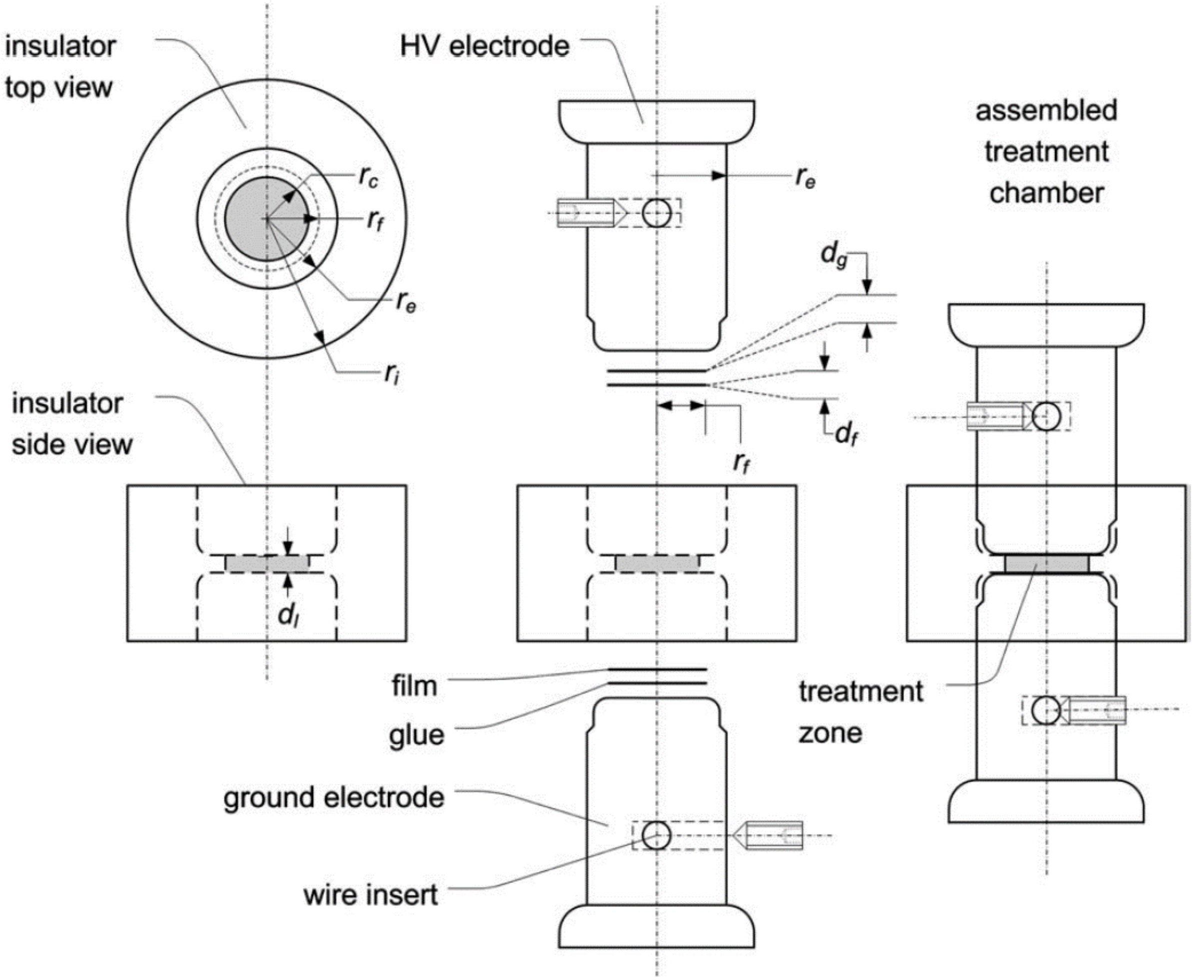


Figure 1-7: Construction drawing of the batch-type processing chamber, which can be equipped with any plastic electrode covering [43]

It should be acknowledged that the dissolved metal ions may participate in secondary reactions affecting food compounds once they are released from the electrodes into the liquid. Sun et al. [44] compared the effect of stainless steel and titanium electrodes on the degradation, spectral characteristics, visual color, and antioxidant capacity of two food compounds: cyaniding-3-glucoside and cyaniding-3-sophoside. Higher degradation levels were measured after PEF processing with titanium electrodes, although titanium electrodes released less metal (titanium concentration increased by 8 ppm) than stainless steel electrodes (iron concentration increased by 33 ppm). Results of the spectral characteristics, visual color, and antioxidant capacity were also favored the use of stainless steel electrodes. It seems that not only the quantity but also the type of ions may influence the food degradation by secondary effects. Hence, a sensory/quality analysis should be performed on PEF-processed products to determine the significance of the released ions.

Finally, May and Ruben [45, 46] patented a high impedance electrode (HIE) for PEF processing to achieve “a significantly higher electric field intensity with a given applied voltage” as well as limiting the electric current flow, ohmic heating, energy consumption, and electrochemical reactions. This HIE was fabricated of a lead-magnesium-lead titanate ceramic material, which had a volume resistivity of 10^{12} Ω•cm and a relative permittivity of 6,000. With the 1 mm gap between the two parallel electrodes filled with liquid (conductivity was not reported), the total resistance and capacitance of the arrangement was measured as 2.5×10^{5} MΩ and 652 pF, respectively. The application of 10 kV to the electrodes resulted in a microbial inactivation of 0.21 $\log_{10}$ CFU/mL. This low reduction in microbial killing may be due to significantly low fields across the liquid since much of the field drops across the HIEs.

It can be seen from Table 1-2 that most of the reported work in the literature involved the use of stainless steel electrodes for the PEF processing. Stainless steel has quite high corrosion resistance, reasonable price, and wide acceptance in the food industry.

Table 1-2: Typical electrode materials used for PEF processing

Electrode material	Ref
Carbon	[11]
Stainless steel	[5, 6, 8, 15-17, 32, 37, 38]
Boron carbide	[24]

1.1.3 Electrode material performance during other electrical processes

Amatore et al. [47] studied extensively the electrochemical ohmic heating of liquids starting from the fundamental principles of this technology. In ohmic heating, the aim is to increase the ohmic drop across the electrolyte and reduce the portion of the voltage drop across the electrode/electrolyte interfaces. Two reasons stand behind this objective: a) the reduction of power dissipated by electrochemical reactions, and b) avoidance of forming undesirable products due to partial electrolysis of the heated solution itself and/or electrode reactions. Based on a simple equivalent circuit, it was shown that all electrochemical reactions can be eliminated by operating the cell under an alternating sinusoidal voltage featuring sufficiently high frequency such that both double-layer capacitors are partially but continuously charged and discharged without reaching the threshold potential (ΔE^{Far}) corresponding to the onset of anodic and cathodic Faradaic reactions. Under such circumstances, the current flowing through the cell is purely capacitive and does not correspond at all to charge creation or annihilation at either electrode but only to a formal displacement of charges from one double-layer to the double-layer of the other electrode. Alternatively, using different electrode materials with high double-layer capacitance and high threshold potential would reduce the minimum frequency required for eliminating such electrochemical reactions. A practical method was presented to compare the performance of some electrode materials using cyclic voltammetry; results obtained from the corresponding voltammograms are listed in Table 1-3 and show that the effect of double layer capacitance is more dominant in such comparison.

Under power frequency (60 Hz) operation, Samaranayake and Sastry [48] compared the performance of four electrode materials during ohmic heating of aqueous solutions with various pH levels. The variables investigated in comparing the performance of stainless steel, titanium, platinized-titanium, and graphite electrodes included the concentration of electrode material dissolved in the heated solution, the amount of hydrogen gas generated during the heating process, and the change of pH level due to the heating process. Table 1-4 shows that both titanium and platinized-titanium electrodes have similar superior performance regarding the release of electrode material. However, the latter did not cause any detectable generation of hydrogen gas, which suggests an inhibition of all undesirable electrochemical Faradaic reactions. This observation was supported by examination of the surface morphology of both electrodes under the scanning electron microscope. Tiny cracks, valleys, and bumps were observed over the surface of the platinized-titanium electrode resulting in a high ratio of microscopic surface area to apparent geometric surface area. Such a high ratio would cause a higher double-layer capacitance at the electrode/electrolyte interface which might result in an entirely capacitive current during the cell operation and hence elimination of any Faradaic reactions. A yellowish-brown oxide surface film was observed on the active titanium electrodes before and during the heating process to provide some protection through partial passivation. On the other hand, stainless steel and graphite electrodes showed relatively high concentration of released electrode material, especially at low pH levels. The chemical reactivity of elements forming the stainless steel alloy and the high affinity of graphite for absorption of oxygen and hydrogen gases in surface functional groups was suggested to explain their inferior performance.

Table 1-3: Comparison of the performance of a series of electrode materials of geometric surface area A tested through cyclic voltammetry (scan rate $\boldsymbol{\nu = 0.1\ V/s}$, 20 g/L NaCl aqueous solution, 20°C) [47]

Aspect of comparison	Stainless steel	Titanium	Glassy carbon	DSA*
$C/_{A}\ (\mu F/cm^2)$	35	50	260	2000
$\Delta E^{Far}\ (V)$	0.325	1.2	0.3	0.5
$C^2\Delta E^{Far}/_{A^2}\ (relative\ units)$	1	7.5	51	5000

*Dimensionally stable anode

Table 1-4: Comparison of corrosion rates of electrode materials in terms of release of their major (surface) elements at different pH values [48]

Electrode type	Corrosion rate*		
	pH 3.5	pH 5.0	pH 6.5
Titanium (Ti)	0.26^{a} (0.21)	0.03^{a} (0.01)	0.05^{a} (0.03)
Stainless steel (Fe)	14.20^{b} (1.95)	8.33^{c} (0.30)	11.43^{b,e} (1.51)
Platinized-titanium (Pt)	0.25^{a} (0.10)	0.07^{a} (0.04)	0.05^{a} (0.02)
Graphite (C)	26.6^{d} (2.2)	7.2^{c} (0.0)	8.4^{c,e} (1.1)

$^{a\text{-}e}$Means with different superscript letters are significantly different ($P \leq 0.05$)

*Numbers in parentheses indicate standard deviation of mean (n=3) corrosion rates

Samaranayake et al. [49] applied the methodology presented in [48] to study the performance of the same four electrode materials but under pulsed ohmic heating. An insulated Gate Bipolar Transistor (IGBT)-based power supply was capable of varying the timing parameters (namely frequency, delay time, and pulse width) of the applied bi-polar voltage pulses while keeping the pulse amplitude fixed. The initial pH of the aqueous solution was fixed at 3.5 but the electrical conductivity was varied in order to obtain the same time–temperature curve through all experimental runs. As expected, titanium and platinized-titanium electrodes performed much better than stainless steel and graphite ones. Interestingly, the use of pulsed operation reduced significantly the electrode material release of only the three metallic materials compared to the conventional power frequency operation. For all three metallic materials, shorter pulses were preferred over longer pulses when operating under high frequency (10 kHz), while the reverse was the case under low frequency operation (4 kHz). However, each material performed differently when the frequency itself was varied. Within each material, stainless steel performed better at high frequency for the same duty cycle, titanium performed almost equally at low and high frequencies, whereas platinized-titanium performed better under low frequency conditions. It can be observed that each material may have its own optimum operating conditions;

however, the authors did not provide any explanation or reasoning for this observation. In a separate experiment with only stainless steel electrodes, the effect of varying the delay time between positive and negative pulses within each cycle, at fixed frequency and pulse width, showed a threshold value below which the electrode performance deteriorated drastically. This threshold value was explained as being the required time for the double-layer capacitors to almost completely discharge between successive pulses.

Chapter 2

Objectives of the Present Study

With the exception of a few attempts [6, 35, 36, 43, 45, 46, 50] to avoid physical/electrical contact between the liquid food and the metallic electrodes, electric pulses are typically applied to the liquid food products via two metallic electrodes. A pulse generator and an electrode pair are the two main components comprising any PEF system operating either in batch- or continuous-mode. The function of such electrode pair is just to introduce the generated pulses into the liquid food under processing. Electrochemistry defines this system as an electrolytic cell where an external source of electrical energy is responsible for driving electric current through the cell. This electric current has an electronic nature (electrons) in the conductive solid electrode and an ionic nature (ions) in the conductive liquid electrolyte. As a result, electrochemical reactions will take place at the electrode/electrolyte interface to satisfy the condition of electric current continuity.

On a microscopic scale, a so-called double-layer of charges is formed in the interface upon immersing the electrode into the electrolyte. If, for example, a negative potential is applied to the electrode, positive ions are attracted and migrate towards the interface as shown in Figure 2-1. The speed of migrating ions depends on their mobility as well as the applied electric field. Since negative and positive charges are separated with a distance x, an equivalent capacitor exists at the interface. The charge density as well as the distance x depend on the applied potential. Increasing the potential beyond a certain threshold value will not attract more charges from the bulk of the electrolyte due to the counter electric field created by the double-layer. Instead, electrochemical reactions involving oxidation/reduction half-cell reactions will commence.

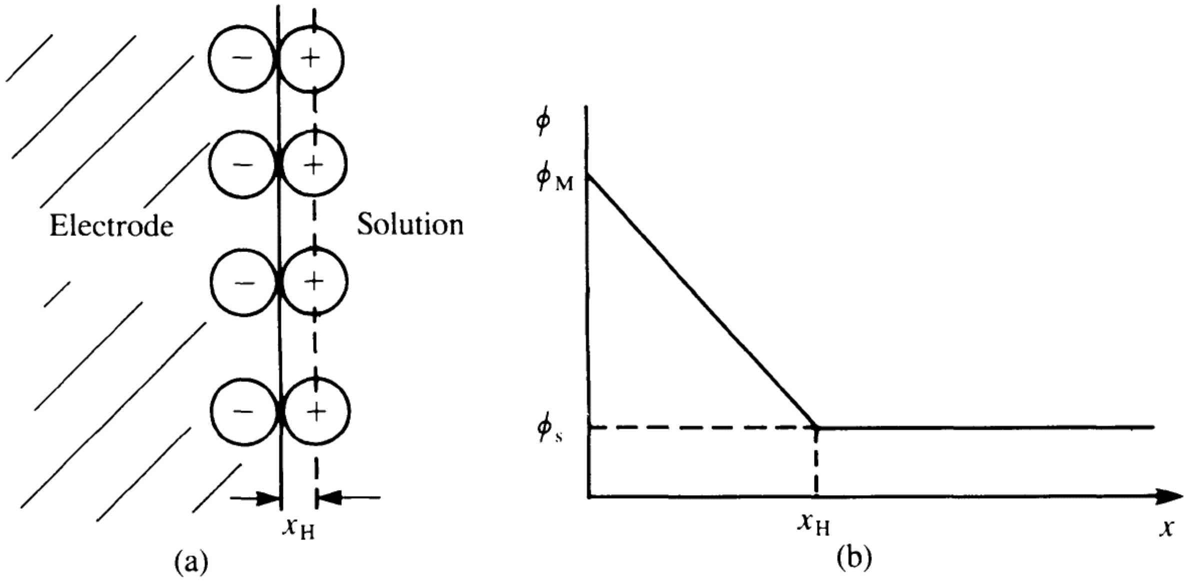


Figure 2-1: The Helmholtz model of the double-layer; (a) rigid arrangement of ions, and (b) variation of the electrostatic potential φ with distance x from the electrode [51]

Since prediction of all possible electrochemical reactions during the PEF processing of any real liquid food falls beyond the scope of this thesis, electrolysis of water will be presented here to explain the nature of electrode reactions. Anodic and cathodic half-cell reactions of water electrolysis have a strong pH dependence as follows:

Cathodic half-cell reactions:

$$2H^{+}{}_{(aq)} + 2e^{-} \xrightarrow{acidic} H_{2(g)}$$

$$2H_2O_{(liq)} + 2e^{-} \xrightarrow{alkaline} H_{2(g)} + 2OH^{-}{}_{(aq)}$$

Anodic half-cell reactions:

$$v_1M_{(s)} + v_2H_2O_{(liq)} \xrightarrow{alkaline} M_{v1}O_{v2} + 2v_2H^{+}{}_{(aq)} + 2v_2e^{-}$$

$$M_{(s)} \xrightarrow{acidic} M^{n+}{}_{(aq)} + ne^{-}$$

where the anodic equations represent a set of equations, for each element $M_{(s)}$ in the electrode material.

On a macroscopic scale, the electric current flowing through the cell is divided among the electrochemical reactions occurring at each electrode. The electrical equivalent circuit of the cell is shown in Figure 2-2, in which a dc source of voltage (V) is applied to a conductive electrolyte of ohmic resistance (R_s) through a pair of electrodes. The ohmic resistance of both electrodes is negligible. However, each electrolyte/electrolyte interface is represented by a double-layer capacitor (C) and a group of parallel branches. The number of parallel branches at each electrode equals the number of all possible electrochemical half-cell reactions that might occur at that electrode. Each branch consists of a normally-open switch in series with a resistance. These switches are controlled through the corresponding double-layer potential, i.e. each switch will close when the double-layer potential exceeds a certain threshold value. The potential drop across the anodic double-layer (E_{anode}) and the cathodic double-layer ($E_{cathode}$) should satisfy Kirchhoff voltage law as follows,

$$V = E_{anode} + IR_s + E_{cathode}$$

Initially, both double-layers are not charged ($E_{anode} = E_{cathode} = 0$) and the current starts to flow through the electrolyte resistance upon the application of the external dc source. This current is a capacitive non-Faradaic current since it is not involved in any electrochemical reactions. However, this current begins the charging process of both double-layers. The onset of a given, for example, anodic half-cell reaction i occurs when the applied double-layer potential E_{anode} exceeds the Nernst potential E_i of the half-cell reaction. Once an electrode reaction occurs, current corresponding to the rate of this reaction flows across the cell. When more than one half-cell reaction occurs at an electrode, the total current passing through the cell is distributed among the half-cell reactions depending on their relative rates.

The current transferred by a given half-cell reaction depends on the electrode potential difference $E - E_i$, the kinetic parameters associated with the reaction, and the temperature. Faraday's law governs the relationship between the number of moles n_{ij} of a species j produced by the reaction i and the cumulative electrical charge Q_i transferred by the reaction over a period of time, i.e.,

$$n_{ij} = \frac{Q_i}{F.z_{ij}} \quad \forall \quad E \geq E_i$$

where the Faraday constant $F = 96{,}485\ C/mol$, and z_{ij} is the number of electrons transferred for each product j formed by reaction i.

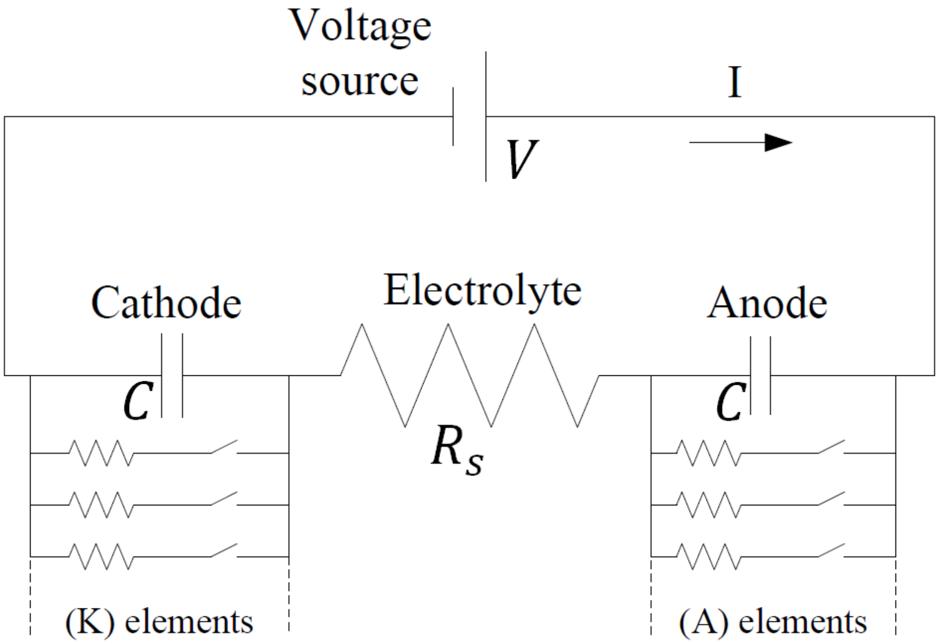


Figure 2-2: Electrical equivalent circuit for the electrolytic cell

This thesis is concerned with the release of metallic ions due to the electrode reactions and their effect on the quality of PEF-processed food products. As shown in Figure 1-1 under electrode reactions, the release of electrode material into the processed food may cause the following:

- Concerns about safety regulations. The dissolved electrode materials themselves may exceed the permissible limits of certain metals in edible food dictated by regulations.
- Loss of flavor/taste. The dissolved electrode material may lead to some changes in the original flavor of the processed food.
- Reduction in the electric field. Changing the dimensions of the processing zone may cause the electric field to be insufficient for microbial inactivation.
- Curtailment of electrode lifetime. Wearing out of electrodes will require frequent replacement at considerable cost.

In the above context, the main objectives of this thesis are as follows:

- Understanding the nature of metallic ion release from the electrodes during the PEF processing so that optimum operating conditions can be recommended for the elimination, or at least the reduction, of this effect. To this end, the validity of the "zero net charge delivery" concept is to be examined by a comparison between two circuit topologies. Then, the effect of the electric pulse parameters (peak voltage and pulse width) on the charging process of the double-layer capacitors will be investigated. The behavior of metallic-coated electrodes could reveal some intrinsic characteristics for each metal. Finally, temperature dependence is investigated by changing the cooling set-point of the processing zone.
- Evaluating the effect of the released metallic ions on the quality of carbonated beer. The immediate (direct) effect on beer quality will be examined by a time-zero sensory panel. In addition, secondary (indirect) effects, which may arise by accelerating the aging process of beer, are taken into consideration through sensory panels over a shelf-life of 12 weeks. Microbial inactivation experiments are performed to evaluate the effectiveness of PEF processing at low processing temperature.

The organization of this thesis can be summarized as follows:

- In Chapter 3, “Materials and Methods”, the experimental setup utilized for the PEF processing and its main components are described in detail. The main characteristics of the liquid food products are presented. Inductively coupled plasma – atomic emission spectrometry (ICP-AES) is utilized to determine the concentration of metallic ions in the PEF-processed food samples. Procedures of microbial inactivation experiments and sensory panels are described as well.
- Chapter 4, “Results – Factors Affecting the Electrode Material Performance”, presents the experimental findings obtained from the ICP-AES measurements under various PEF operating conditions. In addition, the results include voltage and current waveforms, some temperature measurements, and observations from the visual examination of the electrodes.
- Chapter 5, “Results – Microbial/Sensory Analysis of Beer”, presents findings obtained from the quality analysis of beer in terms of microbial inactivation, concentration of metallic ions, and taste attributes.
- Chapter 6, “Discussion”, focuses on the results presented in Chapter 4 and explains the mechanisms behind the metal ion release. In addition, the role of the operating conditions in reducing the rate of metal release is described. Safety concerns about PEF-processed products are discussed in relation to the Canadian and American food regulations.
- Finally, Chapter 7, “Conclusions and Future Work”, provides conclusions to the findings obtained throughout the study and recommendations for future work pertaining to the investigated topics.

