

## **UTILIZATION OF HIGH INTENSITY PULSED ELECTRIC FIELDS (PEF) AS AN UNCONVENTIONAL NON THERMAL METHOD OF LIQUID FOOD PRESERVATION**

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### **ABSTRACT**

*Increasing consumer demand for new products with high nutritional qualities has spurred a search for new alternatives to food preservation. Pulsed Electric Fields (PEF) is an emerging technology for non thermal food pasteurisation and could be an alternative preservation method of food liquids compared to traditional heat pasteurisation preservation method, where the main purpose is to inactive pathogenic bacteria. Using this technology, enzymes, pathogenic and spoilage microorganisms can be inactivated without affecting the colour, flavour, and nutrients of the food.*

*Different studies indicate that pulsed electric fields could be a useful method in liquid food preservation especially in the context of good organoleptic and functional properties of final products. However, the main assessment criterion of that method is the sufficient improvement of microbiological safety especially in the context of spores inactivation.*

*PEF treatment may be provided by applying pulsed electric field to a food product in a treatment zone between two electrodes at ambient, or slightly above ambient temperature.*

*Exposure of microbial cells to the electric field induces a transmembrane potential in the cell membrane, which results in electroporation (the permeabilization of the membranes of cells and organelles) and/or electrofusion (the connection of two separate membranes into one) of the cells.*

*An innovative pulsed electric fields (PEF) unit was developed and constructed in Food Technology research Institute. The system consists of main equipment, the high voltage pulse generator (20 – 80 kV) and the treatment chamber.*

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*The main focus of this work was to develop an innovative PEF system that provides a uniform distribution of electric field, minimum increase in liquid temperature, minimum fouling of electrodes and an energy efficient system and high safety.*

*Designed electric field intensity is in the range of (20-80 kV/cm) applied with square pulses of 2  $\mu$ s duration at least.*

*The idea of PEF treatment, design of the unit and the treatment chambers and the factors affecting microbial inactivation during PEF process were described in the present paper.*

### **INTRODUCTION**

**M**ost of the efforts of the conventional food processing aim to the reduction or inactivation of microbial organisms, that can be achieved by thermal processing (i.e. pasteurization, sterilization, blanching) using water or steam as a means for heat transfer [Knorr, 1998]. Non thermal methods enable the processing of food below the used temperatures during thermal pasteurization, hence flavours, essential nutrients and vitamins undergo minimal [Butz & Tauscher, 2002]. In the past years such preservation methods like aseptic processing, modified atmospheres, pulsed electric fields (PEF), microwave energy were observed to become more popular [Cardello, 2003]. Many studies indicate microbiological effectiveness of these methods at a good level of sensory characteristics of products [Sitzman, 1995]. A success of a food product depends on both product and consumer factors including these social, cultural and attitudinal ones. Consumers in many cases are afraid of some new technologies more than others. Within this context, the application of novel food processing technologies to commercial foods rises high concern among consumers. The consumers' concern is connected, to the greatest extent, with the addition of genetic engineering, pulsed X-rays, and irradiation [Cardello, 2003]. The lowest levels of consumers' fear of food preservation concerns old technologies like heat pasteurisation, cold preservation, thermal energy but also some new technologies like radio-frequency heating, microwave radiation, pulsed electric fields, ultrasounds and oscillating magnetic field [Cardello, 2003]. The most investigated new preservation technologies are non-thermal inactivation technologies such

as high hydrostatic pressure (HHP) and pulsed electric fields (PEF), new packaging systems such as modified atmosphere packaging (MAP) and active packaging, natural antimicrobial compounds and biopreservation. Another investigated inactivation technologies are ionisation radiation, high pressure homogenisation, UV decontamination, pulsed high intensity light, high intensity laser, pulsed white light, high power ultrasound, oscillating magnetic fields, high voltage arc discharge and streamer plasma, but most studies is focused on HHP and PEF [Devlieghere *et al.*, 2004]. One of these new non-thermal methods of food preservation, *i.e.* pulsed electric fields (PEF), was described in the present paper.

