Non-thermal food preservation: Pulsed electric fields

Humberto Vega-Mercado, Olga Martín-Belloso, Bai-Lin Qin, Fu Jung Chang, M. Marcela Góngora-Nieto, Gustavo V. Barbosa-Cánovas and Barry G. Swanson

The use of electric discharges to inactivate microorganisms and enzymes in food products has evolved since the 1920s from the 'ElectroPure process' (ohmic heating process) to the use of high-intensity pulsed electric fields in the 1990s. The non-thermal inactivation of microorganisms and enzymes using electric fields was demonstrated in the 1960s with a variety of microorganisms suspended in simulated food systems. A variety of liquid foods and beverages, including orange, apple and peach juices, pea soup, beaten eggs and skim milk, has been successfully processed during the 1980s and 1990s by several research groups. Little by little, the food industry is demonstrating increasing interest in this promising emerging technology; furthermore, it is expected that it will soon be adopted to process several liquid food products.

Non-thermal processes have gained importance in recent years as a potential technology to replace or complement the traditional thermal processing of foods. Compared with thermal processing, non-thermal processes offer the advantages of low processing temperatures, low energy utilization and the retention of flavors, nutrients and a fresh-like taste, while inactivating the spoilage microorganisms and enzymes.

The inactivation of microorganisms and enzymes contained in food products using electric discharges started in the 1920s with the 'ElectroPure process' for milk[1]; this involves heating the milk to a temperature of 70°C, and then passing it through carbon electrodes in an electric heating chamber, to inactivate *Mycobacterium tuberculosis* and *Escherichia coli*. Beattie and Lewis (1925)[2] demonstrated the lethal effect of electric discharges on microorganisms when the applied voltage used to treat food was 3000–4000 V. The 'Electrohydraulic treatment' was introduced in the 1950s to inactivate microorganisms suspended in liquid foods. The inactivation of microorganisms was attributed to a shock wave generated by an electric arc that prompted the formation of highly reactive free radicals from chemical species in food[3]. In addition, pulsed electric field (PEF) treatment may induce oxidation and reduction reactions, such as those proposed by Gilliland and Speck (1967)[4], within the cell structure. Gilliland and Speck[4] applied pulsed electric discharges at different energy levels to inactivate *E. coli*, *Enterococcus faecalis*, *Bacillus subtilis*, *Streptococcus cremoris* and *Micrococcus radiodurans* suspended in sterile distilled water, as well as the enzyme trypsin and a protease from *B. subtilis*.

Sale and Hamilton (1967)[5] demonstrated the non-thermal lethal effect of homogeneous electric fields on bacteria such as *E. coli*, *Staphylococcus aureus*, *Micrococcus lysodeikticus*, *Sarcina lutea*, *B. subtilis*, *Bacillus cereus*, *Bacillus megaterium* and *Clostridium perfringens*, and on yeasts such as *Saccharomyces cerevisiae* and *Candida utilis*. In general, an increase in the electric field intensity and/or number of pulses was found to increase the inactivation of microorganisms. Other factors that influence microbial inactivation by pulsed electric fields are the treatment temperature, the pH, the ionic strength, and the conductivity of the medium containing the microorganisms[6–12].

The formation of pores in cell membranes by high-intensity pulsed electric fields (HIPEF) is not entirely understood. Zimmermann *et al.* (1974)[13], applying the dielectric rupture theory, concluded that when the transmembrane potential is higher than ~1 V (which is the natural potential of the cell membrane) the membrane will rupture.

The reversible or irreversible rupture or poration of a cell membrane depends on factors such as the intensity of the electric field, the number of pulses and the duration of the pulses[14–17]. The plasma membranes of cells become permeable to small molecules after being exposed to an electric field; permeation then causes swelling and the eventual rupture of the cell membrane (Fig. 1).

Although many sectors of the food industry are considering the application of this technology primarily to process liquid foods, none of the interested sectors is currently using pulsed electric fields in its processing lines. However, because prototype equipment is available, it is expected that very soon some fruit juices, and also liquid eggs, will be industrially processed by this technology.

High-intensity PEF technology and food processing

Microbial inactivation

PurePulse Technologies Co., a subsidiary of Maxwell Laboratories in San Diego, CA, USA, owns three US patents that are concerned with the preservation of fluid

Humberto Vega-Mercado, Olga Martín-Belloso, Bai-Lin Qin, Fu Jung Chang, M. Marcela Góngora-Nieto and **Gustavo V. Barbosa-Cánovas** (corresponding author) are in the Department of Biological Systems Engineering, and **Barry G. Swanson** is in the Department of Food Science and Human Nutrition, Washington State University, Pullman, WA 99164-6120, USA (fax: +1-509-335-2722; e-mail: barbosa@mail.wsu.edu).