Chapter 3

Materials and Methods

The first section of this chapter provides a detailed description of the experimental setup used for the PEF processing and its developments over the course of this thesis. Evaluation methodologies of the PEF-processed samples are discussed in the following sections from the viewpoints of metal concentrations, microbial inactivation, and sensory panels.

3.1 Experimental setup

To assess various aspects of the PEF technology, real liquid food samples were processed by a lab-scale setup, capable of processing various food types by adjusting its operating conditions as per requirements. The setup operates in a continuous mode, i.e. the liquid food is subjected to the high-field pulses, generated by a pulsed-power generator, while flowing through a processing chamber. A detailed description of the pulsed-power generator and the processing chamber will be presented in the next subsections.

Over the course of this thesis, the experimental setup has gone through several modifications. For the preliminary experiments, the liquid food was circulated in a closed-loop configuration as shown in Figure 3-1. Samples were pipetted out from the liquid reservoir at certain time spans corresponding to the input energy density (energy per unit volume) requirement. Closed-loop operation allowed the lab-scale PEF setup to supply the high energy density levels that may be required for inactivating some microbial species [32, 37, 52]. Since the pump speed does not affect the energy density, high speeds (~1000 mL/min) were preferred to avoid excessive temperature build-up in the processing chamber by utilizing the liquid food itself as a cooling fluid by convection.

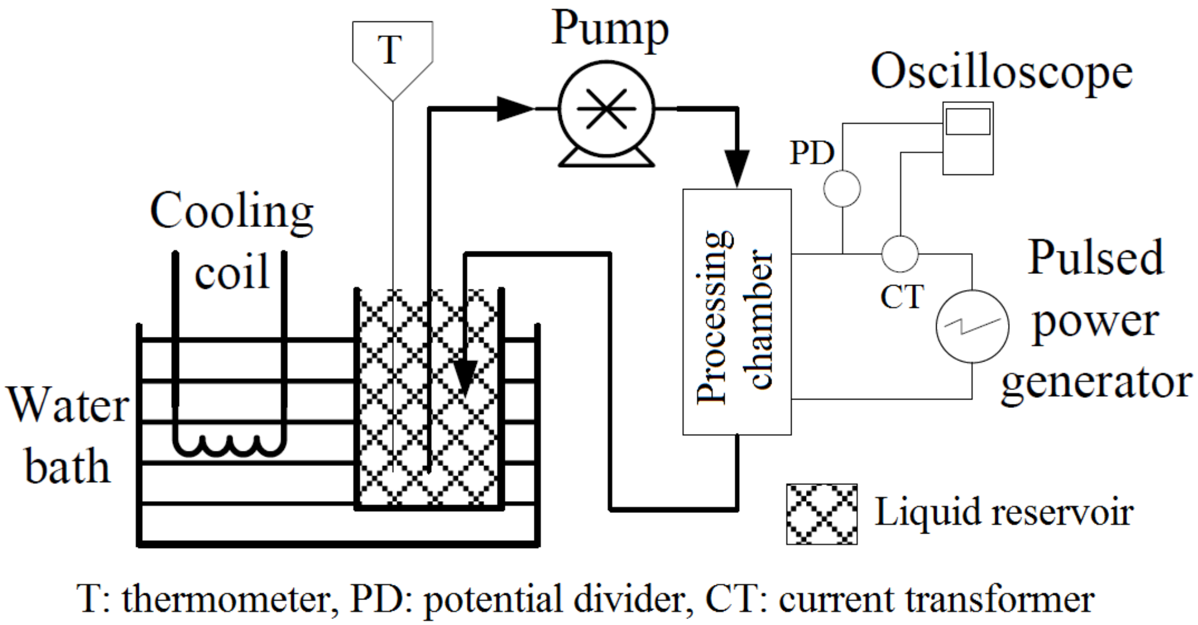


Figure 3-1: Experimental setup for PEF processing (closed-loop configuration)

For the main experiments, the PEF setup operated in an open-loop configuration as shown in Figure 3-2. The open-loop configuration is mandatory in real systems to ensure that all parts of the liquid is sufficiently processed and no microbial contamination occurs. With the lab-scale setup, the pump was operated at low speeds (~50 mL/min) in order to reach the desired energy density levels. To reach high energy density levels, the processed liquid food can go through the PEF system one more time so that it gets subjected to a second (or even a third) dose of the PEF processing. Cooling the liquid food between PEF doses is preferred to avoid excessive temperature build-up in the processing chamber by utilizing the liquid food itself as a cooling fluid. The scheme of multiple PEF doses is meant to simulate the usage of multiple processing chambers in series with cooling coils between them [8].

The aforementioned setup is capable of processing juices as well as milk. However, further developments were adopted in order to process carbonated beer as shown in Figure 3-3. Carbonated beer is a two-phase substance in which carbon dioxide bubbles exist within the beer liquid. This nature of carbonated beer increases the probability of partial discharges within these bubbles, which may lead to a complete arcing event. Operating the PEF setup under relatively high pressure (40–70 psi significantly) reduced the probability of arcing event occurrence since this would cause bubbles to shrink under high pressure and their breakdown strength to increase.

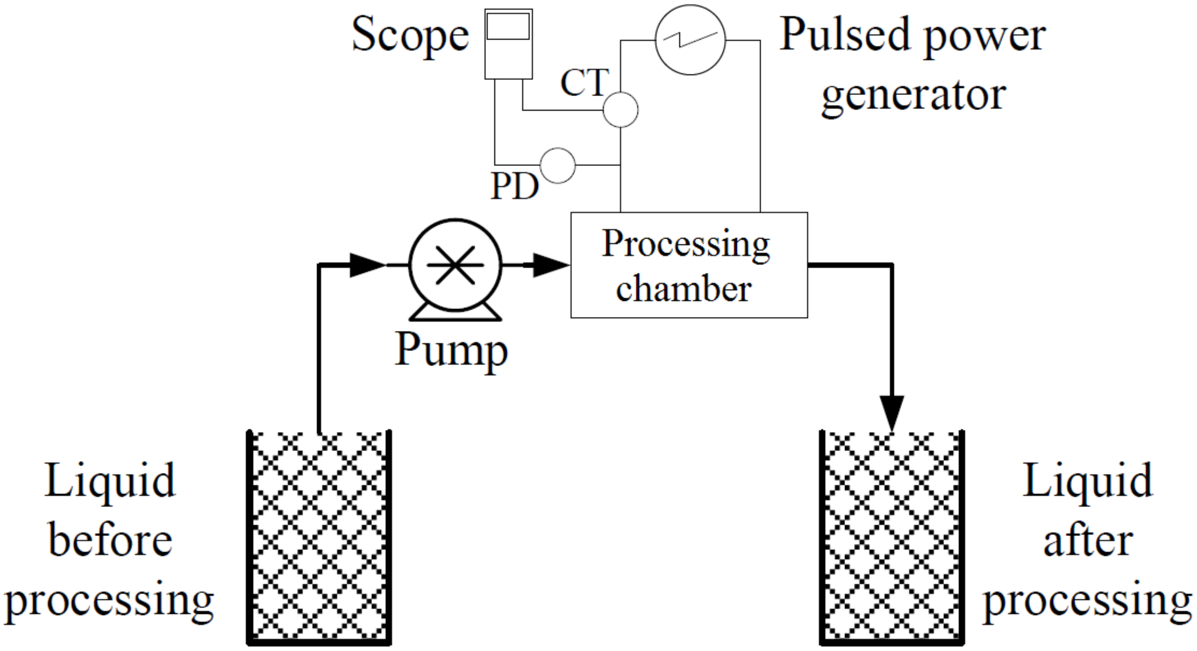


Figure 3-2: Experimental setup for PEF processing (open-loop configuration)

Operating under relatively high pressure is also helpful to avoid foaming of beer within the PEF processing chamber, hence, the carbonation level can be maintained in the processed beer as well. Carbonation charts/tables show that as the beer temperature goes up, a higher head-pressure is required to maintain a certain carbonation level. With the aid of a cooling jacket surrounding the processing chamber, a pressure of 40 psi was sufficient. However, when the cooler set-point was raised from -5°C to 35°C, the pressure had to be increased to 70 psi. For this reason, the PEF setup was designed to operate up to 130 psi. The input/output beer tanks were always immersed in ice. A thermocouple was installed in the outer electrode to measure the temperature of the processing zone, i.e., the processing temperature.

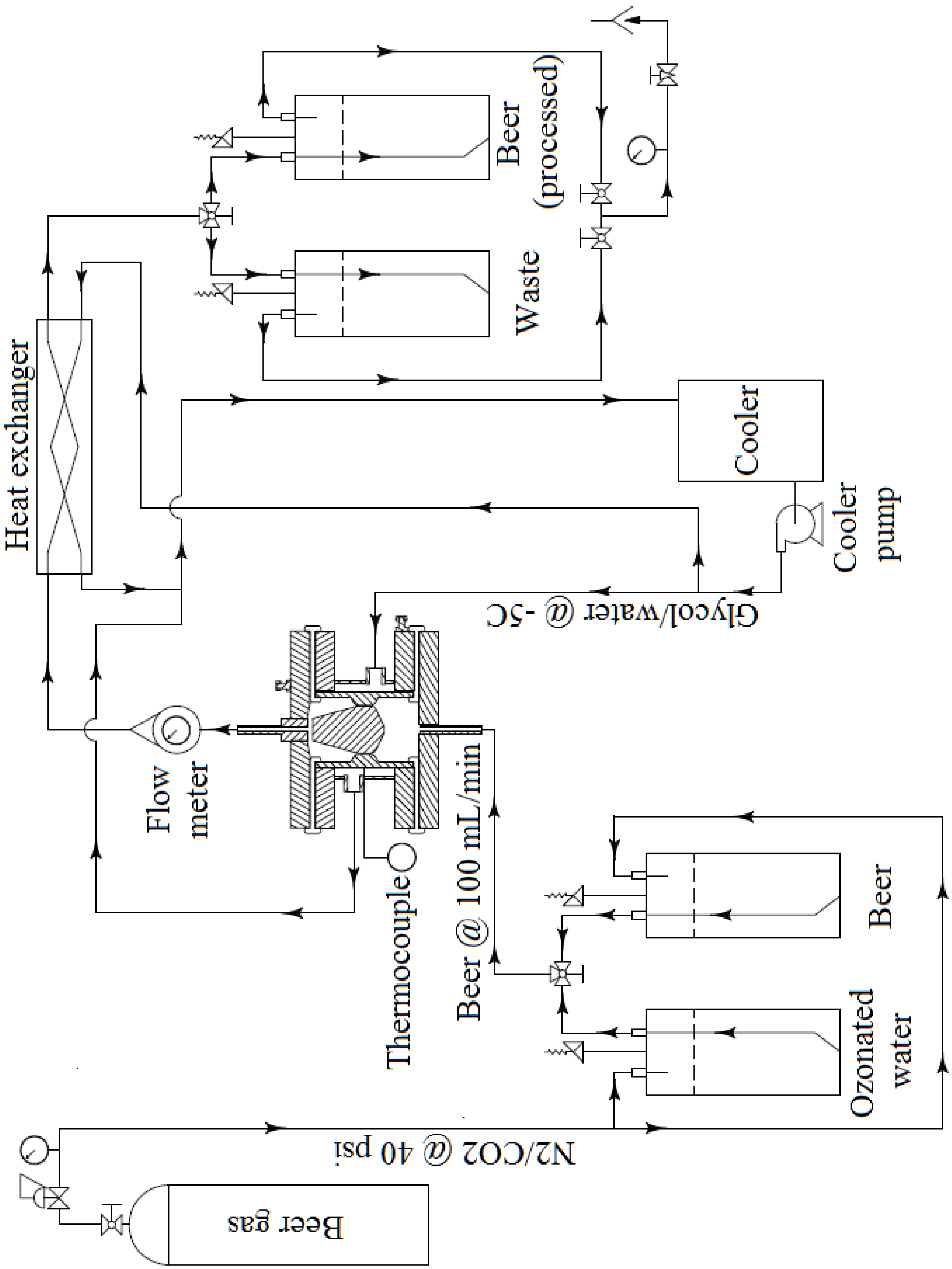


Figure 3-3: Experimental setup for PEF processing (open-loop configuration with high pressure)

Beer was allowed to flow through the PEF setup by maintaining a slight pressure difference between the input and output tanks; the pump was no longer needed and the head-pressure was kept almost the same throughout the system using the beer gas (mixture of nitrogen and carbon dioxide). As discussed earlier, the flow rate with the lab-scale setup was kept low (~100 mL/min in case of beer) in order to inject the typical energy density levels. Applying multiple doses was also possible.

Since PEF-processed beer samples were served in the sensory panels, they should meet the standards for human consumption. Great attention was piad towards the sanitation protocols during beer processing experiments. In the beginning of every set of experiments, the PEF processing chamber was autoclaved and new tubes were installed to eliminate any microbial cross-contamination. Between two successive experiments, the tubing system and the chamber were rinsed by flushing ozonated water to remove any remaining beer and odor.

All experiments for metal detection presented in this thesis were conducted with real food products (i.e., milk, juices, and beer) in order to provide more practical and industrially relevant results unlike those obtained with buffer solutions having the same conductivity and pH [32, 38]. Table 3-1 lists the measured electrical conductivity and pH of milk, orange juice, and beer used in the experiments.

Table 3-1: Electrical conductivity and pH of liquid food products

Liquid	Description	Electrical conductivity (mS/cm)	pH
Milk	2% skimmed – pasteurized	5.52	6.9
Orange juice	No pulp – pasteurized	4.54	4.1
Beer	5% ABV Ale – draught	0.994	4.3

3.1.1 Pulsed-power generator

Electrical pulses were generated through a simple capacitor discharge circuit which produces a repetitive RC exponential-decay voltage waveform across the processing chamber. Due to the high electrical conductivity of the liquid food products [53, 54], the processing chamber acts as a purely resistive load. In the circuit diagram of the pulsed-power generator shown in Figure 3-4, two identical parallel capacitors (0.3 µF each) are initially charged through the charging resistance and the processing chamber load by a 100 mA constant-current capacitor-charging supply. The supply stops injecting current when the capacitor voltage reaches a previously specified value. The charging duration depends however on the required voltage of capacitors and their capacitance. Once the thyratron switch receives a trigger pulse, the capacitors are rapidly discharged through the processing chamber generating the desired high voltage pulse.

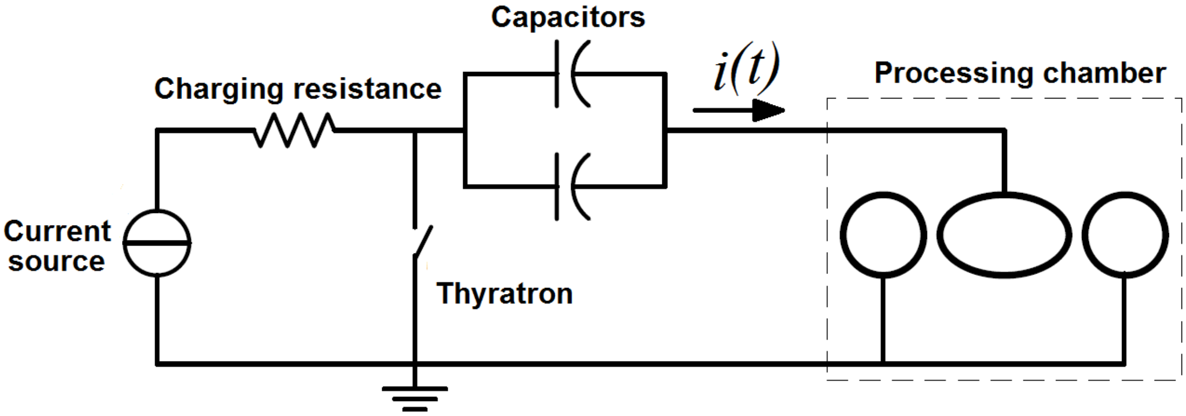


Figure 3-4: Circuit diagram of the RC pulsed-power generator (aka circuit (a))

The above circuit is capable of generating negative pulses with an adjustable peak voltage by controlling the charging voltage of the capacitors. However, the pulse shape and width are dependent on the ohmic resistance of the processing chamber load, and hence on the electrical conductivity of the liquid food under processing. The transient current would be expected to follow first-order behavior during the capacitor discharge. However, due to the stray inductance of the connections and wires, the current decay obeys second-order behavior [55]. By connecting only one capacitor, rather than the two above, it is possible to generate voltage pulses with relatively short pulse width. The pulse repetition rate is also adjustable by controlling the triggering signal of the thyratron switch in the range of 1–35 Hz.

This circuit topology is meant to reduce the electrophoretic and electrochemical effects within the liquid food during processing [33, 34]. This characteristic of the circuit originates from the bi-directional nature of the current waveform as shown in Figure 3-5. The charging current flows in one direction for a certain duration and then the pulse current flows in the opposite direction. As a result, the same electrode will act as an anode during the charging phase and as a cathode during the pulse phase, and vice versa for the other electrode. The charge transferred by the current has the same magnitude during both phases and equals the charge stored in the two capacitors.

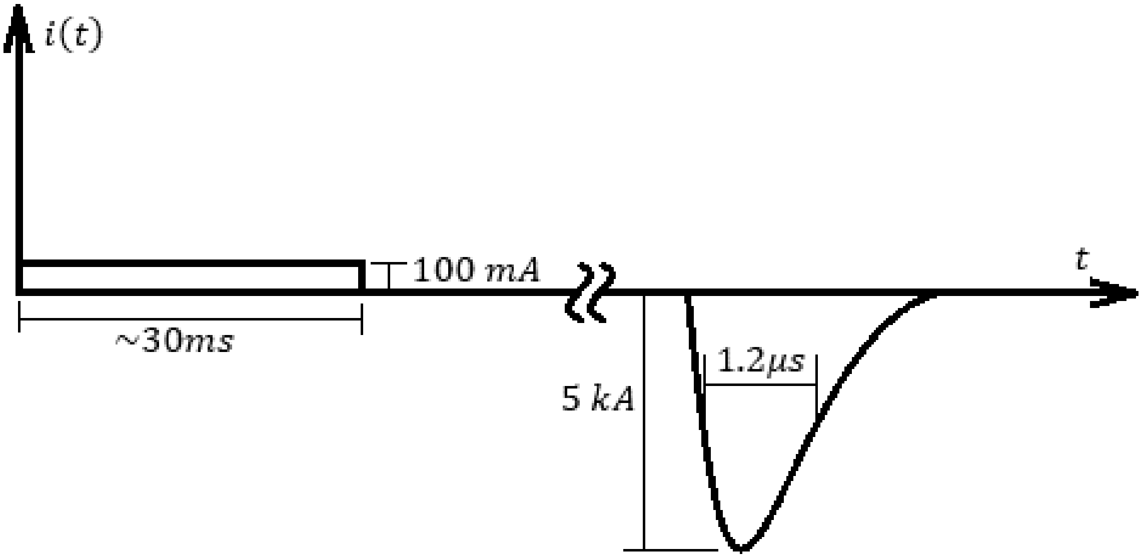


Figure 3-5: Typical current response (not to scale) during both the charging and pulse phases

As a modification to the generator circuit, Figure 3-6 shows the connection of a diode added in parallel to bypass the processing chamber in the charging phase so that the charging current flows through the diode instead. The diode will be reverse-biased in the pulse phase and should not affect the generated high-field pulses.

Voltage across as well as current through the processing chamber were continuously monitored by a Tektronix 3044B oscilloscope (400 MHz, 5 GS/sec) using a high voltage probe (potential divider) and a current transformer, respectively. The oscilloscope is capable of storing the waveforms on all channels with a record length of 10,000 points which were utilized to compute the energy and charge per pulse through simple discrete integration. The voltage waveforms were measured directly across the two terminals of the processing chamber to avoid the inclusion of the voltage drop associated with the inductance of the ground wire.

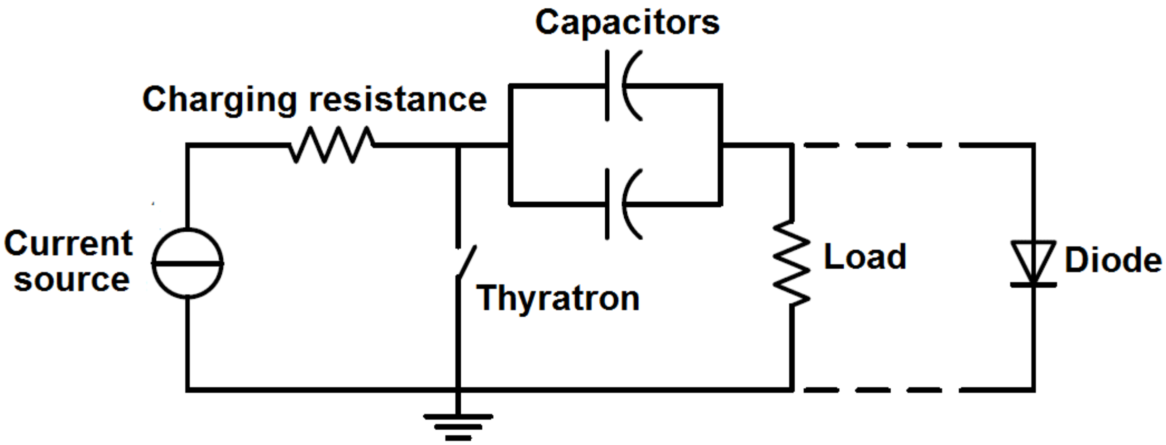


Figure 3-6: Connection of a diode across the processing chamber load (aka circuit (b))

3.1.2 Electric field processing chamber

The processing chamber [27] consists of two electrodes to generate the electric field and an insulating housing to manage the flow of the liquid. The housing comprises an inlet to receive the liquid to be processed and an outlet to collect the processed liquid. The two electrodes have opposing convex electrode surface sections defining a biconcave processing zone to process the liquid with the most intense electric field generated by the electrode assembly, as shown in Figure 3-7. The annular gap between the two electrodes is 1 mm (or 2 mm in a few experiments) in length. The electrode which surrounds the other one to form the processing zone is hereafter referred to as the outer electrode, whereas the other is termed the inner electrode.