### **PULSED ELECTRIC FIELDS (PEF)**

#### **Idea of PEF treatment**

The application of pulsed electric fields (PEF) seems to be an alternative and profitable non-thermal method of food preservation. High intensity Pulsed Electric Fields (PEF) processing involves the application of pulses of high voltage (typically 20 - 80 kV/cm) to foods placed between 2 electrodes. PEF treatment is conducted at ambient, sub-ambient, or slightly above ambient temperature for less than 1 s (in the range of microseconds), and energy loss due to heating of foods is minimized. The effect of PEF is related to the application of high voltage for very short periods of time (in the range of microseconds). Exposure of bacterial cells to the field changes of the sufficient amplitude affects the electrical properties of the cell membrane, reflected in a decrease in its resistance and an increase in conductance. Consequently permeability of the membrane is altered, which is known as electroporation [Knorr *et al.*, 2001; Heinz *et al.*, 2002]. That method is usually applied to liquid foods like orange juice, liquid whole egg, milk, yoghurt. PEF preservation of liquid food helps also to extend the shelf-life of a product. Some important aspects in pulsed electric field technology are the generation of high electric field intensities, the design of chambers that impart uniform treatment to foods with minimum increase in temperature, and the design of electrodes that minimize the effect of electrolysis. The large field intensities are achieved through storing a large amount of energy in a capacitor bank (a series of capacitors) from a DC power supply, which is

then discharged in the form of high voltage pulses [Zhang *et al.*, 1995]. Studies on energy requirements have concluded that PEF is an energy-efficient process compared to thermal pasteurization, particularly when a continuous system is used [Qin *et al.*, 1995a]. For food quality attributes, PEF technology is considered superior to traditional heat treatment of foods because it avoids or greatly reduces the detrimental changes of the sensory and physical properties of foods [Quass 1997].

#### Processing of food using PEF

A continuous flow diagram for PEF processing of foods designed by [Qin *et al.*, 1995] is illustrated in Fig. 1. The test apparatus consists of 5 major components: a high-voltage power supply, an energy storage capacitor, a treatment chamber(s), a pump to conduct food through the treatment chamber(s), a cooling device, voltage, current, temperature measurement devices, and a computer to control operations.

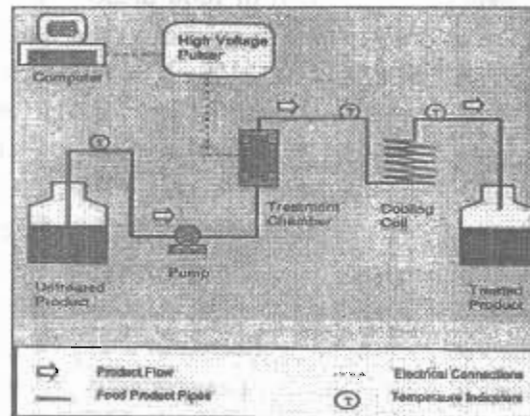
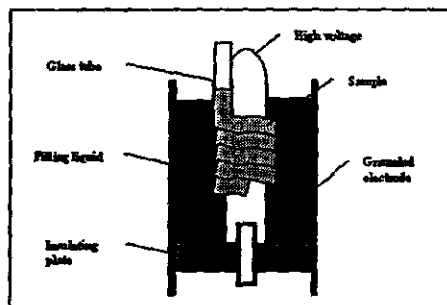


Fig. 1. Continuous PEF Process for Liquid Foods [Qin *et al.*, 1995]

The large field intensities are achieved through storing a large amount of energy in a capacitor bank (a series of capacitors) from a DC power supply, which is then discharged in the form of high voltage pulses [Zhang *et al.*, 1995].

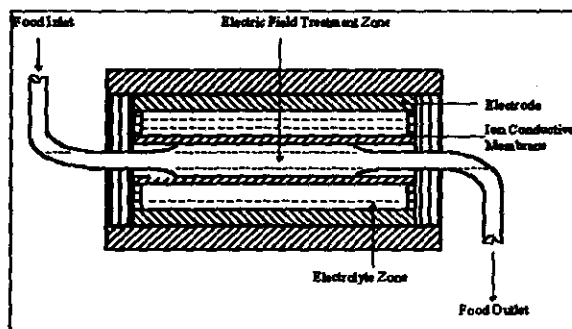
A static treatment chamber designed by [Lubicki and Jayaram 1997] is illustrated in Fig. 2 uses a glass coil surrounding the anode. The volume of the chamber was  $20 \text{ cm}^3$ , which requires a filling liquid with high conductivity and similar permittivity to the sample (media NaCl solution,  $\sigma = 0.8$  to  $1.3 \text{ S/m}$ , filling liquid water  $\sim 10^{-3} \text{ S/m}$ ) used because there is

no inactivation with a non-conductive medium (that is, transformer silicon oil).