 0924-2244/97/$17.00
PII: S0924-2244(97)01016-9

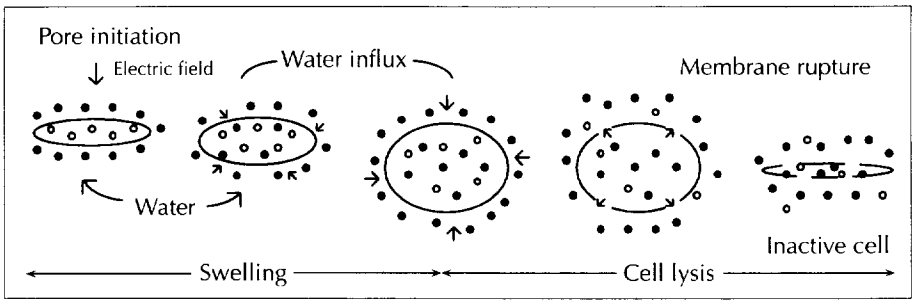

Fig. 1
Mechanism of cell inactivation[16].

foods such as dairy products, fruit juices and liquid eggs by treatment with an electric field strength in the range of 12–25 kV/cm for 1–100 μs[18–20]. The patents describe both a batch and a continuous processing system, using pulsed electric fields, and recommend that these treatment methods are applied to pre-heated liquid foods, to increase both the total log colony-forming unit (cfu) reduction and the shelf-life stability. The lethal effects observed during PEF treatments were remarkably higher than those achieved in a parallel test using just thermal pasteurization. The PEF process is a safe one because no dangerous chemical reactions have been detected; moreover, it is reliable because the same results can be obtained repeatedly.

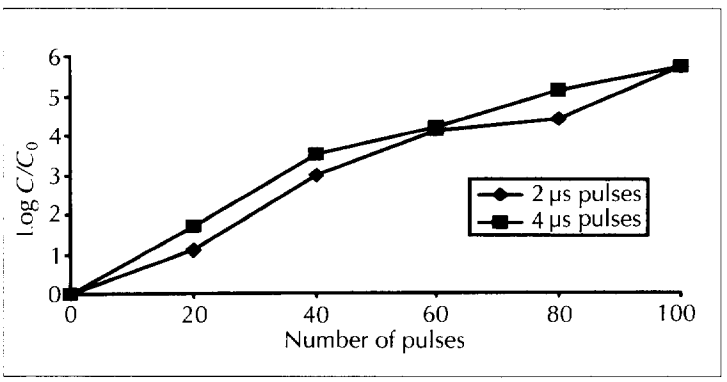

Fig. 2
Inactivation of *Escherichia coli* suspended in liquid egg at 26 kV/cm and 37°C using 2 μs and 4 μs pulses. [Log (C/C_0) = plate count after electric pulse treatment (colony-forming units/ml) / plate count before electric pulse treatment (colony-forming units/ml)]. Data taken from Ref. 28.

Dunn and Pearlman (1987)[18] reported a reduction of more than five log cycles (5D reduction) in the microbial count of naturally occurring microorganisms in orange juice after 35 pulses of 100 μs at a voltage intensity of 33.6–35.7 kV/cm and a process temperature of 42–65°C. The shelf life of the orange juice was increased from 3 d to 1 week with no significant change in odor or taste. A 3D reduction was also reported for *E. coli* (ATTC-10536) inoculated in homogenized and pasteurized milk that was exposed to 23 pulses of 100 μs at 28.6–42.8 kV/cm[18]. When a similar test run was carried out using milk that had been seeded with *Salmonella dublin* before treatment with 40 pulses of 100 μs at 36.7 kV/cm and 63°C, no *Salmonella*, and only 20 cfu/ml of milk bacteria, were found. These results may suggest that microbial deactivation by the PEF treatment process is selective and that *S. dublin* are preferentially deactivated over the milk bacteria. Yoghurt inoculated with *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and *S. cerevisiae* was treated with 20 pulses of 100 μs at 23–38 kV/cm and a process temperature of 63°C; treatment resulted in a 2D reduction of the lactic acid bacteria and *S. cerevisiae*[18].

The 'Elsteril process', which was developed by Krupp Maschinentechnik GmbH (Hamburg, Germany) during the late 1980s and early 1990s, has been used for the sterilization and pasteurization of liquid foods and other liquids[21–23]. Krupp Maschinentechnik GmbH, in association with the University of Hamburg at Harburg, reported microbial inactivation when PEF treatment was applied to fluid foods such as orange juice and milk[22]. Microbial reduction exceeding 4D has been observed for: *Lactobacillus brevis* inoculated in milk and treated with 20 pulses of 20 μs at 22 kV/cm, *S. cerevisiae* inoculated in orange juice and treated with 5 pulses of 20 μs at 6.7 kV/cm, and *E. coli* inoculated in sodium alginate and treated with 5 pulses of 20 μs at 26 kV/cm[21,22]. However, inactivation of the

Table 1. Pulsed electric field inactivation of a mixture of *Escherichia coli* and *Bacillus subtilis* suspended in pea soup[a]

		Field strength: 28 kV/cm		Field strength: 30 kV/cm	
Flow rate; frequency	Number of pulses	Process temperature (°C)	Log reduction (D)	Process temperature (°C)	Log reduction (D)
0.5 l/min; 4.3 Hz	15	43	0.7	55	2.3
	30	39	1.6	55	4.0
0.75 l/min; 6.7 Hz	15	41	0.7	53	4.4
	30	41	0.7	55	4.8
0.75 l/min; 4.3 Hz	10	32	0.8	41	1.1
	20	31	1.0	42	1.0

[a]Data taken from Ref. 29

endospores of *B. cereus* or the ascospores of *Bacillus nivea* has not been reported[22]. A substantial reduction in both ascorbic acid content and lipase activity was observed in milk that had been treated with the 'Elsteril process'[21]. The taste of both the milk and the orange juice had not changed significantly following the electric field treatments[22]. These findings are relevant to the shelf life and quality of the milk. First, rancid odors and flavors would not be produced by the oxidation (mediated by lipases) of the butyric fat content. Second, because milk is not considered to be a source of vitamin C, its inactivation does not diminish the quality of the milk. Finally, the insignificant change in taste is extremely important because today's consumers prefer 'fresh' flavors.