In general, both electrodes were made of stainless steel (Type 316) alloy. Scanning electron microscopy was utilized to analyze the composition of the electrode material. The main elements and their percentage weights were iron (66%), chromium (17%), nickel (12%), molybdenum (3%), and manganese (2%). The concentration of dissolved iron only in the liquid was sometimes used to reflect the performance of the stainless steel electrodes.

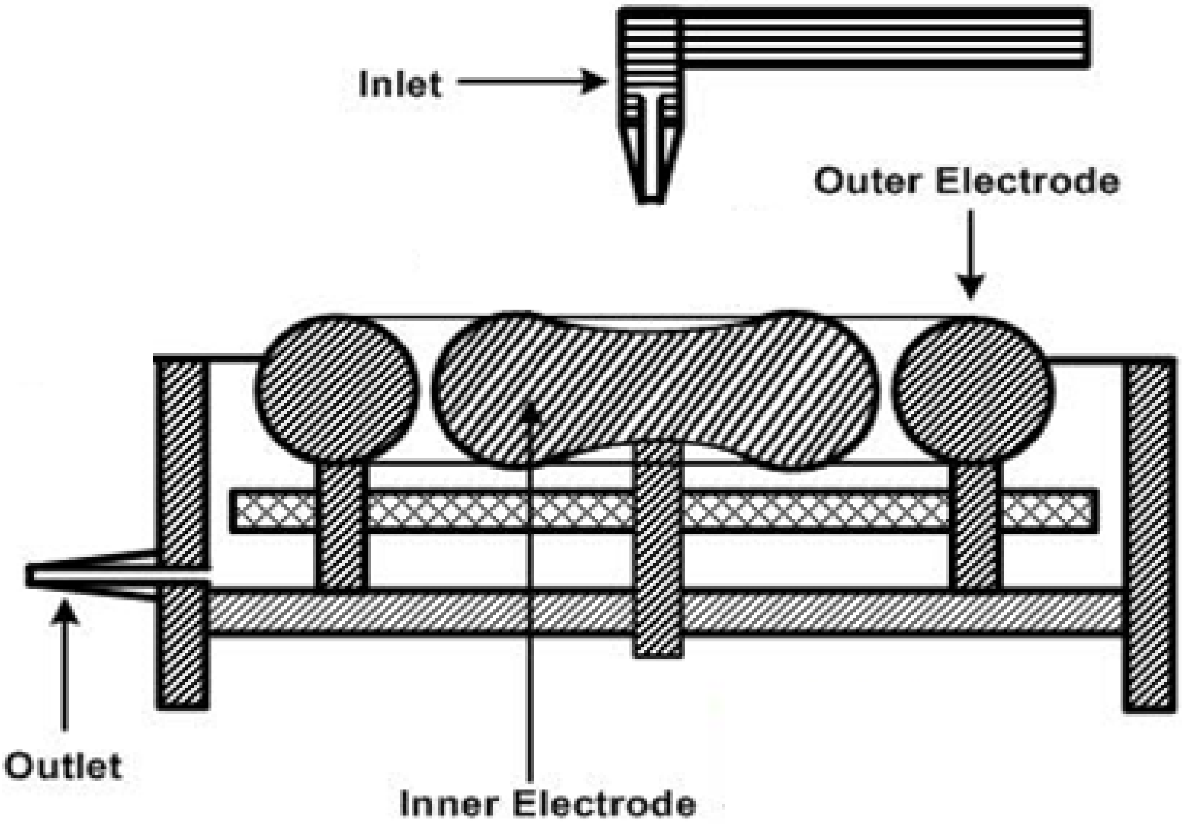


Figure 3-7: A side view of the PEF processing chamber [27]

In the experiments comparing circuit topologies or assessing the performance of various electrode materials, the performance of each material was evaluated by using an inner electrode made of the material under study and the outer electrode made of stainless steel. To ensure a fair comparison could be made, the dimensions of the inner electrode were maintained identical regardless of the material used. In this way, the performance of the material under study during the charging phase could be evaluated when the inner electrode was connected to the capacitors and during the pulse phase by reversing the terminals to connect the inner electrode to the ground.

A preliminary experiment was first performed to evaluate the performance of chromium, nickel, silver, and titanium electrodes. The inner electrode used during the preliminary experiment was a multi-material electrode and was prepared in the following way. First, an aluminum electrode was coated completely with a nickel layer (1 µm) by electroless plating. The peripheral area of this electrode was divided into four equal sections. One section remained as a nickel electrode and did not undergo any further processing. The e-beam evaporation technique was used to deposit a chromium layer (0.25 µm) onto the second section and then silver and titanium, respectively, on the remaining two sections. As a result, the inner electrode consisted of four metals connected together such that the four gaps formed to the outer electrode were connected in parallel, thereby allowing the comparison of the four materials under the same operating conditions. Since the thickness of the coatings was three orders of magnitude smaller than the distance between the electrodes, it was assumed that the electric field remained uniform and the same over the four gaps. The duration of the preliminary experiment was kept short enough to prevent galvanic interactions between the different materials from becoming significant when immersed in the liquid [51]. Figure 3-8 shows a schematic diagram (not to scale) of the inner electrode described above.

Other than the aforementioned multi-material electrode, a solid-aluminum inner electrode was used to compare the different circuit topologies, while a solid-titanium inner electrode was compared to the state-of-the-art stainless steel at various pH levels.

Between experimental runs (except those with the pressurized chamber), the chamber was opened and both the inner and outer electrodes were cleaned as follows: washed with soap and water, immersed in a dilute NaOH solution for 1 hour at 95ºC [56], rinsed with de-ionized water, and finally wiped with two pieces of cotton soaked in acetone and ethanol, respectively. The cleaning procedure described above removed any deposits (whey protein in the case of processing milk) from the electrodes. This cleaning step was important since the presence of these deposits would significantly increase the probability that arcing events will occur during processing.

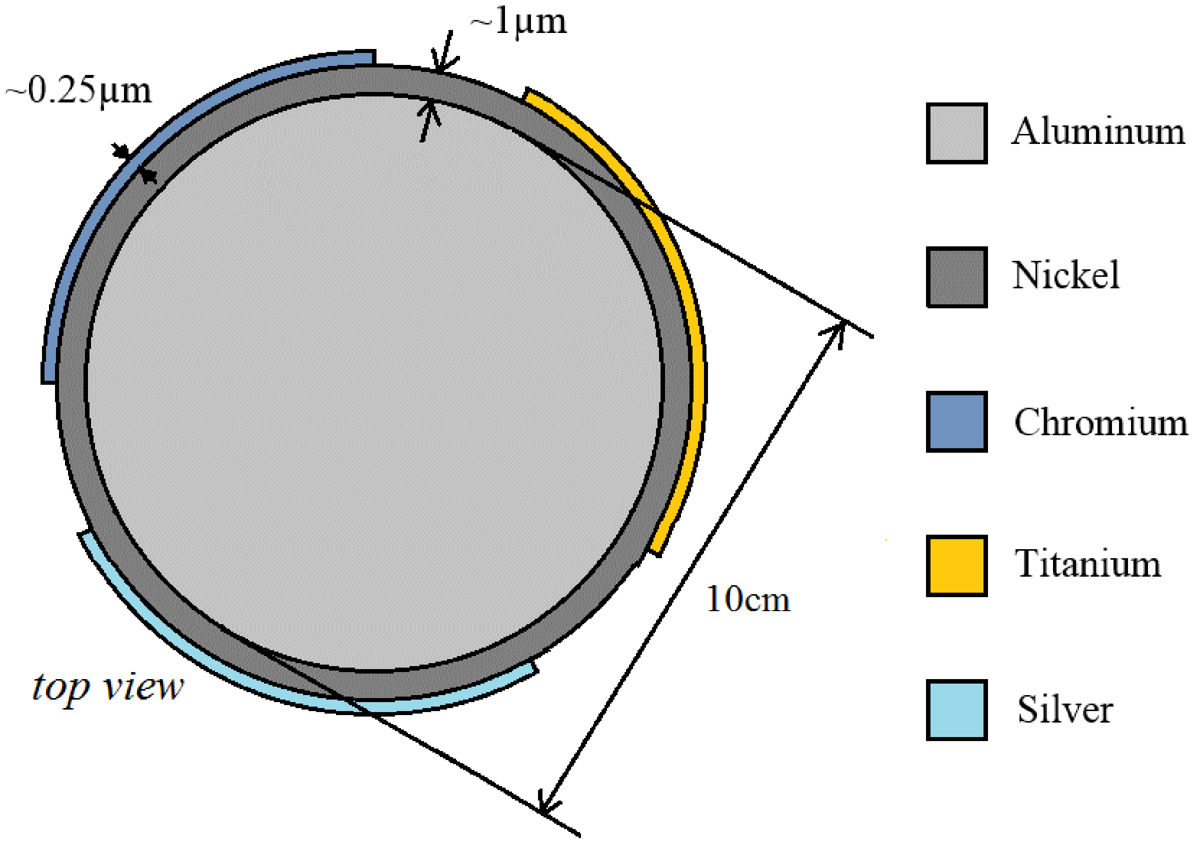


Figure 3-8: A schematic diagram (not to scale) showing the top view of the inner multi-material electrode used in the preliminary experiment

3.2 Metal concentration

To assess the concentration of metal ions that are released during the PEF process, 25-mL samples were collected from the liquid food before and after each experiment. Since the analytical equipment (described below) was capable of measuring the metal concentration accurately only in simple inorganic solutions, liquid food samples first underwent a preparation stage [8, 32] in order to digest all the organic components.

3.2.1 Sample preparation

Food samples were digested through the following wet digestion (1:1 by volume) procedure [57, 58]:

- 10 mL out of each sample were accurately pipetted into a 50 mL polypropylene digestion vessel
- 10 mL of concentrated nitric acid were added
- The vessel was covered with a reflux cap
- The vessel was heated with a hot block at 110ºC for 4 hours
- The vessel was removed from the hot block
- After cooling to room temperature, ultra-pure water (18.2 MΩ•cm) was added to bring up the volume of liquid inside the vessel to 20 mL

The above procedure left the samples completely digested in an acidic medium which perfectly suited the operation of the analytical equipment. It should be noted the contents were diluted by a factor of 2 in this procedure. Samples were collected with the intention of dividing each into two independent digestion vessels so as to reduce any experimental error introduced through the digestion procedure itself.

Samples of ultrapure water were intermittently passed through the same digestion process to serve as blanks. If any metal was detected in these blank samples, the amount would be subtracted from the measurement of all samples (other than the blanks) in that batch.

3.2.2 Analytical equipment

Inductively coupled plasma – atomic emission spectrometry (ICP-AES) with a radial configuration was utilized to determine the concentration of metal ions in the liquid food samples before and after the PEF processing. Prepared samples were fed automatically to the ICP with a radial exposure time of 10 sec repeated 3 times.

Certified standards containing the metals to be analyzed were used to calibrate the ICP spectrometer. As such, each session a new calibration curve was obtained. A certified standard yttrium (Y) solution was fed-in continuously with the samples to serve as an internal standard to correct for any matrix effects [59], especially those introduced at high calcium concentration, as in the case of milk. In addition, any drift in the measurements was corrected by analyzing a quality-control certified standard after every 5 samples. The detection limits for aluminum, chromium, iron, nickel, silver, and titanium are 17, 5, 3, 13, 10, and 2 ppb, respectively.

3.3 Microbial inactivation

The effectiveness (power) of PEF processing as a non-thermal preservation technique was evaluated through a microbial inactivation study. During this study, microbial cells with high concentration were suspended in the beer. Then, the inoculated beer was subjected to PEF processing. The corresponding survival ratio was determined by plating/counting the viable microbial cells in the processed beer with respect to the inoculated cells. A high microbial inactivation should reflect a long shelf-life of the processed products. The microbiological analysis was carried out in collaboration with the Canadian Food and Wine Institute, Niagara College, following the procedure presented in a previous study [30].

3.3.1 Inoculated samples

The microorganisms *Saccharomyces cerevisiae* (ATCC 36026), *Lactobacillus plantarum* (ATCC 14917), *Bacillus subtilis* (ATCC 6051), and *Salmonella enterica* (ATCC 51741) were chosen because of their importance to the brewing industry [30]. Stationary phase pre-inoculation cell concentrations up to 10^8 colony-forming units (CFU) per mL of *S. cerevisiae*, *L. plantarum*, *B. subtilis*, and S. *enterica* were obtained after growth in potato dextrose broth, lactobacilli MRS broth, tryptic soy broth, and brain heart infusion broth, respectively, at 37ºC for 48 hours.

The four cultures were centrifuged at 600 rpm for 5 min and the supernatant was removed. Culture pellets were then added together to a 20 L beer sample (hence, a dilution factor was introduced). A resting period of 30 min was arranged between inoculation and the subsequent processing of the beer to enable the adaptation of the microorganisms to the new environment, and minimize another stress factor in addition to the PEF processing.

3.3.2 Microbial counting

Serial dilutions of unprocessed and PEF-processed beer were prepared in sterile deionized water. Then, 2 μL aliquots were drop-plated from each dilution onto potato dextrose agar for *S. cerevisiae*, lactobacilli MRS agar for *L. plantarum*, tryptic soy agar for *B. subtilis*, and brain heart infusion agar for S. *enterica*. Agar plates were incubated at 37°C for 16 hours before counting the number of grown colonies. Three replicates from each dilution were plated on each type of agar; the values reported are the averages of these replicates.

3.4 Sensory panels

Sensory panels were held to analyze the effect of PEF processing on carbonated beer immediately after processing and over a shelf-life of 12 weeks. Fresh beer (freshly brewed at the brewery) was taken as reference for each panel. The following two types of sensory panels were independently considered in this study.

3.4.1 Quantitative descriptive analysis

On a 15-cm line scale, a panel of 10–12 normal beer drinkers were asked to evaluate quantitatively the organoleptic/quality attributes of the coded samples presented to them. The far left end of the scale represented a weak attribute and the far right end of the scale represented a strong attribute. Participants were also encouraged to provide additional comments regarding any of the presented samples.

Flavor, aroma, color, foam condition, sweetness, and sourness are the quality attributes commonly considered in beer evaluation. In addition, two more attributes were considered here: oxidation to reflect beer aging, and any metallic feeling/taste. A reference sample for the oxidation attribute was prepared by leaving a bottle of beer uncapped overnight. Ferrous sulfate (0.48 mg/mL) was added to fresh beer to create the reference for the metallic feeling attribute.

3.4.2 Difference testing

Following the classical triangle test, participants were served with three coded samples. Unknowns to the participants, two samples were identical and the third was different. Participants were asked to observe, smell and taste the three samples, and identify, according to their opinion, the odd sample. If the panelist makes a random guess because the three samples are difficult to differentiate, the odd sample should be correctly identified ~33% of the time. On the other hand, if the samples are easy to differentiate, the population will tend to select the odd sample correctly ~100% of the time. Difference testing panels consisted of 20–30 untrained participants for each pair of comparison.

To determine the proportion of distinguisher, the guessing model assumes that participants are either distinguishers (P_D) or non-distinguishers ($1 - P_D$). Distinguishers always find the correct answer. Since participants are forced to answer, non-distinguishers would correctly guess the answer with a probability P_G. Someone who does not taste a difference will still have 1 chance out of 3 to identify the odd sample correctly, i.e. $P_G = 33\%$. So with this model, the percentage of correct responses P_C can be written as:

$$P_C = (P_D) * (1) + (1 - P_D) * (P_G) = \frac{2}{3} P_D + \frac{1}{3}$$

$$\rightarrow \ P_D = \frac{(3P_C - 1)}{2}$$

3.5 Statistical analysis

For the statistical analysis and plotting of data, SPSS Statistics v19 software was utilized. The significance of any parameter under study was determined through the Analysis of Variance (ANOVA) tool with a Fisher significance test at P≤0.05. Plotted data include error bars representing two-sided 90% confidence intervals. Except for beer experiments, full factorial design with at least 3 replicates was considered.

It is worth noting that a significant percentage of error in metal concentrations was introduced by the analytical equipment (ICP-AES) itself. The reason behind this error is that the measured concentrations lie in the low range of ICP curves and are comparable to its detection limits. Therefore, each prepared sample was measured 3 times, and the value reported was the average of the two closest measurements.

Chapter 4

Results – Factors Affecting the Electrode Material Performance

Each of the four sections in this chapter focuses on one of the factors that may influence the release of metal ions from the electrodes during the PEF processing. Empirical models/results will first help us understand the nature and mechanisms behind this process; then, optimum operating conditions can be recommended. Each section starts with a brief description of the operating conditions under which experiments are carried out.

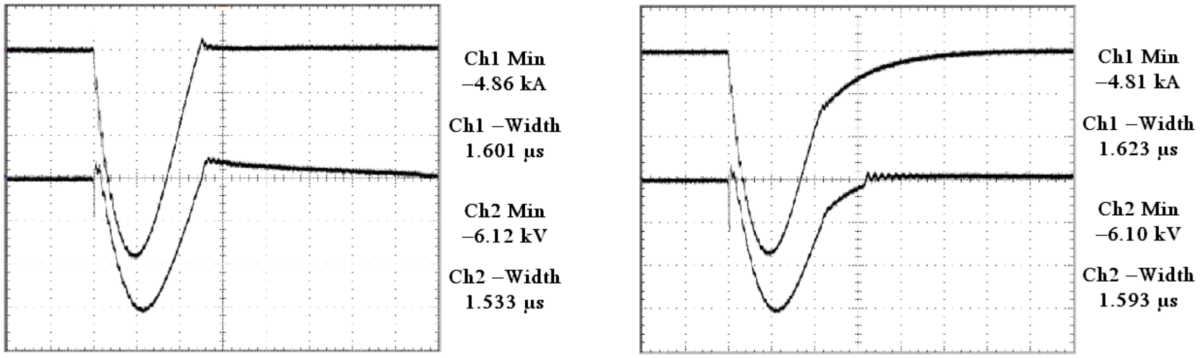
4.1 Circuit topology

The two circuit topologies which are considered for comparison under this section are presented in Figure 3-4 and Figure 3-6 and denoted here as circuits (a) and (b), respectively. Circuit (b) shows the addition of a parallel diode to the original circuit (a) in order to bypass the PEF processing chamber during the capacitor-charging phase so that the charging current flows through the diode instead. The diode is reverse-biased during the capacitor-discharge phase and should not affect the generated pulses.

Milk was PEF-processed in this experiment using the closed-loop configuration with a reservoir volume of 1,000 mL. The outer electrode was always grounded, while electric pulses with 30 kV/cm peak field and 10 Hz repetition rate were applied to the inner electrode. Both electrodes were made of stainless steel, but the inner electrode was sometimes replaced with an aluminum electrode in order to determine the amount of iron released solely from the outer electrode.

Figure 4-1 shows the current and voltage waveforms generated by circuits (a) and (b). It was expected that both circuits would yield responses associated with simple RC transient circuits. However, the relatively high rise time observed can be attributed to the stray inductance of wires. From the dimensions of wires, an inductance of ~1μH was estimated to be in series with the load resistance.

Adding a diode did not affect the pulse-front and peak; the pulse-tail was affected only in the second half with a slower decay due to a free-wheeling effect. The diode takes the charging current and clips any positive voltage that would otherwise appear across the load.



(a) **(b)**

Figure 4-1: Typical voltage (lower) and current (upper) waveforms generated by circuits (a) and (b). Horizontal scale: 1 µs/div; vertical scale: 2 kV/div and 1 kA/div for voltage and current waveforms, respectively

The first part of the experimental work in this section is aimed at comparing the two circuit topologies in terms of the release of stainless steel electrode material. Since iron is the main element in stainless steel, the concentration of iron in the processed milk as a function of the input energy per unit volume (energy density) is shown in Figure 4-2. The iron content shows higher levels when circuit (b) is used as compared to circuit (a).

To understand more about the nature of the electrochemical reactions, the inner stainless steel electrode was replaced in the second part of the experimental work by an aluminum electrode, while the outer stainless steel electrode was retained. After applying the same pulses shown in Figure 4-1, the iron content plotted (solid line) in Figure 4-3 and Figure 4-4 corresponds solely to the release of electrode material from the outer electrode. Both figures also show the iron content evaluated in the first part of this section (dotted line), which corresponds to the release from both inner and outer electrodes. The difference between the two plots will give the contribution of the inner electrode to iron release. Knowing that metallic ions are released only from the electrode having the higher potential (anode), the release of electrode material can be determined to occur during the capacitor-charging and/or –discharging phase.

