# Pulsed Electric Fields (PEF) for Permeabilization of Cell Membranes in Food- and Bioprocessing – Applications, Process and Equipment Design and Cost Analysis.

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# Zusammenfassung

Der Einfluss gepulster elektrischer Felder (PEF) auf Liposomen und Membranen pflanzlicher, tierischer und mikrobieller Zellen wurde untersucht. Eine Reihe von Pulsmodulatoren und Behandlungszellen wurde realisiert, um den Einfluss unterschiedlicher Anlagen-, Prozess- und Produktparameter zu bewerten. Für pflanzliche und tierische Zellen wurde eine kritische Feldstärke im Bereich von 0,3 bis 0,5 kV/cm, für Mikroorganismen von 10 bis 15 kV/cm beobachtet. Das Ausmaß der Membranpermeabilisierung wurde mittels Impedanzanalyse für pflanzliche und tierische Gewebe und mittels Durchflußzytometrie für Mikroorganismen und Liposomen bestimmt. Die Auswirkung auf Stofftransportvorgänge in Geweben wurde im Labor- und technischem Maßstab untersucht. Eine Steigerung der Extrahierbarkeit intrazellulärer Substanzen sowie der Ausbeute von Frucht- und Gemüsesäften bis zu 7 % bei äquivalenter Produktqualität konnte im Vergleich zu Kontrollproben gezeigt werden. Versuche in technischem Maßstab zeigten den Einfluss einer Elektroporation auf die Maischestruktur, eine Anpassung der Parameter der folgenden Fest-Flüssig-Trennung war notwendig. Durch eine Behandlung von Fleischwaren konnte deren Trocknung, Marination oder Pökeln beschleunigt werden, durch verbesserte Verteilung wasserbindender Agenzien innerhalb des Gewebes konnte eine Reduktion des Garverlustes erreicht werden.

Die Inaktivierung von Mikroorganismen wurde am Beispiel von Fruchtsäften und Milch in untersucht, die Anwendbarkeit des Verfahrens zur schonenden Haltbarmachung wurde gezeigt. Eine Kombination mit milder Hitze führte zu einer deutlichen Verbesserung der Energieeffizienz. Anhand von Laktoperoxidase in Milch wurde die Inaktivierung von Enzymen ermittelt, es wurde ein lediglich geringer direkter Einfluss elektrischer Felder beobachtet. Zusätzlich wurde die Eignung zur Reduktion der Überschußschlammenge bei der biologischen Abwasserbehandlung und zur Konservierung von Algenextrakten untersucht.

Der Energiebedarf für eine Permeabilisierung unterschiedlicher biologischer Membranen in Abhängigkeit vom induzierten Membranpotential wurde verglichen. Eine Effizienzanalyse zeigte deutliche Kosten- und Zeitvorteile bei einer Anwendung einer PEF-Behandlung als Zellaufschlussverfahren tierischer und pflanzlicher Gewebe im Vergleich zu konventionellen Technologien. Eine Anwendung als Konservierungsverfahren führte auch nach energetischer Optimierung zu höheren Behandlungskosten als eine thermische Behandlung, jedoch können diese durch Verbesserung der Produktqualität bei Premium- oder thermolabilen Produkten gerechtfertigt werden. Der Zellaufschluss bei Fleischwaren, Frucht- und Gemüsemaischen konnte als vielversprechendste Einsatzmöglichkeit des Verfahrens identifiziert werden um, etwa 50 Jahre nach den ersten empirischen Untersuchungen von Heinz Doevenspeck, eine breite Anwendung des Verfahrens in industriellem Maßstab zu erzielen.

# Abstract

The impact of pulsed electric fields (PEF) on phospholipid vesicles, plant and animal as well as microbial and protozoa membranes was investigated. A series of pulse modulators and treatment chambers was realized in order to examine the diversity of components, materials and processing parameters. Electric field strength, energy input and treatment temperature were identified as key processing parameters. A critical field strength of 0.3 to 0.5 kV/cm for plant and animal and 10 to 15 kV/cm for microbial cells was observed. Degree of permeabilization was investigated by impedance analysis for plant and animal tissue and flow cytometry for microbes and liposomes to optimize processing parameters.

The impact of membrane permeabilization on mass transfer processes was investigated for plant and animal tissue in lab- and technical scale. It was shown that extractability of fruit and vegetable juices or intracellular compounds can be enhanced after a PEF-treatment. An increase of up to 7 % of yield was found in comparison to untreated samples, juice quality was equivalent. Technical-scale treatments revealed the impact of a PEF-treatment on structural properties of fruit mash, an adaptation of liquid-solid separation techniques was shown to be required. A PEF-treatment of meat resulted in enhanced mass transfer during drying as well as brining of products, an improvement of water binding during cooking was found due to improved microdiffusion of brine and water binding agents.

Microbial inactivation was investigated in different liquid media. For fruit juice and milk the applicability to achieve a gentle preservation was shown. The impact of processing parameters was evaluated in order to reduce electric energy requirements. A combined application of PEF and mild heat showed highly synergetic effects and improved energy efficiency. Enzyme inactivation was determined for lactoperoxidase in milk in comparison to thermal inactivation. It was observed that only a minor part of the inactivation was related to electric field effects, whereas at higher treatment intensities mainly thermal effects occurred. In addition the PEF applicability to achieve disintegration of sludge during waste water processing and for preservation of algae extracts was shown.

Energy requirements to induce pore formation in different biological membrane systems were compared dependent on transmembrane potential induced. An analysis of cost efficiency showed that disintegration of plant and animal material by PEF is superior in comparison to a conventional treatment in terms of energy and time requirements as well as costs of operation. For microbial inactivation by PEF even an optimized treatment resulted in higher production costs, but consumer and quality benefits might justify these extra efforts for premium or thermally sensitive products. Meat, fruit and vegetable treatment were identified as the most promising applications to achieve a broad industrial exploitation of the technique, approximately 50 years after first empirical reports by Heinz Doevenspeck.

# Acknowledgements

This thesis is based on experimental work and process and equipment development at the Department of Food Process Engineering and Food Biotechnology at Berlin University of Technology from 2002 to 2006. Numerous people have given advice and encouragement as well as support to my work. First and foremost, my sincere gratitude goes to Prof. Dr. Dietrich Knorr for giving me academic guidance and inspiration as well as support, resources and funds throughout the course of this work. I would like to acknowledge Prof. Dr. Gustavo Barbosa-Cánovas for coming to Berlin and taking his time to be a referee for my thesis and Prof. Dr. Frank-Jürgen Methner for being the head of the commission.

A special thanks to Volker for giving impulses and valuable discussions since my first days working with PEF during my diploma thesis. I hope we can continue the transfer of PEF from research to application. I am grateful to Dr. Sitzmann for providing pictures and insight into the activities and pioneering work of Heinz Doevenspeck and at Krupp Maschinentechnik.

To all present and former colleagues at the Department I am thankful for providing a very comfortable atmosphere during my time at the Department. A particular thanks to the technical assistants Irene, Stefan, Bunny and Gisi, and our former secretary Sybille. My office mate Cornelius as well as Roman and Alex I want to thank for encouraging me and helpful discussions after work. Thanks to the diploma and exchange students Anna, Antje, Bonny, Daniela, Dominik, Giacomo, Guiseppe Henry, Jana, Jeldrik, Julia, Manuel, Manuela, Nico, and Noelia for your support and your activities!

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# List of Abbreviations

| | |
|---|---|
| AC/DC | alternating / direct current |
| $A_F$ / $A_1$/$A_2$/$A_3$ | semi-axis in field direction or x/y/z direction |
| AIJN | European Fruit Juice Association |
| ARMY | |
| C | capacity (F) |
| cF/cFDA | carboxyfluorescine / cF-diacetate |
| Cfu | colony forming units |
| COD | chemical oxygen demand |
| d | distance/electrode gap (m) |
| $E_{(crit)}$ | (critical) electric field strength (kV/cm) |
| EPC | egg phosphatidylcholin |
| EPRI | Electric Power Research Institute |
| f (A) | shape factor for ellipsoidal cells |
| FDA | Food and Drug Administration |
| FL1/FL3 | fluorescence of laser 1(red) and 3 (green) |
| FS/SS | forward/sideward scatter |
| FSTA | Food Science and Technology Abstracts |
| GAE | gallic acid equivalent |
| GTO | Gate Turn off (thyristors) |
| HPLE | high pressure liquid extraction |
| IGBT | Insulated Gate Bipolar Transistor |
| m | sample mass |
| n | pulse number |
| OSU | Ohio State University |
| PFN | Pulse forming network |
| PI | propidium iodide |
| PME | Pectin-methyl esterase |
| RC | resistance-capacitance |
| SCR | Silicone Controlled Rectifier |
| SGCT | Symmetrical gate commutated thyristors |
| TA | total acids |
| TEAC | Trolox equivalent antioxidant capacity |
| TR | tumbling rounds |
| TSS | total soluble solids |
| TUB | Berlin University of Technology |
| U | voltage (V) |
| W | energy input (kJ/kg) |
| $W_{pulse}$ | energy per pulse (J) |
| $W_{Specific}$ | specific energy input (kJ/kg) |
| WSU | Washington State University |
| $Z_p$ | cell disintegration index after Angersbach (1998) |
| ε | dielectric constant |
| κ (T) | electric conductivity (mS/cm) |
| ρ | electric potential |

# 1 Introduction and Objective of Work

The application of electrical currents for microbial inactivation and food treatment has been reported since the beginning of the past century, first applications of pulsed electric fields (PEF) for disintegration of biological material have been described by Doevenspeck (1960) and Flaumenbaum (1968) as well as for microbial inactivation by Sale and Hamilton (1967). The applicability of PEF to enhance, modify or replace a variety of operations during food processing has been shown in an unprecedented amount of publications from approx. 25 groups worldwide (Barbosa-Cánovas *et al.* 1999), but up to present the industrial exploitation of PEF application is limited to one commercial application for premium juice preservation (Clark 2006) and an industrial unit installed to disintegrate fruit mashes prior to juice separation (Kern 2006). In bio-engineering electroporation found wide application for introduction of foreign material into cells in vivo or in vitro, about 14 companies are distributing lab scale pulse modulators and treatment cuvettes commercially (Puc *et al.* 2004). In contrast to food applications processing intensity needs to be maintained at a low, sub-lethal level, the treatment capacity of these devices is often in a range of 200 to 1000 µl, but also batch devices up to 1000 ml are available. The knowledge regarding processing parameters, kinetics of permeabilization and equipment design obtained in this field of application provides the basics for a transfer to food processing, but in addition to the aim of achieving an irreversible permeabilization the production scale as well as reliability and operation time requirements in food industry are substantially different.

After empirical description of effects found on food material and first research studies in the 1960s and 70s, pioneering engineering work was conducted at Krupp Maschinentechnik, Germany in the 1980s. Industrial scale units have been designed, but needed to be dismantled after operability and reliability were poor and the sophisticated demands could not be fulfilled.

During the 1990s the interest in PEF application in universities and research centers increased and until 2006 about 450 research papers are cited in the Food Science and Technology (FSTA) abstracts. The applicability of PEF to successfully achieve membrane permeabilization in plant, animal or microbial cells has been shown in batch as well as in continuous operation, but research work was mainly conducted in a laboratory scale. Knowledge has been obtained regarding key processing parameters and impact of a treatment on microbial and plant cells, but unfortunately no application in an industrial size could be achieved until 2005. Though providing a large potential to achieve a non-thermal, low energy disintegration of plant or animal matrices several hurdles limited the transfer of the technique from research to application, mainly lack of industrial scale treatment systems and, not minor important a lack of innovative motivation from food processors.

During the course of this work applications with a substantial potential for industrial exploitation were identified, and the production scale was increased from laboratory scale to a technical scale, often in cooperation with industrial partners within field tests. Industrial scale equipment for treatment of meat and fruit and vegetable mashes is under development. An evaluation of PEF applicability revealed that a treatment of plant or animal cells is highly competitive in comparison to conventional disintegration techniques such as thermal, mechanical or enzymatic treatments with regard to costs of operation, processing time requirements and detrimental product changes. The treatment can be performed continuously, due to developments in electric and solid state semiconductor engineering a scale up to industrial scale at reasonable costs appears to be feasible at present.

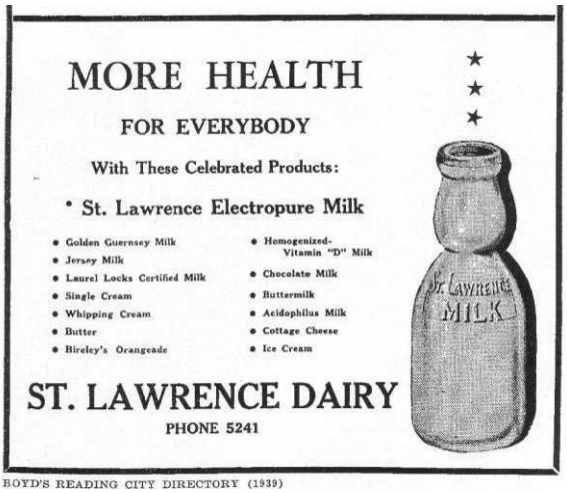
The potential for food preservation was investigated, based on mechanistic studies to determine the impact of processing and product parameters on membrane permeabilization by flow cytometry and kinetic studies in a laboratory scale. A system for PEF application in technical scale has been developed. The research work was concentrated on fruit juice and milk, to identify the potential and eventual benefits of the technique in comparison to conventional processing as well as to perform an evaluation of costs and feasibility in a larger scale. In addition a treatment of waste water and microbial decontamination of algae extracts was investigated to identify the techniques potential beyond food applications. After an introduction to development of food related electrical current applications up to present and an overview of mechanisms of action and inactivation models proposed potential applications will be highlighted with regard to the advantages of a PEF application in comparison to conventional techniques. Systematic studies have been performed to identify key processing parameters and to optimize energy efficiency of an electropermeabilization. General requirements for permeabilization of different biological membranes are compared. Feasibility as well as disadvantages and challenges of the technique will be discussed along with an estimation of costs of investment and operation for selected applications along with design considerations for industrial scale equipment.

# 2 Literature Review

## *2.1 Historical Background*

### 2.1.1 First applications of electrical current for food treatment

The effects of electric current on biological cells have been investigated almost as early as the time when electricity was commercially available. At the end of the 19th century bactericidal effects of direct and alternating electrical current have been investigated the first time by Prochownick et al. (1890). An inactivation of Staphylococcus aureus in suspension was not found after application of direct current of 300 mA. But it was noticed that the treated media showed differences in acidity at different points of the treatment chamber. When microbes where attached on agar gel to investigate the impact of electrically generated pH drop on their viability, it was found that samples taken from the anode were sterile in contrast to samples taken from the cathode. In the 1920s a process called 'Electropure' (see Figure 2.1) was introduced in Europe and the USA (Beattie and Lewis 1925; Fetterman 1928; Moses 1938). Being one of the first attempts to use electricity for milk pasteurization and to improve consumer health it was performed by the application of a 220 – 420 V alternating current within a carbon electrode treatment chamber.



BOYD'S READING CITY DIRECTORY (1939)

Figure 2.1: Advertisement for St. Lawrence Electropure milk in Boyd´s Reading City Directory, 1939

The method was fundamentally a thermal method, using direct heating of milk by electric energy (Joule heating). The electrical chamber consisted of a rectangular tube and opposing carbon electrodes. The milk was preheated to 52°C and subsequently electrically heated to 71°C and held for 15 s. About 50 plants were in operation until the 1950s, serving about 50.000 consumers. Only some researches reported a microbial inactivation below thermal death points (Beattie and Lewis 1925). The technique was accepted as safe pasteurization

step in six states in the US. The units were mainly provided by Trumbell Electric Manufacturing Co. (Getchell 1935; Edebo and Selin 1968). Due to rising energy costs and competition with mild, novel thermal preservation technologies such as UHT, these plants have been replaced (Reitler 1990). In 1949, Flaumenbaum reported the application of direct and alternating current for electroplasmolysis of fruit and vegetable tissue (Flaumenbaum 1949), an increase in juice yield of up to 10 % was found. It took until the 1980s until the application of ohmic heating revived and some industrial applications of the technology have been achieved, including pasteurization of liquid eggs or processing of fruit products. Recently the application of ohmic heating, or also termed as moderate electric field treatment received attention also in the field of pre-treatment prior drying, extraction and expression or the reduction in water use during blanching (Reznick 1996; Cousin 2003; Sensoy and Sastry 2004; Lebovka *et al.* 2005; Praporscic *et al.* 2006). In addition to thermal effects, based on the mechanism of ohmic (joule) heating, sometimes lethal effects of electric current, such as the hydrolysis of chlorine have been reported by (Pareilleux and Sicard 1970), subjecting food to low voltage alternating currents. Tracy (1932) reported a killing effect of low voltage alternating current on yeast cells, at a minimum lethal temperature of 46°C. Formation of free chlorine or other toxic substances was responsible for the killing effect. The inhibition of cell division of *Escherichia coli* has been first described by Rosenberg *et al*. (1965). Further information on impact of electricity on cells and the possibilities of cell electromanipulation can be found in: (Palaniappan *et al.* 1990; Chang *et al.* 1992; Zimmermann and Neil 1996).

### 2.1.2 Electrohydraulic Treatment

Pulsed discharge application of high voltage electricity across two electrodes for microbial inactivation was investigated since the 1950s (Fruengel 1960; Allen and Soike 1966; Edebo and Selin 1968), resulting in a process called electrohydraulic treatment. The electrodes were submerged in the liquid medium within a pressure vessel, electric arcs were generated by high voltage pulses forming transient pressure shock waves up to 250 MPa and ultraviolet light pulses. The method was capable of up to 95 % inactivation of *E. coli, Streptococcus faecalis, Bacillus subtilis, Streptococcus cremoris* and *Micrococcus radiodurans* suspended in sterile distilled water (Gilliland and Speck 1967a). The electrode gap was between 0.16 to 0.64 cm, the peak voltage 15 kV. Using a capacitance of 6 µF and a voltage of 5 kV the greatest effectiveness of the treatment was reported (Allen and Soike 1967). It was concluded that an electrohydraulic treatment was a quick, effective and inexpensive non-thermal method for sterilization of water and sewage. Electrochemical reactions, shock waves and ultraviolet light forming free, highly reactive radicals were claimed to be

responsible for the bactericidal effect. Operating with copper core electrodes resulted in a certain amount of residual toxicity in the treatment media, this effect was not found when using iron or aluminum electrodes. Applying a double chamber system, separated by a diaphragm revealed that mechanical action alone was not responsible for microbial inactivation (Gilliland and Speck 1967b). Edebo and Selin (1968) investigated the impact of plasma photon emission and attributed microbial inactivation to it. Varying electrode material, a higher efficiency was reported for copper than for iron, steel or aluminum electrodes. Though promising results in these early studies, the technology has never been developed to a point where an application in food technology was achieved. Disintegration of food particles and electrodes, causing food contamination appear to have inhibited an industrial application of this process except for wastewater (Jeyamkondan *et al.* 1999).

### 2.1.3 First Application of PEF – pioneering work of Doevenspeck

Secondary effects of electrochemical reactions and hydraulic pressure are less relevant when short, homogeneous pulses without arcing are applied. The first application of pulsed currents of high voltage has been reported (Gossling 1960) with the goal to induce artificial mutation. He reported a partial microbial kill, dependent on treatment intensity for *Streptococcus lactis*, and recultivated the survivors to find mutations. He suggested a batch as well as a continuous treatment chamber in small scale. Pioneering work of experiments of application of pulsed electric fields for food processing has been reported by the German engineer Heinz Doevenspeck, resulting in a patent (Doevenspeck 1960), describing the application of pulsed electric fields for disruption of cells in food material to improve separation of phases (Doevenspeck 1961). In between 1961 to 1971 (Doevenspeck 1975) he investigated the change of pH in a solution subjected to pulsed electric fields, reporting a color change of neutral red at the electrode surfaces. The pH at the anode was measured as 6.8, whereas at the cathode it was increased to a value of 8. After mixing this change showed to be reversible, the initial pH of 7.2 was restored. A treatment of *Lactobacillus delbrückii* in beer stained with methylene blue revealed an uptake of color, indicating a cell permeabilization. Growth of microbes and spoilage of beer samples was prevented after a treatment with pulses of 6 kV discharging a 2.5 µF capacitor. Subjecting cells of *E. coli* to pulsed electric fields, it was found that an application of electric fields with low field strength (“soft pulses”, below 2 kV/cm) lead to an enhanced growth, whereas increasing electric field strength resulted in cell death (“hard pulses”). A treatment of fish tissue revealed an improved separation of solid and liquid phase, a subsequent feeding of PEF-treated fish slurry resulted in a 100 % digestibility in contrast to 97 % for conventionally available fish

meal. No detrimental effect has been found when treating concentrates of vitamin A, B1, 2, 6, 12 and folic acid. The potential to enhance the production of biogas has been investigated at the waste water treatment plant in Nienburg, a 20 % increase has been reported (Doevenspeck 1963). A picture of Doevenspeck and his pulse generator at the facilities of Krupp in the 1980s is shown in Figure 2.2.

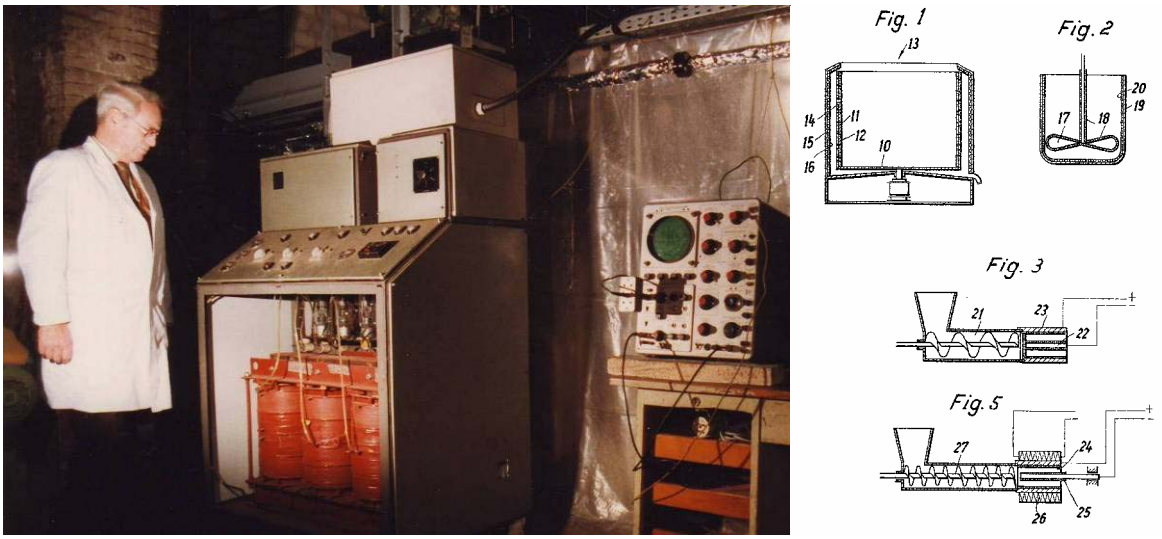



Figure 2.2: Heinz Doevenspeck and his pulsed power generator at the facilities of Krupp Maschinentechnik in the 1980s (Sitzmann 2006), (left); Right: Treatment chamber geometries suggested in a patent by Doevenspeck; Fig 1: rotating carbon coated sieve electrode; Fig 2: carbon coated mixing electrode; Fig 3 and 5: screw press with coaxial treatment chamber of carbon electrodes (Doevenspeck 1960).