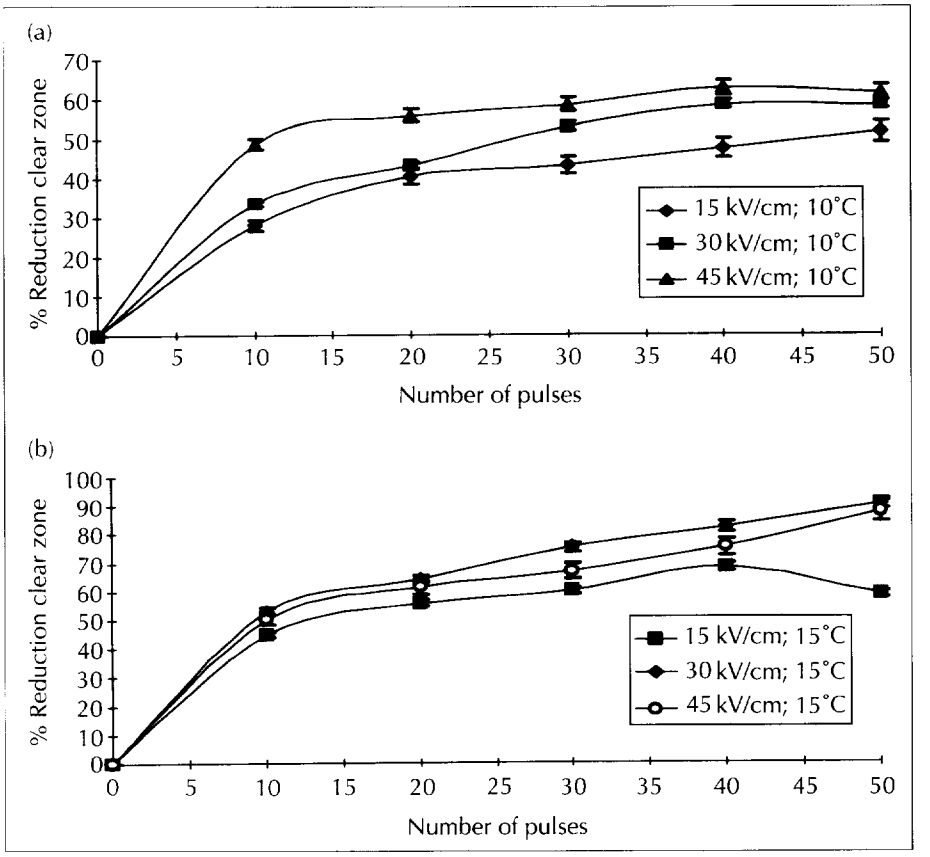

Fig. 3
Pulsed electric field inactivation of plasmin (EC 3.4.21.7) from bovine plasma, suspended in diluted simulated skim milk at (a) 10°C and (b) 15°C, under different field strengths (data taken from Ref. 30).

The Washington State University, Pullman, WA, USA, holds a patent for the design and development of a static PEF chamber and has filed a patent disclosure for the design and development of a continuous PEF chamber intended for processing liquid foods with PEF treatments[24–26]. The static chamber has been used to test the feasibility of the treatment process and its suitability for homogeneous solid media, such as gels.

Raw peach juice, skim milk, beaten eggs, pea soup, apple juice and reconstituted apple juice, exposed to pulsed electric fields of 25–45 kV/cm, were treated using these chambers. A 2D reduction was observed for *E. coli* inoculated in skim milk and exposed to 64 pulses of 2 μs at 45 kV/cm and 35°C[27]. The pulse duration in an exponential-decay wave is the time that corresponds to a 37% reduction of peak voltage. A reduction of 6D was observed for liquid egg inoculated with *E. coli* and treated with an electric field of 25.8 kV/cm, 100 pulses of 4 μs, at 37°C (Fig. 2)[28]. A pulse duration of 2–4 μs has been found to be the most effective range for inactivation, but there is no clear explanation for this as yet.

Only a limited inactivation (<1.5D) was observed for *E. coli* and *B. subtilis* inoculated in pea soup and exposed to pulsed electric fields of 25–33 kV/cm (10–30 pulses of 2 μs) when the process temperature of the pea soup was <53°C, whereas microbial reduction was ~4.4D when process temperatures were in the range 53–55°C (Table 1)[29]. The selected combinations of flow rate and pulse frequency, shown in Table 1, were established in order to give the food product a particular dose, without causing too great an increase in the temperature, and thus assuring non-thermal treatment. It has been observed that the higher the field strength (kV/cm), the greater the number of pulses (n), the higher the pulse frequency (Hz) and the lower the flow rate (l/min), the higher the process temperature, because the PEF process, itself, heats up the food. Thus, a cooling system is needed to keep the temperature down.