**Fig. 2. Static chamber with glass coil surrounding the anode**  
**[Lubicki and Jayaram 1997]**

A continuous chamber with ion conductive membrane designed by [Dunn and Pearlman 1987] is illustrated in Fig. 3 consists of 2 parallel plate electrodes and a dielectric spacer insulator. The electrodes are separated from the food by conductive membranes made of sulfonated polystyrene and acrylic acid copolymers. An electrolyte is used to facilitate electrical conduction between electrodes and ion permeable membranes.



**Fig. 3. Continuous-treatment chamber with ion-conductive membranes separating the electrode and food**  
**[Dunn and Pearlman 1987]**

PEF may be applied in the form of exponentially decaying, square wave, bipolar, or oscillatory pulses. An exponential decay voltage wave is a unidirectional voltage that rises rapidly to a maximum value and decays slowly to zero. The circuit in Fig. 4 may be used to generate an exponential decay waveform. A DC power supply charges a capacitor

bank connected in series with a charging resistor ( $R_s$ ). When a trigger signal is applied, the charge stored in the capacitor flows through the food in the treatment chamber.

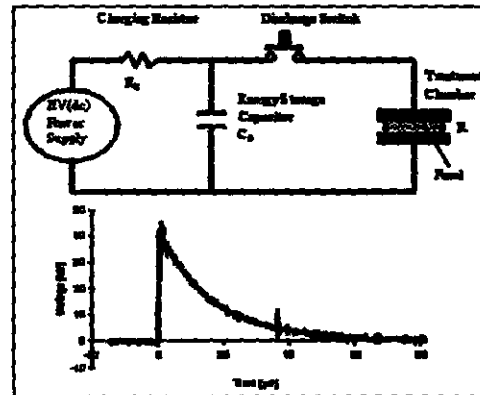


Fig. 4. Electrical circuit for the production of exponential decay waveforms [U.S.FDA 2000]

Square pulse waveforms are more lethal and more energy efficient than exponential decaying pulses. A square waveform can be obtained by using a pulse-forming network (PFN) consisting of an array of capacitors and inductors and solid state switching devices Fig. 5.

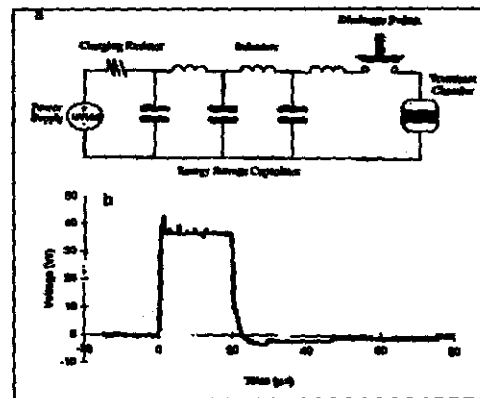


Fig. 5. Square pulse generator using a pulse-forming network of 3 capacitors inductor units and a voltage trace across the treatment chamber [U.S.FDA 2000]

### Mechanisms of microbial inactivation

Two mechanisms have been proposed as the mode of action of PEF on microorganisms: electrical breakdown and electroporation.

#### Electrical breakdown

Zimmermann (1986), as shown in Fig. 6, explains what electrical breakdown of cell membrane entails. The membrane can be considered as a capacitor filled with a dielectric (a). The normal resisting potential difference across the membrane  $V_m$  is 10 mV and leads to the build-up of a membrane potential difference  $V$  due to charge separation across the membrane.  $V$  is proportional to the field strength  $E$  and radius of the cell. The increase in the membrane potential leads to reduction in the cell membrane thickness. Breakdown of the membrane occurs if the critical breakdown voltage  $V_c$  (on the order of 1 V) is reached by a further increase in the external field strength (c). It is assumed that breakdown causes the formation of transmembrane pores (filled with conductive solution), which leads to an immediate discharge at the membrane and thus decomposition of the membrane. Breakdown is reversible if the product pores are small in relation to the total membrane surface. Above critical field strengths and with long exposure times, larger areas of the membrane are subjected to breakdown (d). If the size and number of pores become large in relation to the total membrane surface, reversible breakdown turns into irreversible breakdown, which is associated with mechanical destruction of the cell membrane.