A typical unit for PEF-treatment of food consists of a high voltage pulse generator and a treatment chamber where the media is exposed to the electrical field. In the Patent of Doevenspeck (1960) the setup of a pulse modulator as well as a continuously operated treatment chamber has been described. In general the pulsed power is generated by repetitive discharge of energy stored in a capacitor bank across a high voltage switch; mercury switch tubes have been suggested. As shown in Figure 2.2, right, different treatment chamber geometries have been proposed, a centrifuge coated with carbon, containing a carbon coated sieve as well as a mixing tank with carbon coated agitator have been described for batch treatment. For continuous treatment the product slurry was suggested to be conveyed by a screw press through cylindrical electrodes in a coaxial setup. Application examples presented in the patent range from waste and tap water treatment to cleaning of gasses as well as extraction from animal tissue. In addition the inactivation of pathogenic microorganisms on pathogenic microbes has already been described, reporting a 96 % inactivation of microbes suspended in marination brine as well as inactivation of Salmonella in egg powder suspensions (Doevenspeck 1961). An industrial scale plant with a capacity of up to 2500 kg/h has been erected for processing of beef and pork material as well as fish waste material as early as 1961 in fat smeltery in Germany. On his quest for possible

applicants of the technique Doevenspeck, active as a consulting engineer, came in contact with Münch, technical director for animal material processing at Krupp Maschinentechnik in 1985, recognizing the techniques potential. After a restriction of perchloroethylen use for fat extraction, Krupp Maschinentechnik was seeking for alternative processing techniques to induce cell disintegration and to improve phase separation of fish slurry in a screw press (Sitzmann 2006). Since then, consulted by Doevenspeck, a work group consisting of Münch, Sitzmann as well as other co-workers developed the processes ELCRACK® and ELSTERIL® (Sitzmann and Münch 1988; Sitzmann and Münch 1989).

### 2.1.4 Early PEF applications in the UK and Ukraine

After application of direct and alternating current for electroplasmolysis of apple mash in 1940s by Flaumenbaum (1949) in 1965 an industrial prototype of 6 to 15 t/h capacity was erected in a canning factory in the Soviet Republic Moldawia (Flaumenbaum 1968). At this time also the application of pulsed direct current was reported without mentioning more details on pulse parameters or results regarding the different current applications. An increase in juice yield of 10–12 % was found and the products were described to be lighter in color and less oxidized than after a heat or enzymatic pre-treatment (McLellan *et al.* 1991). The first systematic studies in the UK investigating the nonthermal lethal effect of homogeneous pulsed electric fields on microbes were conducted at Unilever Research Centre, Bedford, UK (Sale and Hamilton 1967). To investigate the effect of pulsed electric fields, a pulse generator connected to a batch treatment chamber was developed. Carbon electrodes were separated by a polyethylene spacer, a U-shaped sample container was obtained. The maximum electric field strength was limited to 30 kV/cm by the electrical breakdown of air above the sample (Sale and Hamilton 1967). The pulse voltage was adjustable up to 10 kV with a pulse length of 2 to 20 µs. They showed that electric field strength and total treatment time, the product of pulse width and number, were the most important factors involved in microbial inactivation. By treating microorganisms in a gel impermeable for electrolytic products, they showed the insignificance of electrolysis on the lethal effect of direct current (DC) pulses. Damage to the cell membrane, causing an irreversible loss of its function as a semipermeable barrier between the cell and its environment, was proposed as the cause of cell death. After treatment, leakage of ions, loss of cytoplasmatic content as well as changes in membrane morphology and cell lysis (Sale and Hamilton 1968) have been reported. The killing effect of PEF was reported to be independent of current density, thus it was concluded that inactivation was due to nonthermal

effects. Electric field intensity has been identified to be one of the most important factors, with a critical field strength of 10 to 15 kV/cm for most microorganisms.

### 2.1.5 Development of industrial equipment at Krupp Maschinentechnik

Based on Doevenspecks work at Krupp Maschinentechnik a technical scale unit with a capacity of up to 200 kg/h for treatment of meat or fish slurry, sugar beet, palm fruit, oil seeds and fruit mashes has been developed in the 1980s and is shown in Figure 2.3 along with the treatment chamber and a picture of Werner Sitzmann. In cooperation with the University of Applied Sciences Hamburg several diploma thesis have been finished, investigating disintegration of oil seeds, degree of cell permeabilization and the potential to optimize treatment chamber geometry and options for process control.

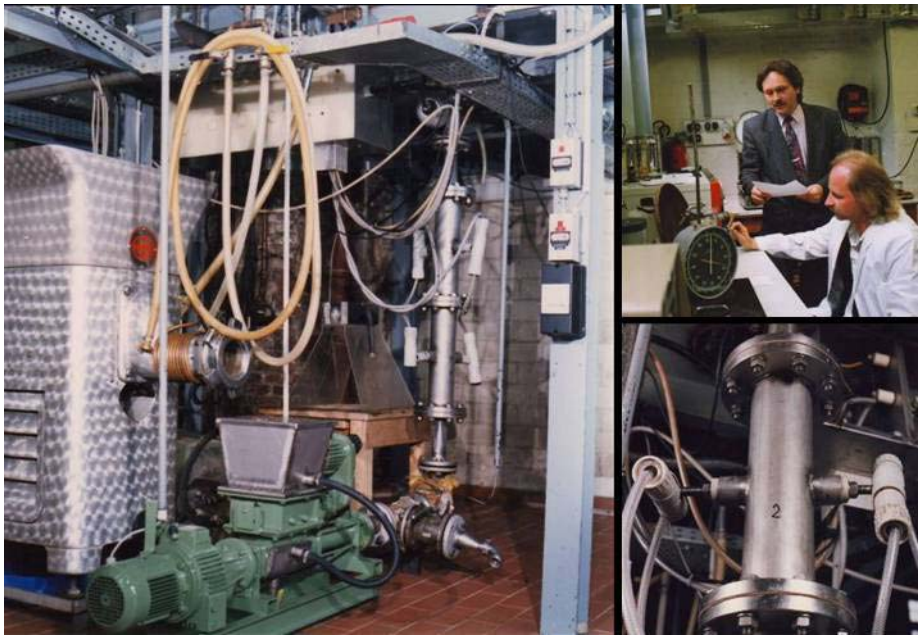


Figure 2.3: Pilot scale PEF system at Krupp Maschinentechnik (left), Werner Sitzmann (top right, standing), diploma student Volker Stemper (top right, front) and ELCRACK® treatment chamber (bottom right).

After performance of very promising technical scale tests, industrial equipment has been realized by Krupp to be installed in a fish factory in Norway. The total process consisted of the ELCRACK® system, a subsequent separation of free liquid and a screw press for slurry separation. The fluid was separated in water and oil phase by a decanter centrifuge and separators. Protein was removed from the aqueous phase using an ultrafiltration. In 1988 a brochure was released (Krupp 1988) to describe the technology as well as the application of ELCRACK® in fish processing. Pictures of the equipment taken from this brochure can be

found in Figure 2.4. After installation of the first equipment many problems arouse concerning the electrode stability, subsequent liquid-solid separation as well as protein recovery after the treatment, the installation was dismantled after a few months of operation. From a today's point of view, the failure of this first installation was not only related to the ELCRACK® technique, which was a small part of the total installation only. Due to design and implementation of to sophisticated separation technology and realization of a whole fish processing unit without prior experience, the equipment had to be taken back by Krupp (Sitzmann 2006).

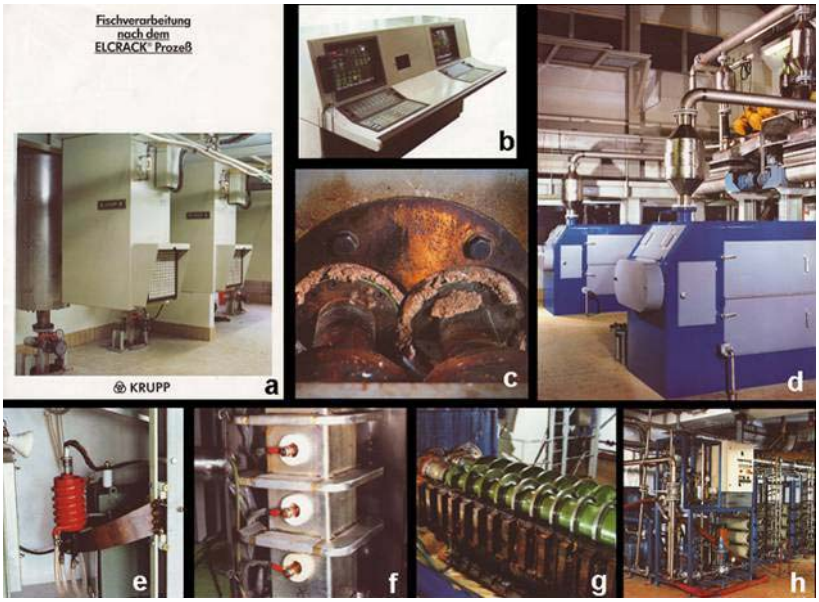



Figure 2.4: Fish processing by ELCRACK® – pictures of industrial equipment installed in Norway from a Krupp Maschinentechnik brochure (Krupp 1988). a) switch boxes; b) control unit; c) press outlet; d) screw presses; e) HV-switch; f) capacitor bank; g; screw press, dismantled; h) ultra filtration unit.

Two further industrial scale equipments have been designed, but, after the experience obtained with the unit installed in Norway were never installed at their destination. Since 1986 an ELSTERIL® pilot plant was developed, consisting of a high voltage pulse generator with a peak voltage of 15 kV and a repetition rate of 22 Hz. The storage capacity was varied between 0.5 and 5 µF, an ignitron was used to discharge the electrical energy stored (Grahl 1994). Five different batch- as well as continuous treatment chambers have been developed, equipped with two parallel plate carbon electrodes, the electrode gap was 0.5 or 1.2 cm, a flow rate of 165 l/h was used (Grahl 1994). In cooperation with FMC Europe in 1990 no detrimental effects on orange juice quality were found. After the failure with the first industrial unit, the financial support by Krupp was reduced substantially, the work group was going to be closed when Krupp and Hoesch merged. Trying to publish and to commercialize the technology during and after their activities at Krupp Maschinentechnik, Sitzmann and Münch were able to waken the interest of international research groups in this technique (Sitzmann 1987; Sitzmann and Münch 1987, 1988, 1989), Sitzmann continued the activity in the field of

PEF applications running his own businesses, DWS and Nafutec GmbH, subsequently (Anonymous 1995; Sitzmann 1995). In 1993 the development of a novel electroshock technique developed in the United Sates was reported (Nöldechem 1993), which was obviously based on prior ELSTERIL® developments at Krupp. The ELSTERIL® unit was placed at Berlin University of Technology and used for experiments concerning the extractability of carrot tissue (Geulen *et al.* 1992) or to enhance potato drying (Angersbach and Knorr 1997). A picture of the ELSTERIL® system at Berlin University of Technology can be found in Figure 2.5.

Figure 2.5: ELSTERIL® system installed at Berlin University of Technology, Mohamed Eshtiaghi, Stefan Boguslawski, Volker Heinz (hidden) and Dietrich Knorr (left to right).

In 1992 (Mertens and Knorr 1992) and 1994 (Knorr *et al.* 1994) potential applications of PEF for food processing have been reviewed. During this time the interest in PEF application increased at a research level, a numerous amount of working groups in Universities as well as commercial activities followed.

### 2.1.6 Research work on PEF application from 1980s to 2000

After empirically exploring the basic mechanisms and effects of pulsed electric fields on biological cells in the 1960s the technique received attention on a university level much later. Developments diverged into reversible and irreversible effects of electroporation. Neumann and Rosenheck (1972) as well as Zimmermann *et al.* (1976; 1982; 1996) investigated the potential to achieve a reversible permeabilization on a cellular level for applications in bioengineering, other groups focused on irreversible, lethal effects for preservation purposes.

In the 1980s the research group led by Hülsheger developed mathematical models to describe microbial inactivation by PEF, dependent on electric field strength and treatment time (Hülsheger and Niemann 1980; Hülsheger *et al.* 1983). A 10 kV pulse generator was developed, discharging a 1 µF capacity across a spark gap with a pulse repetition rate of 1 pulse in 5 s. The treatment chamber was constructed of a cylindrical glass tube closed by two round brass electrodes with a gold coating. Maximum field was limited to 20 kV/cm, as an electrode gap of 5 mm and an electrode area of 8 $cm^2$ was used. In the former German Democratic Republic Jacob *et al.* investigated the microbial implications of electric field effects (Jacob *et al.* 1981) at a maximum field of 35 kV/cm. In the United States, since 1982 Dunn, Hofmann and Bushnell investigated PEF applications, in 1987 Dunn and Pearlman filed a patent assigned to Maxwell Laboratories, San Diego, USA, describing an apparatus for extending shelf life of fluid food products (Dunn and Pearlman 1987). Batch- as well as continuous treatment chambers have been proposed, followed by a later Patent of Bushnell (1996). Since 1988 the ELSTERIL® process has been investigated at the Technical University Hamburg Harburg (Grahl 1994), the impact of treatment intensity required for a variety of microbes has been investigated and compared between batch wise and continuous operation. In Japan in 1980 Sakarauchi and Kondo reported lethal effects of high electric fields on microorganisms, using a disk-shape parallel plate treatment chamber with platinum electrodes. A 2 kV pulse generator was used. In 1988 Mizuno and Hori (1988) reported the destruction of living cells by high voltage, using parallel plate as well as needle-plate, wire-cylinder and rod-rod shaped electrodes. A rotary spark gap system was used for pulse generation, operating at 20 kV peak voltage and a repetition rate of 25 Hz. Investigating the efficiency of different treatment chamber geometries the maximum inactivation was found when using rod-rod shaped electrodes and producing an arc discharge. In 1996 the Japanese Research and Development Association for Application of Electronic Technology in Food Industry was founded, reporting activities in the field of PEF food preservation as well as for meat processing (Anonymous 1998). The work was conducted by Mitsubishi and Toyohashi University of Technology. Since 1992 Berlin University of Technology (Knorr) and Washington State University (Barbosa-Cánovas and Swanson) performed research work in this field, followed by Ohio State University (Zhang) in 1994. First reviews of the technology have been published in the 1990s (Palaniappan *et al.* 1990; Tsong 1990; Mertens and Knorr 1992; Knorr *et al.* 1994; Ho and Mittal 1996; Jeyamkondan *et al.* 1999), a first PEF book was published in 1999 (Barbosa-Cánovas *et al.* 1999). In 1995 Pure Pulse Technologies, a subsidiary of Maxwell Laboratories developed a continuous processing system called CoolPure® for treatment of up to 2000 l/hr. For research use, a pilot system called CoolPure® Jr. was available, to be operated at a flow rate of 6 to 10 l/h at a maximum field strength of 50 kV/cm. A brochure of Pure Pulse described the two non-thermal technologies

PureBright® (Pulsed Light) and CoolPure® (PEF). In the same year, a letter of no objection has been released by the Food and Drug Administration (FDA) for the use of PEF technology for food preservation; in 1996 the treatment of liquid egg has been approved with certain conditions to be accepted.

In the United States in 1997 a collaboration of OSU, WSU, EPRI and US Army was initiated (Mermelstein 1998), and a series of PEF workshops was held in 1997/98. Protocols were developed for microbial challenge tests and a laboratory PEF system designed. Subsequently, in a cooperation of OSU and Diversified Technologies, bench as well as pilot scale systems have been developed and realized. Engineering aspects required for PEF have been reviewed by Zhang *et al.* (1995). Systems developed by OSU have been installed in laboratories in the United States as well as Europe. In Europe, a project funded by the European Community has been initiated in 1997, a cooperation of TU Berlin, KU Leuven, the Universities of Montpellier and Zaragoza, SIK, Icetek, TetraPak, Unilever, Bestfoods and Pernod Richard. During the 1990s also Centralp, Lyon, France was developing pulsed power systems for PEF application. At Unilever own PEF systems have been developed at this time (Wouters and Smelt 1997; Wouters *et al.* 1999; Wouters *et al.* 2001; Abram *et al.* 2003). A European workshop concerning PEF technology has been held in 1998. Since 1998 a prototype system of PurePulse®, which later ceased business and was reintegrated into Maxwell, has been installed at Berlin University of Technology and was in use for numerous research work in field of PEF application for plant tissue until 2005 (Angersbach *et al.* 2000; Eshtiaghi and Knorr 2000; Ade-Omowaye *et al.* 2001; Tedjo *et al.* 2002). In parallel own pulse generators and treatment chambers have been designed by Angersbach, Heinz and Knorr at Berlin University of Technology since 1997, when a national project on PEF applicability in potato starch extraction was initiated. This project wakened interest also at the research centre Karlsruhe, where a workgroup led by Bluhm and Schultheiss was investigating PEF effects on plant and vegetable tissue. Based on an electro-physical model of biological cells Angersbach developed a technique to determine cell permeability and defined a cell disintegration index (Angersbach *et al.* 1997, 1999). First reports on drying enhancement have been published by Angersbach and Knorr (1997) and Rastogi *et al.* (1999). At Berlin University of Technology since 1999 a prototype setup was developed and realized to apply a combined treatment of pulsed electric fields as well as high pressure up to 200 MPa by Volker Heinz (Heinz and Knorr 2000). A simultaneous application of 200 MPa pressure was shown to have a protective effect on susceptibility of *B. subtilis* against PEF. Figure 2.6 shows the amount of relevant publications cited in the Food Science and Technology Abstracts (FSTA) Database since 1987.

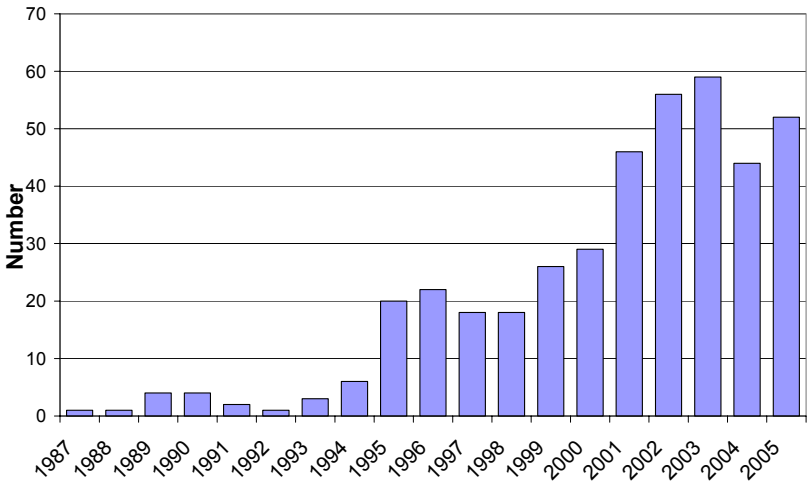



Figure 2.6: Relevant publications of PEF research work as cited in Food Science and Technology Abstracts (FSTA) Database from 1987 to 2005.

## 2.2 Fundamental Effects and Mechanisms of Electropermeabilization

### 2.2.1 Mechanisms of action

Following first empirical descriptions of Gossling (1960) and Doevenspeck (1960, 1961) in the 1980s the interest to use an electroporation in medical science and genetic engineering surged. Whereas empirical emphasis of the disruptive effect of electric fields on biological cells has already been provided in the 1960s, the dielectric rupture theory was introduced in parallel by Zimmermann *et al.* (1974) and Neumann and Rosenheck (1972). Subsequently the electrical breakdown of cellular membranes has been explored based on model systems as phospholipid vesicles and planar bilayers as well as microorganisms (Zimmermann *et al.* 1974; Chernomordik *et al.* 1987; Chang *et al.* 1992; Wouters and Smelt 1997; Barsotti *et al.* 1999; Ho and Mittal 2000) but until now there is no clear evidence on underlying mechanisms at a cellular level. Elucidation of membrane permeabilization is a difficult task as the time sequence of formation of pores is in the sub-microsecond range and the area of pore formation is only in the range of 0.1% of total membrane surface. The permeabilization of a cell membrane requires two key steps: first the formation of a pore has to be induced by the electric field applied and secondly this pore has to be stable enough to allow interaction of the intra- and extracellular media. Very little information is available regarding the time sequence and the dynamics of the electroporation process as well as on reversible-irreversible structural changes of cells during and after PEF-treatments. Two effects have been described to be triggered by the electric field, the ionic punch-through effect (Coster 1965) and the dielectrical breakdown of the membrane (Zimmermann *et al.* 1973). At present

it is generally accepted that the primary effect of PEF on biological cells is related to local structural changes and breakdown of the cell membrane, which is a highly important component of the biological cell as it acts as semipermeable barrier responsible for mass transfer and plays an important role in synthesis of RNA and DNA, protein and cell wall components as well as many other complex metabolic activities (Rogers *et al.* 1980). In addition the disruption of intracellular organelles and other structural changes have been described (Harrison *et al.* 1997).

The cell membrane of biological cells can be considered as a capacitor filled with dielectric material of low electrical conductance, and a dielectric constant in the range of 2 (Zimmermann *et al.* 1974). Accumulation of charges with opposite polarity on both sides of the membrane leads to a naturally occurring, perpendicular transmembrane potential of about 10 mV. In an electrically conductive media placed between a high voltage and a grounded electrode the resulting electrical field can be predicted from the Laplace equation $\nabla^2\varphi=0$, where $\varphi$ denotes the electrical potential.

By exposure to an external electrical field an additional potential is induced by movement of charges along the electric field lines. A schematic depiction of impact of external electric field on cell membranes is shown in Figure 2.7.