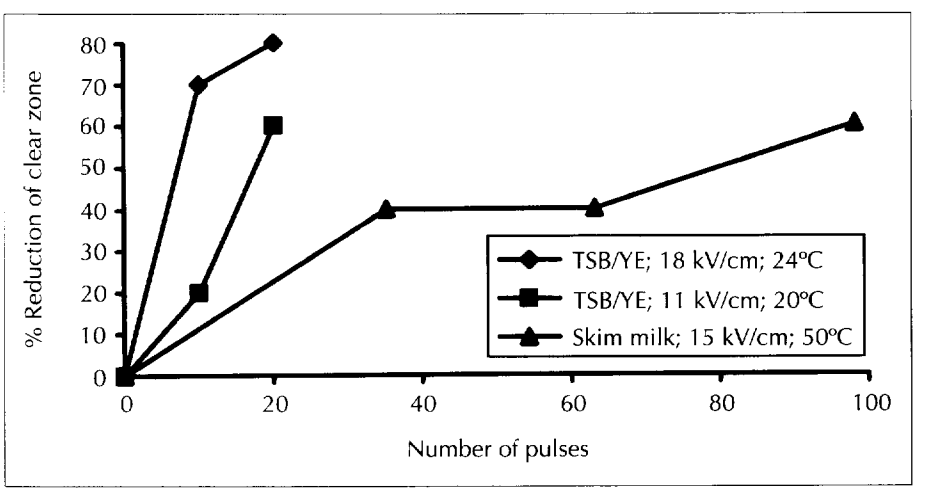

Fig. 4
Reduction of protease activity from *Pseudomonas fluorescens* M3/6 in tryptic soy broth enriched with yeast extract (TSB/YE; pulsing rate of 0.25 Hz) and skim milk (pulsing rate of 2 Hz) using 2-μs pulses (data taken from Ref. 31).

Table 2. Applications of high-intensity pulsed electric fields in food processing

Product	Treatment regime	Inoculate	Maximum inactivation; log reduction (D)	Chamber characteristics	Ref.
Fluid foods	12–25 kV/cm; 45–55°C; 25 pulses; 1–100 μs	Natural microflora	Shelf life extended from 3 d to 1 week	Static chamber; parallel stainless steel electrodes; 2-cm gap; volume of 157 cm^3	18
Orange juice	33.6–35.7 kV/cm; 42–65°C; 35 pulses; 1–100 μs	Natural microflora	Shelf life extended from 3 d to 1 week (5D)	Static chamber; parallel stainless steel electrodes; 2-cm gap; volume of 157 cm^3	18
Orange juice	6.7 kV/cm; 45–50°C; 5 pulses; 20 μs	*Saccharomyces cerevisiae*	Almost 5D	Static chamber; parallel carbon electrodes; 0.5-cm gap; volume of 25 cm^3	22
Milk	28.6 kV/cm; 42.8°C; 23 pulses; 100 μs	*Escherichia coli*	3D	Static chamber; parallel stainless steel electrodes; 2-cm gap; volume of 157 cm^3	18
Milk	36.7 kV/cm; 63°C; 40 pulses; 100 μs	*Salmonella dublin*	3D, resulting in 0 cfu/ml *Salmonella dublin* after treatment	Static chamber; parallel stainless steel electrodes; 2-cm gap; volume of 157 cm^3	18
Milk	22 kV/cm; 45–50°C; 20 pulses; 20 μs	*Lactobacillus brevis*	4.6D	Static chamber; parallel carbon electrodes; 0.5-cm gap; volume of 25 cm^3	22
Skim milk	45 kV/cm; 35°C; 64 pulses; 1.8–6 μs	*Escherichia coli*	2D	Static chamber; parallel stainless steel electrodes; 0.51-cm gap; volume of 13.8 cm^3	27
Skim milk	15 kV/cm; 50°C; 98 pulses; 2 μs	Protease extracted from *Pseudomonas fluorescens*	60% RCZ	Continuous chamber; coaxial stainless steel electrodes; 0.6-cm gap; volume of 28.5 cm^3	31
Tryptic soy broth	11–18 kV/cm; 20–24°C; 20 pulses; 2 μs	Protease extracted from *Pseudomonas fluorescens*	80% RCZ	Continuous chamber; coaxial stainless steel electrodes; 0.6-cm gap; volume of 28.5 cm^3	31
Yoghurt	23–38 kV/cm; 63°C; 20 pulses; 100 μs	*Saccharomyces cerevisiae* *Lactobacillus bulgaricus* *Streptococcus thermophilus*	2D	Static chamber; parallel stainless steel electrodes; 2-cm gap; volume of 157 cm^3	18
Liquid egg	25.8 kV/cm; 37°C; 100 pulses; 4 μs	*Escherichia coli*	6D	Continuous chamber; coaxial stainless steel electrodes; 0.6-cm gap; volume of 11.87 cm^3	28
Pea soup	25–33 kV/cm; <53°C; 10–30 pulses; 2 μs	*Escherichia coli* *Bacillus subtilis*	<1.5D	Continuous chamber; coaxial stainless steel electrodes; 0.6-cm gap; volume of 28.6 cm^3	29
Pea soup	25–33 kV/cm; 53–55°C; 10–30 pulses; 2 μs	*Escherichia coli* *Bacillus subtilis*	4.4D	Continuous chamber; coaxial stainless steel electrodes; 0.6-cm gap; volume of 28.6 cm^3	29
Simulated milk ultrafiltrate	30–45 kV/cm; 10–15°C; 10–50 pulses; 2 μs	Plasmin	90% RCZ	Continuous chamber; parallel stainless steel electrodes; 0.6-cm gap; volume of 8 cm^3	30
Sodium alginate	26 kV/cm; 45–50°C; 5 pulses; 20 μs	*Escherichia coli*	>4D	Static chamber; parallel carbon electrodes, 0.5-cm gap; volume of 25 cm^3	22