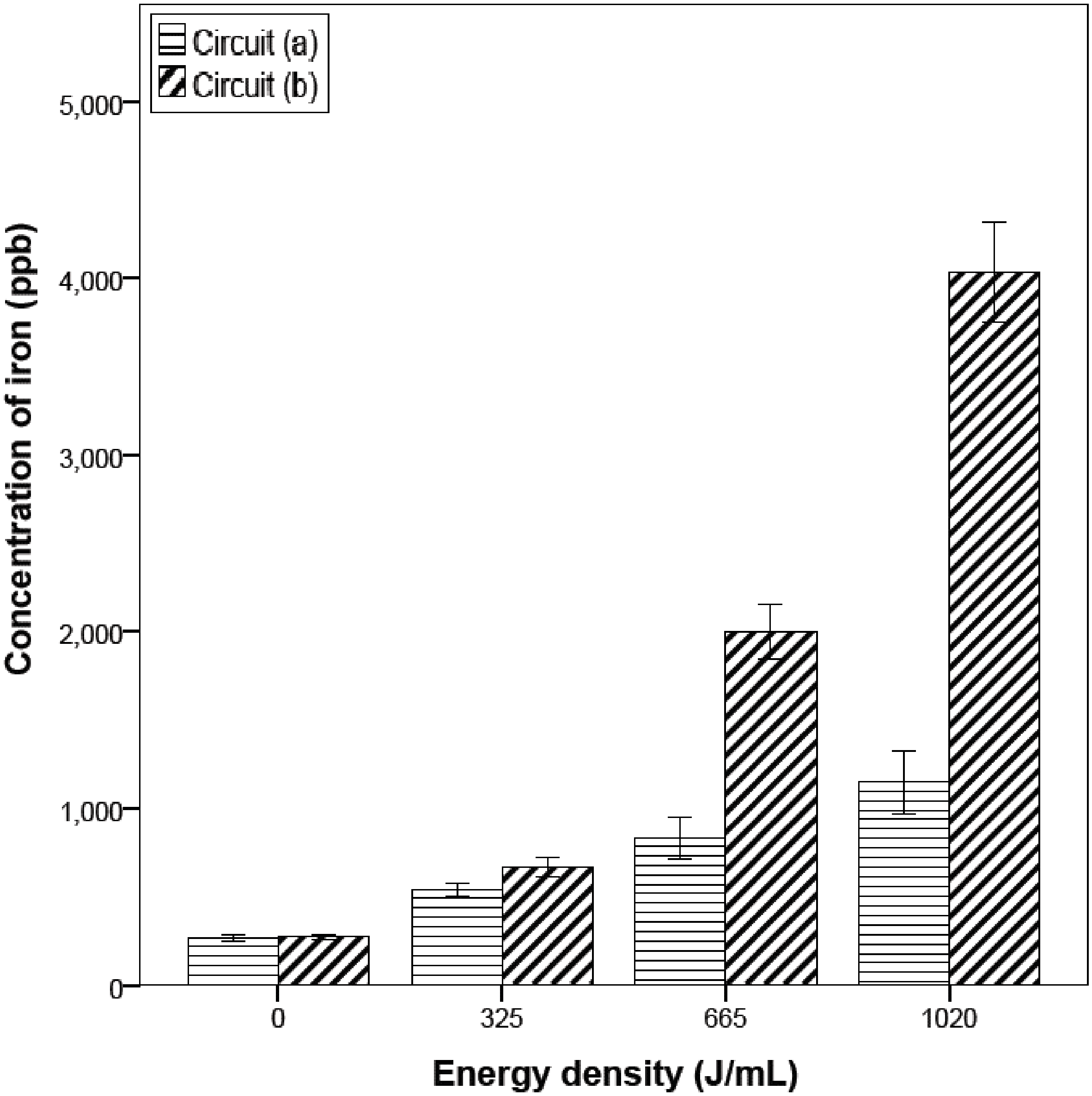


Figure 4-2: Mean concentration of iron when utilizing circuits (a) and (b)

Figure 4-3 shows that for circuit (a), the contribution of the inner electrode to iron release is higher than that of the outer electrode. In other words, the ions are released from the inner electrode during the capacitor-charging phase at a higher rate than from the outer electrode during the capacitor-discharging phase. This observation confirms the advantage of this circuit in reducing the total ions released, but on the other hand, it experiences an additional release during the capacitor-charging phase. The above conclusion is based on the assumption that the contribution of the outer stainless steel electrode to iron release is not affected by the material of which the inner electrode is made.

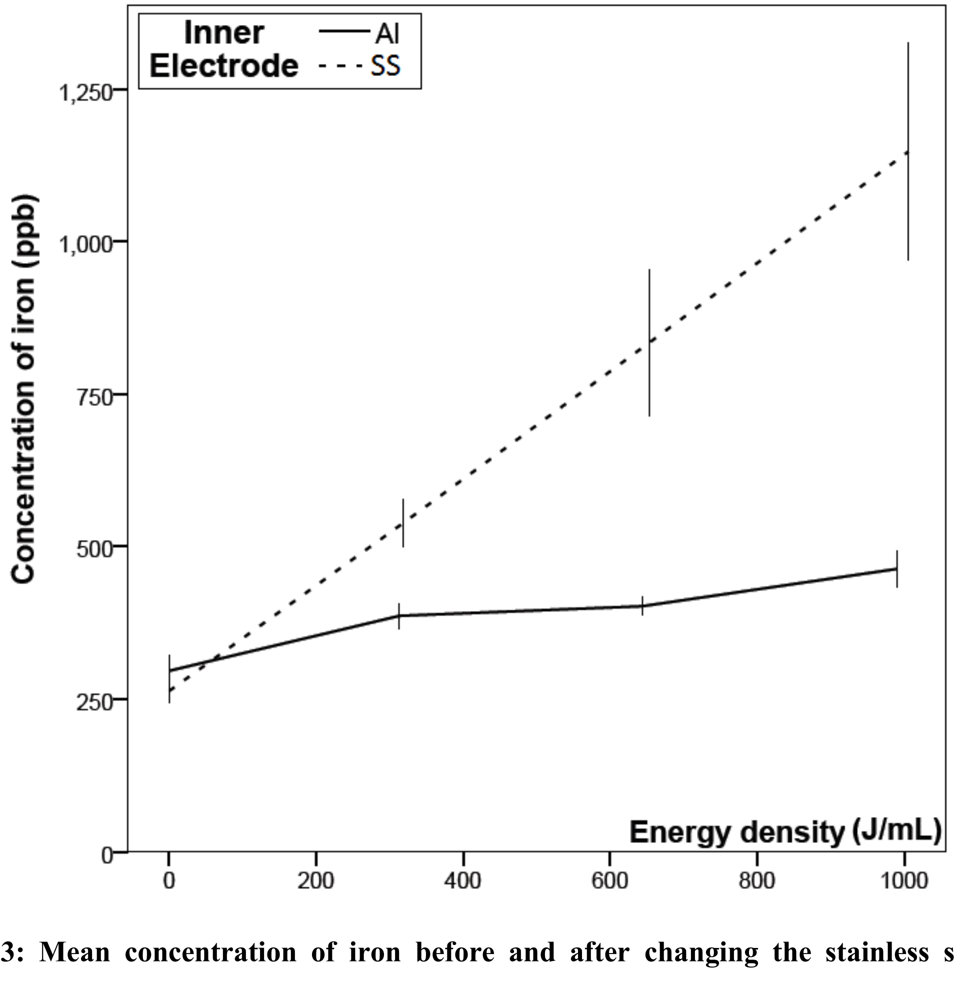


Figure 4-3: Mean concentration of iron before and after changing the stainless steel inner electrode with aluminum electrode when utilizing circuit (a)

For circuit (b), the capacitor-charging current was not flowing through the load, and so only the outer electrode should release material during the capacitor-discharge phase. Figure 4-4 verifies that the contribution of the outer electrode is higher than that of the inner electrode. The difference between the two curves in Figure 4-4 at high energy levels can be attributed to the iron released from the inner electrode due to the physical contact with circulating milk.

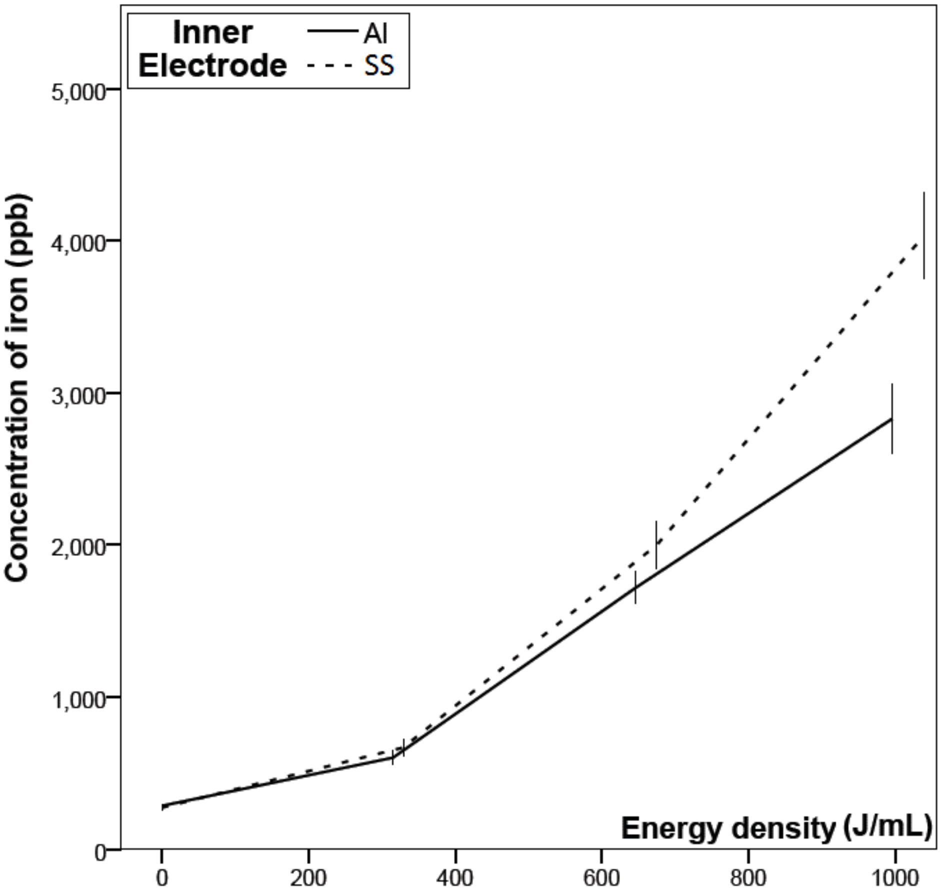


Figure 4-4: Mean concentration of iron before and after changing the stainless steel inner electrode with aluminum electrode when utilizing circuit (b)

In conclusion, the addition of the parallel diode was expected to reduce the concentration of the released ions by ~50%, but instead the concentration increased significantly; therefore, the topology of circuit (a) will be utilized in the remaining sections.

4.2 Electric pulse parameters

This section investigates the effect of varying two parameters (peak voltage and pulse width) of the electric pulses on metal release. Experiments were carried out in the closed-loop configuration with a reservoir volume of 1,000 mL. The pump speed was set to a flow rate of 500 mL/min; the temperature of the reservoir was continuously monitored by a thermometer and controlled within the range of 20–25°C by the cooling water path. This set of experiments was carried out on milk with both electrodes made of stainless steel, with the outer electrode grounded.

Upon the application of high-field pulses, the voltage across and the current through the processing chamber were recorded as in Figure 4-5. Cases (a) and (b) involve relatively shorter pulse widths since only one capacitor has been utilized; in cases (c) and (d), two capacitors are connected in parallel. The charging voltage has been set for a peak voltage to produce a field of 30 kV/cm in (a) and (c) and 40 kV/cm in (b) and (d). The pulse repetition rate was kept at 21 Hz. These four cases differ in their input energy per pulse; hence, the number of applied pulses in each case was adjusted so that the total energy per unit volume (energy density) is the same. At this point, it was assumed that within these four cases, the energy density mainly governs the resulting microbial inactivation of the PEF processing and should be fixed when comparing the amount of metal released in the different cases.

This section considers the total released metals during both the charging phase and the pulse phase. In the above four cases, the charging current has a fixed value of 100 mA which was measured by differentiating the voltage waveform across the capacitor(s). However, the charging duration depends on the voltage of the capacitor(s) and the number of capacitors. In other words, the electric pulse parameters will affect the electrochemical reactions occurring during the charging phase as well.

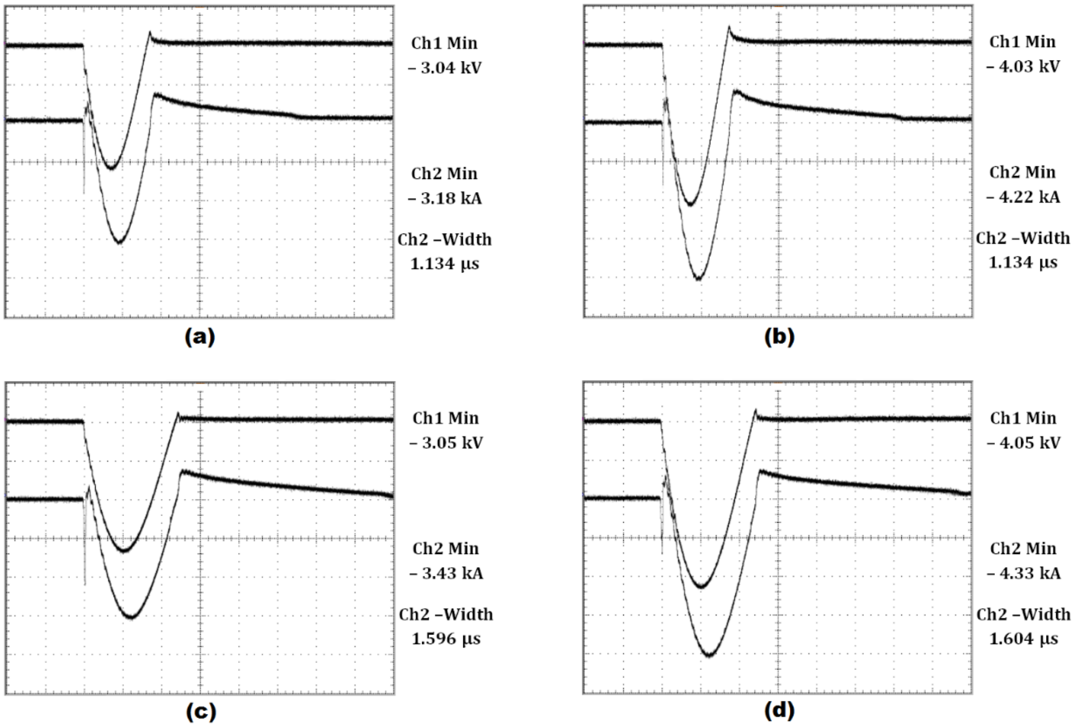


Figure 4-5: Typical voltage (lower) and current (upper) waveforms when utilizing one (a,b) or two (c,d) capacitors. Horizontal scale: 1 µs/div; vertical scale: 1 kV/div and 1 kA/div for voltage and current waveforms, respectively

The concentration of iron in the processed milk is plotted as a function of the input energy density from the PEF system in Figure 4-6. Milk has an initial iron content of slightly below 300 ppb. This iron content rises to 400-450 ppb at about 300 J/mL input energy and reaches 550 ppb at about 900 J/mL input energy. Although energy in the range of 300-600 J/mL may be sufficient for microbial inactivation, a higher range was included in this study for the sake of comparison between the cases. After the first increase from the initial iron content, all cases show a linear relationship between the increase in the iron content and the input energy, or the number of pulses. A linear relationship is expected based on Faraday’s law as long as identical pulses are applied.

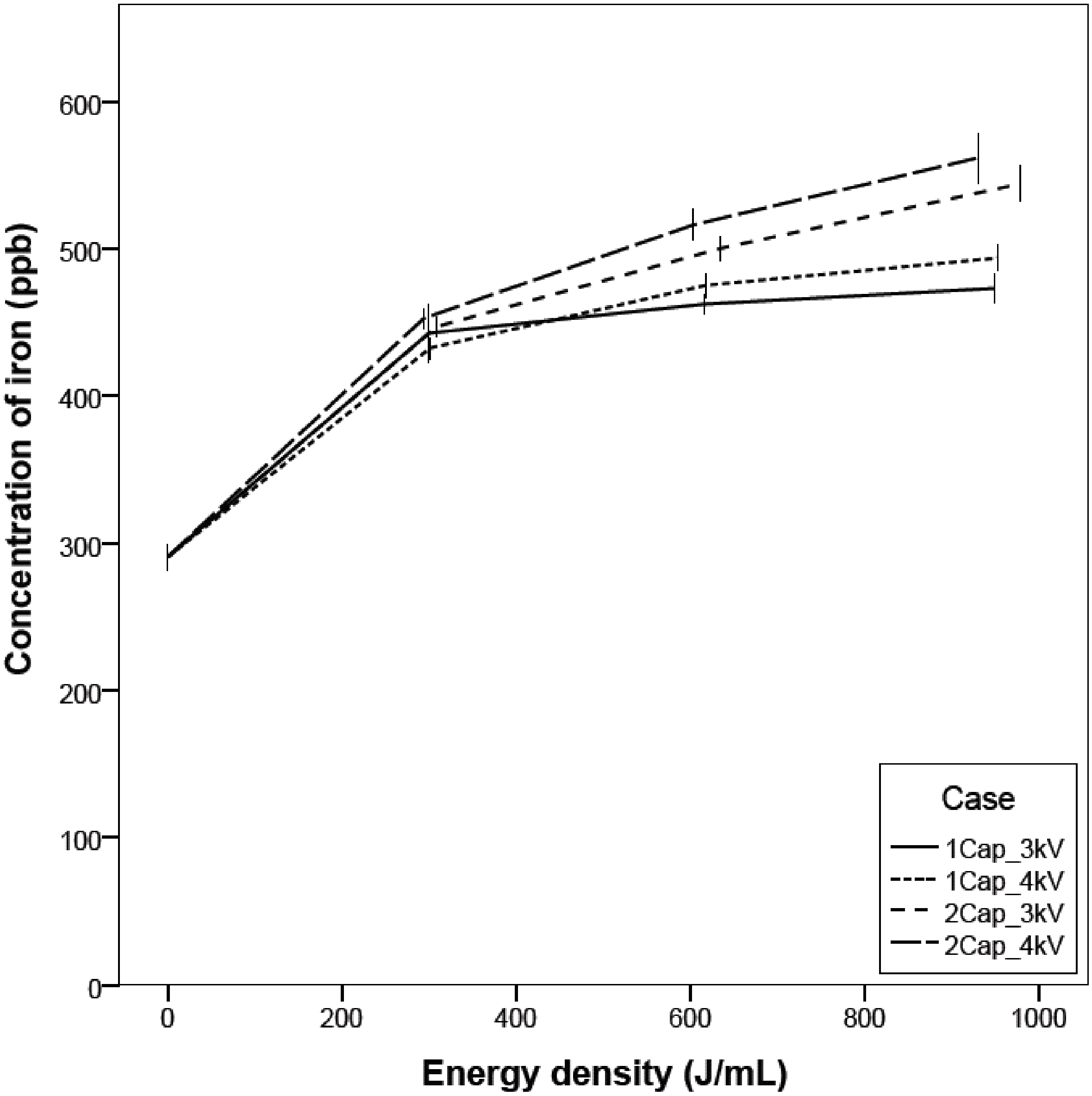


Figure 4-6: Mean concentration of iron versus the PEF energy density

It can be seen from Figure 4-6 that shorter pulses (utilizing one capacitor) release less iron from the electrodes when the peak voltage is kept the same. When comparing the two voltage levels with fixed capacitance, one can observe that increasing the voltage level slightly increases the iron content. The ANOVA test showed significance only for the effect of the number of capacitors. To conclude, in order to minimize iron release, the optimum pulse should feature relatively short pulse width with a peak voltage satisfying the minimum field required for microbial inactivation.

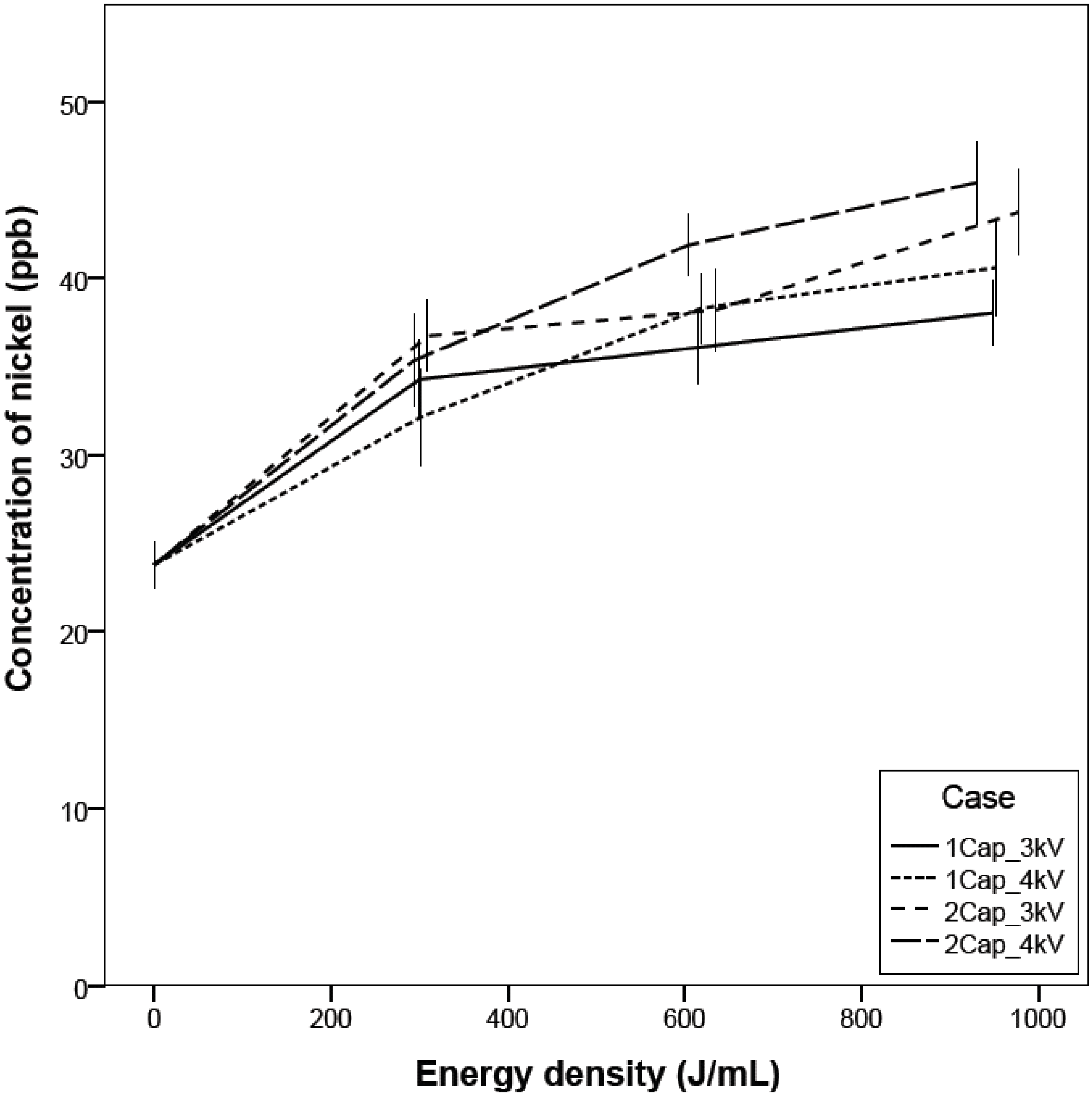


Figure 4-7: Mean concentration of nickel versus the PEF energy density

The same conclusions could be reached on the basis of the nickel content, although much less is released, as shown in Figure 4-7. Although chromium is the second main element in the electrode material, Figure 4-8 shows that energy density has no statistically significant effect on its content. This observation with chromium can be attributed to its higher resistance to electrochemical reactions with respect to iron.