The corresponding electric field is  $E_{\text{critical}} = V_{\text{critical}} / f a$ , where  $a$  is the radius of the cell and  $f$  is a form factor that depends on the shape of the cell. For a spherical cell,  $f$  is 1.5; for cylindrical cells of length  $l$  and hemispheres of diameter  $d$  at each end, the form factor is  $f = l(l - d)/3$ . Typical values of  $V_{\text{critical}}$  required for the lysing of *E. coli* are on the order of 1 V. The critical field strength for the lysing of bacteria with a dimension of approximately 1  $\mu\text{m}$  and critical voltage of 1 V across the cell membrane is therefore on the order of 10 kV/cm for pulses of 10 microsecond to millisecond duration [Schoenbach *et al.*, 1997].

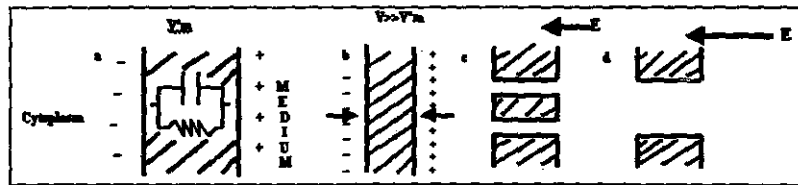


Fig. 6. Schematic diagram of reversible and irreversible breakdown. (a) cell membrane with potential  $V_m$ , (b) membrane compression, (c) pore formation with reversible breakdown, (d) large area of the membrane subjected to irreversible breakdown with large pores

[Zimmermann, 1986]

### Electroporation

Exposure of bacterial cells to the field changes of the sufficient amplitude affects the electrical properties of the cell membrane, reflected in a decrease in its resistance and an increase in conductance. Consequently permeability of the membrane is altered, which is known as electroporation [Knorr *et al.*, 2001; Heinz *et al.*, 2002].

Electroporation is the phenomenon in which a cell exposed to high voltage electric field pulses temporarily destabilizes the lipid bilayer and proteins of cell membranes [Castro and others 1993]. The plasma membranes of cells become permeable to small molecules after being exposed to an electric field, and permeation then causes swelling and eventual rupture of the cell membrane Fig. 7 [Vega- Mercado *et al.*, 1997]. The main effect of an electric field on a microorganism cell is to increase membrane permeability due to membrane compression and poration [Vega- Mercado *et al.*, 1997]. Kinoshita and Tsong (1977; 1979) demonstrated that an electric field of 2.2 kV/cm induced pores in human erythrocytes of approximately 1 nm in diameter. Kinoshita and Tsong (1977) suggested a 2-step mechanism for pore formation in which the initial perforation is a response to an electrical suprathreshold potential followed by a time-dependent expansion of the pore size (Fig. 7). Large pores are obtained by increasing the intensity of the electric field and pulse duration or reducing the ionic strength of the medium.

In a lipid model vesicle (liposome), the electrophoretic movement of ions and water dipoles through the spontaneous hydrophobic pores is postulated to be the first event of electroporation, after which lipid

molecules rearrange to form more stable hydrophilic pores. This could also take place in a cell membrane. In addition, protein channels, pores, and pumps in these membranes are extremely sensitive to transmembrane electric field and become initiation sites for the electropores [Tsong 1990]. In the cell membrane charges to electric dipoles of lipids, proteins, carbohydrates, and ions and the polarizability of these molecules make up the electric field. Therefore, electroporation occurs both in the liposomes and cell membranes, but the molecules affected by the applied field are not necessarily the same in these 2 systems [Tsong 1990]. The gating potentials to the channel constituted by the proteins are in the 50 - mV range [Castro *et al.*, 1993].