cfu, Colony-forming units
%RCZ, Percentage reduction in clear zone

Inactivation of enzyme activity

The proteolytic enzyme plasmin and an extracellular protease from *Pseudomonas fluorescens* M3/6 (from culture) were inactivated using pulsed electric fields. The change in the proteolytic activity of plasmin was determined as the change in its ability to hydrolyse casein in a 'Bio-Rad Protease Activity Gel' contained in a petri dish. The enzymatic ability is related to the width of the clear zone in the gel. The reduction in proteolytic activity is estimated as the percentage reduction in clear zone (%RCZ):

$$\%RCZ = (D_{in} - D_{tr})/(D_{in} - D_{well})$$

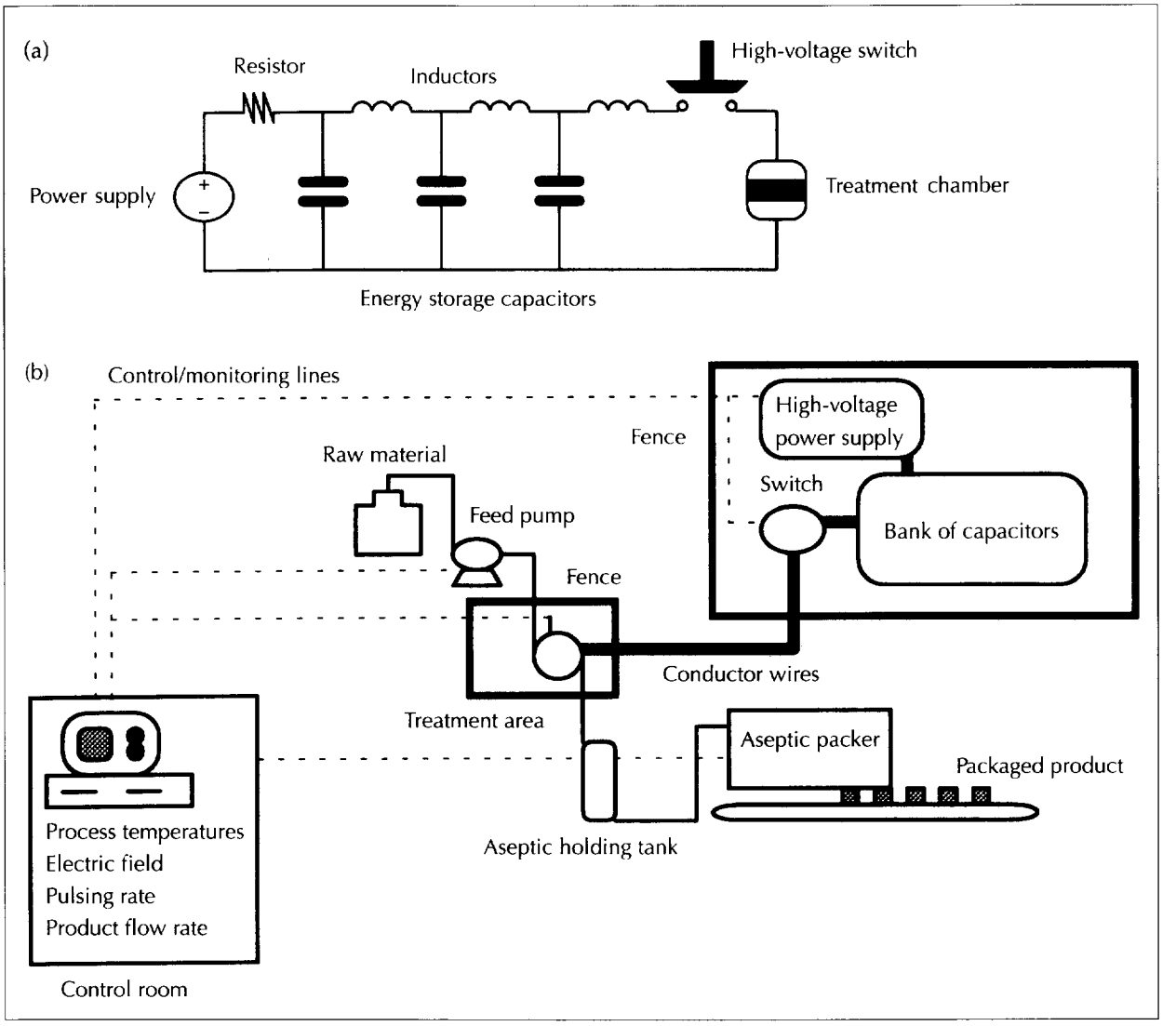

Fig. 5

(a), Layout of a pulse-forming network of three capacitor–inductor units[32]. (b), Schematic diagram of a pulsed electric field equipment configuration[33].