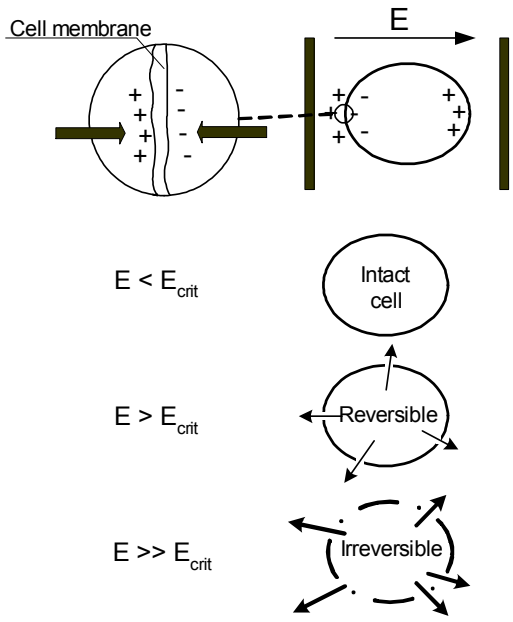



Figure 2.7: Schematic depiction of mechanism of membrane permeabilization by electro-compressive forces induced by an external electrical field. Increasing treatment intensity will lead to formation of large, irreversible membrane pores.

The potential difference $\Delta\varphi_M$ at the membrane of a biological cell with spherical shape and a radius R induced by the external electrical field E can be approximated by Equation 1 which is derived from solving Maxwell´s equations in ellipsoidal coordinates assuming several simplifying (Zimmermann *et al.* 1974):

$$\Delta\varphi_M = -f(A)\cdot A_F E$$

Equation 1

This formula yields the local membrane potential difference at the distance $A_F$ from the centre in direction of the external electrical field. The shape factor f(A) is a function of the three semi-axis ($A_1$,$A_2$,$A_3$) of elliptical cells (Equation 2):

$$f(A) = \frac{2}{2 - A_1 A_2 A_3 \int_0^\infty 1 \Big/ \left(\left(s + A_F^2\right)\left(\sum_{n=1}^{3} \sqrt{s + A_n^2}\right)\right) ds}$$

Equation 2

When the overall potential exceeds a critical value of about 1 V, depending upon the compressibility, the permittivity, and the initial thickness of the membrane (Crowley 1973; Zimmermann 1996) the electro-compressive force causes a local dielectric rupture of the membrane inducing the formation of a pore, acting as a conductive channel (Schoenbach *et al.* 1997). Taking into account a membrane thickness of 5 nm this translates to a dielectric strength of 2000 kV/cm. A drastic increase in permeability re-establishes the equilibrium of the electrochemical and electric potential differences of the cell plasma and the extracellular medium forming a Donnan-equilibrium (Glaser, 1998). This phenomenon was termed dielectric breakdown, borrowing the expression from solid state physics (Zimmermann *et al.* 1976). An electro-mechanical model was developed, implying that the presence of an electrical field across the membrane results in a mechanical compression. The cell membrane was considered as a capacitor, containing a perfectly elastic dielectric. Compressive forces are balanced by the restoring mechanical force, increasing the compression by increasing the transmembrane potential a mechanical instability can occur. Crowley (1973) reported a good agreement between predicted breakdown voltage and assumed elastic parameters for a model system of phosphatidylcholin bimolecular lipid layers. Electrical breakdown has been shown for algal cells as well as for bacteria and human red blood cells measuring size distribution with a Coulter counter, concluding that probably the most important application will be to load cells with substances the cell membrane is normally impermeable for (Zimmermann *et al.* 1976).

Up to now the electro-mechanical instability is still being used to explain the effect of external electrical fields on biological cells and is one of the most accepted theories. The electric breakdown is reversible if the pores induced are small in comparison to the membrane area. Increase of electric field strength and treatment intensity by increasing pulse width and/or number will promote formation of large pores and the reversible damage will turn into irreversible breakdown. Experimental evidence is supporting this electro-mechanical

compression model, a critical electric field strength was found and was dependent on the size and geometry of a cell in the range of 1 – 2 kV/cm for plant cells and 10 - 14 kV/cm for microbial cells as *E. coli*. However as subsequent behavior such as resealing of pores, membrane conductance course and transport phenomena are not taken into account, several other models have been proposed to predict the mechanisms at a molecular level, as the fluid mosaic model of a lipid bilayer with protein units embedded (Jacob *et al.* 1981).

In contrast to the electric compressive forces, other theories include the occurrence of membrane deteriorations and reorientations on the lipid bilayer and the protein channels as cause of increase in permeability.

Dimitrov (1984) presented an extension of the electromechanical model taking into account the viscoelastic properties of the membrane, membrane surface tension and molecular rearrangements as well as pore expansion to describe the time course of field induced breakdown of membranes. Other alternative concepts are based on molecular reorientation and localized defects within the cell membrane which are expanded and destabilized by exposure to an electric field. The presence of small fluctuating hydrophobic pores in the lipid matrix was suggested to be the initial structural basis of electroporation (Chernomordik 1992). By external electrical stress these may be transformed into hydrophilic pores by reorientation if the pore radius is increased above the value where the pore energies of both orientations coincide. The pore energy is the change of free energy resulting from the formation of a pore within the lipid bilayer. As long as the pore radius is small the formation of hydrophobic pores is more favorable, but at a range of 0.5 nm the pore energies of hydrophobic and hydrophilic pores become equal and pore inversion may occur (Glaser *et al.* 1998). Dipolar reorientation of phospholipids and transition from hydrophobic to hydrophilic pores has been described by Tsong (1991), assuming a change in membrane structure by Joule heating within a conductive pore. These pores might also cause a loss of ability to regulate the intracellular pH (Simpson *et al.* 1999) and short circuit of protein-pumps (Chernomordik 1992). Membrane rupture was related to osmotic imbalances and cell swelling after opening of pores, and defined a two-step mechanism defined (Tsong 1990): an initial perforation of the cell membrane after a dielectric breakdown is followed by a time-dependent pore expansion.

Electroporation could take place both in lipid domains and protein channels, in particular as their functionality is influenced by the transmembrane potential. The gating potential for protein channels is in the 50 mV range, considerably smaller than the dielectric strength of a phospholipid bilayer. However, though opening of protein channels is induced it may not be sufficient to prevent the development of a transmembrane potential above the breakdown potential of the lipid bilayer. Based on experiments with model systems such as liposomes or protoplasts large eukaryotic cells and microbes several theories have been developed or

proposed to explain the underlying mechanism of pore formation and resealing (Neumann and Rosenheck 1972; Zimmermann *et al.* 1974; Sugar and Neumann 1984; Weaver and Powell 1989; Chang *et al.* 1992; Ho and Mittal 1996; Kinosita and Tsong 1997; Neumann *et al.* 1998; Weaver 2000). Pore formation might also occur as a consequence of structural defects within the cell membrane, expanding spontaneously formed pores in the presence of an electric field (Tsong 1991). An unprecedented amount of research work has been published in the field of genetical and bioengineering (Prasanna and Panda 1997; Pavlin *et al.* 2002; Valic *et al.* 2003; Puc *et al.* 2004), a comprehensive review on the (lack of) knowledge regarding cell permeabilization mechanisms has been published by Teissie *et al.* (2005). Pulse generators as well as batch treatment cuvettes in micro-liter scale are commercially available from Eppendorf, Bio-Rad, BTX as well as Genetronics, exemplarily (Puc *et al.* 2004). Recently a review has been published, comparing micro-fluidic devices available for electroporation (Fox *et al.* 2006). Even if underlying mechanisms of action are the same and micro-fluidic units are very helpful for mechanism elucidation, in contrast to food application treatment intensity is much lower and in most cases a very small volume in a range of µl to ml per min is treated.

### 2.2.2 Models proposed to describe microbial inactivation

Several empirical and phenomenological models to describe the relation between microbial inactivation and electric field strength have been proposed. The high number of processing and product parameters, differences in treatment systems, experimental conditions and definitions limit the generality of the relations found. Lack of knowledge concerning mechanisms of action and dependence of processing and product parameters prevent the development of inactivation models based on the physiological meaning of obtained parameters. Models proposed have been based on empirical observations, but precautions need to be taken to avoid limitations to specific experimental design or PEF equipment used. Whereas application of static chambers seems to be preferable for study of inactivation kinetics, it is shown in sections 4.2.1, 4.2.2 and 4.2.3 that inactivation efficiency of PEF is different in batch or continuous treatment chambers.

The model described by Hülsheger (1981) is based on assumption of a linear relation between log survival fraction and electric field strength as well as a linear relation between fraction of survivors and the treatment time (Equation 3):

$$s = \left( \frac{t}{t_c} \right)^{-\frac{(E-E_e)}{k}}$$

Equation 3

Where s is the number of survivors, t is the treatment time and E is the field strength. $E_c$ and $t_c$ denote the threshold values of E and t, k is an independent factor specific for each microorganism. In view of our own results this model overestimates the impact of electric field strength, in comparison to treatment time. We found that exceeding a certain level of field strength no further increase in efficiency can be found when energy input is kept constant (see 4.2.2, 4.2.1 and 4.1.1). From an engineering point of view the use of energy input instead of treatment time appears to be more adequate to describe process intensity.
The model proposed by Peleg (1995) is based on Fermi´s equation, representing percentage of survivors as a function of electric field strength and the pulse number applied Equation 4:

$$s = \frac{100}{\left( 1+\exp\left( \frac{\left( E-E_c(n) \right)}{a(n)} \right) \right)}$$

Equation 4

Where E and $E_c$ are field strength and critical field strength, n is the pulse number and a is a factor characterizing the steepness of inactivation curve in a field strength range close to $E_c$. Microbial inactivation has been described as basic first order kinetic (Martín-Belloso *et al.* 1997; Reina *et al.* 1998) as well non-linear curves (Peleg 1995; Peleg and Cole 1998; Barbosa-Cánovas *et al.* 1999; Rodrigo *et al.* 2003). Non-linear inactivation has been related to distributions of sensitivity against electroporation, protective effects of food constituents, presence of air bubbles, insulating particles or inhomogeneities in electric field distribution as well as distribution of cell size, geometry and random orientation in the electric field.
The presence of sublethal injury of bacteria exposed to pulsed electric fields PEF-treatment was described by Garcia *et al*. (2005), whereas using fluorescent viability staining no sublethal damage was found by Yaqub *et al*. (2004). Also Wuytack *et al.* (2003) observed no significant sublethal damage by investigating growth of *Salmonella entericar* on different selective media after PEF-treatment. Inactivation of microorganisms by PEF was regarded as ”all or nothing” process, i.e. a single target mechanism based on irreversible membrane permeabilization.

## 2.3 Processing Parameters

### 2.3.1 Electric field strength

Applying an external electrical field with sufficient strength to cells suspended in an electrically conductive media, an accumulation of charges at the nonconductive microbial membranes will be induced. Pore formation will occur when a certain threshold value of the transmembrane potential formed is exceeded, which was found to be in the range of 1 V (Zimmermann 1996).

It was shown that the critical external field strength is highly dependent on cell size as well as cell orientation in the field (Heinz *et al.* 2002). With decreasing cell size the required field strength sharply increases, and variations in cell shape can cause considerable increase of E. Increasing the electric field strength was reported to lead to a further increase in treatment efficiency (Hülsheger *et al.* 1983; Boyko *et al.* 1998; Heinz *et al.* 1999; Heinz and Knorr 2000; McDonald *et al.* 2000; Alvarez *et al.* 2003; Heinz *et al.* 2003) but is limited to the dielectric strength of the food material (Ho and Mittal 2000) in a range of 60 to 80 kV/cm typically. Breakdown and associated arcing will cause current flow in a narrow channel and promote undesired electrochemical reactions, bubble formation and electrode erosion. This can be prevented by optimizing the field distribution in the chamber excluding hot spots as well as avoiding presence of air bubbles by degassing or operation under back pressure.

### 2.3.2 Treatment time, specific energy and pulse geometry

Apart from the peak electric field strength the product of pulse width and the average number of pulses applied has often been used to evaluate treatment intensity. Increasing treatment time results in higher microbial inactivation (Sale and Hamilton 1967). The pulse width is defined as the time where the peak field is maintained for square wave pulses or the time until decay to 37% for exponential decay pulses. Typically increasing the number of pulses causes increase in treatment time, as the pulse width is fixed by the impulse generation setup. In general increasing inactivation has been found when treatment time is increased, but in some cases saturation has been reported after a certain amount of pulses. While for batch chambers the number of pulses per volume element is well defined for continuous flow chambers the average number has to be considered. Besides in many cases the treatment zone with an electric field above the critical electric field strength is different from the treatment chambers geometry. This can in particular be found in case of co-linear configuration where the distribution of the field intensity has to be taken into account when

calculating the medium residence time in the treatment zone. Therefore the specific energy input was suggested as intensity parameter and it can be estimated by product temperature increase and specific heat capacity of the media by assuming an adiabatic system where the energy delivered to the treatment media is totally converted to heat. Most accurately it can be calculated for all waveforms based on voltage and current signals determined close to the electrodes by:

$$W_{Pulse} = \int U(t)I(t)dt$$

Equation 5

For exponential decay pulses the specific energy input can also be estimated by the energy stored in the capacitor bank (Equation 6), where U denotes charging voltage and C the storage capacity, but the ratio of losses in an eventual protective resistor or pulse forming network and cables needs to be taken in account.

$$w_{specific} = \frac{U^2 C}{2} \cdot f \cdot \dot{m}$$

Equation 6

Based on media conductivity and electric field strength measured the specific energy input for exponential decay and rectangular pulses can also be calculated by:

$$w_{specific} = f \cdot \frac{1}{\dot{m}} \cdot \int_0^\infty \kappa(T) \cdot E(t)^2 dt$$

Equation 7

where E, $\kappa$(T), f and $\dot{m}$ denote the electric field strength, the media conductivity, the repetition rate and the mass flow rate, respectively. The temperature increase due to energy dissipation can be utilized for process evaluation by comparing energy input with bulk product temperature after treatment.

From a processing point of view the energy input required to achieve a given microbial inactivation rate or cell matrices disintegration seems to be advantageous to be used as treatment intensity parameter, as it can indicate the costs of operation. However neither treatment time nor specific energy is adequate to describe processing parameters sufficiently, as no information is given about the energy delivered per pulse or the number of pulses per volume element.

Although many different waveforms are applicable for PEF technology, the pulse shapes commonly used are either exponential decay or square wave pulses. Square wave generating systems require a switch with turn-off capability or a pulse forming network. As

switches with turn off capability are hardly available for high power applications systems (Mohan *et al.* 1995), serial or parallel connections of switches (Gaudreau *et al.* 2001) or lumped or distributed pulse forming networks with several sections of capacitors and inductive elements have to be used. In this case the pulse generation system, in particular the impedance of the line has to be adapted to the resistive load of the treatment chamber. A comparison of energy performance of different pulse generation systems has been conducted by De Haan *et al*. (Willcock 2002), concluding an exponential decay system will not exceed an energy efficiency of 38 %. They compared square wave pulses with a certain peak voltage $U_{peak}$ and duration $t_p$ to exponential ones by fitting blocks of $U_{peak}$ and $t_p$ under an exponential pulse, assuming that excess voltage of the exponential pulses results in excess losses.

The impact of pulse rise times of rectangular pulses of 1 ms from 2 to 100 µs on permeabilization of cell membranes was investigated by Kotnik *et al.* (2003). No significant difference was found and treatment efficacy was correlated to the time with above-critical pulse amplitude. The impulse characteristics of an exponential decay pulse are highly dependent on the parameters of the charging and the discharging circuit. Peak voltage and capacity of the energy storage determine the energy input per pulse, resistance and inductivity of the discharge circuit and influence pulse rise time and pulse width. The impact of impulse characteristics on microbial inactivation has been discussed extensively, but since they are not independent parameters their influence has not been fully elucidated up to now.

### 2.3.3 Treatment temperature

Treatment temperature has a highly synergetic effect on treatment efficacy, as it has significant influence on cell membrane fluidity and stability. Whereas at low temperatures the phospholipid structure is packed in a gel-like structure their order decreases with increasing temperature. The temperature dependent phase shift from gel to a liquid crystalline structure affects cell membrane stability (Stanley 1991). Dunn and Pearlman (1987) observed an increase of inactivation of *S. dublin* in milk from 1 to 4 log-cycles when increasing treatment temperature from 40 to 50°C. Jayaram (1993) reported an enhanced inactivation of *L. brevis* when increasing treatment temperature from 24 to 60°C, assuming the phase transition of the phospholipids being responsible for this effect. The effect of increasing efficiency of PEF application at elevated treatment temperatures has been reported in several studies (Evrendilek and Zhang 2003; Heinz *et al.* 2003; Li *et al.* 2005). The effect of treatment temperature on textural properties of apple tissue has been investigated by Lebovka *et al*.

(2004), showing that preheating to 50°C resulted in more effective tissue damage than PEF-treatment alone and better juice extraction by pressing.

## 2.4 Treatment Media Factors

Product properties and constitution have significant influence on microbial growth as well as resistivity against different inactivation techniques. Similar to heat treatment, a strong dependency of susceptibility against pulsed electric fields on product parameters has been reported, and physical and chemical parameters as pH or water activity of the product strongly influence microbial inactivation. The media itself represents the aim of the treatment if a PEF-treatment is applied for tissue disintegration. Tissue properties such as cell size and conductivity will have an impact on treatment efficacy, product properties such as pumpability, fruit, tuber or piece size will influence product handling during a continuous PEF application.

### 2.4.1 Conductivity

One of the key parameters for pulsed electric field processing is the media conductivity κ(T), which is also a function of media temperature (Reitler 1990). Media rich in ionic species such as for example tomato juice present problems to achieve a sufficient voltage for a supercritical field strength, since a smaller peak field strength is generated across the treatment chamber. This effect is important for the treatment of plant or animal cells as well as to achieve microbial inactivation for liquid food preservation. Conductivity is the inverse of resistivity and is measured in Siemens per unit length (S/m). As the conductivity of most food materials is fixed by its intrinsic properties or recipes only the choice of an electrode configuration and geometry with high load resistivity helps to diminish this effect and to improve voltage division in the discharge circuit. Nevertheless the temperature increase will lead to changes in conductivity during treatment. Apart from its influence on field strength the conductivity is supposed to determine the difference between ionic strength in the media and the cytoplasm (Jayaram *et al.* 1993). The membrane will be weakened and more susceptible to an electric pulse in media with higher ionic strength, causing higher permeability and structural changes. The relation between inactivation rate and media conductivity was investigated by Hülsheger *et al.* (1981) and Vega-Mercado *et al.* (1996), showing significant increase in inactivation at lower ionic strength and conductivity, whereas Alvarez *et al.* (2000)

did not confirm this. Heinz *et al.* (2002) calculated the influence of media conductivity on charging time constant and showed a negligible influence of the media conductivity on transmembrane potential build up.

### 2.4.2 Effect of air bubbles and particles

Apart from electric conductivity the dielectric strength of the food matrix has a significant influence on the applicability of PEF, as a dielectric breakdown has to be prevented. Air bubbles, which cannot withstand high electric field strengths may be present in case of sparkling products or be released due to temperature increase or electrochemical reactions. In particular for microbial inactivation, where an electric field strength in a range of 30 – 50 kV/cm is required, air has to be removed from the product. Apart from dielectric breakthrough, where a high current will flow within a narrow channel within the bubble instead of the liquid, the different dielectric properties of air will influence treatment efficacy. The perturbating effect of air bubbles present within the treatment chamber has been reported by Góngora-Nieto *et al.* (2003), indicating that in boundary regions of bubbles a significant drop in field strength will cause food safety problems. A similar effect has been found when agglomerations of microorganisms and/or particles with different dielectric properties such as fat globules are present (Toepfl *et al.* 2004). Therefore product constitution has to be taken into account for choice of processing parameters for a certain product. For treatment of solid foods as plant or animal material or fruit mashes air encapsulations have to be removed to avoid electric discharges, foam forming products might be unsuitable for a PEF-treatment.

### 2.4.3 Microbial cell characteristics

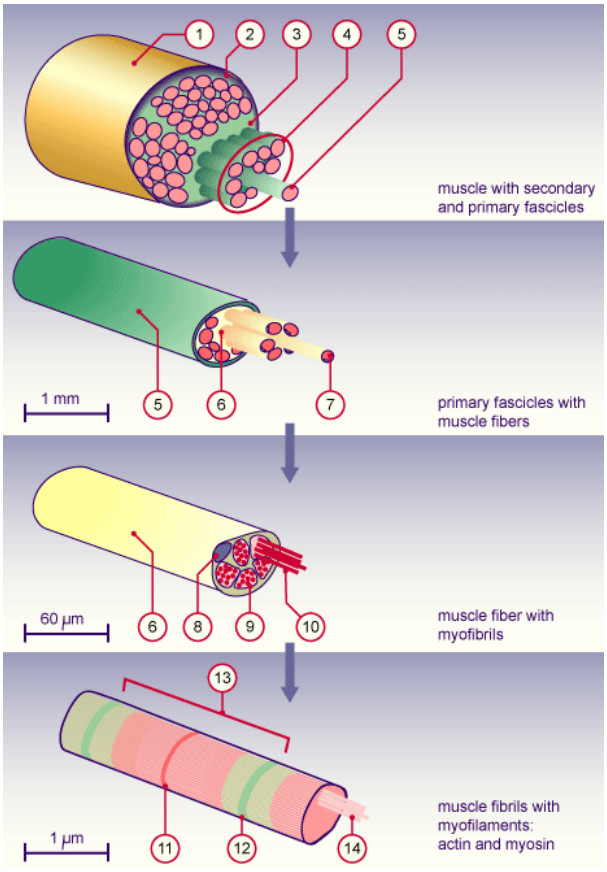
Already in the 1960´s Sale and Hamilton (1967; 1968) observed that yeasts were more susceptible to a pulsed electric field treatment than bacteria, Hülsheger *et al.* (1983) showed a broad variety in inactivation of different microbes. Apart from the effect of electric field strength as described before the membrane constitution of different microbes influences their resistivity. In general gram-positive organisms seem to be less sensitive. An influence on PEF-treatment of cells at different growth phases was not observed by Sale and Hamilton, but Jacob *et al.* (1981), Pothakamury (1995), Gaskova *et al.* (1996) and Alvarez (2000) Alvarez reported higher efficacy for cells in logarithmic growth phase. Even if some research

groups described a slight inactivation of some types of spores (Raso *et al.* 1998) after a PEF-treatment, it is important to note that PEF is not an efficient tool for inactivation of endo- or ascospores, and that a germination after a treatment is not induced. Hence it may not be possible to sterilize food products unless a combination with other techniques such as heat or previous germination and inactivation of vegetative cells is performed.
It is noteworthy that resistance of cells against PEF does not correlate to their resistance against other thermal or non-thermal treatments, for example strains of *Listeria*, which are highly sensitive to heat showed to be highly resistant against PEF-treatment (Unal *et al.* 2001; Toepfl *et al.* 2004; Aronsson *et al.* 2005). Lado and Yousef (2003) compared inactivation of nine different strains of *Listeria monocytogenes* after a treatment at 25 kV/cm and 25°C, which ranged from less than 1 up to 3.5 log-cycles. No correlation to heat sensitivity or genotype was found. This variety emphasizes that still there is a need to identify resistant target strains to evaluate and prove process efficacy and safety.