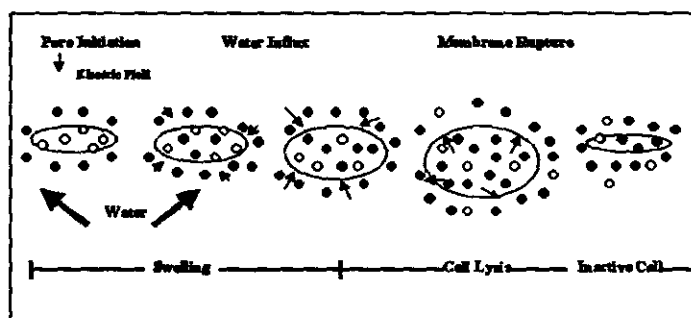


Fig. 7. Electroporation of a cell membrane  
[Vega-Mercado, 1997]

#### Factors affecting microbial inactivation

Factors affecting microbial inactivation described below have been provided by San Martin *et al.* [2003]. The microbial inactivation is determined by three main factors, *i.e.* electrical treatment, microorganism and suspending medium. Factors dependent on the electrical treatment are PEF pulse waveshape, electric field strength and treatment time. The correlation between the microbial inactivation and electric field strength is simply: the higher the electric field strength, the higher the inactivation achieved [Grahel & Märkl, 1996]. Treatment time is equal to the number of pulses applied times the pulse width. In general, the microbial inactivation increases with an increasing number of pulses, but the significant heating of products is likely to occur. Factors dependent on

the microorganism are cell size, growth stage, microbial concentration and the presence of spores. Larger microbial cells will require less intense field strengths to undergo an equivalent inactivation as compared to smaller cells. Cells in the exponential growth phase are more sensitive to PEF treatment than the cells in lag or stationary phase [Alvarez, 2000]. Although PEF treatment is rather not an effective method for spore inactivation there are some research indicating that spores may be inactivated by PEF and that the type of pulse may also play a key role [Marquez, 1997]. Factors dependent on the suspending medium are temperature, pH, ionic strength, conductivity and medium composition. To achieve the same amount of inactivation, lower electric field strengths are needed at higher temperatures. The effects of pH, ionic strength and conductivity should be taken into consideration when selecting a suspending medium, but these aspects need to be further investigated. There is no general agreement about medium composition and PEF treatment, nevertheless certain components of food, such as protein or lipids, may have a protective effect over microorganisms [Barsotti & Cheftel, 1999].

#### **Effectiveness of PEF treatment in food preservation**

The effectiveness of PEF treatment depends on many previously described factors. Authors report the effectiveness of PEF for specified processing conditions. Below there are described some examples for that aspect of food preservation. The inactivation of *Escherichia coli* by PEF was studied in liquid, solid and semisolid foods or model systems by Manas *et al.* [2001] where it was found that agitation of the inoculated liquid samples (16 mmol/L sodium phosphate buffer) during pulse processing resulted in efficient microbial inactivation – 5 log cycles at 33 kV/cm and about 25°C after 261 µs of cumulated pulses. The highest extent of *Listeria innocua* inactivation in liquid

whole egg (LWE) was 3.5 log cycles for an electric field intensity of 50 kV/cm, 32 pulses and total time duration of 64 µs [Calderon-Miranda, 1999]. The presence of 37 IU nisin/ ml in LWE under the same PEF

conditions resulted in a better effectiveness of *Listeria innocua* inactivation (4.4 log cycles). It points to better results achieved upon the application of combined methods for food preservation, which was confirmed in the next studies where liquid whole egg (LWE) with 0.15% addition of citric acid and after PEF treatment ( $E=30$  kV/cm,  $t=489$  ms,  $W=6331$  J/ml) had the shelf-life of 20 days at 4°C. The same LWE but with 0.50% addition of citric acid and after a slightly different PEF treatment ( $E=30$  kV/cm,  $t=55$  ms,  $W=357$  J/ml) was characterized by 30-day shelf-life at a temperature of 4°C [Gongora-Nieto *et al.*, 2003]. The above examples indicate that a skilful combination of preservation methods results in a safer product with extended shelf-life. Although some studies have concluded that PEF preserves the nutritional components of the food, effects of PEF on the chemical and nutritional aspects of specific foods should be better recognized [Qin *et al.*, 1995]. The effectiveness of PEF treatment on different microorganisms and food systems was shown in Table 1 [Vega-Mercado *et al.*, 1997]. The maximum microbial reduction of the presented examples was 6 log cycles for *Escherichia coli* in liquid whole egg. We have also evaluated the efficiency of pulsed electric field (PEF) against *Escherichia coli* contaminating the liquid whole egg (LWE) in our studies [Malicki *et al.*, 2004]. The samples of LWE were inoculated with the test bacteria and subsequently treated for 30  $\mu$ s by the different number of pulses (20-180) of PEF (32.89 kV  $\times$  cm<sup>-1</sup>). The application of PEF resulted in a statistically significant reduction of the test microorganisms, proportional to the number of pulses used. Depending on the studied strain, the treatment with 150-160 PEF pulses was required to obtain the reduction of initial bacteria level by 4 log units. Considering the obtained results, PEF seems to be an effective technique that improves the microbiological status of LWE. Its industrial application is, therefore, highly advisable. Moreover, we have studied functional and rheological properties of LWE after the PEF treatment [Oziębłowski *et al.*, 2005].