where D_{in} is the clear-zone diameter of a 100 μg/ml plasmin control, D_{tr} is the diameter of the clear zone for treated plasmin after 24 h incubation, and D_{well} is the well diameter. Plasmin activity was reduced by 90% following treatment with 50 pulses of 2 μs at 50 kV/cm and 45 kV/cm, and a process temperature of 15°C[30]; under the same conditions, but at 10°C[30], only a 60% reduction in activity was achieved (Fig. 3). Analysis of variance demonstrated that the number of pulses, the strength of the electric field and the temperature were significant in the inactivation of plasmin, at a confidence level of 0.001. Furthermore, results suggested that these electric factors and the temperature act synergistically in the inactivation of enzymes.

In other studies, 80% reduction (%RCZ) was found for a protease extracted from *P. fluorescens* that was dispersed in tryptic soy broth and exposed to 20 pulses of 2 μs at 11–18 kV/cm and 20–24°C. A 60% reduction (%RCZ) was observed when the protease was inoculated in sterilized skim milk and exposed to 98 pulses of 2 μs at 15 kV/cm and 50°C (Fig. 4); no reduction occurred when the protease was inoculated in a sterilized casein–tris buffer and exposed to a PEF treatment similar to that for skim milk (data not shown). The decreased effectiveness of PEF treatment for the inactivation of the protease in skim milk and the casein–tris buffer is attributed to the substrate (i.e. casein) playing a protective role and thus preventing the enzyme from undergoing conformational changes induced by the electric fields[31].

The susceptibility of casein to proteolysis varies as a function of treatment conditions. A HIPEF treatment of 25 kV/cm at 0.6 Hz and 30°C was found to increase the proteolytic activity in skim milk inoculated with a protease from *P. fluorescens* M3/6. However, 14–15 kV/cm at 1–2 Hz and 30°C had no significant effect on the susceptibility of casein in skim milk to proteolysis, and no significant change was observed in the susceptibility of casein suspended in a casein–tris buffer when exposed to treatment conditions similar to those for skim milk[31]. In conclusion, the pulse frequency, regardless of the number of pulses applied, is another important factor to consider in the study of this process.

Table 2 is a compilation of all of the experimental results already discussed in this article, indicating the food

product, type of microorganism, conditions of PEF treatment, and type of treatment chamber used in each experiment.

Because the technology has not yet been standardized, owing to the fact that the local bactericidal effects of electric shock in the suspension volumes treated are not well understood and the electric field intensity is difficult to assess, the lethal effects measured in tests depend quite specifically on the apparatus used. Hence, any comparison of results between different research groups should be done very carefully, taking into consideration all of the experimental conditions such as electric field strength, number of pulses, wave shape, pulse width and batch, step-wise or continuous circulation processing.

Fat extraction

HIPEF has been used not just for microbial and enzymatic inactivation, but also for fat extraction. The disruption of cell membranes to release fat from animal cells was carried out using the 'Elcrack process' (Krupp Maschinentechnik GmbH). This process consists of exposing a slurry of comminuted fish or slaughterhouse offal to high-intensity electric pulses that break down cells, leading to increased fat recovery during the separation step after it is pumped through one or more treatment chambers[21].

Design and construction of a PEF facility

A typical pulser configuration has a high-voltage power supply to charge the capacitors, and a discharge switch that releases the stored electric energy from the capacitor(s) in the form of an electric field through the product (Fig. 5a). The inductors modify the shape of the pulse (triangular, exponential, square, etc.), while the total capacitance affects the time constant of each pulse as a function of the product conductivity. Exponential-decay pulses are widely used owing to the simplicity of the circuit. Although the circuits are more complex, the square and bell-shaped wave forms are potentially more energy efficient (almost 30%)[32]. Figure 5b is a schematic illustration of a commercial PEF facility, in which the power supply, capacitors and treatment chamber must be confined in a restricted-access area with interlocked gates because of the potential hazard of high voltage.

In this type of facility, the pulser must automatically turn off when the gates are opened for any reason; the emergency switches and discharging bars must be accessible to discharge any element in the circuit before maintenance or inspection can be carried out. To prevent energy leakage through any fluid food or refrigerant that is in contact with the treatment chamber, connections to the chamber must be isolated; the pipes carrying materials to and from the chamber must be connected to the ground.

The design and construction of a PEF facility for food processing requires both state-of-the-art equipment and common sense. Hazard analysis and critical control points (HACCP) principles are key elements in the preparation of a strategy for manufacturing a PEF product[33]. HACCP focuses special attention on product handling, treatment parameters and equipment cleanliness.