## 2.5 PEF as Disintegration Technique

When a PEF-treatment is applied for tissue disintegration the product itself and its properties will determine the applicability and efficacy of an electropermeabilization. Treatment of a variety of plant and animal raw materials has been reported in literature and will be described in section 2.7.2 and results in discussion (4.1). Raw material properties such as cell size and tissue structure will determine the processing parameters required (Chalermchat 2005). Plant and animal cells commonly have a diameter in a range of 20 to 140 µm, about one order of magnitude larger than microbial cells. Within this work the raw material properties and constitution of different plant and meat material can not be described in detail, but properties relevant to PEF application will be discussed in section 4.
For meat tissue it is noteworthy, that each skeletal muscle fiber is a single, longitudinal muscle cell. An individual skeletal muscle is consisting of muscle fibers bundled together, surrounded by a connective tissue covering. Each muscle is surrounded by a connective tissue sheath called the epimysium. Connective tissue outside the epimysium surrounds and separates the muscles. Muscle fibers (cells) have a diameter of 10 to 100 µm, but a length of up to 30 cm, so the impact of a PEF-treatment might show a significant dependence on the direction of electric field application. Cutting a muscle across fiber direction will result in a mechanic destruction of cells, dependent on size of resulting pieces. Cutter application will cause a high degree of mechanic cell destruction of meat cells in comparison to plant cells, where cells are smaller, separated structural elements. In addition the structure and textural properties of meat are mainly determined by the high protein and connective tissue content,

whereas tissue strength of plants or fruit is, in addition to structural units formed by hydrocolloids such as cellulose, hemicelluloses or lignin, often a result of turgor pressure, which is lost after an electropermeabilization. The impact of a PEF-treatment on textural properties of plant or animal tissue was expected to be different with regard to this aspect. Besides raw material also a treatment of product formulations or final products such as sausage meat or marinated meat was performed, ingredients such as salt or hydrocolloids will influence tissue conductivity as well as protein swelling and water binding activity. A schematic depiction of the muscle structure and dimensions is shown in Figure 2.8.




1. Fascicle
2. Epimysium
3. Perimysium
4. Secondary bundle
5. Primary bundle
6. Endomysium
7. Muscle fiber
8. Nucleus of muscle cell
9. Myofibrils
10. Myofilament
11. M-line
12. Z-disk
13. Sarkomer
14. Myofilaments actin and myosin

Figure 2.8: Schematic structure of a skeletal muscle (source: http://www.wikipedia.com).

## 2.6 PEF Equipment Design

The main components required for a pulsed electric field application are an impulse generation system and a treatment chamber. A crucial prerequisite for an economic and efficient production is a continuous operability with high flow rate capacity, which led to the development of continuous treatment chambers, where the food is pumped through while being exposed to the electrical field at ambient or refrigerated as well as elevated temperatures. Before treatment heat exchangers might be used to preheat the media, after

the treatment the dissipated electrical energy resulting in a temperature increase has to be removed before aseptic packaging. An aseptic packaging is required to prevent recontamination. One of the main advantages of PEF-treatment is its continuous operability with very short processing times; therefore a system can easily be implemented into existing processing lines.

### 2.6.1 Generation of Pulsed Electric Fields

The pulse modulation system transforms the electric power from a low utility level voltage to pulsed high intensity electric fields. Simplified circuits for generation of exponential decay and rectangular pulses are shown in Figure 2.9, consisting of a charging and a discharging circuit. In the first one an energy storage device is charged across a charging resistor by a DC high voltage power supply. The generation of pulsed electric fields requires slow charging and a fast discharging of the energy, as the pulse width is short in comparison to the time between pulses. The charging voltage $U_0$ required to generate pulses of sufficient electric field strength is highly dependent on the electrode distance, for two parallel plate electrodes the electric field strength E is given by Equation 8:

$$E = \frac{U}{d}$$

Equation 8

where U is the Voltage (kV) and d (m) the gap between the electrodes. Voltages in the range of 10 – 60 kV have been commonly used for food treatment. Increasing the gap d to obtain high flow rate capacity imposes increasing the charging voltage and therefore the switching system stress. The electric power is most commonly stored in a bank of capacitors connected in series or parallel and discharged into the treatment chamber across a high voltage switch and protective resistors within microseconds.

As discharge switch many devices are applicable, including vacuum or gas spark gaps, electron tubes as thyratrons, high power transistors or other semiconductor switches, each with its drawbacks and limitations. The type of switch will determine the maximum repetition rate, and the maximum current and voltage rating that it can withstand. The current is highly dependent on the resistance of the treatment chamber and can reach several kA when operating with a low resistivity treatment chamber configuration. Increasing resistive load by appropriate chamber design e.g. co-linear configuration will result in currents far below kA range. A spark gap, which is basically a two electrode system with arc-over when the

breakdown voltage is exceeded, provides a very simple and reliable pulse generation which can handle high voltage and current ratings but is limited to repetition rates below 100 Hz and maximum lifetime of $10^6$ shots. Whereas a simple spark gap cannot be triggered and repetition rate is controlled by the charging current the other types of switches allow generation of pulses with a defined frequency. Implementation of a trigger electrode to a spark gap to promote a controlled arc-over leads to a trigatron switch. Thyratron switches found wide application for generation of pulses, providing high, triggerable repetition rates up to kHz range but are limited regarding the maximum energy delivered per pulse as sufficient cooling of grids can not be maintained (Wenske 2005).

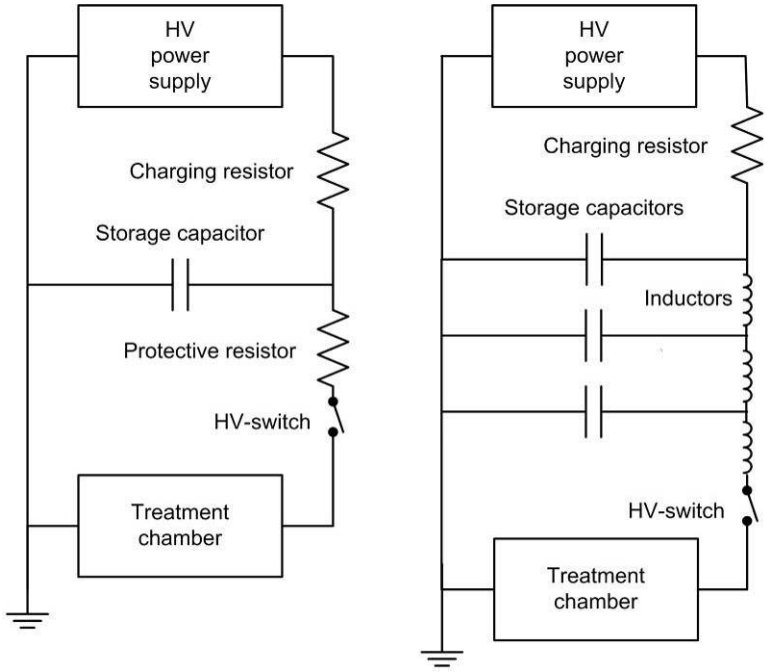



Figure 2.9: Simplified electrical circuits of impulse generation systems for exponential decay (left) and square wave pulses (right).

Solid state switches as for example SCR (Silicone Controlled Rectifier) GTO (Gate Turn-off) or IGBT (insulated Gate Bipolar Transistor) switches require less complex driving circuits and are easy to handle and control by external triggering and optimum variability of pulse parameters. Pulse repetition of standard switches can reach up to several kHz, suitable for application in industrial scale. Limitations have been found in the reliability in long term use, in particular when exposed to reversal current in case in underdamped situations such as arcing, treatment chamber blocking or electromagnetic interference. For high voltage pulses greater than 100 kV a “Marx”-generator is commonly used, invented by Marx (1923). In this configuration capacitors are charged in parallel and switched into series by spark gaps for discharge to increase the output voltage above the charging voltage. Though improvements and developments of Marx Generator designs with higher spark gap lifetime and inclusion of

trigger possibility as patented by KEA-Tec (Kern 2004) the maximum pulse repetition rate is still limited to a range of 30 Hz. Overviews of different high voltage pulse power systems have been given by Mankovski (2000) or Bluhm (2006).

In a capacitive storage system, when the high voltage switch is closed by a trigger signal or after its breakdown voltage has been achieved, the energy stored is discharged to ground across the discharge circuit with a protective resistor and the treatment chamber with the food material. Dependent on the type of switch and the configuration of the discharge circuit several pulse waveforms are possible, but mainly two have been used: exponential decay and square wave pulses.

Generation of an exponential decay pulse requires a switch with turn on capability only, as the total energy stored in the capacitor bank will be discharged. Square wave pulses can be realized either by an incomplete discharge of a capacitor with high capacity by a switch with on/off capability or a more complex pulse forming network, providing a relatively constant voltage during the pulse width. As switches with turn off capability are hardly available for high power application systems (Mohan *et al.* 1995), serial or parallel connections of switches (Gaudreau *et al.* 2001) or lumped or distributed pulse forming networks with several sections of capacitors and inductive elements have to be used. In this case the pulse generation system, in particular the impedance of the line, has to be adapted to the resistive load of the treatment chamber, which is difficult to realize in practice (Zhang *et al.* 1995) and possibly leading to an inflexible system with regard to applicability for different treatment media which may have a wide range of conductivity. Square wave pulses maintain a high voltage level for the total impulse width, whereas exponential pulses have a long tail with low electric field. Studies comparing the efficacy of different pulse waveforms for PEF inactivation have been conducted (De Haan and Willcock 2002; Góngora-Nieto *et al.* 2002; Kotnik *et al.* 2003), which concluded that both are effective for microbial inactivation, but square wave pulses would save energy and require less cooling effort.

For an industrial exploitation with high flow rates high pulse repetition rates up to several kHz are required at high voltage (40 – 100 kV) and current levels (>100 A), which are operation conditions hard to handle, in particular if the lifetime of the impulse generation system is taken into account. Lifetime ratings vary from $10^6$ for spark gaps, $10^8$ for thyratrons up to approximately $10^{12}$ pulses for semiconductor switches when operated under nominal conditions, arcing or operation under non-optimal conditions will drastically reduce the lifetime. At repetition rates of 1 kHz a lifetime of $10^9$ pulses translates into a continuous operation of 11 days. The reliability in case of malfunction, pump failure, arcing and protection against short circuit will be a crucial parameter for the applicability of a switch and the total pulse modulator setup.

## 2.6.2 Treatment Chamber Design

The treatment chamber, wherein the food is exposed to the electric field pulses consists of at least two electrodes, one on high voltage and the other on ground potential, separated by insulating material in different geometric configurations. Parallel plates, coaxial or co-linear cylinders have been commonly used. A large number of studies have been performed with parallel plate systems in batchwise and later on in continuous flow operation. Batch chambers provide many advantages for laboratory use, small volumes of treatment media are required and treatment temperature is easy to maintain by cooling the electrodes and by slow repetition rates. Above all the pulse number for each volume element is well known. Apart from niche products for an industrial application continuous chambers will be necessary to achieve high volume capacity and an easy integration into already existing food processing lines.

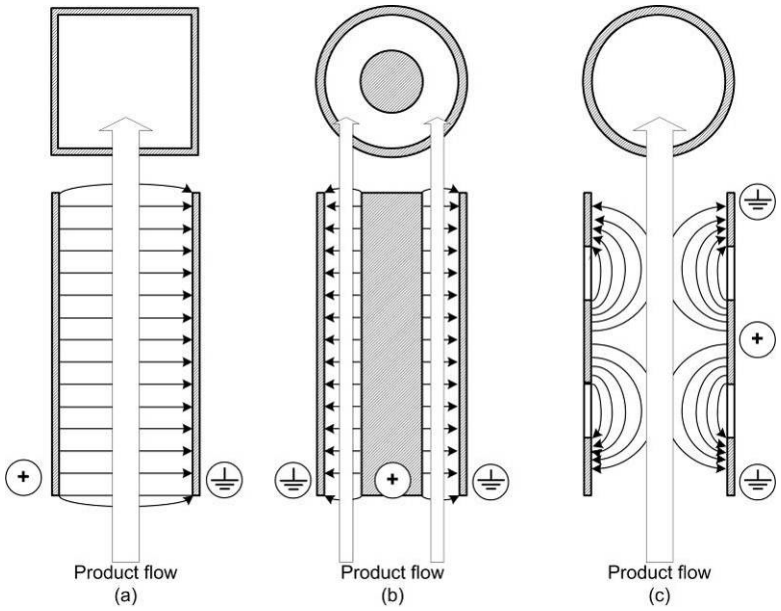



Figure 2.10: Configurations of treatment chambers for continuous PEF-treatment; (a) parallel plate, (b) coaxial and (c) co-linear configuration.

Among the different electrode configurations presented in Figure 2.10 parallel plates provide the most uniform electric field in a large usable area between the plates, but treatment intensity is reduced in boundary regions. In batch chambers without mixing or product flow leading to changes of position a considerable part of the volume may remain under-processed, noticeable i.e. as tailing effects in microbial inactivation kinetics. In continuous treatment chambers, this can be prevented by adding multiple treatment zones in line or baffled flow channels (Zhang *et al.* 1995). To achieve high flow rate as required for industrial applications, the pulses have to be applied with high repetition rate, leading to a fast temperature increase of the media. Maintaining constant temperature may require high

cooling efforts, implementation of cooling channels in the electrode material or intermediate cooling between multiple treatment zones. The electrode and insulator material have to be food grade and autoclavable, furthermore its electrochemical properties have to be taken into account. Bushnell *et al.* (1996) suggested gold, platin, carbon and metal oxides as alternative to commonly used stainless steel electrodes. Electrode erosion and release of metallic particles into the food material has been reported lately (Morren *et al.* 2003; Roodenburg *et al.* 2003; Toepfl *et al.* 2003; Mastwijk 2004). To avoid product exposure to the electrode surface Lubicki and Jayaram (1997) developed a system consisting of a glass coil surrounding the anode and confirmed that microbial inactivation can be obtained even without direct contact. PEF-treatment of packaged food without direct contact to the electrodes has been discussed in the last few years, questions such as how efficient a sufficient (and homogenous) electric field strength can be induced or if the required pulse energy can be transferred into the product, will have to be addressed.

A colinear chamber consists of a set of hollow, cylindrical electrodes separated by insulators, in which the product is pumped through the drilling; the flow is not perturbed by any impediments. The geometry of the treatment chamber (together with media conductivity) has a decisive impact on its total resistance, and therefore on the discharge circuit. The ratio of the total resistance of the treatment chamber and the protective resistor is highly important, as the applied charging voltage is divided between them. At the protective resistor, which is necessary to prevent breakdown of the switching system in case of short circuit, a considerable amount of energy might be dissipated if its resistance is in the same range as the one of the treatment chamber.

## 2.7 Applications and Aims

Many applications of PEF have been investigated in food- as well as biotechnology or medicine within the last decades, utilizing its impact on biological cell membranes. Dependent on treatment intensity electropermeabilization of membranes leads to a reversible or irreversible pore formation and cell disintegration, which is often a key processing step in food and bioengineering operations. As after a high intensity treatment cell vitality is lost, a non-thermal inactivation of microbes can be achieved. The following sections will review different applications of this novel, non-thermal and short-time technique. An overview of possible applications and processing intensities required is shown in Figure 2.11.

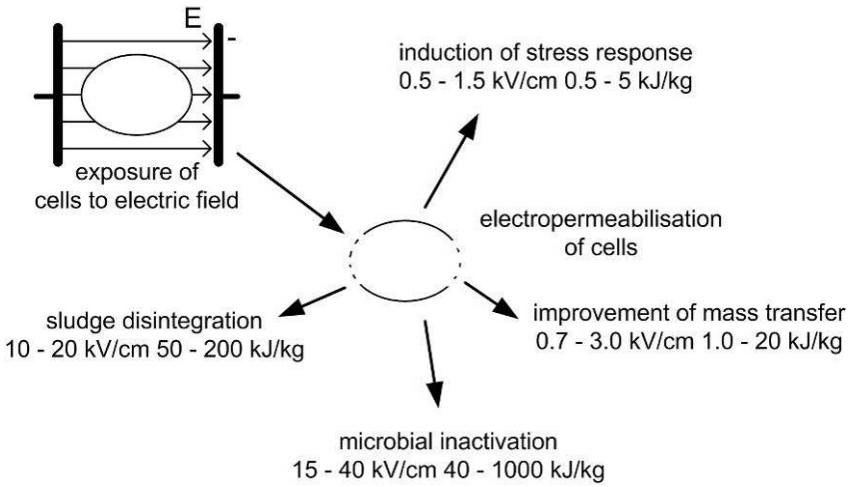



Figure 2.11: Exposure of biological cells to an electric field and applications in food, bio- and waste water processing with typical electric field strength and energy input requirements.

### 2.7.1 Stress Induction

The application of a low intensity treatment at low electric field strength and/or pulse number, though initiating a conductive channel across the membrane, does not necessarily cause irreversible cell rupture, this effect is used in bioengineering (Puc *et al.* 2004). Reversible electroporation has also been reported for potato tissue, after a time of 0.7 µs for membrane charging and a membrane potential of 1.7 V a pore is formed, but electrically insulating properties can be recovered within seconds, restoring vitality and metabolic activity (Angersbach *et al.* 2000). This provides a potential to induce stress reactions in plant systems or cell cultures as previously described for high pressure techniques (Dörnenburg and Knorr 1998). An airlift bioreactor with a coaxial electrode configuration has been developed to investigate sublethal stress on cultures of *vitis vinifera* for recovery of resveratrol, it was shown that metabolic activity can be stimulated and extractability of intracellular compounds was improved. A PEF-treatment at low temperatures does not damage enzymes or proteins, thus a potential to extract valuable components is provided. For example a mild, sublethal treatment of maize germs increased oil yield and phytosterol production, resulting in a plant oil with higher phytosterol concentration. In subsequent studies with soy beans and olives increased oil yield and isoflavonoid content were found (Guderjan *et al.* 2005), providing an eminent potential to develop processing concepts activating cells as ‘bioreactors’ to produce high quality food with high concentration of functional constituents.

### 2.7.2 Disintegration of Biological Material

Many operations in food and bioengineering such as extraction, pressing or drying include an enzymatic or thermal treatment or mechanical grinding for disruption of cellular material. These techniques may require a high amount of mechanical or thermal energy as well as holding times and storage tanks for an enzymatic maceration. Furthermore side activities of natural or added enzymes and thermal degradation lead to significant losses of nutritionally and physiologically valuable substances. When applying PEF to cellular tissues an increase in mass transfer coefficients could be observed (Doevenspeck 1960; Flaumenbaum 1968; Knorr *et al.* 1994; Knorr and Angersbach 1998; Bazhal and Vorobiev 2000; Fincan 2004). Based on this possibility conventional processing can be supported or replaced. As a PEF-treatment allows a defined degree of tissue permeabilization, biological systems can be stimulated in terms of metabolic activity and the recovery of valuable components can be improved. Operating at ambient temperatures a treatment with sufficient electric field strength, pulse repetition rate and/or energy input results in formation of large, permanent pores, while retaining product quality contrast to thermal or enzymatic treatments. In juice processing a similar juice yield with fresh like quality and a higher concentration of functional components was found. Short-time and continuous operability allows a continuous liquid-solid separation e.g. by a decanter centrifuge (Knorr *et al.* 2001). For carrot juice an increase of juice yield from 60.1 to 66.4 % was found in comparison to an untreated sample, in the same way the dry matter of the pomace was increased from 13 to 15 %, resulting in fewer efforts for drying. For grapes a juice yield of 87%, similar to that after an enzymatic maceration, and an increased content of soluble solids and pigments was reported after cell disintegration by PEF (Eshtiaghi and Knorr 2000). In the context of consumer demand for functional food with composition and mineral content close to fresh products and high content of physiologically valuable compounds, an increased extractability of anthocyans from grapes or phenolic substances provides an enormous potential for product development.

Increase in extractability of black tea and mint leafs by moderate electric fields was investigated by Sensoy and Sastry (2004), showing an increased leaching of solutes. The applicability of PEF to enhance pressing and extraction rate from beet cossettes has been shown by Bouzrara and Vorobiev (2000). It was shown that high temperature thermal degradation (70 – 120°C, 10 – 20 min) became obsolete after a PEF-treatment of sugar beet while maintaining sugar quality and yield (Eshtiaghi and Knorr 1999).

Textural changes such as tissue softening for apple, potato and carrot by PEF have been reported (Lebovka *et al.* 2004). For apple tissue a disintegration similar to freeze-thawed tissue was found after a treatment with 0.5 kV/cm for 10 ms. Whereas a low temperature PEF-treatment led to an almost complete membrane destruction, while the effective stress

relaxation time for carrot and apple tissue was still much higher than for freeze-thawed tissue (Lebovka *et al.* 2002).

Drying is one of the most energy consuming steps in food and bioengineering, as large amounts of water have to be transported from the inner of the product its surface for removal. A PEF-treatment can be utilized to improve mechanical water removing by pressing if applicable and facilitate a subsequent air drying by enhancing mass transport (Toepfl and Knorr 2006). For fluidized bed drying of red pepper slices a reduction of drying time from 360 to 220 min was found (Ade-Omowaye *et al.* 2001). Osmotic drying rates and diffusion coefficients of carrots were found to be increased (Rastogi *et al.* 1999). For apple slices an increased osmotic drying rate and improved rehydration capacity and reduced rehydration times were reported (Taiwo *et al.* 2002). Drying time or drying temperature reduction will result in a reduction of costs of operation and increase in production capacity.