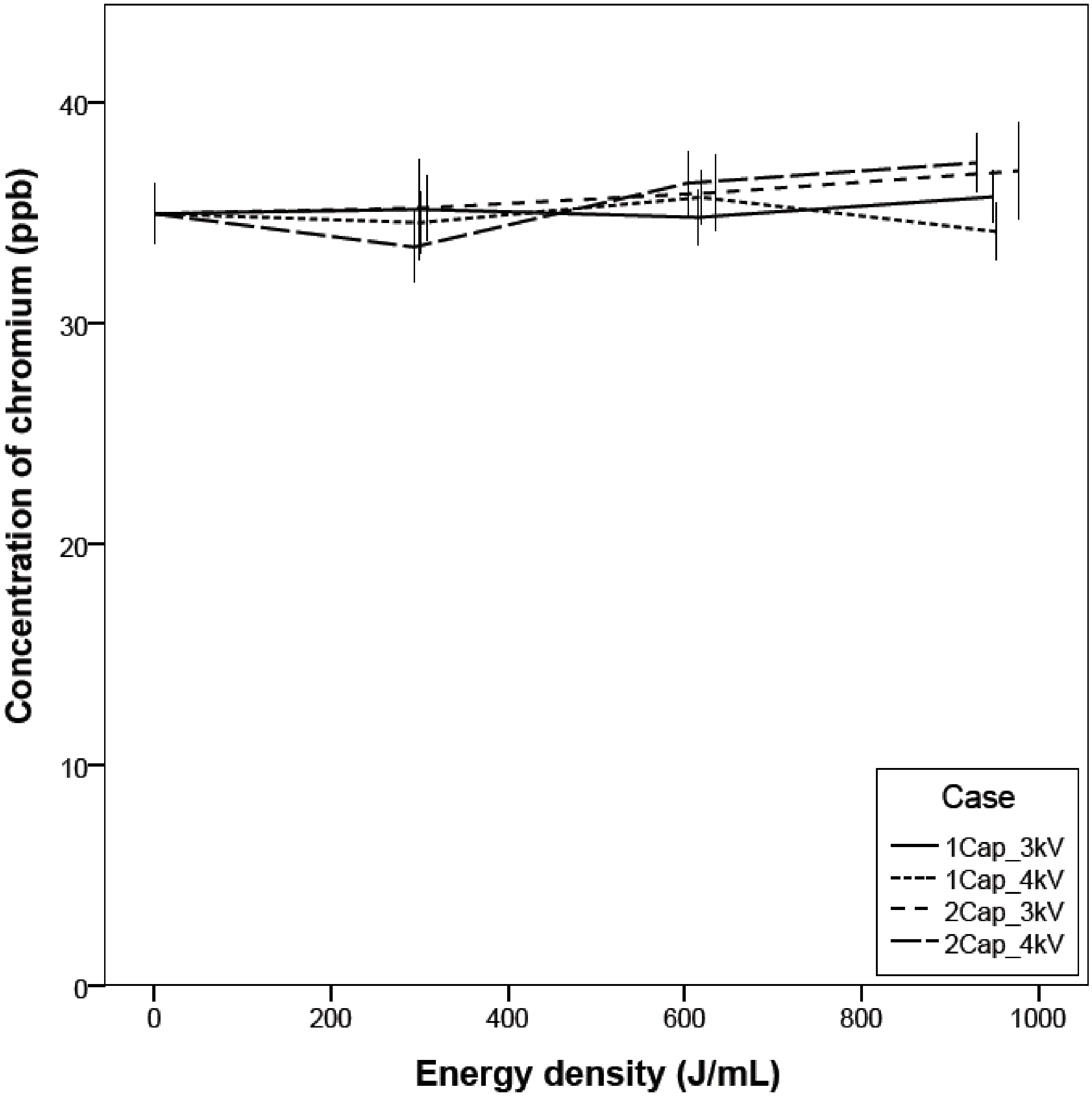


Figure 4-8: Mean concentration of chromium versus the PEF energy density

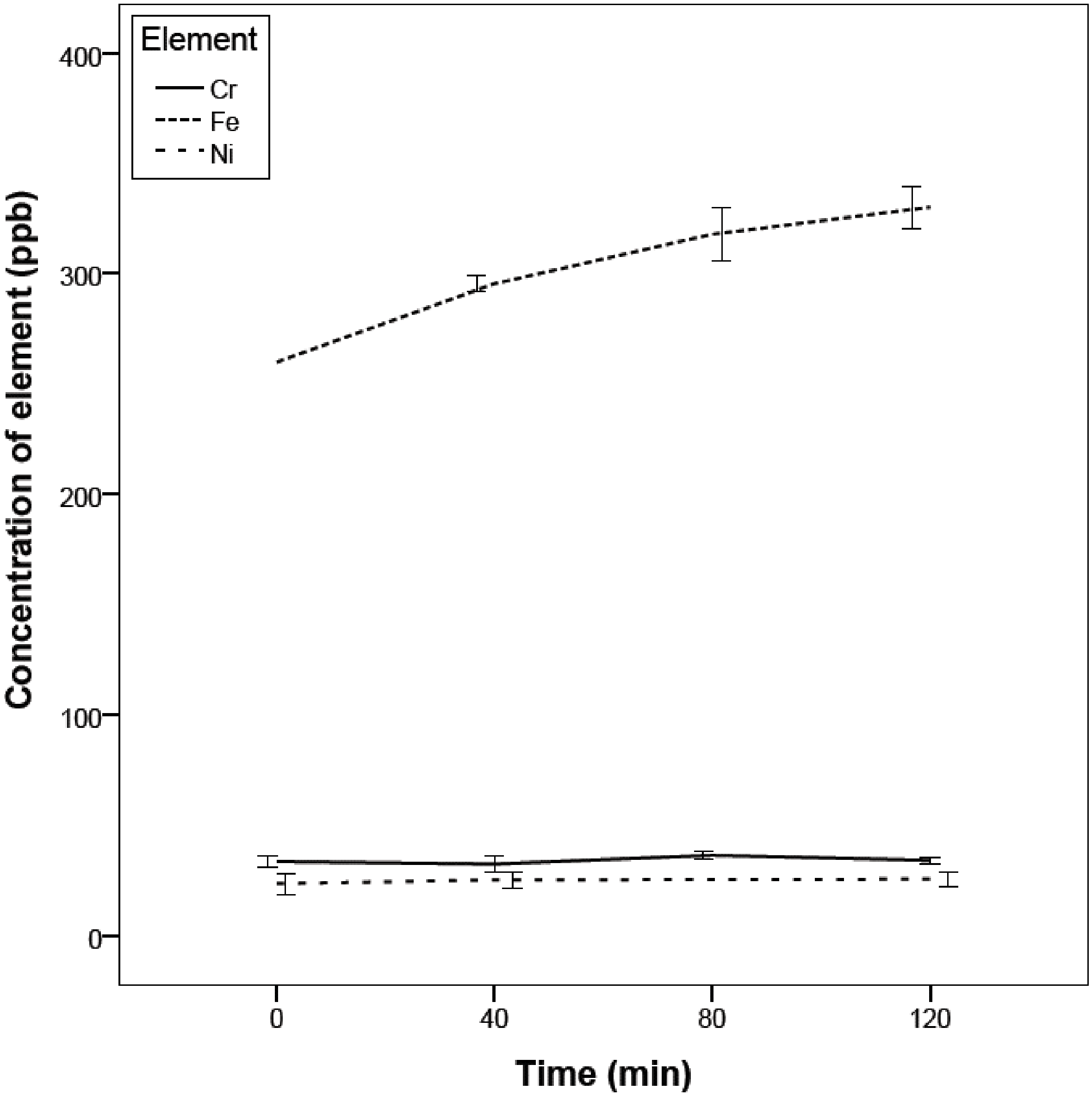


Figure 4-9: Mean concentration iron, chromium, and nickel during circulation in the absence of electric pulses

In a separate experiment, the effect of just the physical interaction between the milk and the electrode material and all galvanic-type reactions was tested by circulating the milk through the PEF system without applying any electric pulses. In this experiment, the residence time of milk between the two electrodes exceeded 1.5 times the maximum residence time for any experimental run in the previous experiment. Only iron in Figure 4-9 shows a significant increase in its content. Nickel and chromium content remains unchanged. The observed increase in iron content is much less than that observed in the main experiment when electric pulses were applied. This difference confirms that the release of metallic particles during the PEF processing is due primarily to electrically-driven electrochemical reactions at the electrodes and not due to just the physical contact between the milk and the electrodes and/or galvanic-type reactions at the temperature range considered.

4.3 Electrode materials

The objective of this section is to study the release of metallic ions using electrode materials that may have higher resistance to the PEF processing. Preliminarily, the electrodes coated with chromium, nickel, silver, and titanium were compared to each other under the same PEF conditions. Then, a solid-titanium electrode was tested against the conventional stainless steel electrode during the processing of milk (neutral) and orange juice (acidic).

The open-loop configuration was used and the liquid underwent 3 consecutive doses of PEF processing. The PEF conditions were maintained constant during all experiments. Before each pulse, the two capacitors were charged to 5.5 kV and discharged at a repetition rate of 25 Hz. The unprocessed liquid was pumped at a speed of 50 mL/min so that each PEF dose would inject energy at 272 J/mL. Samples were collected from the unprocessed feed as well as after processing with different PEF doses. Consequently, the performance of electrode materials was studied over an energy density range of 272–816 J/mL.

In the previous section, it was shown that the amount of iron released due to the physical contact between stainless steel electrodes and milk in the absence of any pulses was small compared to the amount released due to PEF processing. So, a reasonable assumption was made here that any ions released are mainly due to PEF processing. As described in Chapter 3, a preliminary experiment was carried out with milk to test the performance of four metals (chromium, nickel, silver, and titanium) coated on the inner electrode. The outer electrode was made of stainless steel and was connected to ground. Figure 4-10 and Figure 4-11 show the concentrations of the metallic ions in the unprocessed milk and in the milk that has received the PEF doses.

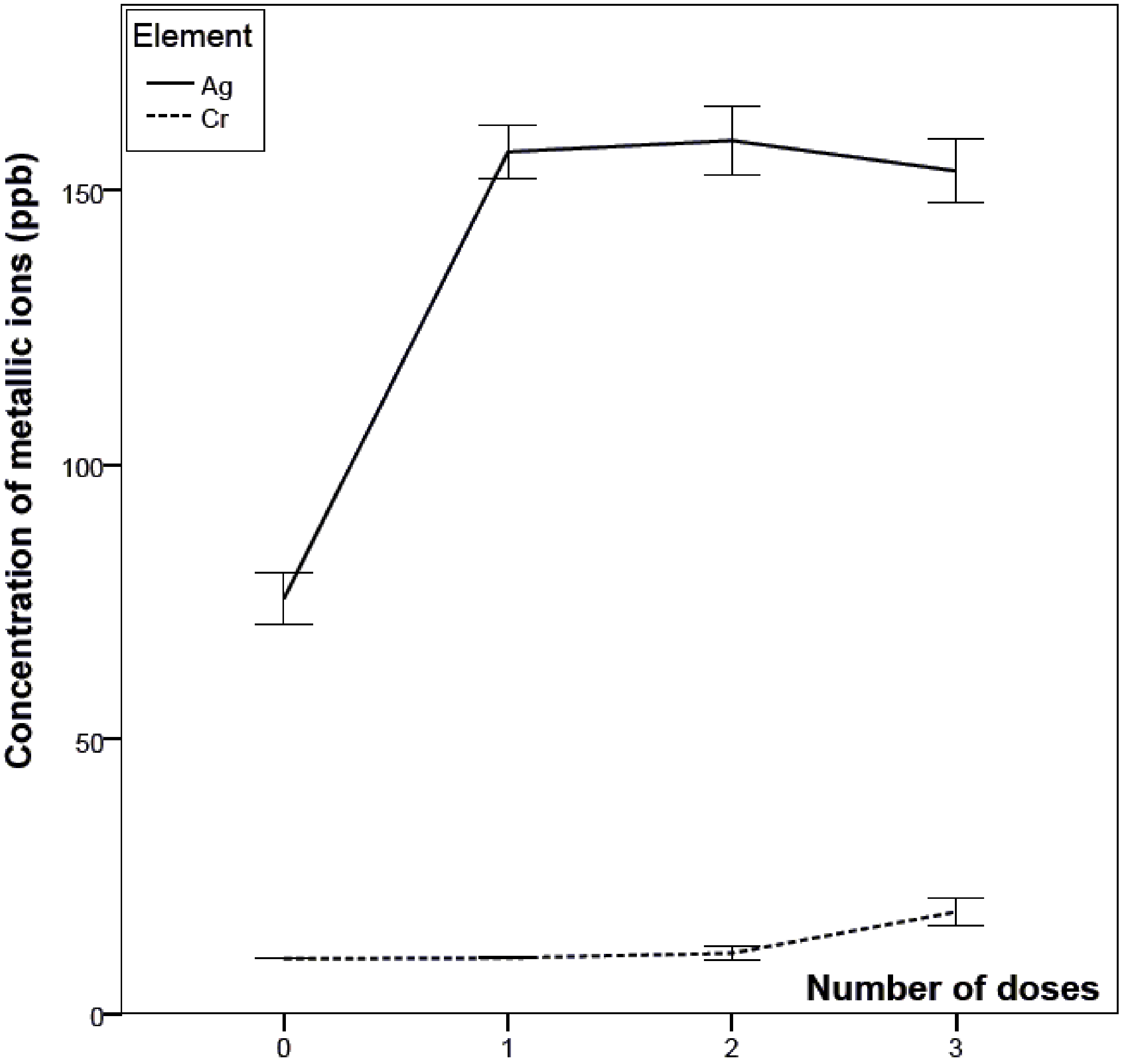


Figure 4-10: Concentration of chromium and silver in the feed and after 1, 2, and 3 PEF doses during the preliminary experiment

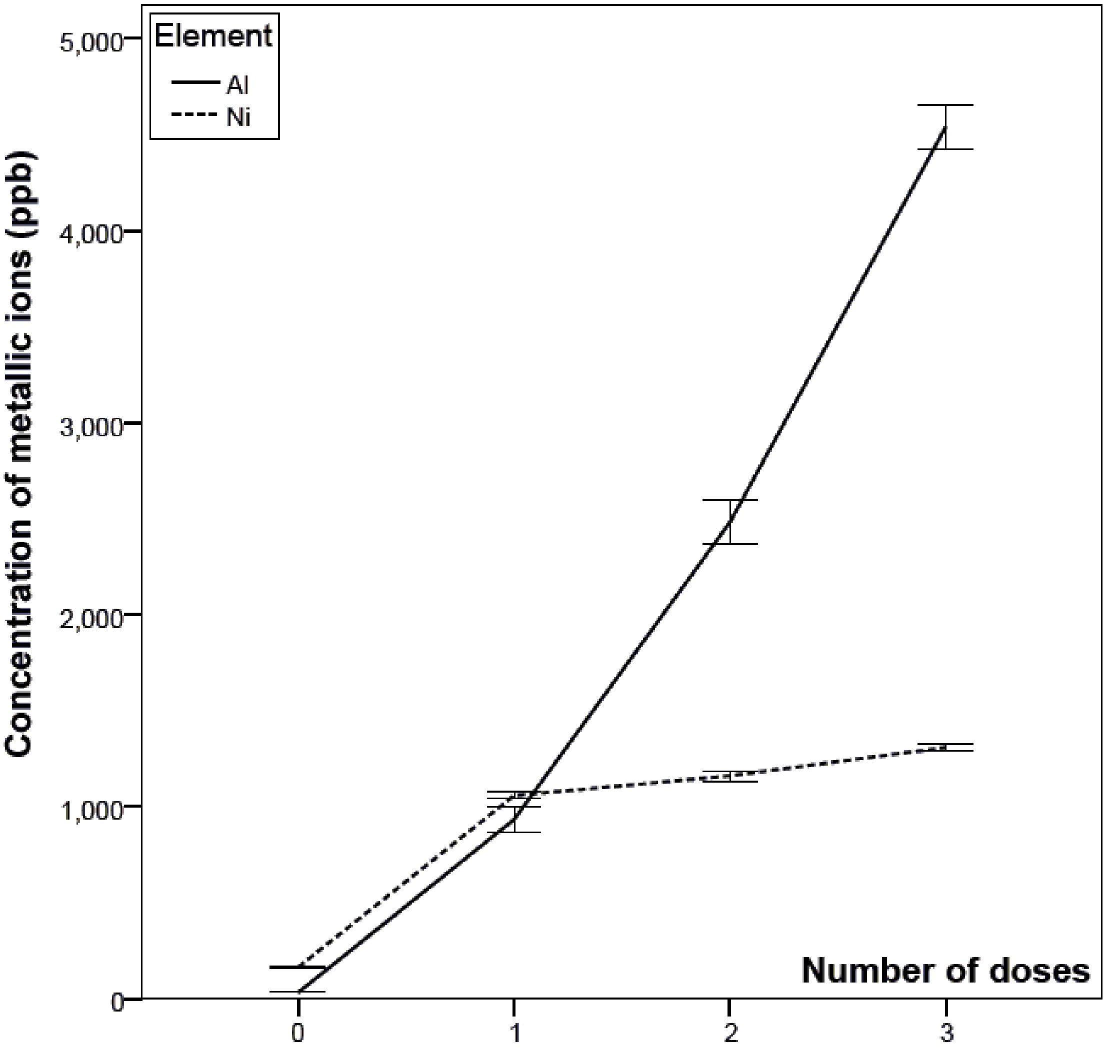


Figure 4-11: Concentration of aluminum and nickel in the feed and after 1, 2, and 3 PEF doses during the preliminary experiment

Titanium concentration is not shown since it always remained below the detection limit of the ICP. The chromium section was very resistant and showed evidence of attack only after the third dose (Figure 4-10). On the other hand, much more silver was observed in the milk. Virtually all of the attack occurred during the first dose and leads to almost complete removal of the silver layer. It was difficult to evaluate the performance of nickel from the results shown in Figure 4-11 because some of the nickel may have originated from the nickel layer of the sections containing the other three metals. However, aluminum was detected in the milk and its concentration increased as the number of doses rose. It is most likely that this came from the nickel section being removed to expose the underlying aluminum.

Visual examination of the electrodes indicated that most of the nickel and silver layers dissolved during the first dose. On the other hand, very little chromium and titanium was removed even by the end of the 3 doses with no evidence of pits. Since titanium is a popular metal for biomedical applications [60] and shows the best chemical resistance in the preliminary experiment, it is the only metal considered hereafter.

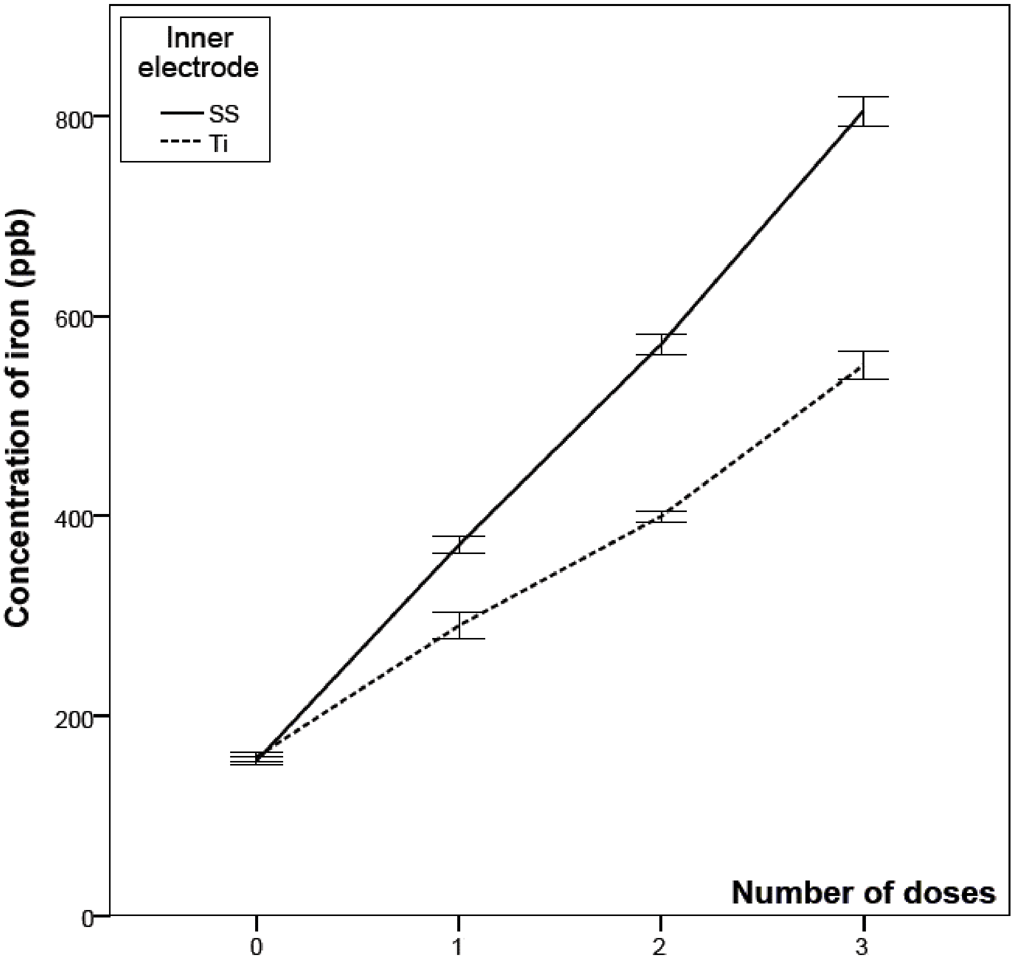


Figure 4-12: Variation of iron concentration with PEF dose during milk processing using stainless steel and titanium inner electrodes with the outer electrode connected to ground

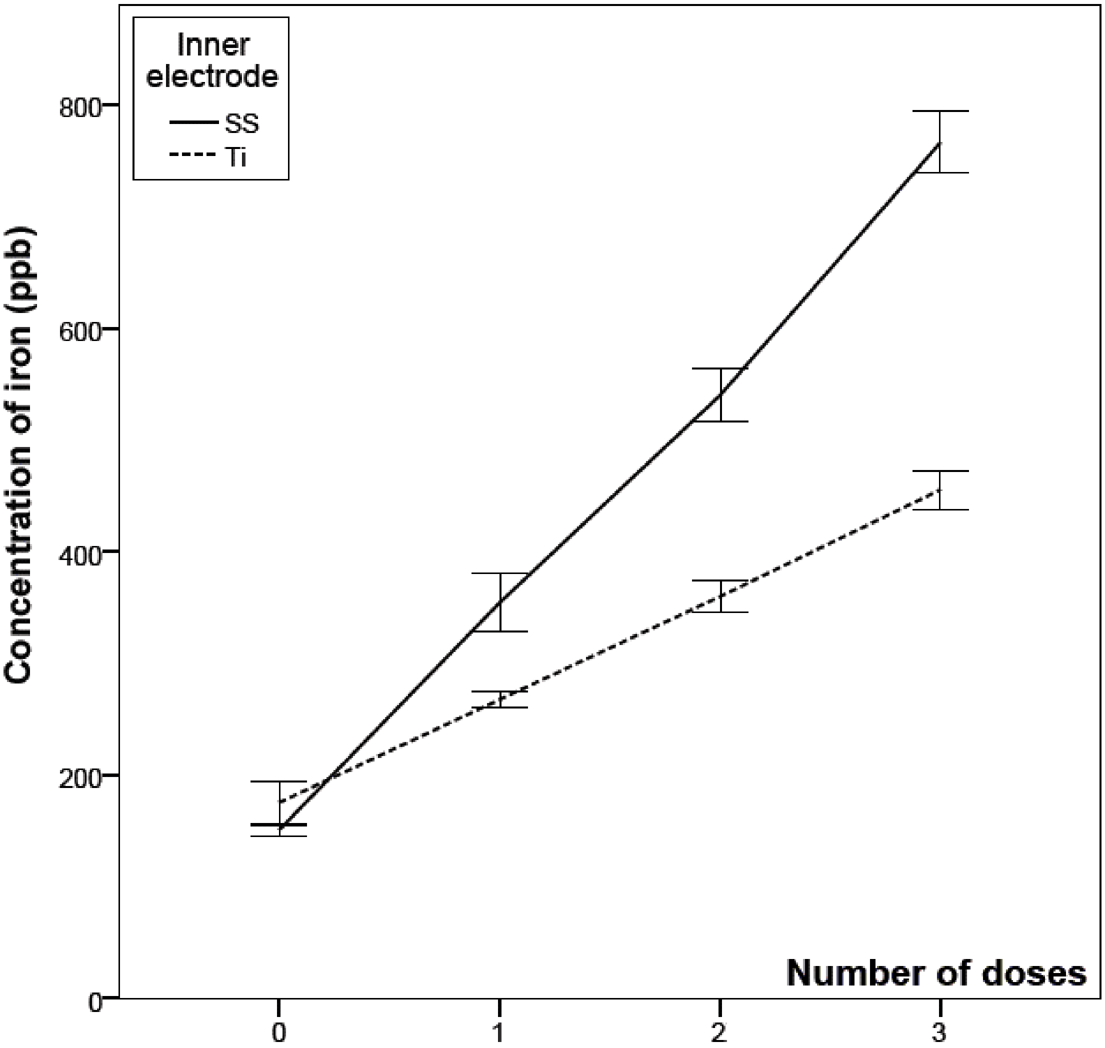


Figure 4-13: Variation of iron concentration with PEF dose during milk processing using stainless steel and titanium inner electrodes with the inner electrode connected to ground

Consequently, the performances of titanium and stainless steel as the entire inner electrode were compared during milk processing in the subsequent experiments. The outer electrode was always made of stainless steel. To assess the resistance of both titanium and stainless steel electrodes to the charging current, the outer electrode was connected to ground. As shown in Figure 4-12, the rise in iron content with each dose decreased by ~40% when stainless steel was replaced by titanium as the inner electrode. In other words, the charging current contributed ~40% of the total released iron when both electrodes were made of stainless steel. The same conclusion was reached when each of inner electrodes was tested against the pulse current by connecting it rather than the outer electrode to ground. The rise in iron content with dose was reduced by ~60% with titanium as the inner electrode (Figure 4-13). Therefore, the pulse current contributed ~60% of the iron released when both electrodes were made of stainless steel.