The future of PEF technology

The research on pulsed electric fields as a non-thermal process needs to consider not only the inactivation of microorganisms, but also the inactivation of enzymes, the retention of vitamins, and the effects of PEF treatments on other food components. The reported inactivation of enzymes, as well as the increased proteolysis of casein following PEF treatment, should encourage detailed research in other areas besides preservation. Pulsed electric fields can be used as a single technology, as a 'hurdle' in combined methods, or as a complementary step with mild thermal processes.

Acknowledgements

This work was funded by the AASERT Program (US Department of Defense), Natick Research Development and Engineering Center (US Army) and Bonneville Power Administration (US Department of Energy). Olga Martín-Belloso would also like to express appreciation for the support received by NATO (North Atlantic Treaty Organization) during her sabbatical leave at the Washington State University in Pullman.

References

1 Fetterman, J.C. (1928) 'The Electrical Conductivity Method of Processing Milk' in *Agric. Eng.* 9, 107–108

2 Beattie, J.M. and Lewis, F.C. (1925) 'The Electric Current (Apart From the Heat Generated). A Bacteriological Agent in the Sterilization of Milk and Other Fluids' in *J. Hyg.* 24, 123–137

3 Sitzmann, W. (1995) 'High-voltage Pulse Techniques for Food Preservation' in *New Methods of Food Preservation* (Gould, G.W., ed.), pp. 236–252, Blackie

4 Gilliland, S.E. and Speck, M.L. (1967) 'Mechanism of the Bactericidal Action Produced by Electrohydraulic Shock' in *Appl. Microbiol.* 15, 1038–1044

5 Sale, A.J.H. and Hamilton, W.A. (1967) 'Effect of High Electric Fields on Microorganisms. I. Killing of Bacteria and Yeast' in *Biochim. Biophys. Acta* 148, 781–788

6 Jacob, H.E., Foster, W. and Berg, H. (1981) 'Microbial Implication of Electric Field Effects. II. Inactivation of Yeast Cells and Repair of Their Cell Envelope' in *Z. Allg. Mikrobiol.* 21, 225–233

7 Hülsheger, H., Potel, J. and Niemann, E.G. (1983) 'Electric Field Effects on Bacteria and Yeast Cells' in *Radiat. Environ. Biophys.* 22, 149–162

8 Sato, M., Tokita, K., Sadakata, M. and Sakai, T. (1988) 'Sterilization of Microorganisms by High-voltage Pulsed Discharge Under Water' in *Kagaku Hogaku Ronbunshu* 4, 556–557

9 Mizuno, A. and Hori, Y. (1988) 'Destruction of Living Cells by Pulsed High-voltage Application' in *IEEE Trans. Ind. Appl.* 24, 387–394

10 Zhang, Q., Monsalve-González, A., Barbosa-Cánovas, G.V. and Swanson, B.G. (1994) 'Inactivation of *E. coli* and *S. cerevisiae* by Pulsed Electric Fields Under Controlled Temperature Conditions' in *Trans. ASAE* 37, 581–587

11 Zhang, Q., Qin, B.L., Barbosa-Cánovas, G.V. and Swanson, B.G. (1995) 'Inactivation of *E. coli* for Food Pasteurization by High-intensity Short-duration Pulsed Electric Fields' in *J. Food Process. Preserv.* 19, 103–118

12 Vega-Mercado, H., Pothakamury, U.R., Chang, F.J., Barbosa-Cánovas, G.V. and Swanson, B.G. (1997) 'Inactivation of *E. coli* by Combining pH, Ionic Strength and Pulsed Electric Field Hurdles' in *Food Res. Int.* 29(2), 117–121

13 Zimmermann, U., Pilwat, G. and Riemann, F. (1974) 'Dielectric Breakdown on Cell Membranes' in *Biophys. J.* 14, 881–899

14 Benz, R. and Zimmermann, U. (1980) 'Pulse-length Dependence of the Electrical Breakdown in Lipid Bilayer Membranes' in *Biochim. Biophys. Acta* 597, 637–642

15 Knorr, D., Geulen, M., Grahl, T. and Sitzmann, W. (1994) 'Food Application of High Electric Field Pulses' in *Trends Food Sci. Technol.* 5, 71–75