### 2.7.3 Preservation of Liquid Media

Microbial Inactivation by PEF has been extensively investigated (Sale and Hamilton 1967; Qin *et al.* 1994; Zhang *et al.* 1995; Gaskova *et al.* 1996; Grahl and Märkl 1996; Jeyamkondan *et al.* 1999; Ho and Mittal 2000; Cserhalmi *et al.* 2002) within the last decades, initially in batch treatment systems and model foods which are free of fat or proteins. Even if the underlying mechanisms of action have not been fully elucidated up to now, key processing parameters have been identified and inactivation of a broad variety of vegetative cells has been shown. In general yeasts have shown to be very sensitive against a PEF-treatment (Barbosa-Cánovas *et al.* 1999), as cell size seems to play an important role in addition to cell membrane constitution. Effective inactivation for most of the spoilage and pathogenic microorganisms has been shown, but it has to be emphasized that, in comparison to the treatment of plant or animal cells the treatment intensity in terms of field strength and energy input is much higher. As a treatment with this field strength will disintegrate the tissue of solid food, PEF-treatment for preservation therefore seems to be virtually impossible for solid food if product textural properties need to be mainteained and is limited to liquid media. The potential to achieve sufficient reduction of microbes has been proven in a broad variety of food products, fruit or vegetable juices (Zhang *et al.* 1994; Qui *et al.* 1998; Evrendilek *et al.* 2000; McDonald *et al.* 2000; Hodgins *et al.* 2002; Heinz *et al.* 2003; Molinari *et al.* 2004), model beer (Ulmer *et al.* 2002) as well as milk (Reina *et al.* 1998; Bendicho *et al.* 2002), liquid egg (Martín-Belloso *et al.* 1997) and nutrient broth (Selma *et al.* 2004).

Apart from microbial inactivation, the reduction of enzymatic activity is critical in food processing and preservation, but there are only limited reports about effects of PEF on enzymes. Due to different experimental setups and processing parameters it is sometimes difficult to compare the results from different research groups, as conclusions drawn are often inconsistent. Yang *et al.* (Yang *et al.* 2004) investigated the inactivation of five different enzymes after a PEF-treatment, and the results varied by enzyme to enzyme. Whereas lysozyme was not affected by an electric field strength below 38 kV/cm, a significant reduction of pepsin activity was achieved. It was concluded that PEF and PEF-induced heat contribute to an enzyme inactivation. Van Loey *et al.* (2001) reported that lipoxygenase, polyphenoloxidase, pectin-methylesterase (PME) and peroxidase are resistant towards a PEF-treatment in distilled water and inactivation found in more complex products was based on temperature effects. Schuten *et al.* (2004) found a reduction of PME activity of 24 % after a PEF-treatment, which was sufficient to increase shelf life from 5 to 21 days for orange juice in refrigerated storage. It has to be taken into account that the higher resistivity of enzymes might be a restriction for preservation of liquid food by PEF, unless utilizing thermal effects. On the other hand it provides a high potential for the development of new processes and products, as many enzymes are positively used in food processing.

## *2.8 Current State of Technique*

At present there are approximately 25 research groups working on PEF applications for food production. Though the technology has been explored almost 50 years ago, only a limited amount of technical or industrial scale prototypes or commercial applications is available at present. A transfer of successful results from laboratory to industrial scale showed to be a difficult task. Research work is performed on microbial and enzyme inactivation mainly, PEF applications as disintegration technique for plant or animal tissue are limited to France, Canada and Germany at present. The workgroup of Vorobiev (Université de Technologie de Compiègne) has been investigating the impact of a PEF-treatment on structural properties as well as mass transport coefficients (Bazhal *et al.* 2001; El-Belghiti *et al.* 2005; Lebovka *et al.* 2006; Praporscic *et al.* 2006). At McGill University the impact of PEF on porosity of plant tissue and drying was investigated (Bazhal *et al.* 2003; Ngadi *et al.* 2003; Arevalo *et al.* 2004). In 2001 for potato starch extraction a prototype was developed by ProPuls, Germany. Commercial prototypes for this application based on a patented Marx Generator design (Kern 2004) have been developed since 2001 by a spin-off of the research center Karlsruhe, KEA-Tec in Germany. A mobile demonstration unit has been designed, industrial prototypes have been done for sugar processing (Kraus 2003, 2004), an industrial scale prototype PEF

system has been installed at a fruit juice company in Germany in 2006 (Kern 2006). Solid state pulse modulators for PEF preservation and treatment chambers have been developed by Thomson CSF, France as well as Diversified Technologies Inc, USA (Gaudreau *et al.* 2001; 2004), still active in this field. These OSU-called systems are also installed in Europe at the Universities of Salerno and Lleida, Spain as well as Budapest, Hungary. In 2003 the installation of a technical scale system at Stork Food Systems in the Netherlands was reported for preservation of fruit juices (Braakman 2003). In addition to turn-key systems, pulse modulators designed for other applications could be combined with PEF-treatment chambers to reduce investment costs. The costs for investment have been estimated to be in between 1 and 2 Mio. Euro for an industrial installation, resulting in extra production costs of 1 to 2 Euro-cents per liter of product. A comparison of costs of investment as well as operation for different applications will be discussed in section 4.5. In good agreement with Braakman (2003), the treatment costs for preservation applications have been estimated in between 1 and 2 Euro-cents per liter, whereas the treatment of fruit or vegetable mashes results in total costs of approximately 30 cents per ton of product. In addition to significant costs of operation within the last years, other challenges and pitfalls of a PEF application for liquid food preservation have been identified. Distribution of treatment intensity was shown to be dependent on many product and processing parameters, such as medium conductivity and constitution, presence of particles, treatment chamber geometry and flow pattern (Fiala *et al.* 2001; Lindgren 2001; Heinz *et al.* 2002; Lindgren *et al.* 2002; Mastwijk 2004; Toepfl *et al.* 2004). The presence or formation of air bubbles by electrolysis also was shown to have a detrimental impact on treatment homogeneity and product safety (Góngora-Nieto *et al.* 2003). In addition to variation of size and resistivity of microbial cells, an inhomogeneous intensity distribution may result in deviations from first order kinetics and tailing effects found (Lebovka and Vorobiev 2004). Within the European Community funded project “NovelQ”, starting in 2006, modeling of intensity distribution as well as identification of target microorganisms for sensor development is one main point of emphasis. At Saligus AB, Sweden, a turn-key PEF system for liquid food preservation was developed since 2004, based on a pulse modulator provided by ScandiNova, Sweden. A treatment chamber design based on a gear pump was patented (Lindgren 2002), ensuring a sharp residence time and treatment intensity distribution, but at the end of 2005, the company ceased to exist, transferring its prototype to SIK, Sweden. Also corrosion of electrodes and release of metal particles have been reported for PEF application (Tomov and Tsoneva, 2000; Morren *et al.* 2003; Roodenburg *et al.* 2003; Toepfl *et al.* 2003; Mastwijk 2004; Roodenburg *et al.* 2005, 2005b), indicating that stainless steel can be subjected to erosion. Roodenburg *et al.* (2005, 2005b) reported that when applying short pulses, the release of metal particles can be limited to an amount still in agreement with European tap water legislative. The formation of

bactericidal and mutagenic compounds has been reported (Reyns *et al.* 2004), but the treatment intensity applied was much higher than required for microbial inactivation in many other reports. Occurrence of sublethal damage has been investigated within the last years (Wuytack *et al.* 2003; Yaqub *et al.* 2004), Garcia *et al.* (2005) reported the presence of sublethal damage using stress nutrient agar, whereas Simpson *et al.* (1999) and Russel *et al.* (2000) considered the underlying mechanism as an “all or nothing effect”. Susceptibility of microbes against PEF and applicability for pasteurization appears to be dependent on product type and properties.

Since 2005 the first commercial PEF application has been reported (Clark 2006) for fruit juice preservation in the US. In a scale of 200 l/h premium quality juices are treated at Genesis, Eugene, US, a fruit juice cooperative using an OSU system. Genesis used to distribute unpasteurized, premium fruit juices, but in 2003 a warning letter of FDA was published (FDA 2003), initiating the quest for a non-thermal preservation technique. After a PEF pasteurization a shelf life of four weeks is obtained, clearance of FDA is available since 1996, indicating the potential of the technique for safe and gentle preservation. In Europe an approval is still pending, but the Novel Food legislative opens a possibility to commercialize PEF-treated products if substantial equivalence to a commercially available, conventionally processed product is proven by the manufacturer.

# 3 Materials and Methods

During the course of this work lab and pilot scale pulse modulators and treatment chambers have been developed and realized for multiple purposes. To identify the equipment used a letter has been given to the different modulator and chamber designs. For mechanistic studies a micro batch system has been developed to use dyes, for determination of microbial and enzyme inactivation kinetics lab-scale continuous systems have been realized. Treatment of plant and animal material was performed in technical scale batch and continuous treatment chambers, using variable setups of pulse modulation systems. Pulse modulators have been designed based on spark gap as well as semiconductor and pulse transformer designs. Dependent on unit design a computer or analogue control interface was used, for determination of process intensity high voltage and current probes connected to an oscilloscope have been used. A detailed description of PEF equipment used and realized can be found below.

## *3.1 Pulse Modulators*

***A) Exponential decay micro- pulse modulator, 16 kV, 100 J/s, TUB 1***

A setup was designed to provide a flexible pulse modulator for mechanistic and flow cytometry studies. Key requirements were PC-control and high variability of processing parameters. A power supply FUG HCK 800M-20000, 20 kV, 80 mA (FUG, Rosenheim, Germany) was used to deliver the electrical energy to a capacitor bank (see Figure 2.1). This consisted of a setup of Ceramite Y5U 6800Z (Behlke, Kronberg, Germany) capacitors, up to 6 capacitors of 6.8 nF each could be placed in parallel to obtain a capacity up to 40.8 nF. In addition packs of 2 or 3 parallel capacitors could be introduced into the bank to add a capacity of 2.3 and 4.5 nF, therefore variation was possible in steps of 2.3 nF. This high variability was required to maintain energy delivered per pulse while pulse rise time by increasing discharge circuit inductivity. Pulse energy was variable between 0.34 and 2.04 J at a charging voltage of 10 kV, exemplarily. A HTS 160-500 SCR, 16 kV, 5 kA, 2 kHz (Behlke, Kronberg, Germany) was used as switching unit, connected in series to the storage capacitors and a protective resistor with 2.5 Ohm (Stervice, France). The switch was protected against current reversal by a free wheeling diode: FDA 150-200, 20 kV, 1.5 kA (Behlke, Kronberg, Germany) and triggered by a TTL signal from a frequency generator (AFG 320 Sony Tektronix, Beaverton, US). As switch power supply an A400, 5 V, 2 A (EMS

Power, Basingstoke, U.K.) was used. By addition of inductive elements (cable coils) the inductivity of the discharge circuit could be changed to achieve a pulse rise time in between 55 and 750 ns at a peak voltage of 10 kV. Pulse width to achieve a drop to 37 % of peak voltage was in a range of 2 to 20 µs, dependent on treatment media conductivity and treatment chamber used. Pulse parameters where controlled by a high voltage and a current probe, connected to a TDS220 (Sony Tektronix, Beaverton, US) oscilloscope. Data acquisition and control was performed on a PC connected by GPIB, using a software developed based on TestPoint (Keithley Instruments, Cleveland, USA). The unit was used to supply pulsed power to micro batch cuvettes, the PEF microscope and a combined high-pressure-PEF unit (data not shown).

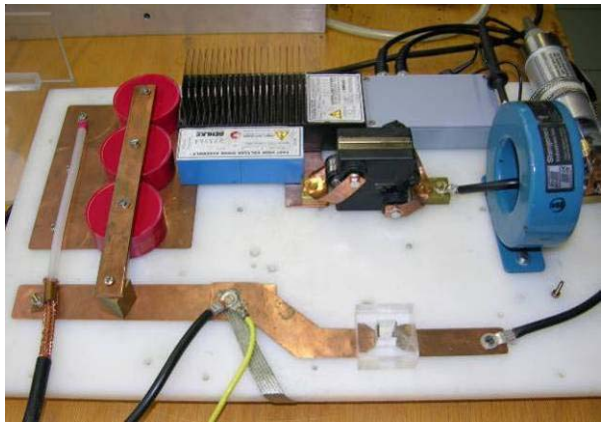


Figure 3.1: Micro-pulse modulator, 16 kV, 100 J/s

***B) Exponential decay lab scale pulse modulator, 16 kV, 800 J/s, TUB 2***

The system was designed similar to the previous system, but a variable storage capacity (34 - 136 nF) was used, charged by a stabilized high voltage charging unit with maximum 20 kV and 80 mA output (see Figure 3.2). A thyristor switch HTS 160-500SCR (Behlke Electronic GmbH, Kronberg, Germany) is used to discharge the stored electric energy in exponential decay pulses across the electrical load to ground. Three 886AS (Kanthal, Amherst, USA) with 15 Ohms in parallel have been used for switch protection. A 400 MHz digital storage oscilloscope (TDS 430A, Tektronix, Wilsonville, OR) was used for acquisition of voltage and current at the treatment chamber. For signal conditioning a 75 MHz high voltage and a 100 MHz current probe connected to an amplifier system were used. The pulse rise time typically was in the range between 70 and 100 ns, the pulse width, defined as time needed to decrease voltage to 37% of its peak value between 1.5 and 6 µs, depending on the number of capacitors, the load voltage and the electric properties of the media. Maximum pulse repetition rate was 100 Hz. The unit was used to study microbial and enzymatic inactivation in laboratory scale.

Figure 3.2: Pulse modulator with 16 kV peak voltage and 100 J/s average power (right rack) and fibre optic temperature measurement unit (left).

***C) Exponential decay lab scale pulse modulator, 36 kV, 800 J/s, TUB 3***

To improve reliability of switching system for applications in waste water processing a system based on a spark gap has been designed. A storage capacity of four Ceramite Y5U 6800Z (Behlke, Kronberg, Germany) with a total capacity of 27.2 nF was charged by a FUG HCK 500M-65000, 65 kV, 50 mA (FUG, Rosenheim, Germany). Three types of OGP 75 (Perkin Elmer, Salem, USA) with breakdown voltages of 18.5, 25 and 36 kV were used to discharge the energy stored across three parallel 15 Ohm resistors AS886A (Kanthal, Amherst, USA). The system was used for waste water treatment and inactivation experiments where pulse voltages above 20 kV were required. As untriggered spark gaps have been used the pulse frequency was adjusted by the charging current level, the peak pulse voltage was determined by the breakdown voltage of the respective spark gap. Data acquisition was performed using a 400 MHz digital storage oscilloscope (TDS 430A, Tektronix, Wilsonville, OR) and a 75 MHz high voltage and a 100 MHz current probe connected to an amplifier system. Spark gap lifetime was dependent on media conductivity and pulse repetition rate applied and varied between approx. $10^5$ to $10^8$ pulses. During gap lifetime the breakdown voltage decreased, after a decrease of 10 % the spark gap was replaced. The maximum repetition rate, limited by the recovery time of the gap was 70 Hz.

***D) Exponential decay lab scale pulse modulator, 24 kV, 1600 J/s, TUB 4***

To allow a solid state pulse modulation up to 24 kV a thyristor system was assembled, using a FUG HCK 500M-65000, 65 kV, 50 mA (FUG, Rosenheim, Germany) capacitor charger and five parallel Ceramite Y5U 6800Z (Behlke, Kronberg, Germany) with a total capacity of 34

nF. A HTS 240-800 SCR (Behlke, Kronberg, Germany), protected by a diode FDA 240-500 was used for repetitive discharge of the energy stored. Maximum pulse repetition was dependent on peak voltage and subjected to average power of the power supply. At 20 kV the maximum repetition was 212 Hz, at this voltage the energy delivered per pulse was 6.8 J. Due to the low storage capacity and limited pulse energy this system was used for microbial inactivation experiments in treatment chambers with low volume and was not applicable for treatment of fruit mashes or whole fruits, where a larger cross section and higher pulse energy was required.

***E) Exponential decay technical scale pulse modulator, 20 kV, 5500 (40000) J/s, TUB 5***

In cooperation with the University of Applied Sciences, Gelsenkirchen and Barmag, Germany a capacitor charger based on standard components available for motor drive control or other industrial equipment was developed (see Figure 3.3). The design is based on a 400 V frequency converter and subsequent transformation to 20 kV. A 2 A constant charging current at 20 kV maximum was delivered by the unit, the maximum charging power was 40 kJ/s. The unit was controlled using a CTherm PLC (ESD, Hannover, Germany) with internal trigger (+5 V TTL signal). The power supply has a size of 250 x 215 x 80 cm and a weight of 1.5 t. A capacitor bank of four DP 30560 (GA, San Diego, USA), 15 kV, 2 µF in series has been used to achieve a total capacity of 0.5 µF and a maximum voltage above 15 kV. Wire resistors have been used to balance the voltage drop across the capacitors. The charging time required to obtain a voltage of 14 kV was 1.1 ms, whereas the recovery time of the control unit and the charging system was up to 16 ms. The time required to discharge the energy stored was in a range of 200 to 500 µs, dependent on media conductivity. A total cycle time of 18 ms was required for delivery of a pulse, limiting the maximum repetition rate of the charging process to 56 Hz. During capacitor discharge an inhibition of charging was required for switch recovery, the maximum current to allow recovery of the HTS 240-800 was 50 mA. At a charging voltage of 14 kV and 20 kV the maximum power output was therefore limited to 2700 J/s and 5500 J/s, respectively. One way to work around this limitation would be the use of a larger capacitor, which would require a treatment chamber with larger volume to achieve similar specific energy input per pulse. As by increase of electrode gap the voltage required to achieve sufficient electric field strength is increasing the maximum output voltage of the charger of 20 kV was limiting. At present a control unit with shorter recovery time is implemented into the capacitor charging unit to reduce total cycle time and obtain higher power output.

Figure 3.3: 20 kV, 40 kJ/s power supply (left) and pulse modulator internal view (right), showing thyristor switch in the front, five parallel protective resistors and a setup of capacitors in series.

***F) Exponential decay technical scale pulse modulator, 24 kV, 8000 J/s, TUB 6***

For treatment of fruit or vegetable mashes and oil seeds a technical scale pulse modulator with an average power of 8 kJ/s and a maximum voltage of 24 kV was developed. A solid stated design based on a thyristor switch was used. A capacitor bank of four DP 30560 (GA, San Diego, USA), 15 kV, 2 µF in series has been used to achieve a total capacity of 0.5 µF and was charged using an ALE802 (Lambda-Emi, Neptune, USA), 40 kV power supply. The maximum charging current was 400 mA, an inhibition of charging was required during capacitor discharge to allow switch recovery. For trigger and charging inhibition a 110GP (Thurlby Thandar Instruments, Huntingdon, UK) function generator was used, supplying 1 ms rectangular pulses of + 5 V. The pulse repetition rate was adjusted by variation of rectangular trigger pulse frequency. For prevention against current reversal a FDA 240-500 (Behlke, Kronberg, Germany) was used, for current limitation 5 parallel 889AS (Kanthal, Amherst, USA) with a total resistance of 15 Ohms and a total maximum power dissipation of 500 W have been used. Current and voltage have been monitored by a TDS 220 Oscilloscope (Tektronix, Beaverton, USA). For load balancing and discharge of stored energy four 10 MOhm resistors have been connected in parallel to the capacitors, a high voltage relay E25NC (Ross Engineering Corp., Campbell, USA) has been used for safety discharge in case of emergency off pressed or interlock circuit signal. The unit proved to be stable in long term trials in industrial environments and well protected against operation with open load or short circuit.

Figure 3.4: 24 kV, 8000 J/s pulse modulator (left), power supply (right) and frequency generator (top of power supply)

***G) CoolPure, 10 kV, 8000 J/s***

The CoolPure® pilot scale equipment developed by PurePulse since 1995 was available to the Department of Food Biotechnology and Food Process Engineering; it is consisting of a power supply with a maximum voltage of 10 kV and an average power of 8000 J/s. An internal storage capacitor of 4 µF is periodically discharged across a thyristor disk switch and protective resistors. The maximum pulse repetition rate was 30 Hz, the pulse width in a range of 100 to 600 µs, dependent on treatment chamber geometry and media conductivity. An internal function generator was used for switch triggering. A PM 3335 (Philips, The Netherlands) oscilloscope was used for monitoring of pulse waveform. The equipment was used for preliminary experiments for fruit and vegetable processing in a batch-wise operation.

***H) Exponential decay technical scale pulse modulator, 40 kV, 8000 J/s, TUB 7***

For treatment of meat products such as salted, minced meat, pork haunches or shoulders a PEF system with large energy per pulse and over-current and short circuit protection was required. The minimum treatment chamber size and electrode area was determined by the meat piece dimensions, a gap of at least 15 cm was required and the impedance of the treatment chamber typically was in a range of a few Ohms only. For this purpose a solid state system was decided to be to sophisticated and a simple and reliable setup using an air spark gap was designed Figure 3.4. The storage capacity could be varied between 1 and 3 µF at a maximum voltage of 40 kV, an ALE802 (Lambda-Emi, Neptune, USA), 40 kV power supply was used for capacitor charging. A spark gap with tungsten electrodes with an adjustable electrode gap between 1 and 40 mm was used to discharge the electrical energy into the treatment chamber. A cuvette filled with salted water to achieve a 0.5 Ohm

resistance was used as current limiting resistor when required. The maximum pulse repetition was dependent on charging voltage, at 30 kV and a capacity of 1 µF a maximum repetition of 17 Hz was obtained. The energy per pulse was 450 J at this voltage. The pulse width was dependent on type of product treated, but was typically within a range of 10 to 400 µs. The maximum peak current was up to 35 kA for treatment of salted minced meat. The lifetime of the spark gap was dependent on pulse ratings, in particular when operating at high repetition rate surface erosion was noticed. A manual discharge device was used to discharge residual energy prior to servicing.

***l) Rectangular pulse modulator, 50 kV, 7000 J/s, TUB 8***

For comparison of exponential decay and rectangular pulse shape efficiency for microbial inactivation a modified rectangular pulse modulator based on an M2 standard design (ScandiNova Systems, Uppsala, Sweden) was used (Figure 3.5). The modulator consists of a high voltage power supply with 1 kV maximum voltage and 12000 J/s average power, six energy storage capacitor banks and six parallel IGBT units for rectangular pulse generation. Using a 1:50 pulse transformer at the secondary side up to 50 kV pulses can be achieved, the maximum current is limited to 200 A, the maximum repetition rate is 400 Hz, subjected to average power. Pulse width is adjustable between 3 and 8 µs, the system is IP 65 protected and suitable for industrial environments. Voltage and current monitoring is performed using a TDS 430 (Tektronix, Beaverton, USA). Trigger and control system are included, the unit can be operated as stand-alone system or controlled by analogue input signals. The system could be used for continuous treatment with production rates of up to 200 l/h.