**Table 1.** Parameters of PEF and effectiveness of microbial inactivation in different liquid food products [Vega-Mercado, 1997].

Product	PEF parameters				Effectiveness of microbial inactivation	
	Electric field strength E (kV/cm)	Total time t (s)	Number of pulses n (s)	Temperature of product after PEF treatment T (°C)	Microflora	log cycles (D (or description))
Orange juice	6.7	20	5	43-50	<i>Saccharomyces cerevisiae</i>	5 D
Milk	28.6	100	23	42.8	<i>Escherichia coli</i>	3 D
Milk	55.7	100	40	63	<i>Salmonella typhimurium</i>	3 D
Milk	21	20	20	45-50	<i>Lactobacillus thermophilus</i>	4.6 D
Yoghurt	23.38	100	20	63	<i>Lactobacillus acidophilus</i> <i>Streptococcus thermophilus</i>	2 D
Liquid whole egg	25.8	4	100	37	<i>Escherichia coli</i>	6 D
Skim milk	45	1.8-6	64	35	<i>Escherichia coli</i>	2 D
Fluid food	12-25	1-100	25	45-55	Natural	Shelf life extended from 5 to 7 days

LWE was obtained from eggs of 27-week-old layer hens, Tetra SL. Parameters of the treatment were chosen according to earlier experimental results where a significant reduction of microflora was observed at 32.89 kV/cm using 20, 60, 100 pulses. It was concluded that the functional properties of liquid whole egg after PEF were not worse than those of the control sample. Furthermore, foam ability and emulsifying capacity of LWE after PEF were significantly better with an increasing number of impulses compared to the control sample. Viscosity of LWE at a shear rate 250 (1/s) was higher after the PEF treatment: 112 mPa s (20 pulses) and 106 mPa s (100 pulses). For the control sample, the apparent viscosity was 102 mPa s. The results obtained indicate that pulsed electric fields could be useful in the preservation of the liquid whole egg, especially in the context of functional and rheological properties. It is obvious, however, that the sufficient improvement of microbiological safety was the main assessment criterion of that method.

### CURRENT STUDY

A laboratory size prototype PEF treatment system shown in Fig. 8 was developed and constructed at Food Technology Research Institute. The test apparatus in the batch system consists of 3 major components: high voltage generator, pulse shaper and triggering unit and static treatment chamber.

The high voltage generator contains 8 groups of 10 kV each with a total capacity of 80 kV, each group is assembled from 40 capacitors (400V 480N) shown in Fig. 9 and 40 diodes (1000V 6A) shown in Fig. 10 connected together as shown in Fig. 11 for generating a square pulses in the pulse shaper and triggering unit of 2  $\mu$ s at least. The eight groups are connected using high voltage reed switch. The generator was developed to obtain higher volt and power factor.

The treatment chamber shown in Fig. 12 is made as designed by [Dunn and Pearlman, 1987] consists of 2 electrodes of different materials (stainless steel, copper and aluminum) and a cylindrical teflon spacer. The chamber is 1 – 3 cm high with an inner diameter of 10 cm and the electrode area is 78 cm<sup>2</sup>. The electrodes are polished to mirror surface.