16 Tsong, T.Y. (1990) 'Review on Electroporation of Cell Membranes and Some Related Phenomena' in *Biochem. Bioenerg.* 24, 271–295
17 Tsong, T.Y. (1991) 'Electroporation of Cell Membranes' in *Biophys. J.* 60, 297–306
18 Dunn, J.E. and Pearlman, J.S. (1987) 'Methods and Apparatus for Extending the Shelf-life of Fluid Food Products', United States Patent US 4 695 472
19 Dunn, J.E. *et al.* (1991) 'Methods for Preservation of Foodstuffs', United States Patent US 5 034 235
20 Bushnell, A.H., Dunn, J.E., Clark, R.W. and Pearlman, J.S. (1993) 'High Pulsed Voltage Systems for Extending the Shelf-life of Pumpable Food Products', United States Patent US 5 235 905
21 Sitzmann, W. (1995) 'High-voltage Pulsed Techniques for Food Preservation' in *New Methods of Food Preservation* (Gould, G.W., ed.), pp. 236–252, Blackie
22 Grahl, T., Sitzmann, W. and Märkl, H. (1992) 'Killing of Microorganisms in Fluid Media by High-voltage Pulses' in *10th Dechema Biotechnology Conference Series 5B* (Kreysa, G. and Drisel, X., eds), pp. 675–678, Verlagsgesellschaft, Hamburg, Germany
23 Mertens, B. and Knorr, D. (1992) 'Developments of Nonthermal Processes for Food Preservation' in *Food Technol.* 46, 124–133
24 Martín, O., Zhang, Q., Castro, A.J., Barbosa-Cánovas, G.V. and Swanson, B.G. (1994) 'Pulse Electric Fields of High Voltage to Preserve Foods. Microbiological and Engineering Aspects of the Process' in *Span. J. Food Sci. Technol.* 34, 1–34
25 Zhang, Q., Barbosa-Cánovas, G.V. and Swanson, B.G. (1995) 'Engineering Aspects of Pulsed Electric Field Pasteurization' in *J. Food Eng.* 25(2), 268–281
26 Zhang, Q., Qin, B., Barbosa-Cánovas, G.V., Swanson, B.G. and Pedrow, P.D. (1996) 'Batch Mode Food Treatment Using Pulsed Electric Fields', United States Patent US 5 549 041
27 Martín, O., Qin, B.L., Chang, F.J., Barbosa-Cánovas, G.V. and Swanson, B.G. 'Inactivation of *Escherichia coli* in Skim Milk by High Intensity Pulsed Electric Fields' in *J. Food Eng.* (in press)
28 Martín, O. *et al.* 'Inactivation of *Escherichia coli* Suspended in Liquid Egg Using Pulsed Electric Fields' in *J. Food Process. Preserv.* (in press)
29 Vega-Mercado, H., Martín-Belloso, O., Chang, F.J., Barbosa-Cánovas, G.V. and Swanson, B.G. (1997) 'Inactivation of *Escherichia coli* and *Bacillus subtilis* Suspended in Pea Soup Using Pulsed Electric Fields' in *J. Food Process. Preserv.* 20(6), 501–510
30 Vega-Mercado, H., Powers, J.R., Barbosa-Cánovas, G.V. and Swanson, B.G. (1995) 'Plasmin Inactivation with Pulsed Electric Fields' in *J. Food Sci.* 60, 1150–1154
31 Vega-Mercado, H. *et al.* (1997) 'Effect of Pulsed Electric Fields on the Susceptibility of Proteins to Proteolysis and the Inactivation of an Extracellular Protease From *Pseudomonas fluorescens* M 3/6' in *Engineering and Food ICEF7 (Seventh International Congress on Engineering and Food)* (Jowitt, R., ed.), Part 1, pp. C73–C76
32 Zhang, Q., Monsalve-González, A.M., Qin, B.L., Barbosa-Cánovas, G.V. and Swanson, B.G. (1994) 'Inactivation of *Saccharomyces cerevisiae* in Apple Juice by Square-wave and Exponential-decay Pulsed Electric Fields' in *J. Food Process. Eng.* 17, 469–478
33 Vega-Mercado, H., Luedecke, O.L., Hyde, G.M., Barbosa-Cánovas, G.V. and Swanson, B.G. (1996) 'HACCP and HAZOP for a Pulsed Electric Field Processing Operation' in *Dairy Food Environ. Sanit.* 16(9), 554–560

Review

The use of *in vitro* models for assessing the presence and safety of residues of xenobiotics in food

L.A.P. Hoogenboom and H.A. Kuiper

In order to give a more scientific basis to the health risk assessment of residues of environmental contaminants, agrochemicals and natural toxins in food products, it is important to increase our knowledge of the mechanisms behind the toxic effects of both single and, especially, mixtures of compounds, as well as of actual exposure levels. In addition, it is important to improve the techniques that are currently used to extrapolate animal data to humans by performing proper species comparisons. *In vitro* models offer new opportunities for us to fill in some of these gaps in our knowledge. Furthermore, in view of the ever-increasing number of chemicals that need to be monitored, *in vitro* models can be used as bioassays, thereby detecting compounds or groups of compounds by their biological activity rather than by their physicochemical properties. In this review article, we describe some of the recent advances in this field, including the development of bioassays for environmental contaminants with oestrogenic and dioxin-like activities.

L.A.P. Hoogenboom and **H.A. Kuiper** are at the State Institute for Quality Control of Agricultural Products (RIKILT-DLO), Bornsesteeg 45, 6708 PD Wageningen, The Netherlands (fax: +31-317-417717; e-mail: l.a.p.hoogenboom@rikilt.dlo.nl).

The presence of environmental contaminants or residues of veterinary drugs, pesticides or mycotoxins in the food chain has attracted broad scientific interest from both analytical chemists involved in the development of analytical detection methods, and toxicologists attempting to understand the potential health risks for humans of exposure to such compounds. Based on the fundamental concept formulated centuries ago by the Swiss physician Paracelsus, 'Every compound may be toxic, it is the right dose which differentiates the remedy from its toxicity'[1], the risk assessment of chemicals requires knowledge of exposure levels, and of the toxic potential, that is the

0924-2244/97/$17.00
PII: S0924-2244(97)01025-X