Figure 3.5: Rectangular pulsed power modulator, 50 kV peak voltage, 200 A peak current, 400 Hz.

## 3.2 Treatment Chambers

### *A) Micro batch PEF-treatment cuvettes*

For flow cytometric investigation of permeabilization mechanisms using fluorescent dyes and to study the impact of pulse rise time variation micro cuvettes with aluminum parallel plate electrodes, 20 x 2 mm, 2 mm gap, 400 µl volume (Eppendorf, Hamburg, Germany) have been used. An amount of 405 µl sample (stained with propidium iodide if applicable) was filled into the cuvette, a preheating within a water bath was performed for investigation of treatment temperature impact. The cuvettes have been treated using the micro pulse modulator (A). Temperature rise during treatment was observed using a fibre optic sensor (Takoaka, Tokyo, Japan). After treatment the cuvettes have been placed on ice, in case of immediate propidium iodide staining the dye was given into the sample after treatment. Subsequently the cuvettes have been cleaned with distilled water, sterilized in ethanol and rinsed with distilled water. The load resistance of the chamber was 50 Ohm in case of Ringer solution adjusted to a conductivity of 2 mS/cm

### *B) PEF microscope*

A PEF-treatment chamber suitable for placement on a microscope (Zeiss Optik, Jena, Germany) was developed, using copper foil electrodes with an electrode gap of 0.5 mm, a length of 3 mm and a thickness of 0.2 mm. The electrode area was 0.6 $mm^2$, the chamber volume was 0.3 µl. The impedance of the electrode system was approx. 4 kOhm when using a buffer solution with a conductivity of 2 mS/cm. The maximum magnification was 400 fold. The chamber was used to visualize the impact of a PEF-treatment on plant tissue, electrophoretic effects and to investigate pH changes during treatment using a pH indicator.

### *C) Continuous lab-scale parallel plate system*

A setup of two parallel stainless steel electrodes with a gap of 2.5 mm and an electrode area of 2 $cm^2$ was used for microbial inactivation studies in fruit juices (Figure 3.6). The spacer was made of Teflon. With apple juice at 55°C ($\kappa \approx 0.3$ mS/cm) a load resistance of approximately 40 Ω is obtained, exemplarily, the maximum flow rate was 5 l/h.

Figure 3.6: Parallel plate continuous treatment chamber with stainless steel electrodes and Teflon insulator, 2 mm electrode gap.

***D) Continuous lab scale co-linear system***

To increase the treatment chamber resistance and to improve matching between pulse modulator and electrical load treatment chambers with small electrode area were required. When a solid state switch is used a current limiting, protective resistor is required, typically in a range of 10 to 20 Ohms. Parallel plate electrodes commonly have a typical resistance in a range of 30 to 50 Ohm, the ratio between energy dissipated in the resistor and delivered to the load is therefore in a range of 1:3 to 1:5 and up to 33 % of the electrical energy needs to be removed from the protective resistors by forced air cooling. A co-linear treatment chamber design was developed with the aim to increase its load resistance even with highly conductive media, to provide a sufficient field homogeneity and treatment intensity distribution and to be suitable for particulate or highly viscous food. The chamber has been used for microbial inactivation in Ringer solution, fruit juices, milk as well as treatment of algae extracts and waste water, the maximum flow rate was 10 l/h. Subsequently different modifications and improvements have been performed; chambers with an internal diameter of 10 mm and electrode gap of 10 mm and different sealing and insulator materials have been used. The maximum flow rate was increased to 25 l/h with the 10 mm chamber and suitability for waste water and sludge treatment improved. To investigate the stability against erosion the initial stainless steel electrodes were replaced, copper beryllium (Unipress, Warsawa, Poland), graphite and titan-covered platinum (Metakem, Germany) have been used as electrode material. Electrode erosion was determined by long-term runs for 500 h and gravimetric determination of weight loss. As sealing material silicone foils have been cut to shape, in later models o-rings have been used to improve leak tightness when working with back-pressure.

***E) Continuous technical scale co-linear system for fruit mashes and minced meat***

For continuous treatment of fruit mashes using a solid state pulse modulator a treatment chamber with a cross section of 7 $cm^2$ was required to prevent clogging and to maintain a flow velocity below 2 m/s. A system with a minimum diameter of 30 mm was designed, allowing a maximum transport capacity of up to 5 t/h (Figure 3.7). To improve treatment

homogeneity the insulators where pinched in comparison to the electrodes inner diameter of 34 mm. To avoid local field enhancement the edges where chamfered with a radius of 2 mm. The insulators were made of Marcor® ceramic material, but the mechanical resistance of the material showed to be not sufficient for field tests in technical scale, a second set of insulators from Acetal Delrin® was realized. Pressure resistance up to 4 bar was required, as the unit was designed for continuous treatment of apple and carrot mash as well as olive treatment directly to decanter centrifuges. A support clamp of high density polyethylene was designed. The chamber was placed into a steel box for shielding and protection against splash water, a door interlock switch was used. The exterior dimensions of the chamber are 60 x 60 x 25 cm at a weight of 12 kg.

A similar design with an inner diameter of 60 mm was realized for treatment of minced meat and sausage meat for production of Salami-type raw sausages. The chamber was installed at Kemper Fleischwaren, Nortrup, Germany during a field test and operated at a production rate of up to 400 kg/h prior to filling of sausages. A peak voltage of 36 kV was used to obtain an average field strength of 4.7 kV/cm in the treatment chamber, the repetition rate was adapted to the flow rate in a range of 10 to 50 Hz.

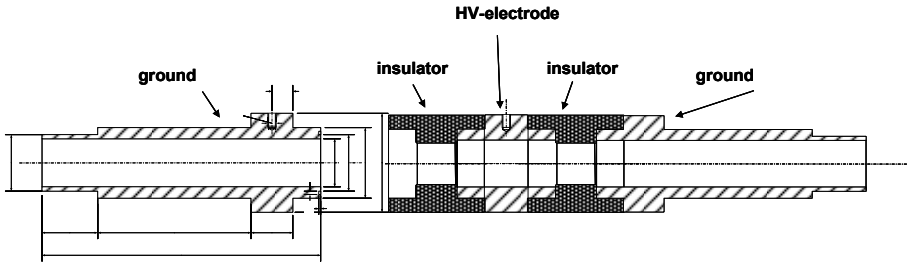



Figure 3.7: Schematic view of colinear PEF-treatment chamber with a diameter of 30 mm and an electrode gap of 30 mm.

### *F) Continuous chamber for potato processing*

A treatment chamber designed by Alexander Angersbach was modified and used for treatment of whole potato tubers, the system consisted of a conveyor belt with a width of 9 cm, transporting potatoes in a water bath through a treatment channel of 9 cm width, 9 cm height and a length of 38 cm. In this channel four stainless steel electrode pairs were placed, two of the in horizontal and two in vertical alignment, the electrode size was 9 x 2 cm, the electrode gap was 9 cm. Dependent on media conductivity and number of electrode pairs connected the resistance of the chamber was in a range of 50 to 450 Ohm. The belt speed was adjustable to achieve a total residence time in the treatment channel from 2 to 60 s. The maximum production capacity was dependent on the size of the tubers in a range of 200 to 500 kg/h. The unit was used for continuous treatment of potatoes for drying improvement and to induce structural changes.

### *G) Batch chambers for meat and plant material processing*

To investigate the impact of a PEF-treatment on drying of Serrano-type ham a treatment chamber for whole pork haunches was realized. The typical size of a pork leg is in a range of 50 x 28 x 15 cm. A parallel plate treatment chamber for batch-wise operation was built with an electrode size of 49,5 x 32 cm and 45 x 29,5 cm for the upper and lower electrode, respectively (Figure 3.8). The electrode gap was variable between 8 and 18 cm (max. 25 l volume). When filled with tap water with a conductivity of 0.6 mS/cm the resistance of this chamber was measured as 3.8 Ohms at an electrode gap of 15 cm. Filled with a haunch and water the resistance dropped to approx 1.5 Ohm only, requiring a pulse generator able to stand high peak currents of up to 27 kA at a peak voltage of 40 kV. The treatment chamber was used for treatment of haunches as well as other meat products such as shoulders or filets of different origin. In addition a treatment of minced pork meat and chicken or duck meat has been performed. The impact of a PEF-treatment on drying, brining, pickling and meat curing was investigated. Dependent on product properties the addition of water was avoided if applicable. A treatment of pork shoulders was shown to be possible in direct electrode contact without water addition if the chamber was properly filled. A total amount of 25 kg could be treated at one time, filling and unloading required one minute in total, the treatment time was one minute, so the maximum production capacity during field tests was 750 kg. Subsequently also several larger chamber designs have been realized, the largest having an inner dimension of 59 x 40 x 40 cm, suitable for treatment of up to 100 kg meat per treatment. Smaller treatment chambers with an electrode size of 20 x 7 cm and an electrode gap of 3 (420 ml volume) or 5 cm (700 ml volume) have been realized.

Figure 3.8: Batch PEF-treatment chamber with a volume of 25 l

## 3.3 PEF-Application for Disintegration of Biological Tissue

### 3.3.1 Raw materials used

#### 3.3.1.1 Apple treatment

For lab scale tests apples of the varieties Royal Gala, Braeburn, Jona Gold, Golden Delicious and Granny Smith were obtained from a local grocery store. In addition Royal Gala and Boskoop were obtained from Werder Frucht (Werder, Germany) and a grocery store in Scharnhausen, Germany for pilot tests at the Department. The raw material was stored at + 4 to + 8°C, a coarse grinding of 2 kg of apples was performed by a UMC 12 (Stephan Machinery, Hameln, Germany), when appropriate ascorbic acid (1 g/kg of mash) was added and mixed for 15 s to prevent browning. The apple mash was randomly divided into three lots of 400 g each. Two lots (samples a and b) were each filled into the PEF-treatment chamber, treated with pulsed electric fields and subsequently pressed manually (Hafico HP 2, Tinkturenpressen Schwanke, Neuss, Germany). During pressing, the hydraulic pressure was raised up to 250 bar three times. Manual pressing was discontinued after the juice flow had stopped. The third lot of the same mash was pressed without previous treatment (control). Electric field strength was varied from 1 to 5 kV/cm, pulse number from 10 to 40 and treatment temperature between 10 and 30°C. Alternatively, two lots of the mash were macerated using a pectolytic enzyme preparation (100 µL/kg of mash) for 1 h at 30 °C instead of the PEF-treatment before pressing, while a third lot of the mash provided the control without enzymatic or PEF-treatment. The maceration preparation Fructozym MA (Erbslöh, Geisenheim, Germany) was used according to the supplier.

For technical scale tests at the Department of Food Biotechnology and Food Process Engineering the batch treatment chamber (G) and pulse modulator (G) and the continuous treatment chamber with a diameter of 30 mm (E) have been used at a flow rate of 200 kg/h. Grinding of apples (Royal Gala and Jona Gold varieties, Werder Frucht, Werder, Germany) was performed using a fruit mill with a maximum capacity of 1 t/h, pressing was performed using a Hollmann (Remscheid, Germany) baling press with sample sizes of 50 kg. The maximum pressure applied was 20 bar, the pressing was performed using a pressure time regime of 2 min initial phase without pressure and subsequently increasing the pressure every 2 min by an increment of 4 bar. After 12 min the pressing was stopped and the yield determined gravimetrically.

For field tests at the University of Applied Sciences in Geisenheim the 20 kV 5500 J/s pulse modulator has been used with the continuous treatment chamber with a diameter of 30 mm (E). Electric field strength was set to 3 kV/cm, the specific energy input was varied between 5

and 15 kJ/kg by adjusting pulse frequency. At a flow rate of 290 kg/h a pulse repetition of 40 Hz was required, exemplarily, corresponding to an average number of 21 pulses applied per volume element. The temperature increase after PEF-treatment never exceeded 3°C. Apples of variety Boskoop (Kirsch Obstbau, Rüdesheim, Germany) and a mixture of industry apples (Eckes-Granini, Nieder-Olm, Germany) have been used. For experiments with stored apples Jona Gold and Golden Delicious (Gottschalk, Ingelheim, Germany) have been used. Grinding of fruits has been performed using a BTM 5 (Seepex, Bottrop, Germany) or a fruit mill (Amos Engineering, Heilbronn, Germany), for liquid solid separation a HPL 200 press (Bucher-Guyer Foodtech, Niederweningen, Switzerland) or a decanter centrifuge Z23-3 (Flottweg, Vilsbiburg, Germany) was used. Juice yield was determined gravimetrically for application of the decanter centrifuge and by determination of the press piston position for juice pressing. After juice winning the juices have been pasteurized, clarified, filtrated and filled into 0.7 l glass bottles for conductance of storage tests and juice quality analytics. The residue was dried in a fluidized bed convective air dryer TG 100 (Retsch, Haan, Germany) for subsequent extraction of pectin and pectin quality analysis. An overview of analytical methods used for juice quality evaluation in cooperation with the University of Hohenheim, Stuttgart, Germany can be found in Table 1.

Table 1: Analytical methods used for determination of apple juice quality parameters

| **Parameter** | **Method** |
|---|---|
| Soluble dry matter (° Bx) | Refractometry |
| pH | Porentiometric (IFU-Method Nr. 11) |
| Density | Density Measurement |
| Total acid | Titrimetric (IFU-Method Nr. 3) |
| Sugar (glucose, fructose, saccharose) | Enzymatic (Testkit; R-Biopharm AG, Darmstadt, Germany) |
| Ascorbic acid | Enzymatic (Testkit; R-Biopharm AG, Darmstadt, Germany) |
| Sorbit | Enzymatic (Testkit; R-Biopharm AG, Darmstadt, Germany) |
| L-malic acid | Enzymatic (Testkit; R-Biopharm AG, Darmstadt, Germany) |
| Phenolic compounds | HPLC (DAD, MS) |
| Total phenolics | Folin-Ciocalteu |
| Antioxidant capacity | TEAC, FRAP, DPPH |
| Browning index | Photometric (420 nm) |
| Color | L*a*b*-values (Photometric) |
| Turbidity distribution | Mastersizer |
| Turbidity stability | Centrifugation, storage tests |

#### 3.3.1.2 Potato treatment

Potatoes of the varieties Bintje and Russet Burbank obtained from a local grocery store have been treated to determine a PEF impact on tissue integrity and softening by impedance and textural analysis. Both varieties have also been used to investigate the impact of PEF on convective air drying in a fluidized bed dryer (ATP, Berlin, Germany) with a maximum heating

power of 16 kW. A drying temperature of 40 to 130°C was used for potato drying, the air velocity was set to 2 m/s. The PEF-treatments have been performed using the PurePulse (G) 10 kV pulse modulator and the 420 and 700 ml batch treatment chambers as well as using the 25 l (G) when a larger amount of samples was required.
Extractability of an anthocyanin-rich pigment from purple-fleshed potato cubes with a size of 1 x 1 x cm *(Solanum tuberosum L.)* (Elbländische Pflanzengarten, Lenzen, Germany). after a PEF-treatment was investigated by water and alcoholic extraction. After blanching at 70°C for 5 min the PEF-treatment (1.5 kV/cm, 150 pulses) was applied to the extraction media adding an amount of ethanol to the blanching media to obtain a ratio of 50 % ethanol. Extraction was performed at 60 °C for 3 hours in a shaker (Certomat U, Braun, Melsungen, Germany). Subsequently the extracts were concentrated to 65°C and a color and stability analysis was performed, determining A) determining the L*a*b*- values, anthocyanin content, turbidity value and stability and a transmission measurement at a wavelength of 660 nm

#### 3.3.1.3 Garden Huckleberry and grapes

In addition to purple-fleshed potatoes the anthocyanin extraction from eight varieties of Garden Huckleberry (*Solanum scabrum*) supplied by Genebank IPK Gatersleben, Germany (1: SOL 28/2 67 15.3 50 – 80 and 2: SOL 402/76 80 10.0 70 – 90), Genebank Nijmegen, The Netherlands (3: 99 4750 011 20 26.5 0 – 50, 4: 99 4750 005 73 11.5 60 – 80 and
5: 99 4750 019 80 10.0 70 – 90), Botanischer Garten Bonn, Germany (6: 53 15.3 40 – 70 and 7: 19 700 56 6.8 50 – 60) and VERN, Greiffenberg, Germany (8: 47 11.5 40 – 60) has been investigated. *Solanum scabrum* fruit samples of 1000 g have been grinded using a UMC 12, (Stephan Machinery, Hameln, Germany) for 15 s. The mash was divided in two lots, each 500 g. One of them was subjected to a pulsed electric field treatment (PEF) at a field strength of 1.3 kV/cm and 120 pulses prior to pressing. The Cool Pure® pulsed electric field treatment system was used with the 420 ml batch chamber. A lab scale press (Hafico HP 2, Schwanke Tinkturenpressen, Neuss, Germany) was used for juice winning, a pressure of 8 bar was obtained at a hydraulic pressure of 200 bar. The pressing time was 2 min. The anthocyanin content of the juice was determined by photometric analysis at a wavelength of 520 nm using a Spectrophotometer U-3000 (Hitachi, Tokio, Japan).

The extractability of anthocyanin, total polyphenolics and the impact of antioxidant capacity of a PEF-treatment of grapes pomace (*vitis vinifera spp.)* was investigated in cooperation with the Federal Research Centre for Nutrition and Food, Germany in comparison to a conventional solvent extraction and a high hydrostatic pressure extraction. The PEF-treatment was performed using the Cool Pure® modulator (E) and the 420 ml (G) batch

treatment chamber at an electric field strength of 3 kV/cm, 30 pulses and a specific energy input off 10 kJ/kg. The pulse repetition rate was 2 Hz, the time required for the treatment was 15 s. As treatment media a mixture of ethanol and water (40 % Ethanol) was used. A ratio of 4.5 ml extraction solvent for 1 g grape skins has been used. The subsequent extraction was performed at a temperature of 60°C for 1 h. High-pressure extractions were conducted in thermostated microautoclaves with a volume of 5mL at a pressure of 600MPa and a temperature of 70°C, the ethanol concentration used for extraction was 50 %.

#### 3.3.1.4 Treatment of meat and meat products, fish and seafood

Different types of meat and meat products have been used to investigate PEF effects on meat texture and mass transport. For drying improvement lab scale tests have been performed using pork shoulders (Bahlmann, Berlin, Germany). The PEF-treatment has been performed using a 40 kV 8000 J/s (H) pulse modulator and a 25 or 100 l batch treatment chamber (G). Electric field strength was varied from 0.5 to 5 kV/cm, applying up to 1000 pulses and a total energy input between 1 and 25 kJ/kg. Drying was performed using a climatic cabinet (Weiss Klimatechnik, Reiskirchen, Germany) at an air temperature of 8°C and a relative humidity of 95 %. Additionally 5 % of salt was added as commonly applied during raw ham production, either by manual hand salting of the meat surface or injection of 10 % saturated salt brine, using a IR 28 injector (Rühle, Grafenhausen, Germany).
For technical scale tests approx. 30 whole pork haunches and one ton of pork shoulder (Gebrüder Abraham Schinken, Edewecht, Germany) have been used, salting and drying were performed at the facilities of Abraham, a 100 l batch PEF-treatment chamber was used.

The impact of PEF during production of cooked ham was investigated using a 40 kV 8000 J/s (H) pulse modulator and a 25 l batch treatment chamber (G). Pork haunches were obtained from Bahlmann (Berlin, Germany) and Metro Store (Berlin, Germany) for tests performed at the Department of Food Biotechnology and Food Process Engineering. Pork haunches were weighed and subject to a PEF-treatment with an electric field strength from 0.5 to 5 kV/cm, a pulse number of 50 to 1000 pulses and a specific energy input from 1 to 25 kJ/kg. The PEF-treatment was either performed prior or subsequent to brine injection. Salt brine was injected using a IR 28 (Rühle, Grafenhausen, Germany) industrial or a PB1 (Rühle, Grafenhausen, Germany) manual injector. A 10 % salt brine was used, the effect of addition of polyphosphate EldoLak (HageSüd, Hemmingen, Germany) or SchinkenQuick (Beck, Schnaitach, Germany) as well as soy protein, carragenaan and starch (Hahn, Lübeck, Germany) was investigated. A tumbling step for kneading of meat pieces and to improve brine dispersion using a MKR 150 (Rühle, Grafenhausen, Germany) has been applied after

PEF-treatment and injection. During tumbling a temperature of 4°C set, tumbling time was selected between 15 min and 8 h, the impact of vacuum application during tumbling was investigated. The tumbling intensity was characterized by the tumbling time or the number of tumbling rounds (TR).

For production of prosciutto type ham an ER 1 (Rühle, Grafenhausen, Germany) was applied for introducing meat pieces into a collagen foil and a net. A 4 h at 4°C curing was applied after PEF-treatment if not stated otherwise. Cooking was performed until a core temperature of 65°C using a steam oven (Rational, Landsberg a. Lech, Germany) with automatic cooking program for ham or a climatic cabinet (Weiss Klimatechnik, Reiskirchen, Germany) set to 90°C air temperature and 85 % relative humidity. After cooking and cooling of the samples the drip loss was investigated and a texture as well as a sensorial analysis performed.
Field tests have been performed at Kemper Wurstwaren (Nortrup, Germany) as well as Hahn Stabilisierungstechnik (Lübeck, Germany) and Beck (Schnaittach, Germany), except the PEF equipment using equipment available at the respective facilities but with a similar procedure.

Minced meat (MeMa, Berlin, Germany) and sausage meat (Kemper Fleischwaren, Nortrup, Germany) were treated in batch-mode, using the 700 ml and 25 l treatment chamber as well as continuously, using the 60 mm collinear chamber. The impact on lactic acid fermentation, drying and curing was investigated by pH measurement and weight determination.

The impact of a PEF-treatment on fish tissue as well as different aquatic species was investigated in cooperation with the Icelandic Fishery Institute (IFL), Reykjavik, Iceland. Frozen cod fillets, shellfish loins and scallops have been obtained by the IFL, fresh cod and Pollack fillets from a local store (Lindenberg, Berlin, Germany). The samples were treated with different intensities, by variation of the pulse number n (20, 40, 80 or 120) and the electric field strength E (1.2 kV/cm or 2.0 kV/cm). The pulse width (to a decay to 37 %) was 400 microseconds. Samples were weighed before and after PEF-treatment. After treatment salt brine (approx. 10 %) was manually injected by using syringes with a volume of 20 ml, or using a commercially available injection machine IR 28 of Rühle. After injection the fish samples were packed in vacuum bags, stored at 4°C for 1 to 4 h and cooked in a water bath at 95°C. After cooking the water loss was evaluated. After PEF-treatment of scallops a tumbling step was performed to investigate its impact on textural properties, a Rühle MKR 150 tumbler was used. Texture analysis was performed using a texture analyzer TA-XT2 (Stable Micro Systems, Godalming, U.K.)