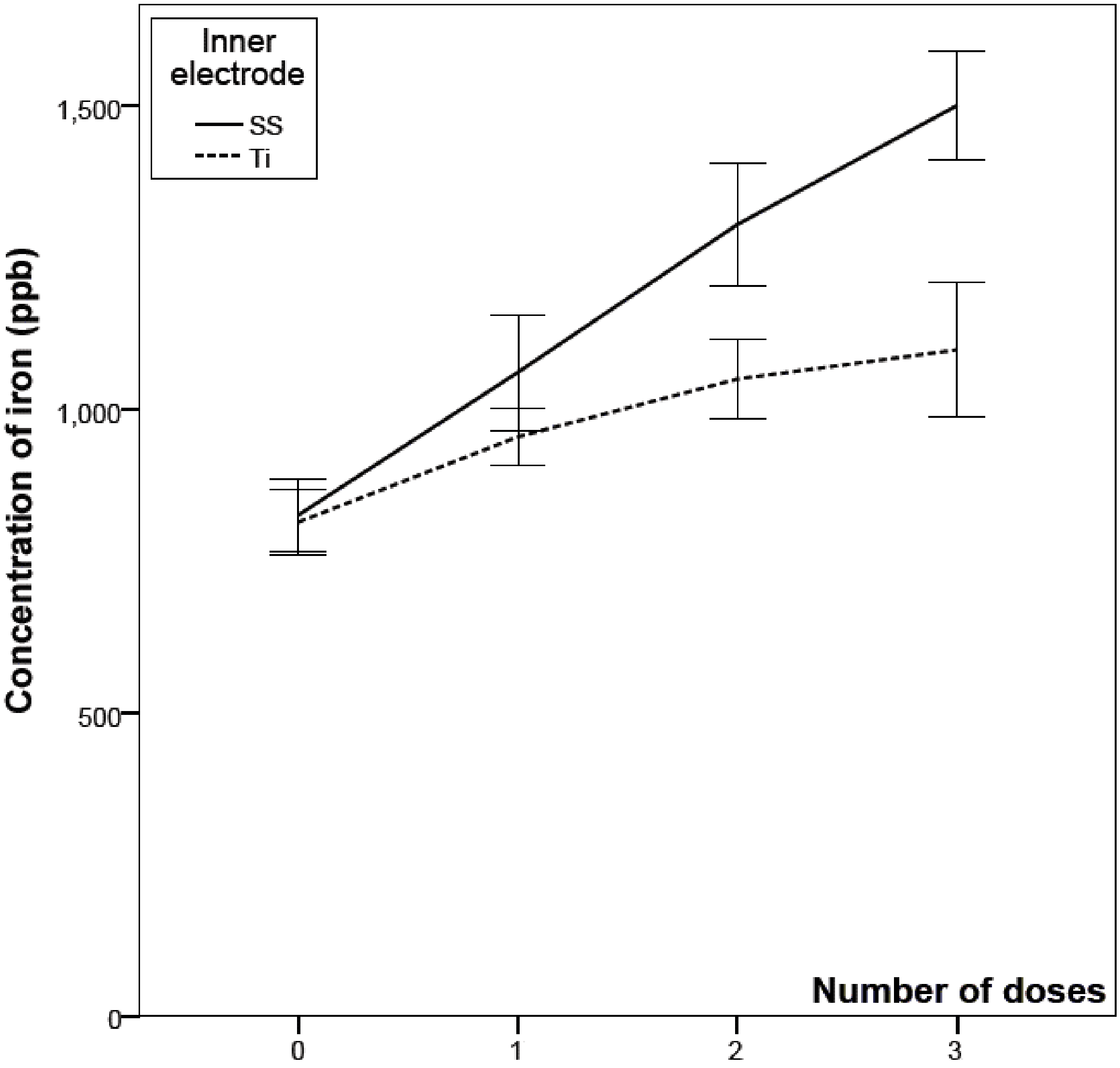


Figure 4-14: Concentration of iron when processing orange juice with the outer electrode connected to ground

In the above experiment, the titanium content always remained below the ICP detection limit in all analyzed samples. Hence, the titanium electrode performed better than the stainless steel one during both the charging and pulse phases of the current.

To study whether the observed behavior in Figure 4-12 was affected by the acidity of the liquid, experiments were carried out using low-pH orange juice. As shown in Figure 4-14, the incremental increase in the iron content per dose was not significantly different from that observed during the processing of milk (Figure 4-12). This suggests that pH may not play a critical role on the performance of the stainless steel electrodes. As before, the titanium content was always found to be below the ICP detection limit in all processed orange juice samples.

4.4 Processing temperature

Finally, the effect of the temperature inside the PEF processing zone was studied by varying the set-point of the cooler in the open-loop setup accommodated for carbonated beer processing with both electrodes made of stainless steel. Beer (ale type) was allowed to flow at a speed of 100 mL/min, and the two capacitors were charged to a voltage corresponding to an electric field of 30 kV/cm and discharged at a rate of 30 Hz. The thermocouple read 1°, 15º, and 30ºC when the cooler was set at -5°, 15º, and 35ºC, respectively. The corresponding current pulses had peaks of 1.9, 2.0, 2.2 kA, respectively. A pressure of 40 psi was sufficient to avoid foaming with the first two set-points, but it was raised to 70 psi with the 35ºC setting. Samples were collected after each of the 4 PEF doses; the energy density was computed to be 48.6 J/mL per dose.

It can be seen in Figure 4-15 that the processing temperature did not significantly affect the amount of iron released. Although the electrochemical reactions are temperature dependent (both thermodynamically and kinetically), the effect of the range studied here appeared to be insignificant. In conclusion, the elevated temperatures inside the PEF processing zone, which may synergistically enhance the microbial inactivation power of the technology, did not adversely release more metallic ions from the electrodes.

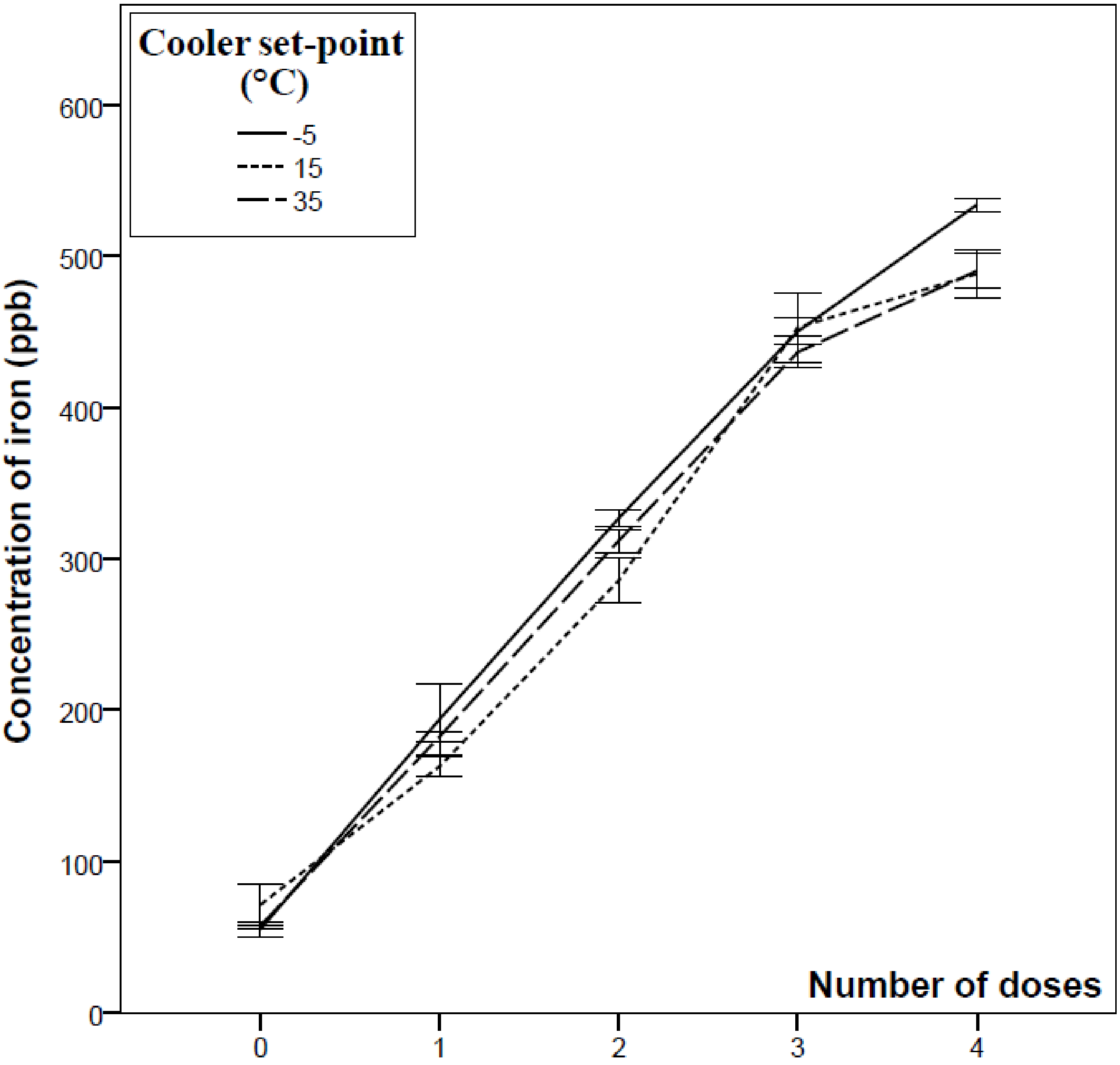


Figure 4-15: Concentration of iron when processing carbonated beer with the cooler set at -5, 15, and 35º C

Chapter 5

Results – Microbial/Sensory Analysis of Beer

In this chapter, carbonated beer (ale type) was processed by PEFs in the open-loop configuration under a head pressure of 40 psi with both electrodes made of stainless steel. Beer was allowed to flow at a rate of 100 mL/min, and the cooler was set to a temperature of -5°C. The two capacitors were charged to a voltage corresponding to an electric field of 40 kV/cm and discharged at a rate of 30 Hz. As a result, the thermocouple read 2.5–5°C and the current pulse reached a peak of ~2.5 kA and a width of 1.3 µs. Samples were collected after 1, 2, and 4 doses of processing; the energy density was computed as 86.4 J/mL per dose.

Aside from the PEF processing, a group of bottles of beer underwent steam thermal pasteurization (TP) at 60°C for 20 min. This TP protocol matches the recommendations for tunnel pasteurization in the brewing industry. In the following sections, PEF-processed beer samples are compared to TP processed samples from both microbial and sensory aspects.

5.1 Microbial inactivation

The microbial counts of the four microorganisms under study are shown in Table 5-1 following PEF processing and in Table 5-2 following TP processing of inoculated beer. The data obtained after repeating each experiment 3 times show consistency and reproducibility. A side experiment (results are not reported here) was carried out to ensure that the bacterial cells survive safely in the carbonated beer with an alcohol level of 5%.

Table 5-1: Microbial counts (log$_{10}$ CFU/mL) of the four microorganisms after the PEF processing of inoculated beer with 1, 2, and 4 doses

Microorganism	**Processing level**	**Microbial counts (log$_{10}$ CFU/mL)**		
		Run #1	**Run #2**	**Run #3**
S. cerevisiae	Untreated	5.044 ± 0.212	5.894 ± 0.119	5.965 ± 0.446
	PEF-1	4.849 ± 0.004	5.748 ± 0.210	4.392 ± 0.078
	PEF-2	4.687 ± 0.200	5.708 ± 0.497	4.163 ± 0.537
	PEF-4	4.079 ± 0.205	4.993 ± 0.013	4.452 ± 0.009
L. plantarum	Untreated	5.221 ± 0.084	6.238 ± 0.034	5.623 ± 0.048
	PEF-1	4.924 ± 0.110	5.736 ± 0.903	4.837 ± 0.027
	PEF-2	4.788 ± 0.097	5.683 ± 0.042	4.666 ± 0.146
	PEF-4	4.531 ± 0.140	5.404 ± 0.168	4.591 ± 0.074
B. subtilis	Untreated	4.988 ± 0.150	6.080 ± 0.036	6.339 ± 0.216
	PEF-1	5.420 ± 0.090	6.097 ± 0.526	5.954 ± 0.813
	PEF-2	4.839 ± 0.378	5.656 ± 0.200	4.972 ± 0.319
	PEF-4	4.204 ± 0.151	5.491 ± 0.227	4.735 ± 0.031
S. *enterica*	Untreated	5.467 ± 0.009	6.928 ± 0.748	5.979 ± 0.630
	PEF-1	5.301 ± 0.174	5.580 ± 0.120	5.415 ± 0.030
	PEF-2	5.329 ± 0.376	5.760 ± 0.373	4.591 ± 0.050
	PEF-4	4.552 ± 0.197	5.439 ± 0.354	4.853 ± 0.335

The average microbial inactivation is computed for both preservation techniques and presented in Table 5-3. It can be observed that the microbial inactivation for the four microorganisms in general after PEF-4 ranges are close to 1 log$_{10}$ CFU/mL reduction whereas TP achieves 2.7 log$_{10}$ CFU/mL. In this chapter, the low PEF processing temperature is preferred so that the sensory panels evaluate the changes in taste (if any) due solely to the PEF, and not by the elevated temperature.

Table 5-2: Microbial counts ($\log_{10}$ CFU/mL) of the four microorganisms following the TP of inoculated beer

Microorganism	**Processing level**	**Microbial counts ($\log_{10}$ CFU/mL)**		
		Run #1	**Run #2**	**Run #3**
S. cerevisiae	Untreated	6.570 ± 0.773	5.477 ± 0.094	5.501 ± 0.166
	TP	2.802 ± 0.208	2.602 ± 0.111	3.054 ± 0.096
L. plantarum	Untreated	5.778 ± 0.039	5.663 ± 0.025	5.650 ± 0.147
	TP	3.041 ± 0.128	2.669 ± 0.088	3.067 ± 0.159
B. subtilis	Untreated	6.824 ± 0.790	5.678 ± 0.038	6.097 ± 0.426
	TP	3.420 ± 0.200	3.125 ± 0.150	3.329 ± 0.085
S. *enterica*	Untreated	6.472 ± 0.963	5.623 ± 0.236	5.255 ± 0.115
	TP	3.684 ± 0.598	3.447 ± 0.000	3.477 ± 0.213

Table 5-3: Average microbial inactivation ($\log_{10}$ CFU/mL) of the four microorganisms under study following the PEF processing with 1, 2, and 4 doses as well as the TP of inoculated beer

Microorganism	**Average microbial inactivation ($\log_{10}$ CFU/mL)**			
	PEF-1	PEF-2	PEF-4	TP
S. cerevisiae	0.638	0.782	1.126	3.030
L. plantarum	0.528	0.648	0.852	2.771
B. subtilis	0.021	0.647	0.992	2.908
S. *enterica*	0.693	0.898	1.177	2.247

5.2 Sensory evaluation

Sensory evaluations, in this thesis, are intended to study the effects of PEF processing on carbonated beer taste, especially that affected by the release of metal ions from the electrodes into the beer. Analytically, the concentration of iron shows roughly a linear increase of ~ 110 ppb for each dose as shown in Figure 5-1. Chromium and nickel (not shown here) follow the same trend as iron but with concentrations less than 100 ppb.

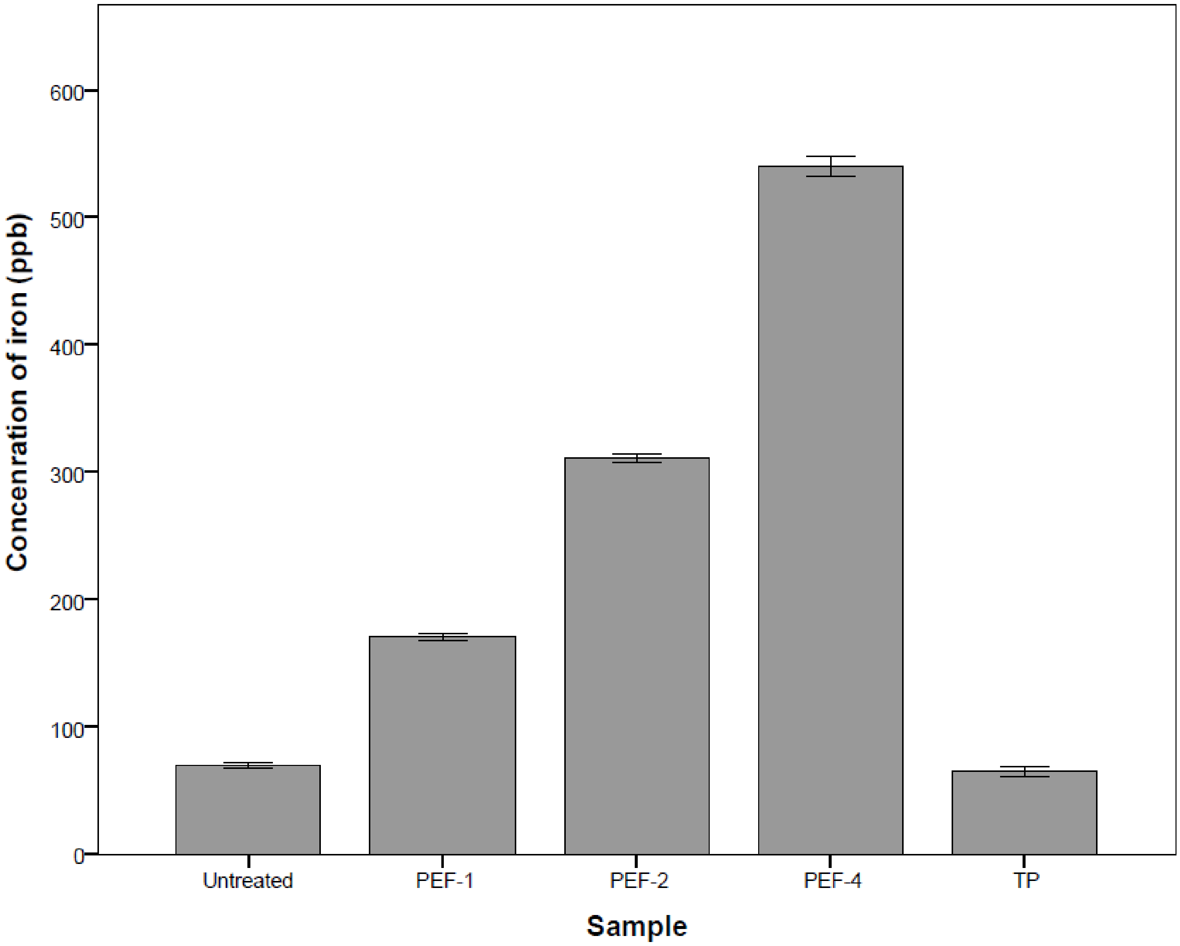


Figure 5-1: Concentration of iron in carbonated beer after PEF processing with 1, 2, and 4 doses as well as TP processing

The direct (immediate) effect of the released iron ions shown in Figure 5-1 can be evaluated through a time-zero study performed within a few days of processing. Although the concentration of iron will not change over time, iron may accelerate the aging process of beer. This indirect (secondary) effect of the released iron was evaluated through a shelf-life study over a period of 12 weeks with the beer stored at room temperature.

5.2.1 Time-zero study

For the time-zero study, a quantitative descriptive analysis was performed on various beer samples to yield the results summarized in Table 5-4. All 3 PEF samples are preferable over the TP sample in terms of aroma and foam condition. PEF-1 and PEF-2 samples have a better flavor than PEF-4 and TP ones. It is noticeable that increasing the dose of PEF causes the beer color to become darker; however, no change in color is observed with TP. Although the sweetness and sourness of PEF samples are below the fresh reference, they are very comparable to the TP sample.