Fig. 8. laboratory size prototype PEF treatment system



Fig. 9. Capacitors

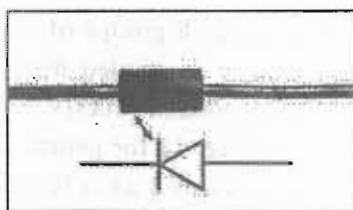


Fig. 10. Diodes

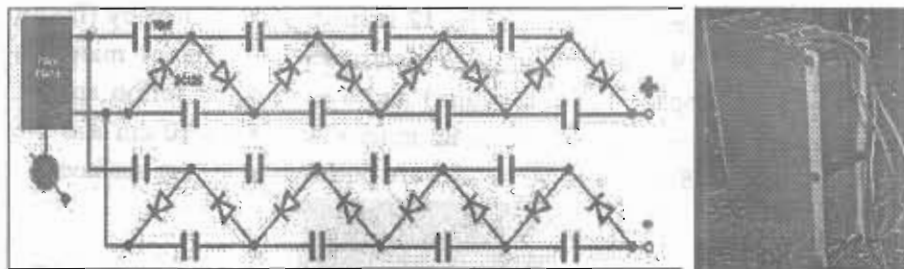


Fig. 11. Groups of capacitors and diodes

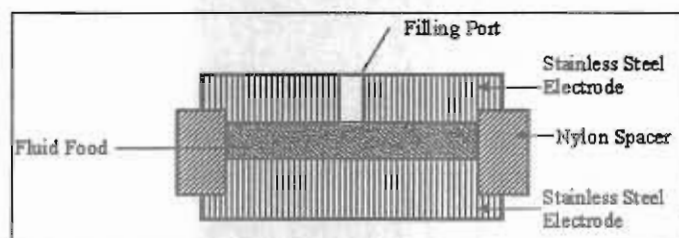


Fig. 12. Static treatment chamber

[Dunn and Pearlman, 1987]

The apparatus was constructed for applying the study of the effect of pulsed electric fields (PEF) as a non-thermal method of food preservation on different liquid foods (milk and juices) against different types of microorganisms using different voltages up to 80 kV/cm) with square pulses of (2, 3 and 4  $\mu$ s) in a static treatment chamber with different volumes and different electrodes materials (stainless steel, copper and aluminum).

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#### الملخص العربي

#### إستخدام المجالات الكهربائية عالية الشدة ذات النبضات كأحد طرق الحفظ الغير تقليدية الغير حرارية للأغذية المسائلة

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تجري عمليات الحفظ التقليدية للأغذية المسائلة بالمسترة بإستخدام المعاملات الحرارية بهدف إطالة فترات الحفظ عن طريق تثبيط عمل الإنزيمات و القضاء على الكائنات الحية الدقيقة و الميكروبات المسببة للأمراض أو للفساد ، حيث يتم تعريض المادة الغذائية إلى درجة حرارة مرتفعة لفترة زمنية محددة الأمر الذي يؤدي إلى حدوث تغيرات بالخصائص الحسية و القيمة الغذائية.

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و قد أدى تزايد الطلب على المنتجات الغذائية ذات القيمة الغذائية المرتفعة إلى تحفيز البحث عن طرق غير تقليدية للحفاظ لا تؤثر على القيمة الغذائية و الحسية .

و يعتبر أسلوب الحفظ باستخدام المجالات الكهربائية عالية الشدة ذات النبضات احد تقنيات الحفظ الحديثة الغير حرارية ، حيث يتم القضاء على الكائنات الحية الدقيقة و الميكروبات المسببة للأمراض و الفساد دون التأثير على القيمة الغذائية و الخصائص الحسية مثل اللون و النكهة .

و يتم معاملة الأغذية المسائلة بالمجالات الكهربائية عن طريق تعريض المادة الغذائية إلى فرق جهد مرتفع بالمرور خلال قطبين كهربيين عند درجة حرارة الجو المحيط أو أعلى قليلاً ، و يرجع تأثير استخدام هذا النظام في القضاء على الكائنات الحية الدقيقة إلى حدوث تغيرات بالأغذية الخلوية و حدوث انهيار لتلك الخلايا .

و الهدف من هذه الدراسة هو تطوير و تصنيع جهاز يعمل بتقنية المجالات الكهربائية عالية الشدة ذات النبضات لبسترة الأغذية المسائلة دون رفع درجة حرارتها للحفاظ على القيمة الحسية و الغذائية مع تحقيق معدل أمان مرتفع ، حيث تم تصنيع وحدة للبسترة باستخدام المجالات الكهربائية بمعهد بحوث تكنولوجيا الأغذية تتألف من مولد نبضات ذي فرق جهد مرتفع (٢٠ - ٨٠ كيلوفولت) و غرفة معالجة حيث يتم تعريض المواد للمجالات الكهربائية عالية الشدة ذات نبضات مربعة لأزمنة تبدأ من (٢) ميكروثانية.