### 3.3.2 Cell permeabilization index, Impedance analysis

A method developed by Angersbach *et al.* (1997, 1999) was used to determine the impact of PEF on integrity of biological tissue. This method is based on interfacial polarization effects of the Maxwell-Wagner type at the intact membrane interfaces. Measurement of the frequency-dependent passive electrical properties of biological cell systems (for the vegetable and muscle tissues characterized frequency ranges from $10^3$ to $10^7$ Hz) allows the simple quantification of the degree of membrane disintegration. An impedance analyzer (Biotronix, Henningsdorf, Germany) was used to apply a frequency sweep to cylindrical samples of 5 mm diameter and 10 mm length placed within a stainless steel electrode system. Cell disintegration Index $Z_p$ was calculated by:

$$Z_p = 1 - b_k \frac{(K_{HF} - K_{NF})'}{(K_{HF} - K_{NF})}$$

Equation 9

Where $K_{HF}$ and $K_{NF}$ denote the untreated sample conductivity in high and low frequency range, respectively, $K_{HF}'$ and $K_{NF}'$ the treated sample and $b_k$ denotes a correction factor. The index has a value between 0 (intact tissue) and 1 (totally disintegrated tissue).

### 3.3.3 Moderate electric field treatment

For comparison of high intensity pulsed electric field treatments with high energy per pulse and effects of moderate electric field applications described by (Jemai and Vorobiev 2002; Cousin 2003; Lebovka *et al.* 2004; Sensoy and Sastry 2004) a power supply was realized. The treatment was applied using a parallel plate treatment chamber with an electrode gap of 3 cm and an electrode size of 5 x 8 cm. The resistance of the chamber was in a range of 60 to 100 Ohm. The power supply was designed to deliver 120 to 200 V alternating, sinusoidal current at a frequency of 50 Hz, the maximum electric field strength was 40 to 66 V/cm. Current flow and power output were observed using a clamp meter IPM 3000 (RS components, Morfelden, Germany), the energy input was varied between 2 to 40 kJ/kg, corresponding to a temperature increase between 0.25 and 10.5°C for fruit mash with a specific heat capacity of 3.8 kJ/(kg K). The device was used for treatment of apple samples (Braeburn and Royal Gala) and potato samples (Bintje). After treatment cell disintegration index $Z_p$ was determined, for potato samples also textural properties were investigated.

## 3.4 PEF-Application for Microbial Inactivation

Kinetic studies have been performed using a continuous lab scale parallel plate and co-linear chamber. The electric field strength was varied between 5 and 50 kV/cm, the treatment temperature between 20 and 70°C, the specific energy input between 10 and 3,000 kJ/kg. The flow rate for kinetic studies was 5 l/h, if not stated otherwise. Field tests have been performed at higher flow rates, as indicated in the results and discussions section. The impact of pulse width, energy per pulse, pulse rise time and pulse shape has been studied. The inactivation of different microbes in a variety of products has been investigated using synergetic effects of temperature. Enzymatic inactivation was studied for lipoperoxidase in milk and polyphenoloxidase in apple juice. Liposomes have been used as a model system to show the effect of PEF application on phospholipid bilayers. Temperature measurement during and after a PEF-treatment was performed using a fibre optic temperature sensor TAKAOKA 1110 (Takoaka, Tokyo, Japan), which was not susceptible to electric fields. The sensor has a diameter of 1.2 mm and a reaction time of approx. 1 s.

### 3.4.1 Liposome generation as a model membrane system

To study the impact of pulsed electric fields on artificial phospholipids bilayers and comparison to biological cell membranes liposomes charged with carboxyfluorescine diacetate (cFDA) have been used as model systems. Egg phosphatidylcholin solved in chloroform (Lipid Products, South Nuffield, UK) was dried in nitrogen stream and stored under vacuum for 24 h for residual chloroform removal. Subsequently the lipid was resuspended in 1 ml of carboxyflourescine solution (Molecular Probes, Leiden, The Netherlands) and unilamellar liposomes were produced by manual extrusion of an egg phosphatidylcholin suspension through a polycarbonate membrane of 1,000 nm pore size using a hand-held LiposoFast Extruder (Avestin, Mannheim, Germany) for 15 times. In preliminary experiments an optimum concentration of 1,000 mM CF was found. To remove excessive carboxyflourescine a washing step was applied, using a NAP-5-column (Amersham Biosciences, Freiburg, Germany). A total liposome concentration of 10 mg/ml lipid was obtained. To investigate the uptake of dye uncharged vesicles have been produced, CF was added to the media prior to treatment. PEF-treatment of liposome solutions was performed using the micro system (A) and a 400 µl batch treatment chamber (A), for subsequent data of stained liposomes acquisition flow cytometry was used.

### 3.4.2 Flow Cytometry

*Staining procedure for microbial cells:* PEF- treated cells were initially incubated with 50 mm cFDA (Molecular Probes Inc., Leiden, NL) at 37°C for 10 min to allow intracellular enzymatic conversion of cFDA into cF. Cells were then washed to remove excessive cFDA. This step was followed by addition of 30 mm PI (Molecular Probes Inc., Leiden, NL) and by incubation in ice bath for 10 min to allow labeling of membrane-compromised cells. To measure the performance of treated cells in extruding intracellular accumulated cF activity, cF-stained cells were incubated together with glucose 20mm for 20 min at 37°C. To monitor the kinetics of cF-release from glucose energized cells, cF-labeled cells were incubated at 37°C in the presence of glucose 20 mM and the progress was measured every 5 min.

*Flow cytometric measurement:* Analysis was performed on a Coulter EPICS XLMCL flow cytometer (BeckmanCoulter Inc., Miami,FL, USA) equipped with a 15 mW, 488 nm air-cooled argon laser. Cells were delivered at a low flow rate, corresponding to 400 – 600 events/s. Forward scatter (FS), sideward scatter (SS), green (FL1) and red fluorescence (FL3) of each single cell were measured, amplified, and converted into digital signals for further analysis. cF emits green fluorescence at 530nm following excitation with laser light at 488 nm, whereas red fluorescence at 635 nm is emitted by PI-stained cells. A set of band pass filters of 525 nm (505 – 545 nm) and 620 nm (605 – 635 nm) was used to collect green fluorescence (FL1) and red fluorescence (FL3), respectively. All registered signals were logarithmically amplified. A gate created in the dot-plot of FS vs. SS was preset to discriminate bacteria from artifacts. Data were analyzed with the software package Expo32 ADC (Beckman- Coulter Inc., Miami, FL, USA). All detectors were calibrated with FlowCheck Fluorospheres (Beckman-Coulter Inc., Miami, FL, USA).

*Data Analysis:* Dot plot analysis of FL1 vs. FL3 was applied to resolve the fluorescence properties of the population measured by flow cytometry. The population was graphically differentiated and gated according to its fluorescence behavior. Based on the shift of cF-stained population upon glucose addition from gate A4 into gate A3 after a 20 min incubation period the cF-extrusion was able to be monitored as an indicator for active transport across cell membranes.

### 3.4.3 Microbial growth conditions and analysis

To investigate microbial inactivation by PEF application the following strains were obtained by the culture collection of the Department of Food Biotechnology and Food Process engineering or the source mentioned: *E. coli* K12 DH5$\alpha$, *Rh. Rubra* (0805, VLB, Berlin, D), *L. rhamnosus* GG ATCC 53103, *L. acidophilus* ATCC 4356, *Candida utilis, B. subtilis, Erwinia carotovorum (*ATB, Potsdam, Germany)*, B. megaterium* DSM 322 (DSMZ, Braunschweig, D*), Pseudomonas fluorescens* DSM 50090 (DSMZ, Braunschweig, D*), A. niger* ATCC 16404, *S. cerevisae* DSM 70451 and *L. innocua* NCTC 11289. The pathogenic strain *L. monocytogenes* ATCC19114 was obtained from the Federal Institute of Risk assessment, Berlin, Germany, treatments and analysis were performed at place. Inocula were prepared from stock cultures 24h before each experiment by inoculating 50 ml of nutrition broth. As media were used: Standard I Nutrient Broth (Oxoid, Basingstoke, UK) for *E. coli*, *E. carotovorum, B. subtilis, B. megaterium, Ps. flourescens*, Tryptose-Soy-Broth (Oxoid, Basingstoke, UK) for *L. innocua and L. monocytogenes,* MRS-Bouillon (Merck KgaA, Darmstadt, D) for *L. rhamnosus* and *L. acidophilus* and Malt Extract Broth (Merck KgaA, Darmstadt, D) for *S. cerevisiae, C. utilis* and *Rh. Rubra.* Cells were incubated for 24 h at 37°C to obtain cultures in stationary growth phase. Pellets of *A. niger* ATCC 16404 (Fa. Microbiotics, St. Cloud MN, 56303 USA) were suspended in the provided dilution liquid according to preparation instructions. Before PEF-treatment, 10 ml/l cell suspension were added to the treatment media, if not stated otherwise.

Collected samples were placed on ice immediately after treatment and dilutions were made right after finishing the experiment using sterile Ringer Solution (Merck KgaA, Darmstadt, Germany). Viable counts of vegetative cells were determined using drop plating method (Baumgart, 1986) on Standard I Nutrient Agar (Merck KgaA, Darmstadt, D) for *E. coli, E. carotovorum, B. subtilis, B. megaterium, Ps. flourescens,* Yeast Extract Glucose Agar with addition of Oxytetracycline (Oxoid, Basingstoke, UK) for *Rh. rubra*, Malt Extract Agar (Merck KgaA, Darmstadt, D) for *S. cerevisiae* and *Candida utilis* and Malt Extract Agar with addition of tartaric acid for *A. niger*. Pour plates with MRS-Agar (Oxoid, Basingstoke, UK) were used for *L. acidophilus*, with Rogosa-Agar (Oxoid, Basingstoke, UK) for *L. rhamnosus*. Plates were incubated at 37°C for 48 h, *A. niger, S. cerevisiae, C. utilis* and *Rh. rubra* were incubated for 48 h at 30°C. All microbial analysis was done at least in duplicate. The inactivation of vegetative organisms was evaluated by calculating the log reduction in viable cell counts compared to the untreated sample.

### 3.4.4 Treatment media for microbial inactivation experiments

| | |
|---|---|
| Apple juice: | Euro-Shopper apple juice, made from concentrate, 100% fruit content, Elmenhorster Fruchtsaftgetränke, Rostock, Germany |
| | Juice produced from concentrate obtained from Eckes-Granini, Nieder-Olm, Germany (0.170kg concentrate + 0.830kg dist. water) |
| | Freshly squeezed apple juice from Braeburn apples, Werder Frucht, Werder, Germany |
| Orange juice | Granini orange juice without pulp, 100 % fruit content, Eckes-Granini, Nieder-Olm, Germany |
| Blood orange juice | Granini blood orange juice, from concentrate, Eckes-Granini Nieder-Olm, Germany |
| Red grape juice | Beisiegel red grape juice, made from concentrate, 100% fruit content Trinkfrucht Kelterei, Reidesheim, Germany |
| White grape juice | Beisiegel white grape juice, made from concentrate, 100% fruit content, Trinkfrucht Kelterei, Reidesheim Germany |
| Ringer solution | Merck, #3979,Darmstadt, Germany, adjusted to an electrical conductivity of 2 mS/cm by addition of distilled water unless stated otherwise |
| Milk | Full fat milk, 3.9 % fat, skimmed milk, 1.5 % fat and skimmed milk, 0.3 % fat, UHT-treated, A&P, Tengelmann, Viersen, Germany |
| | Raw milk, full fat, obtained from German Institute of Risk Assessment, Berlin, Germany |
| Cream | 30 % fat, UHT-treated, A&P, Tengelmann, Viersen, Germany |
| Microalgae extracts | *Spirulina platensis, Chlorella vulgaris* and *Porphyrridium purpureum* extracts, approx. 3 % dry matter, IGV, Potsdam, Germany |

### 3.4.5 Calculation of cook- and PU-value

The Cook (C) -Value, a key benchmark for the thermal load and degradation of ascorbic acid and flavor during a treatment with variable temperature-time-regime changes can be calculated using Equation 10 with a medium z-value of 25 °C (Ohlson 1980) for quality losses and a reference temperature of 100 °C.

$$C\text{-}value = \int_0^t 10^{\frac{T-T_{ref}}{z}} dt$$

Equation 10

The Pasteurization Unit (PU), a key benchmark for thermal load in relation to Microorganisms can be calculated using Equation 11 with a z-value of 10°C and a reference temperature of 80 °C (Shapton *et al.* 1971). The z-value is the increase or decrease in temperature required to increase the decimal reduction time by one order of magnitude.

$$PU = \int_0^t 10^{\frac{T-T_{ref}}{z}} dt$$

Equation 11

### 3.4.6 D-value determination using glass capillaries

For comparison of impact of PEF and thermal effects microbial and enzymatic inactivation experiments have been performed in a temperature range of 50 to 75°C and residence times in between 5 to 300 s. A sample volume of 60 µl was filled into the sterile AR-glass capillaries of 10 mm length, 1.0 mm inner and 1.3 mm outer diameter (Kleinfeld Labortechnik GmbH, Gehrden, Germany).The capillaries were pre-cooled in an ice bath and heated in a thermostat (Huber GmbH, Offenburg, Germany) with silicon oil (Huber GmbH, Offenburg, Germany) as heating medium. After the defined heating time the samples were immediately cooled in an ice bath again. Survivors were measured by plate count in three replicates or enzymatic activity was determined from a sample volume of 30 µl obtained from the capillary after treatment.

# 4 Results and Discussion

## *4.1 Disintegration of Biological Tissue by PEF*

### 4.1.1 Induction of cell permeabilization by PEF application

The impact of a PEF-treatment at different electric field strength and pulse number on Royal Gala apples is shown in Figure 4.1. The cell disintegration index $Z_p$ was measured after treatment of half apples in comparison to untreated control samples. Typical sigmoid curves dependent on pulse number have been obtained, similar than reported for potato tissue (Knorr and Angersbach 1998). Increase of electric field intensity showed higher PEF efficiency for tissue disintegration at same pulse number, a disintegration index of 0.6 can be obtained after 20 pulses at 4 kV/cm or after approx. 150 pulses at 1 kV/cm. It is noteworthy that also at very low electric field strength of 0.3 kV/cm resulted in a permeabilization after a sufficient number of pulses. From a technical point of view such data can provide the basis for selection of appropriate processing parameters and suitable field strength and pulse number combinations for scale up.

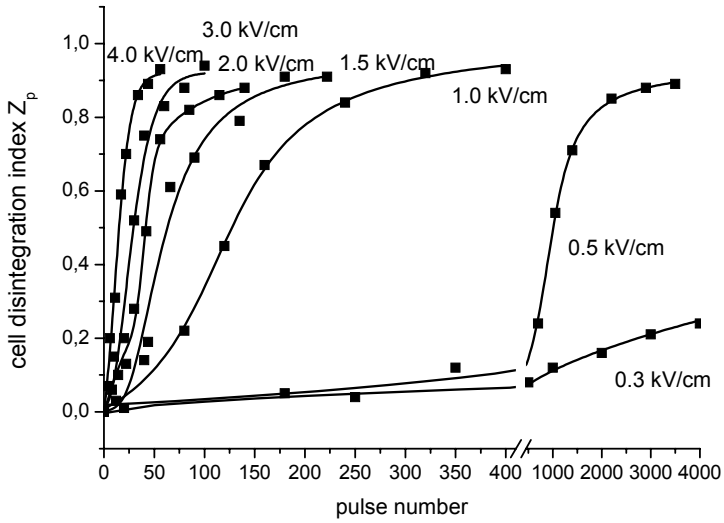



Figure 4.1: Impact of PEF-treatment at different electric field strength on tissue integrity of apple (Royal gala) samples dependent on pulse number. Cell disintegration index $Z_p$ has been determined by impedance analysis.

The electrical energy delivered per pulse has an exponential dependency on peak voltage (and therefore field strength) applied. In Figure 4.2 the cell disintegration index after treatment at different field strength has been related to the total specific energy input. The scatter diagram shows the impact of electric field strength and energy input on tissue integration. It can be seen that exceeding a sufficient level of electric field strength no further

improvement of energy efficiency is found. An YZ-projection of data points and average for all samples treated at 1 kV/cm or higher is shown by the red squares and line.

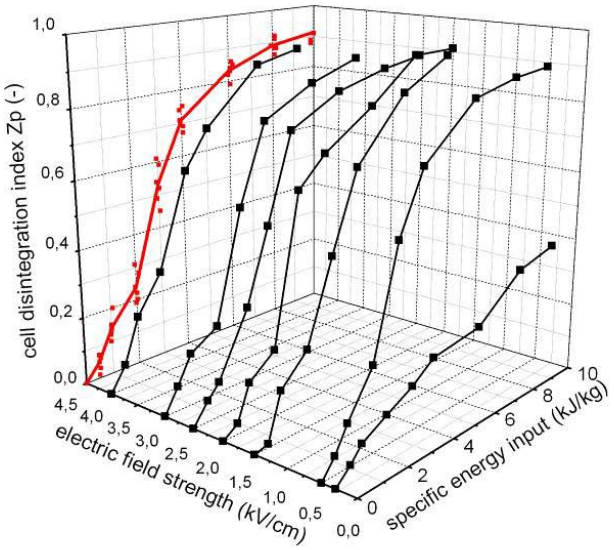



Figure 4.2: Scatter diagram of cell disintegration index $Z_p$ of apple (Royal gala) samples after a PEF-treatment at a field strength of 0.3 to 4 kV/cm and a specific energy input of 0.5 to 10 kJ/g. The red line denotes the average of an YZ-projection of data points of the treatments at a field strength between 1 and 4 kV/cm.

The cell disintegration level obtained is then dependent on total energy input only. For royal gala apples this minimum level of electric field strength was in a range of 0.5 kV/cm, an operation at 0.3 kV/cm resulted in cell permeabilization, but a higher total energy input was required to achieve a similar level of permeabilization. After application of 5000 pulses and a total energy input of 10 kJ/kg a disintegration index of 0.37 was obtained, which could also be obtained with an energy input of 3 kJ/kg when operating above 1 kV/cm. Up to an energy input of a range of 10 kJ/kg an increase in cell disintegration was found. From an engineering point of view an operation at low field strength could be interesting, as then pulse modulation and high voltage switching could become obsolete and the parameters of treatment would approach to ohmic heating or moderate electric field processing.

In Figure 4.3 the impact of a moderate electric field, alternating current on apple (Royal gala) samples is shown dependent on specific energy input in comparison to a PEF-treatment at 0.3, 0.5 and 1 kV/cm. The treatment was performed using sinusoidal (50 Hz) current, the field strength applied was 60 V/cm, the energy output was 430 J/s, corresponding to a specific energy input of 3.6 kJ/kg and a temperature increase of approx. 0.9°C per second of treatment at a chamber volume of 120 ml. The maximum energy input after 9 s was 32.4 kJ/kg, resulting in a temperature increase of 8°C. Sample temperature has been measured after treatment and showed good agreement to expected increase.

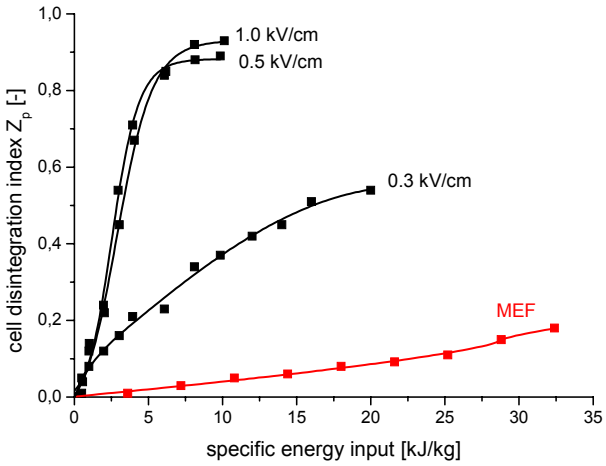



Figure 4.3: Impact of a moderate electric field (MEF) and a PEF-treatment on tissue integrity of apple (Royal gala) samples. MEF was applied for 1 to 9 s at a field strength of 60 V/cm, the specific energy input per second was 3.6 kJ/kg. PEF-treatment was applied using 1 to 10000 pulses dependent on peak voltage.

A treatment at a field strength of 60 V/cm showed to be effective to induce cell disintegration, the maximum sample temperature was below 30°C. In accordance to the patent of Cousin (2003) after a MEF treatment a tissue softening was visible similar than after a low intensity PEF-treatment when a similar $Z_p$ is obtained, but juice release from apple samples was lower in comparison to samples treated at higher field strengths. A very high number of 10,000 pulses with a pulse energy of 0.8 J was necessary to achieve a 50 % cell disintegration at a PEF-treatment of 0.3 kV/cm, indicating the transition between pulsed power and alternating current application. Considering energy efficiency of the treatment a the energy input required to achieve similar cell disintegration index than after a PEF-treatment showed to be more than ten times higher. Further increase of MEF treatment time will cause a linear increase of media temperature and a transition to a thermal treatment will occur. With the equipment used an increase above 10 s treatment time was not possible as cooling of the current limiting resistors in the power supply was necessary. A MEF device can be expected to cause much lower costs of investment, as primarily a high intensity power supply will be needed. Within a PEF modulator the power supply typically amounts for 50 % of the component costs but electrical energy required and the potential to achieve higher level of cell disintegration seem to justify the efforts for HV-pulse modulation.

For treatment of potato samples similar results have been found. The energy input required to achieve a disintegration index of 0.8 was in a range of 5.5 kJ/kg for Russet Burbank and 3.7 kJ/kg for Bintje. An MEF treatment also resulted in a tissue disintegration, a $Z_p$ of 0.10 was obtained after a 6 and an 10 s treatment or 21.6 and 36 kJ/kg for Bintje and Russet Burbank, respectively (data not shown). Applicability of impedance measurement for other

tissues was dependent on tissue properties, for carrots a similar typical, sigmoid curve shape was found than for potato and apple, whereas measurement of inhomogeneous samples such as rape seed or meat did not show good dependency of cell disintegration index on treatment intensity.