As expected, the metallic feeling is higher for PEF samples than in case of TP. The metallic feeling gets stronger when increasing the dose of PEF, which is consistent with the concentration of released iron shown in Figure 5-1. The significance of this metallic feeling can be further examined in the additional comments provided by the participants, which are listed in Table 5-5; one participant commented on the metallic feeling of the PEF-4 sample only. No comments were made regarding the PEF-1 sample demonstrating its closeness to fresh beer.

Table 5-4: Time-zero quantitative descriptive analysis of carbonated beer following the PEF processing with 1, 2, and 4 doses as well as the TP

Sample	Flavor	Aroma	Color	Foam	Sweetness	Sourness	Metallic feeling
Fresh	-	-	7.20	7.20	7.20	7.20	-
TP	30%	50%	7.20	4.20	6.14	5.71	5.41
PEF-1	40%	60%	7.36	5.35	6.21	6.69	6.11
PEF-2	60%	70%	7.50	6.13	5.00	6.03	6.50
PEF-4	30%	70%	7.78	5.63	5.58	5.56	6.90

Table 5-5: Additional comments associated with the time-zero quantitative descriptive analysis of carbonated beer following the PEF processing with 1, 2, and 4 doses as well as the TP

Sample	Additional comments provided by participants
TP	▪ Slightly sweet, but better mouth feel compared to PEF-4 ▪ No taste – bland ▪ Delicious, well balanced, good aroma ▪ Sour, not appealing to your average consumer ▪ Increased bitterness, not hops ▪ Do not like metallic aftertaste ▪ Slightly sour with a green apple aroma ▪ Tastes closest to the reference fresh sample but still was not un-sour. Could be from 2 previous beers but was the closest
PEF-1	(no comments have been made on this sample)
PEF-2	▪ Had a touch of bitter edge I like ▪ Just was not good
PEF-4	▪ More flavor with no back flavor ▪ Tastes like wet dirt and stones ▪ Is flat and unappealing ▪ Increased bitterness ▪ Metallic aftertaste but not as dominant as TP

5.2.2 Shelf-life study

Table 5-6 shows the ratio of correct responses to the total population from a difference testing in which the PEF samples are compared over a shelf-life of 12 weeks to freshly-brewed beer. Based on observations from the week 8 panel, only PEF-4 samples were considered by the week 12 panel. The corresponding proportion of distinguishers is computed for the ratios in Table 5-6 and is plotted against the weeks in Figure 5-2. PEF-1 sample is the least distinguishable at week 0 because it experienced minimal processing and the aging process had not taken place yet. On the other hand, PEF-4 is the least distinguishable at week 12 because it experienced higher processing, so it is expected to have a longer shelf-life. On average, the proportion of distinguishers increases over time due to beer aging.

Table 5-6: Shelf-life difference testing of carbonated beer following the PEF processing with 1, 2, and 4 doses over a period of 12 weeks

Sample	Correct responses/population size			
	Week 0	Week 4	Week 8	Week 12
PEF-1	11/30	14/31	15/20	-
PEF-2	12/30	16/29	14/20	-
PEF-4	16/30	15/32	13/20	16/30

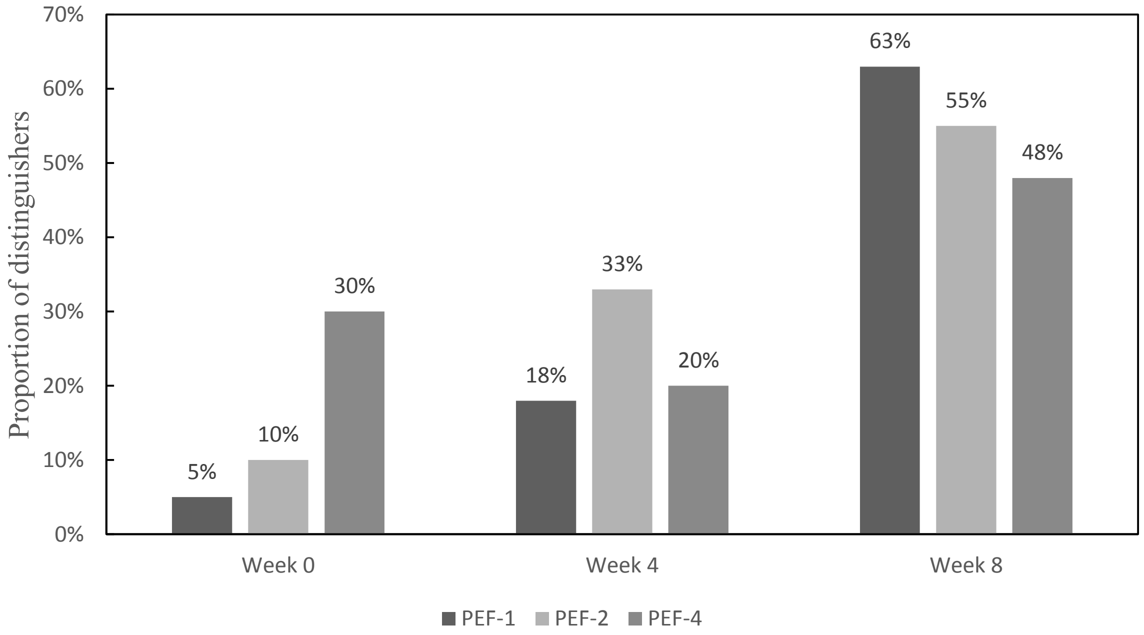


Figure 5-2: Proportion of distingushers in shelf-life difference testing of carbonated beer following the PEF processing with 1, 2, and 4 doses over a period of 12 weeks

Along with the difference testing, a quantitative descriptive analysis was performed on the same samples to identify the possible reasons for the differences reported. It can be seen from Table 5-7 that the color of PEF samples, in general, does not follow a clear trend over the weeks; it is possible that freshly-brewed beer has a slightly different color every batch. Although the concentration of iron in PEF processed samples should not change over time, the metallic feeling tends to diminsh. It can be caused by other taste attributes (e.g., oxidation) which masks the metallic feeling, or because the iron forms a new compound through its secondary reactions and is no longer detectable by participants. Oxidation was expected to increase over time to reflect the aging process of beer, but the data below do not reflect this.

Table 5-7: Shelf-life quantitative descriptive analysis of carbonated beer following the PEF processing with 1, 2, and 4 doses over a period of 12 weeks

Sample	Color (fresh=7.20)				Oxidation				Metallic feeling			
	Week 0	Week 4	Week 8	Week 12	Week 0	Week 4	Week 8	Week 12	Week 0	Week 4	Week 8	Week 12
PEF-1	7.36	5.76	6.90	-	8.44	6.74	5.90	-	6.11	6.03	5.00	-
PEF-2	7.50	5.33	7.60	-	8.08	7.65	5.70	-	6.50	6.69	5.30	-
PEF-4	7.78	6.50	7.30	5.72	9.78	7.01	7.60	6.69	6.90	6.75	4.90	5.95

Chapter 6

Discussion

The acceptability of PEF processing as an alternative method for liquid food preservation requires a deep understanding of the mechanisms causing the undesirable release of metal ions accompanying the flow of high current through the liquid. These metal ions migrate into the liquid food and may impede the commercialization of the PEF technology due to safety, quality, and/or cost aspects. First, the type and amount of metal ions, which may exist in PEF-processed food, must be within health/safety regulations before it can be introduced as a processed food to the market. Second, the "taste" quality of PEF-processed food should be comparable to that obtained using other conventional preservation methods; the presence of any metallic mouth-feeling may affect consumer acceptance to this technology. Third, the price and lifetime of electrodes should be included in the economical assessment studies of PEF systems. Electrodes with lower price and/or longer lifetime will enhance the feasibility of PEF technology, and consequently, attract more investment.

Arcing events can be considered a cause of metal release into the liquid by a thermal erosion mechanism that produces "cathode spots" [61]. It is worth noting that efforts have been made throughout the experiments in this thesis to minimize the number of arcing events that occur. At a certain voltage, arcing events become more likely when the electrodes are not cleaned well between experiments. The erosion severity of an arc depends on the electric charge stored in the capacitor(s) at the instant of arcing. In one of the experimental runs described in section 4.3 with both electrodes made of stainless steel, a few (7 or 8 times) arcing events were observed during the first dose of PEF processing. An increase of ~2,000 ppb evident in Figure 6-1 is caused by the cluster of arcing events. The volume of milk processed in this run was 4 L indicating that each arcing event produced ~1 mg of iron, which corresponds to a concentration increase of ~250 ppb.

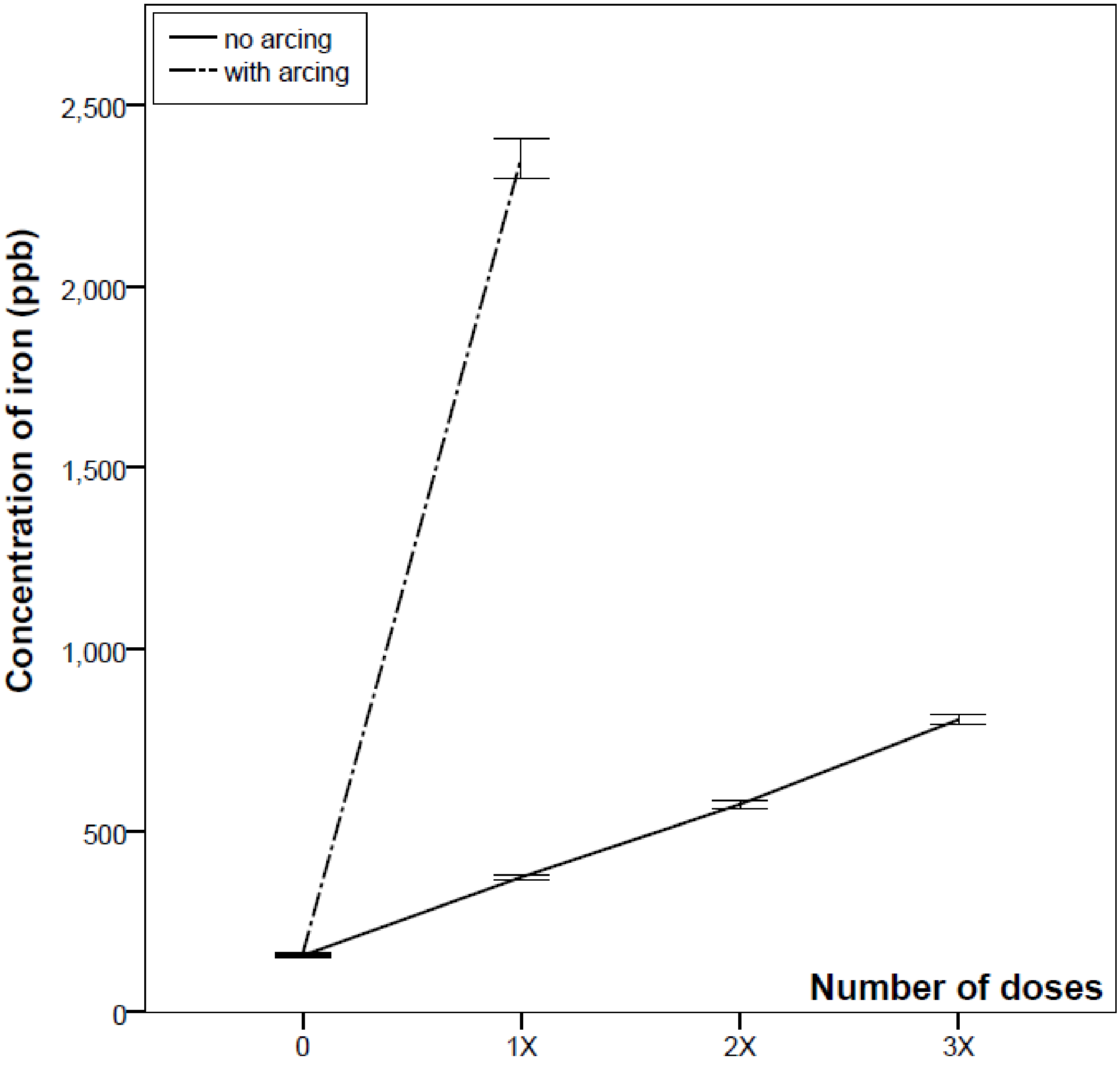


Figure 6-1: Variation of iron concentration with PEF dose during milk processing using stainless steel in the presence and absence of arcing events

In addition to the cleanliness of electrode surfaces, the design of the PEF processing chamber can play a critical role in reducing the likelihood of arcing events. The chamber introduced in [27] and used throughout this thesis features a processing zone free of insulating materials. Among the designs introduced in the literature [62], the so-called Ohio State University (OSU) chamber is widely used [8, 19, 21, 32, 37, 38, 42]. The distribution of the electric field intensity inside the OSU chamber is shown in Figure 6-2; it shows an enhancement in the field at the points where the metal, insulator, and liquid contact each other. Liquid speed is always lowest near the walls so the liquid is heated which perhaps initiates an arcing event.

Gaouda et al. [64] introduced a technique of detecting the discharge activities within a PEF processing chamber using wavelet transforms. Through features extraction, the technique is capable of detecting the discharge events preceding the occurrence of a complete arcing event. As a result, continuous monitoring of the PEF processing chamber can automatically guide the operator for required maintenance/cleaning.

The electrode pits, caused by arcing events, were always observed to affect the cathode but not the anode. Therefore, the release of metallic ions due to arcing events cannot be attributed to electrochemical oxidation (anodic reactions), but instead likely arises from the erosion of the metal by the arc. Electrochemical reactions are the second source of metal ion release, as explained in the next section.

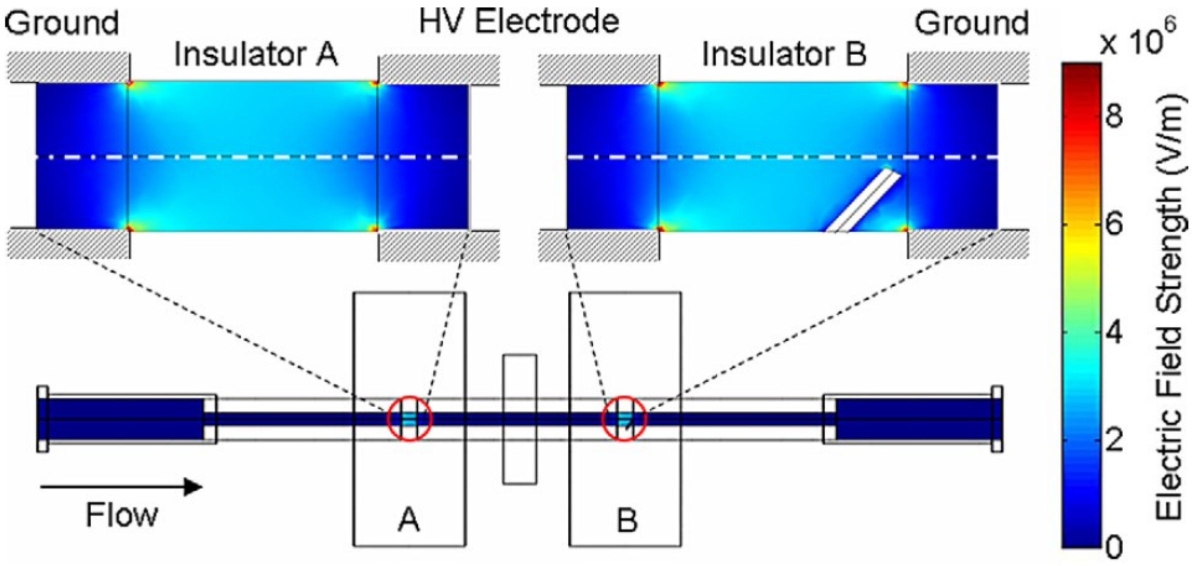


Figure 6-2: Distribution of the electric field intensity inside the OSU chamber [63]

6.1 Electrochemical release of metallic ions

In PEF processing, the scenario described in Chapter 2 for charging the double-layer occurs during each pulse. The width of each pulse can be divided from the perspective of each half-cell reaction into two parts. In the first part, the double-layer is charged as long as its potential is less than the Nernst potential of the reaction. The charging rate depends mainly on the magnitude current flowing through the cell and the capacitance of the double-layer. The second part starts when the Nernst potential is reached and thus the half-cell reaction occurs. If a train of identical pulses is applied, a linear relationship is expected between the mass of products formed and the number of pulses. In the results presented in Chapters 4 and 5, deviation from linearity can be attributed to variations in temperature, liquid composition, and/or electrode surface over the course of each experiment.

Between two consecutive pulses, the electric charges in the double-layer can be depleted by either forced or free double-layer discharging schemes, which are represented by circuits (a) and (b), respectively. Two reasons can be given for the superior performance of circuit (a) in Figure 4-2. First, the current flowing through the load during the capacitor-charging phase removes any charge remaining on the double-layer from the capacitor-discharge phase. Thus, the accumulation of charge on the double-layer from one pulse to another can be avoided. The current during the capacitor-charging phase also charges the double-layer with an opposite polarity. As a result, the current during the capacitor-discharging phase has to discharge the double-layer from any residual charge, and then charge it with the opposite polarity until the Nernst potential is reached. Since the capacitor-discharge phase is relatively short in time, reducing the duration of the final portion where electrode reactions take place will significantly reduce the concentration of metallic ions released. Second, circuit (b) undergoes a slower potential decay in the second half of the pulse-tail, which increases the duration during which current flows in the capacitor-discharge phase and therefore allows more electrode reactions to take place.

The forced double-layer discharging scheme was first introduced in [34] based on the concept of “zero net charge delivery”, which substantially reduces the rate of electrochemical reactions. Forced double-layer discharging scheme can also be satisfied with bi-polar or oscillatory pulses featuring an interval of reversed current. This idea agrees with the observation of small bubbles and depositions on the electrodes in [15] with only mono-polar pulses. The ratio of the electric charges in the current before reversal to the charges after reversal was used in [38] to conclude pulse shapes with the ratio closer to unity were preferable, which is a reflection of the “zero net charge delivery” concept. The two pulse shapes with ratios of 0.86 and 0.98 performed remarkably better than the shape featuring no reversal of current. The authors in [38] explained their observation in terms of complementary reactions that will re-deposit the metal back on the electrodes during the interval of reversed current. However, our observations with the two circuit topologies as well as the low concentration of metallic ions do not support this explanation.

If the “zero net charge delivery” is satisfied, bi-polar pulses are preferred over the mono-polar pulses because the interval of reversed current in the latter does not contribute to the microbial inactivation but instead releases metallic ions. However, the effectiveness of microbial inactivation using bi-polar and mono-polar pulses seems to be dependent on the pulse width. Jayaram and Boggs [65] stated that their computational analysis provides no basis for the idea that polarity reversal provides a benefit to the electroporation process, especially at high frequencies of the damped sinusoidal waveforms utilized. Experimentally, it was shown in [25] that mono-polar pulses resulted in higher microbial inactivation with pulse widths less than 3 μs, whereas bi-polar pulses in [15] of 50 μs width resulted in higher microbial inactivation.

When the peak voltage is kept the same, Figure 4-6 showed that shorter pulses (utilizing one capacitor) release less iron from the electrodes. This observation agrees with the concept discussed above of dividing the pulse width into two parts. The duration of the first part depends on the current (i.e., peak voltage) and remains unchanged. With shorter pulses, the second part of the pulse (when electrode reactions occurs) is shortened considerably. In addition, the capacitor-charging phase is indirectly almost halved when utilizing one capacitor to generate the shorter pulses.

Figure 6-3 shows the electric charge passed through the PEF chamber when the four pulses shown earlier in Figure 4-5 were applied. The charge was determined by discrete integration of the current waveform. This charge is the same appearing on the double-layers and causing their potential to rise ($E = {}^{Q}/_{C}$). The charge, at which a certain half-cell reaction is initiated, can be computed by multiplying the Nernst potential of the reaction by the double-layer capacitance. For simplicity, we will assume that the double-layer capacitance does not change with the applied voltage, and the experiments are carried out at the standard conditions of pressure and temperature. As a result, the charges corresponding to iron and titanium oxidation are computed in Table 6-1 and shown as horizontal lines in Figure 6-3.

Table 6-1: Standard Nernst potentials and double-layer capacitances for stainless steel and titanium electrodes and the corresponding electric charge for reaction initiation

Material	Oxidation half-cell reaction	Nernst potential [47]	Double-layer capacitance [51]	Electrode area	Electric charge
Stainless steel	$Fe \rightarrow Fe^{+2} + 2e^{-}$	0.44 V	35 µF/cm^2	95 cm^2	1.45 mC
Titanium	$Ti \rightarrow Ti^{+2} + 2e^{-}$	1.63 V	50 µF/cm^2	95 cm^2	7.68 mC

For the stainless steel electrode, the first part of any pulse is defined from the beginning of the pulse up to the intersection point between the charging curve and the horizontal iron line. Beyond this point, the second part of the pulse, which is responsible for the electrode reactions, starts and continues to the end of the pulse. For example, the pulse generated with 1 capacitor and having a peak of 4 kV has its first part from 0 to 0.5 µs and the second part from 0.5 to 1.7 µs, i.e. electrode reactions will last for 1.2

μs during each pulse. It can be seen that the peak voltage does not have a significant effect on the duration of the second part of the pulse; whereas, the number of capacitors does, i.e. pulse width. For titanium electrode, none of the curves reached the titanium line; as a result, the electrode reactions were never initiated. The experimental results presented in sections 4.2 and 4.3 agree with this explanation.

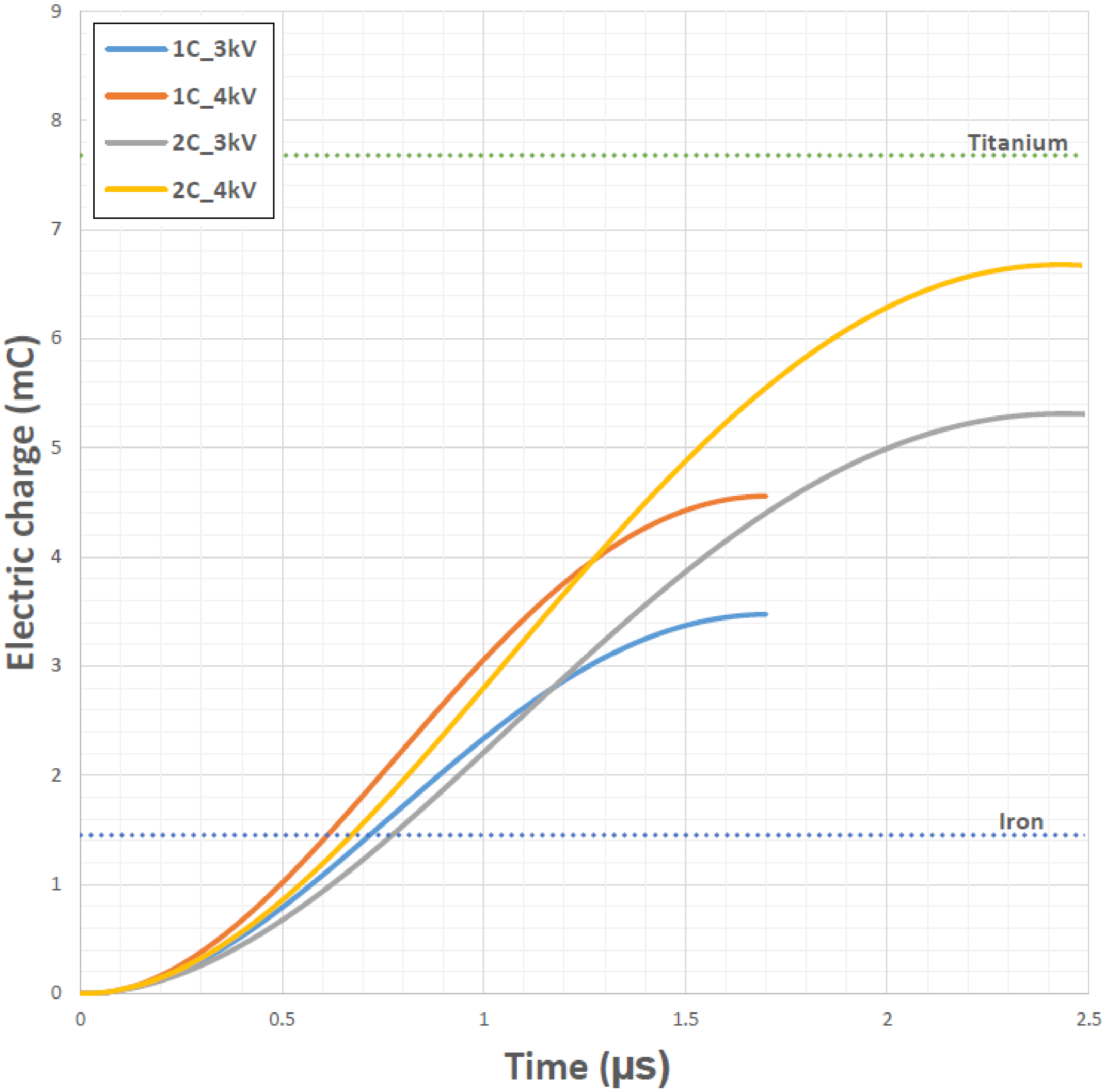


Figure 6-3: The electric current passing through the PEF processing chamber with different pulse parameters. Iron and titanium lines correspond to the double-layer charge at which the electrode reactions are initiated

Experimental results with orange juice presented in [32] indicate the contribution solely due to PEF electric pulses to be 14.98, 0 and 0.69 ppb for iron, chromium, and nickel, respectively. One can attribute these low values to the fact that they applied pulses with only 50 J/mL energy density (Table 1-1). An increase from 258 to 2859 ppb and from 0 to 526 ppb has been reported in [8] for iron and chromium after PEF processing of beer with a total energy density of 589[*] J/mL; nickel content was not measured. Although the energy density was within the range used here, the reported values of the increase in the iron and chromium contents are much higher than the results obtained in this work. The difference in the measured iron concentrations can be related to the differences in pulse width (1.3 μs versus 4 μs), and the electrode geometry of cross-field versus co-field processing chamber. Releases of 8 ppm titanium and 33 ppm iron when using titanium and stainless steel electrodes, respectively, reported in [44] agrees with the superiority of titanium indicated in this work. However, the concentrations (8 and 33 ppm) are much higher than the obtained results reported in the literature and the present study. The authors in [44] did not provide details about the conductivity of their liquid and the flow speed; as such, the energy density utilized could not be computed.

In our experiments with the titanium electrode, a gradual brownish-darkening of the color was observed on the titanium surface facing the processing zone, reflecting the formation of an oxide that passivates and protects the metal [48, 66].

6.2 Regulations on food industry

By reviewing the regulations for food industries dictated by governments in North America, they have been mainly concerned with the presence of heavy/toxic metals, namely cadmium, lead, and mercury. None of the metals which could be used as electrodes for PEF processing is mentioned in American regulations [67], while only two of them (aluminum and copper) are mentioned in Canadian

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[*] computed in Table 1-1

ones [68]. For other metals, guidelines could be taken from drinking water regulations [69, 70] as shown in Table 6-2.

Table 6-2: Maximum contamination levels of metallic materials dictated by Canadian and American regulations

Concentration levels of metallic materials (ppm)						
Aluminum (Al)	**Chromium (Cr)**	**Copper (Cu)**	**Iron (Fe)**	**Manganese (Mn)**	**Silver (Ag)**	**Zinc (Zn)**
Pest control products Act [68]						
100	-	50	-	-	-	-
Actions levels for poisonous or deleterious substances in human food and animal feed [67]						
-	-	-	-	-	-	-
Canadian drinking water guidelines [70]						
0.1-0.2	0.05	1	0.3	0.05	-	5
National primary drinking water regulations [69]						
0.05-0.2*	0.1	1.3	0.3*	0.05*	0.1*	5*

*Secondary drinking water regulations are non-enforceable guidelines regarding contaminants that may cause cosmetic effects (such as skin or tooth discoloration) or aesthetic effects (such as taste, odor, or color) in drinking water.

In European legislations, Roodenburg et al. [32] reported that no standards are given for the maximum concentration of dissolved metals except for the same three heavy metals. For iron concentration, the authors referred to a reference value of 5 ppm given by the Association of the Industry of Juices and Nectars (AIJN). It is worth noting that Figure 4-14 shows that pasteurized orange juice sold in the Canadian market has a concentration of ~800 ppb iron prior to PEF processing, which gives confidence that this range is safe for human consumption.

6.3 Microbial inactivation of inoculated beer

During the doses of PEF processing, *S. cerevisiae* and *S. enterica* show relatively higher inactivation because of the first being a yeast and the second being a gram-negative bacteria. Being gram-positive, *L. plantarum* and *B. subtilis* have thicker cell membranes and, hence, are more resistance to the PEF processing. *B. subtilis* is also capable of forming endospores to survive against ultraviolet radiation, desiccation, high temperature, extreme freezing and chemical disinfectants. The low temperature (<5ºC) in the PEF processing zone is the main reason for the low microbial inactivation power obtained in this work although the applied electric field lies within the typical range shown in Table 1-1; processing temperature has a synergistic effect on the PEF microbial inactivation power [27, 29, 30, 50]. Future work should consider improving the PEF inactivation power by either increasing the number of doses or elevating the processing temperature as long as its effect on taste is insignificant.

Chapter 7

Conclusions and Future Work

7.1 Conclusions

The increasing consumer demand for high-quality, minimally processed, and microbiologically safe foods has stimulated the investigation of non-thermal methods of food preservation. Since the major concern of food preservation is to control the microbial growth, PEF processing is a promising non-thermal method for inactivating microorganisms. The inactivation of vegetative forms of bacteria, yeast, mold, and some enzymes after PEF processing has been extensively demonstrated by several research groups over the last few decades. However, few studies have been conducted to investigate the quality, safety (other than microbial), and processing cost of PEF-processed products, which is essential before the PEF technique can be accepted by the food industry. One critical aspect, the release of metal ions from the electrodes, has been reported in the literature and obviously raises concerns about PEF processing.

It follows that understanding the effects of the released metallic ions during the PEF processing is important. Such understanding can be classified into two stages: (1) understanding the process of metal ion release and its dependence on operating parameters such as pulse shape, voltage peak and pulse width, electrode material, and processing temperature, and (2) relating the measured quantities of the metal ions in PEF-processed products to their safety and quality over their shelf-life. The outcome of these two aspects leads to an optimization of the PEF operating parameters and a better estimate of the processing cost when PEF is carried out on a commercial scale.

In this study, the concentrations of released metal ions in PEF-processed milk, orange juice, and beer have been determined using the ICP-AES under various operating parameters. It has been found that mitigation of arcing events protects the electrode surface from experiencing “pits”, which is one major source of metal ion release. For electrochemical reactions, satisfying a “zero net charge delivery” reduces the metallic content of PEF-processed foods considerably. Allowing the charging current of the generator’s capacitor to flow through the PEF processing chamber was used here to ensure zero net charge delivery with mono-polar pulses. The usage of bi-polar pulses can be another alternative. Lower metallic content has been obtained with pulses of shorter width; this observation agrees with the nature of an electrically-driven electrochemical process. Variation in the voltage peak, within the typical PEF conditions, did not have a remarkable effect on the metal ion release.

The comparison of electrodes coated with chromium, nickel, silver, and titanium has revealed the superior performance of titanium. A solid titanium electrode did not cause any detectible metallic content after PEF processing of milk (neutral) and orange juice (acidic); whereas, the concentration of iron from stainless steel electrodes was detectible in both liquids. Besides its electrochemical characteristics, titanium forms a passive layer to protect its surface. The temperature of the PEF processing zone did not influence metal ion release in the range from 1° to 30ºC.

American, Canadian, and European regulation do not impose certain limits on the presence of metal ions in consumable food products. When comparing to secondary drinking water guidelines, the concentration of iron in PEF-processed liquid food products was higher. However, the difference between PEF-processed and unprocessed products in this work had a maximum of ~800 ppb iron, which is the same as the concentration of iron in the pasteurized orange juice available in the market.

The study also highlights the applicability of PEF processing of carbonated beer, in particular through a sealed and pressurized processing chamber. Brewery regulations in Canada do not necessitate the thermal pasteurization of carbonated beer; hence, PEF processing finds a potential application in the beer industry. Sensory panels has shown that PEF-processed beer is preferred when compared to

TP beer with respect to flavor, aroma, and foam condition. Trained panelists indicated a metallic feeling in the PEF-processed beer in direct relation to the measured concentration of metallic ions. A compromise in the PEF dosage has to be reached because untrained panelists preferred samples subjected to low-dosage of pulses in week 0, but they preferred samples with a high-dosage of pulses in week 12 of the shelf-life.

7.2 Future work

The achievements of this study provide a solid foundation for further investigation of the quality aspects of PEF processing and optimization of the operating conditions. Optimum conditions should minimize the cost of energy and replacement to achieve an extended shelf-life from microbial and quality viewpoints. Sensory panels to perform a quantitative descriptive analysis over the shelf-life and/or an analytical evaluation of taste attributes will further our understanding of the secondary effects of released metal ions on the PEF-processed products. They will also allow a fair comparison between electrode materials on the basis of quality attributes instead of quantitative measurement of ions only.

Development of a solid-state pulsed-power generator, capable of producing bi-polar square pulses, would be useful since it is expected to halve the metal content in PEF-processed samples for the same microbial inactivation. It will also provide a better control of the pulse width for accurate optimization. Through a feedback loop, the peak voltage can be increased to maintain the required electric field, without introducing arcing events, if the electrode dimensions change with time.

Appendix A

List of Publications

Papers in refereed journals

- Ahmed Gad, Shesha H. Jayaram, and Mark Pritzker ,“Performance of Electrode Materials during Food Processing by Pulsed Electric Fields,” to appear in IEEE Transaction on Plasma Science, Oct 2014.
- Ahmed Gad, and Shesha H. Jayaram, “Effect of Electric Pulse Parameters on Releasing Metallic Particles from Stainless Steel Electrodes during PEF Processing of Milk,” IEEE Transaction on Industry Applications, Vol. 50, No. 2, pp. 1402-1409, Mar/Apr 2014.

Papers in conference proceedings

- Ahmed Gad, and Shesha H. Jayaram, “Migration of Electrode Material during Food Processing by High-Field Pulses,” Proceedings of the IEEE Conference on Electrical Insulation and Dielectric Phenomena, Monteal, Quebec, Vol. 1, pp. 170-173, Oct 2012.
- Ahmed Gad, and Shesha H. Jayaram, “Effect of Food Composition and pH on Electrode Material Migration during PEF Application,” Proceedings of the International conference on Bio and Food Electrotechnologies, University of Salerno, Fisciano, Salerno, Italy, pp. 46-50, Sep 2012.
- Ahmed Gad, and Shesha H. Jayaram, “Electrode Material Migration During Pulsed Electric Field (PEF) Treatment,” Proceedings of the Electrostatic Society of America Annual Meeting, Case Western Reserve University, Cleveland, Ohio, Jun 2011.

Appendix B

Inductively Coupled Plasma – Atomic Emission Spectroscopy

The concept of operation of the inductively coupled plasma – atomic emission spectroscopy (ICP–AES) used for the determination of trace elements in a solution is briefly discussed in this section [71]. The technique is based upon the spontaneous emission of photons from atoms and ions that are excited in an RF discharge. The sample solution is converted to an aerosol and directed into the central channel of the plasma. At its core, the inductively coupled plasma (ICP) sustains a temperature of approximately 10,000ºK, so the aerosol is quickly vaporized. Sufficient energy is often available to convert the atoms to ions and subsequently promote the ions to excited states. Both the atomic and ionic excited state species may then relax to the ground state via the emission of a photon. These photons have characteristic energies that are determined by the quantized energy level structure for the atoms or ions. Thus the wavelength of the photons can be used to identify the elements from which they are originated. The total number of photons is directly proportional to the concentration of the originating element in the sample.

A portion of the photons emitted by the ICP is collected with a lens or a concave mirror that forms an image of the ICP on the entrance aperture of a wavelength selection device (monochromator). The particular wavelength exiting the monochromator is converted to an electrical signal by a photodetector. The signal is amplified and processed by the detector electronics, then displayed and stored by a personal computer.

As shown in Figure B-1, the ICP ‘‘torch’’ is usually an assembly of three concentric fused-silica tubes. A water-cooled, three-turn copper coil surrounds the top section of the torch, and is connected to an RF generator. The outer argon flow sustains the high temperature plasma, and positions the plasma relative to the outer walls and the induction coil. The plasma under these conditions has an annular shape. The sample aerosol carried by the inner argon flow enters the central channel of the plasma and helps to sustain the shape.

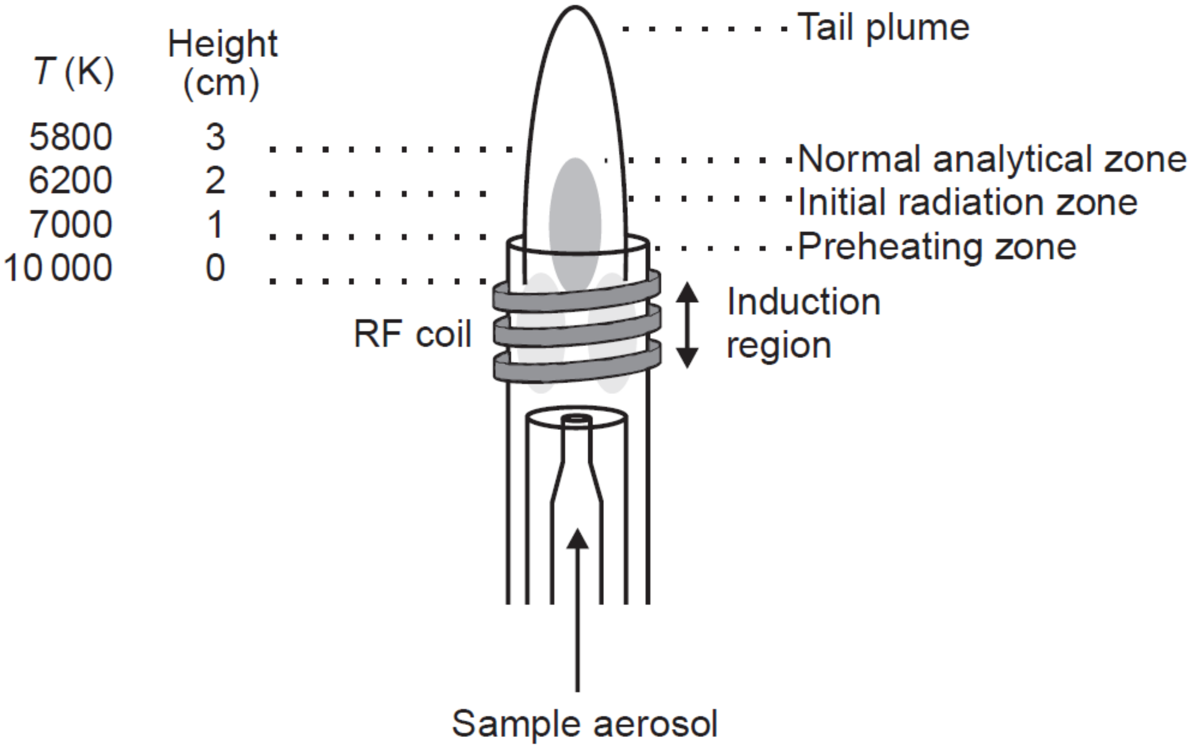

Figure B-1: Schematic diagram of an ICP assembly showing the three concentric tubes composing the torch, the RF coil, the different plasma regions, and the temperature as a function of height above the load coil [71]

Among the commonly used analytical atomic spectrometry techniques, ICP is probably the one with the fewest interferences. The argon plasma is inert when compared to the chemical reactivity of a flame. Also, the high temperature of the plasma helps to reduce chemical interferences. The temperature is high enough to break down most species into atoms or ions for excitation and subsequent emission.

The most common interference problem in ICP is spectral interference (also referred to as background interference). This type of interference arises because of the multielement nature of the plasma. Since the ICP is capable of exciting almost any element that is introduced into the plasma, spectra are likely to be rich especially for highly complex and concentrated samples. If some spectral overlap exists, advanced background correction techniques are employed or a different analytical wavelength for the element(s) of interest is chosen.

Appendix C

PEF Processing of Carbonated Beer

Carbonated beer is a two-phase substance in which carbon dioxide bubbles exist within the beer liquid. Aging of beer accelerates significantly if it is exposed to oxygen or air in general. In addition, beer is a very delicate substance which can lose the carbonation (aka foaming) when experiencing a mechanical shock or temperature rise. In order to accommodate these specific requirements, the PEF processing setup used is completely sealed and pressurized. Figure 3-3 shows the details of the processing facility in which beer is allowed to flow by creating a slight difference in the head pressure between the input and output tanks.

The input 3-way switch position is set to allow the aqueous ozone to flow through the system, and is collected in the waste tank. With water as the treatment fluid, the pulse voltage and frequency conditions are adjusted, then the 3-way switch is changed to connect the beer tank. Sufficient time is allowed to flush the system completely with beer, while collecting the processed fluid in the waste tank. At this point, the output 3-way switch is adjusted to collect the processed beer in an empty sterilized tank for further evaluations. The output tanks were initially purged with carbon dioxide gas, while beer gas is used to push the beer from the input tank. This procedure ensured to avoid any cross-contamination as the unprocessed beer does not flow in the tubing system beyond the processing chamber.

The two-phase nature of carbonated beer increases the probability of partial discharges within the bubbles, which may lead to complete arcing events. Operating the system under relatively high pressure (40–70 psi) reduces the probability of arcing events occurrence; bubbles shrink under high pressure and their breakdown strength increases. To control the temperature, the input/output beer tanks are immersed in ice and cooling jackets surround the processing chamber. Since PEF processing is always associated with ohmic heating of liquid, the cooling jackets as well as the relatively high pressure help the beer maintain its carbonation level.

As PEF-processed beer samples were served in the sensory panels, they should meet the standards of human consumption. Great attention was made towards the sanitation protocols during beer processing experiments. In the beginning of every set of experiments, the PEF processing chamber is autoclaved and new tubes are installed to eliminate any microbial cross-contamination. Between two successive experiments, the tubing system and the chamber are rinsed by flushing aqueous ozone to remove any remaining beer and the odor as well. Bottling is carried out using a manual counter pressure bottle filler at 10–12 psi carbon dioxide to remove any air residue from the bottle neck.

Appendix D

Date: Thu, 26 Apr 2012 11:53:04 -0400 [26/04/2012 11:53:04 EDT]

From: Luc Pelletier <luc.pelletier@hc-sc.gc.ca>

To: agad@uwaterloo.ca

Cc: Kelly Hislop <Kelly.Hislop@hc-sc.gc.ca>, Food-Aliment <Food-Aliment@hc-sc.gc.ca>, santé-health1005 <sante-health1005@hc-sc.gc.ca>

Subject: Regulations on processed food - Metal MLs (ADDCP12042002)

Dear Ahmed Gad,

This is in response to your email inquiry from February 1, 2012, regarding Canadian maximum levels for metal contaminants in food. We apologize for the delay in responding.

In Canada, maximum levels (ML) for chemical contaminants, such as metals, in food are expressed as either regulatory tolerances, which are found in Table 1 of Division 15 of the *Food and Drug Regulations*(http://laws-lois.justice.gc.ca/eng/regulations/C.R.C.%2C_c._870/), or as standards, which are not found in the regulations but can be viewed on the Health Canada website (http://www.hc-sc.gc.ca/fn-an/securit/chem-chim/contaminants-guidelines-directives-eng.php). Regardless of what they are called, all MLs are applicable to all foods sold in Canada (manufactured domestically and imported) and are enforceable by the Canadian Food Inspection Agency (CFIA).

You will notice that Health Canada has established a very limited number of MLs for contaminants in food. The reason is because the finding of a contaminant in food does not automatically lead to the conclusion that there is an unacceptable health risk. Most contaminants found in food are present at such low concentrations that they do not pose a safety concern. Even in situations where a potential health risk has been identified, the establishment of MLs is only one of several possible risk management approaches to consider. MLs are only set when this has been deemed to be the most effective approach to mitigate an unacceptable health risk when one has been identified.

However, the absence of an ML for a particular contaminant in a particular food does not mean that there is no regulatory oversight. Part 1, Section 4 of the *Food and Drugs Act* (http://laws-lois.justice.gc.ca/eng/acts/F-27/page-2.html#h-5) states that no person shall an article of food that has in or on it any poisonous or harmful substance or is adulterated. Both Health Canada and the CFIA have surveillance programs in place to monitor the levels of contaminants in retail foods. Elevated levels are assessed by Health Canada on a case-by-case basis to determine if there is a health risk.

We hope this information is helpful.
Luc Pelletier
Bureau of Chemical Safety / Bureau d'innocuité des produits chimiques
Food Directorate / Direction des aliments
Health Canada / Santé Canada

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