## 4.1.2 Lab scale treatment of apple and other fruits

### 4.1.2.1 PEF-impact on apple juice extraction during lab scale pressing

To correlate the impact of PEF application on tissue disintegration and a possible enhancement of juice winning lab scale tests have been performed using different apple varieties. Electric field strength was varied between 1 and 5 kV/cm, energy input was selected between 1 and 15 kJ/kg by adjusting pulse number, a treatment temperature of 10 to 30°C was used. The impact of PEF-treatments at different intensities on apple juice (Royal gala) yield is shown in Figure 4.4. The calculation of the juice yield was based on the weight of the mash and of the juice obtained. Yield of PEF-treated variants ranged from 67.9 to 71.3 %. Significant differences in juice yield were observed also for the control samples (64.5 - 68.5 %). For this reason, the effects of mash treatment on juice yield shown are expressed relative to the respective control of the same mash. Application of pulsed electric fields increased juice yields in the range of 1.7 to 7.7 %, whereas enzymatic mash treatment resulted in a 4.2 % increase. Though an increase in juice yield for all PEF-treated samples was found, no dependence was found on processing parameters. It is assumed that this effect is based on raw material variability. Accelerated release of the juice, as reported by McLellan *et al.* (1991), was not observed, but the mash became squashier as a result of the PEF-treatment. Textural characteristics were in accordance with previous observations by Lebovka *et al.* (2004) who described changes of the force relaxation curves of apples due to the PEF-induced damage of the apple cells after PEF-treatment. Treatment temperature did not show an effect on PEF efficiency during juice winning in a range of 10 to 30°C (data not shown).

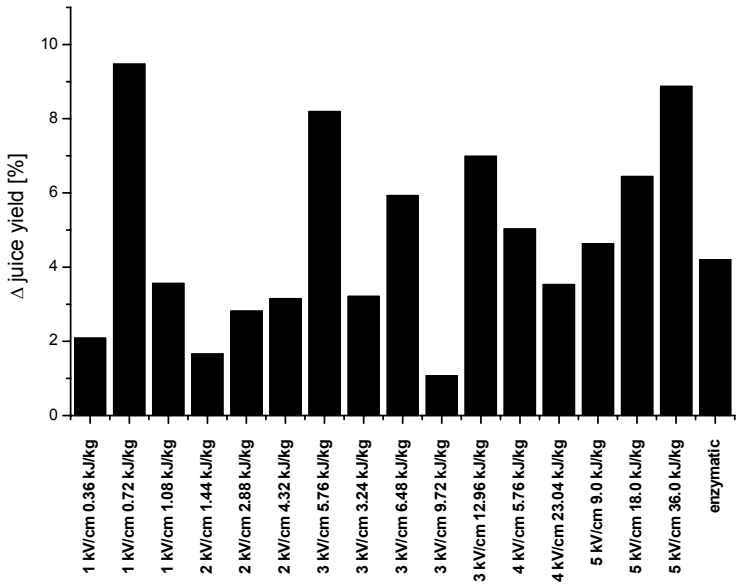



Figure 4.4: Impact of PEF-treatment at different intensity on juice yield from apple (Royal Gala) after lab scale pressing. The average increase in yield of two samples in comparison to control sample from same mash lot is shown for each parameter.

However, it should be taken into consideration that juice yield is only one aspect, even though the most important, determining the profitability of juice production. In contrast to the pomace obtained after enzymatic mash treatment, press residues resulting from PEF-treatment can still be exploited for pectin extraction, which is not depolymerized by enzyme application.

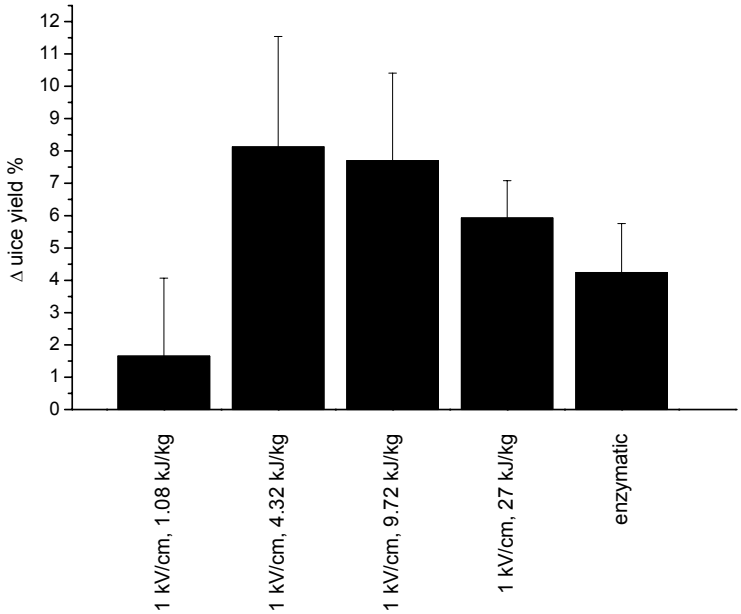



Figure 4.5: Increase in juice yield from Royal gala apples by PEF application and enzymatic maceration in comparison to untreated control samples. Each treatment has been repeated three times.

Three treatment parameters have been selected to perform replications to overcome or at least limit the impact of raw material variability for Royal gala, Granny Smith and Braeburn. A treatment of 30 pulses has been applied at a field strength of 1, 3 and 5 kV/cm, resulting in a specific energy input of 1.08, 9.72 and 27.0 kJ/kg, respectively. A repetition of three times 2 kg mash has been performed, similar to the previous experiments each lot has been separated into three parts, a control and a PEF sample a and b. Results for Royal gala are shown in Figure 4.5, indicating an increase in juice yield after PEF application. The average juice yield for control samples was 69.2 %. A treatment with an energy input of 4.32 showed the highest efficiency for this variety, whereas increasing energy input resulted in a decrease of juice yield. Considering the impact of PEF on tissue disintegration (Figure 4.2) a 4.32 kJ/kg treatment is related to a $Z_p$ of approx. 0.75. A further increase in disintegration appeared to result in a mash structure with adverse effect on liquid-solid separation. Similar results were found for other varieties in lab scale experiments. A comparison of juice yield for Granny smith, Braeburn and Royal gala is shown in Figure 4.6, as PEF-treatment parameters 30 pulses at 2 kV/cm have been selected. The PEF impact was most pronounced for Royal gala, whereas Braeburn showed a minor increase in juice yield only. Differences in juice extractability after a PEF-treatment may be related to structural properties of different varieties.

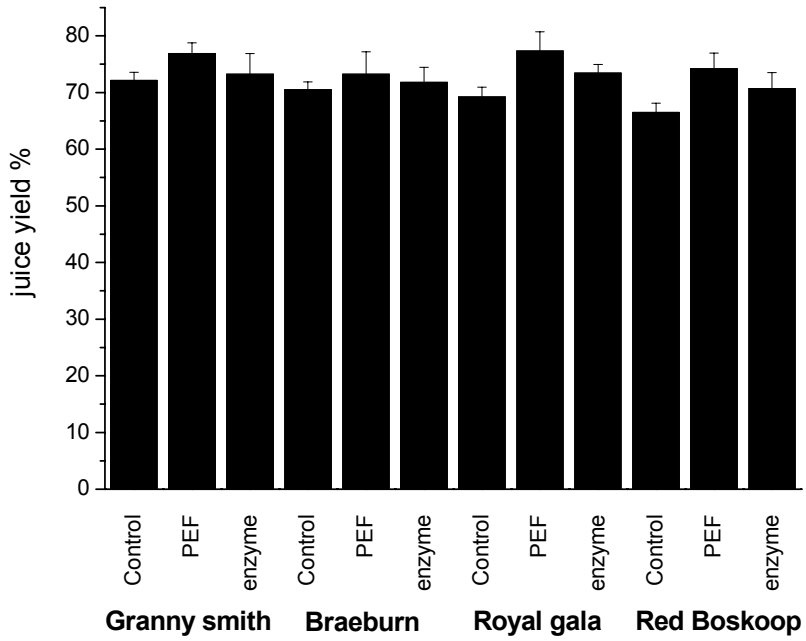



Figure 4.6: Comparison of juice yield after PEF or enzymatic treatment for three different apple varieties Pressing in lab scale, 3 replications per treatment, PEF-treatment: 2 kV/cm, 30 pulses, 4,32 kJ/kg.

#### 4.1.2.2 Impact on polyphenolic compounds, antioxidative capacity and quality parameters

Red Boskoop was used to investigate the impact of a PEF-treatment on polyphenol extractability in lab scale in a cooperation with University of Hohenheim, Germany (Schilling *et al.* 2006). Ascorbic acid (1 g/kg of mash) was added during mashing to prevent oxidation in all processing variants. The profile and contents of individual phenolic compounds were in accordance with literature data for this cultivar. Differences in the phenolic composition between the control juices and PEF-treated samples a/b for field intensities of 1 kV/cm and 3 kV/cm were insignificant (Figure 4.7). In contrast, enzymatic maceration of the apple mash resulted in a marked increase in quercetin glycosides. The flavonols are mainly localized in the apple peels. As a consequence of enzymatic depolymerization of the cell wall, their release into the juice appeared to be enhanced. In conclusion, an increased yield of valuable ingredients using pulsed electric fields as shown for anthocyanins from grapes (Anonymous 2006; Balasa *et al.* 2006) could not be corroborated for polyphenolic compounds from apples.

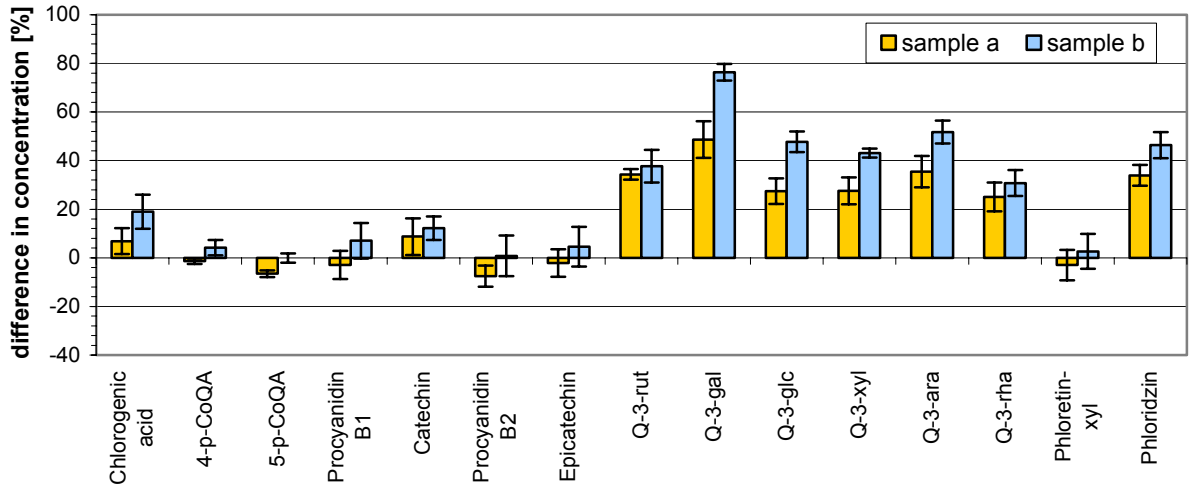



Figure 4.7: Difference in concentration of selected phenolic compounds in juice of Red Boskoop after a PEF-treatment at 3 kV/cm, 30 pulses (samples a and b) in comparison to untreated control.

For the determination of the antioxidative capacity, three assays (TEAC, FRAP and DPPH assays) were applied at University of Hohenheim. Since the antioxidative capacity of apples is mainly attributed to their polyphenolic compounds and significant changes of their contents upon PEF-treatment were not observed, the antioxidative capacity of the juices was expected to remain unchanged. Insignificant differences were found between the control groups and the PEF-treated samples, irrespective of the assay used (data not shown). The addition of ascorbic acid during grinding of the apples prevented phenolic compounds from

oxidation. Residual ascorbic acid in the juices was oxidized using ascorbate oxidase prior to the determination of antioxidative capacity. Due to the protective effect of ascorbic acid during maceration, the longer process time of the enzymatic variant relative to the faster PEF technology did not affect the antioxidant capacity of the juices.

Further investigations with apples of the cultivar Royal gala confirmed that the antioxidant capacity of apple juices was not affected by PEF of the mash at different field intensities (data not shown). However, since these experiments were carried out without addition of ascorbic acid, a significant decrease of antioxidant capacity after enzymatic treatment due to oxidation during mash maceration was observed. Thus, because of the shorter process time, PEF appears to be advantageous to enzymatic maceration if no ascorbic acid is added to the mash. PEF-treatment might be supportive of radical formation due to the high energy input, leading to a rise in antioxidative capacity. The fact that the antioxidative potential was not affected indicates that radical formation has not taken place. This aspect is of utmost interest, in particular with regard to substantial equivalence and acceptance by consumers.

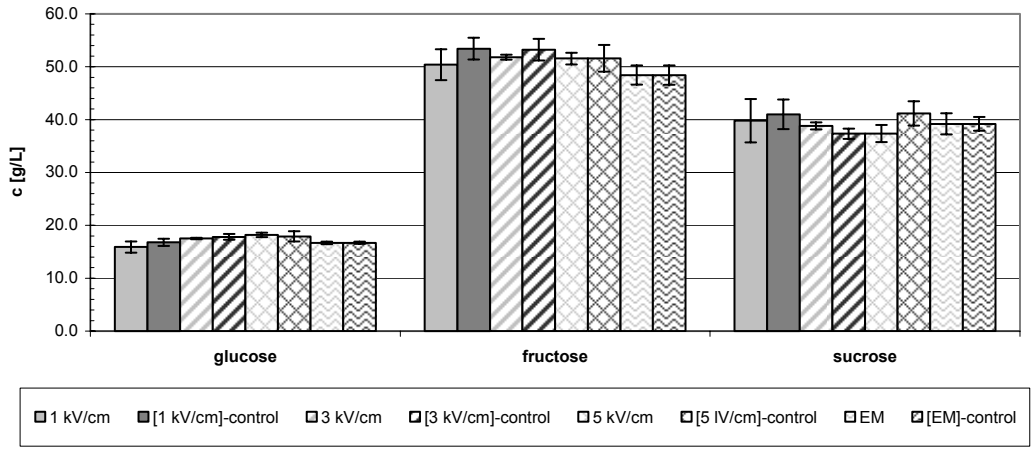



Figure 4.8: Influence of PEF and enzymatic treatments of apple mash on glucose, fructose and sucrose contents of apple juices (PEF-treatment at 20 °C, n = 30; enzymatic maceration (EM) 1 h at 30 °C; control: untreated sample of the respective mash), (Schilling et al., 2006).

Considering pH, TSS (total soluble solids), TA (total acids) and the sugar-acid-ratio, the juices obtained after PEF-treatment of apple mash did not differ from the respective controls. Uniformly, total soluble solids amounted to 13.0 ± 0.2 °Brix, and densities were 1.052 ± 0.001 g/cm³. TA, calculated as malic acid, was approximately 10.1 ± 0.4 g/L. The data are in accordance with the AIJN Code of Practice. Since the acid content was comparatively high, the sugar-acid ratio (11.1 ± 0.4 : 1) did not reach 12 : 1 to 18 : 1, which is considered a prerequisite of harmonic sensory appearance. This is attributed to the apple cultivar used in the present study. The concentrations of glucose, fructose and sucrose are presented in Figure 4.8. Sugar contents of the juices were not affected by different mash treatments.

Analogous results were found for malic acid (10.8 ± 0.4 g/L). Also, the pectin contents of the juices were not diminished by PEF-treatment amounting 1.1 ± 0.1 g GA/L. Due to the pectolytic activity, the pectin content of the macerated samples was only 0.08 ± 0.01 g GA/L.

As the viscosities of the juices are influenced by their sugar and pectin contents, no difference between PEF apple mash treatment and conventional processing without mash enzymation was expected. PEF-treatment had no effect on the flow properties of the apple juices relative to their controls (10.1 ± 1.4 mPas). A shift of viscosity depending on the shear-rate was only observed for the juices after pectolytic enzymation of the apple mash. The marked decrease in viscosity to 0.8 ± 0.1 mPas may be attributed to the pectin degradation. Consequently, the enzymatic formation of GA caused a slight pH decrease (3.14 to 3.11) and an increase in TA (10.4 to 10.8 g/L, calculated as malic acid).
In accordance to studies of PEF application for preservation (Zárate-Rodriguez and Ortega-Rivas 2000; Ayhan *et al.* 2002; Lechner and Cserhalmi 2004) it was confirmed that also a treatment of apple mash does not lead to substantial changes in the chemical composition of the resulting juices. All parameters are in agreement with specifications of EEC food regulations, the samples where equivalent to control samples.

#### 4.1.2.3 PEF-impact on pressing and extraction of other fruits and vegetables

The extractability of anthocyanins from purple fleshed potatoes (*Solanum tuberosum spp.),* Garden Huckleberry *(Solanum scabrum)* and grape pomace after a PEF-treatment was investigated. Eight varieties of *Solanum scabrum* were obtained; one kg of mash was divided in two lots, each 500 g for control and PEF sample. One of them was subjected to a PEF-treatment at a field strength of 1.3 kV/cm and 120 pulses prior to pressing, using the CoolPure® (G) pulse modulator and the 720 ml treatment chamber (G). Anthocyanin concentration in juice obtained is shown in Figure 4.9. Dependent on variety a significant increase in anthocyanin content could be achieved. For accessions 6, 7 and 8 no impact of a PEF-treatment was found, the extractability of intracellular compounds appears to be very good as well from an untreated sample. The extract obtained from accession 1, 2, 6 - 8 showed high color stability, whereas for the other accessions a fast browning was observed. A stabilization of extracts was possible by thermal enzyme inactivation or ascorbic acid addition (data not shown).

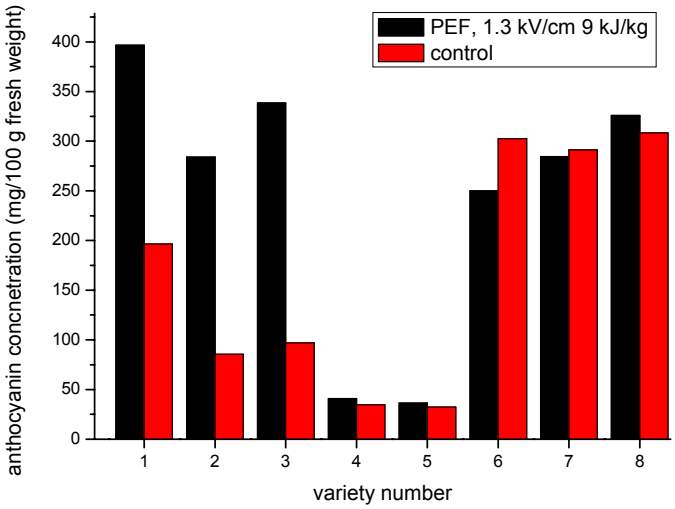



Figure 4.9: Anthocyanin concentrations of freshly extracted *Solanum scabrum* berries after Pulsed Electric Field (PEF) application in comparison to control. A pooled sample per accession was analyzed.

Purple fleshed potatoes have been used to investigate the effect of PEF during extraction using aqueous and ethanol extraction, the impact of a PEF pre-treatment on anthocyanin extraction is shown in Figure 4.10. It is noteworthy that in comparison to Solanum scabrum the pigment concentration in purple fleshed potatoes was much lower. Blanching at 70°C was required for all samples to retain typical pigment color by preventing enzymatic browning. If no blanching was applied browning after a PEF-treatment was faster than for control samples. In a preliminary work four different varieties have been screened regarding their anthocyanin content and the potential of PEF application to increase extraction yield, for all varieties an increase was found after a PEF-treatment (data not shown). In addition the impact of convective air and freeze drying prior to extraction was investigated, whereas air drying at 70°C for 3 h caused a reduction of pigment content an increase of extractability was found after freeze drying.

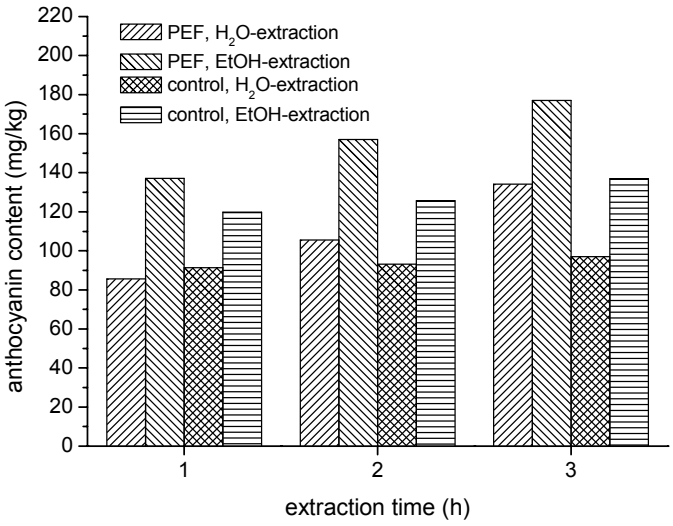



Figure 4.10: Impact of PEF-treatment on aqueous and ethanol extraction of anthocyanins from purple fleshed potatoes. PEF parameters: 1.5 kV/cm, 150 pulses.

Content of phenolic compounds as gallic acid equivalent and antioxidant capacity as Trolox equivalent of extracts from grape pomace after different pretreatments has been investigated in a cooperation with the Federal Research Centre for Nutrition and Food, Karlsruhe, Germany, results are shown in Figure 4.11. The highest phenolic content recovery was obtained after a PEF-treatment, whereas an approx. 3% lower yield was obtained with high pressure liquid extraction (HPLE) and 35% lower content with a conventional extraction method. In addition differences in antioxidant capacity of the extracts are shown. A PEF-treatment at 3 kV/cm, 30 pulses and an energy input of 10 kJ/kg increased the anthocyanin extraction yield up to 10% in comparison with HPLE and up to 30% compared with the conventional extraction (data not shown). It was shown that a PEF-treatment can be utilized to improve extraction of anthocyanin monoglucosides, for acetylglucosides the application of HPLE showed better results. This effect may be based on different solubility and molecular size and weight. The application of HPLE showed to be the most efficient for the extraction of anthocyanin acetylglucosides in comparison with the other methods, showing that mechanisms of permeabilization and impact on cell membrane structure of both techniques are different. To improve diffusion rates of high molecular weight anthocyanins a HPLE treatment appeared to be advantageous, whereas for soluble compounds a PEF-treatment proved to be highly effective.

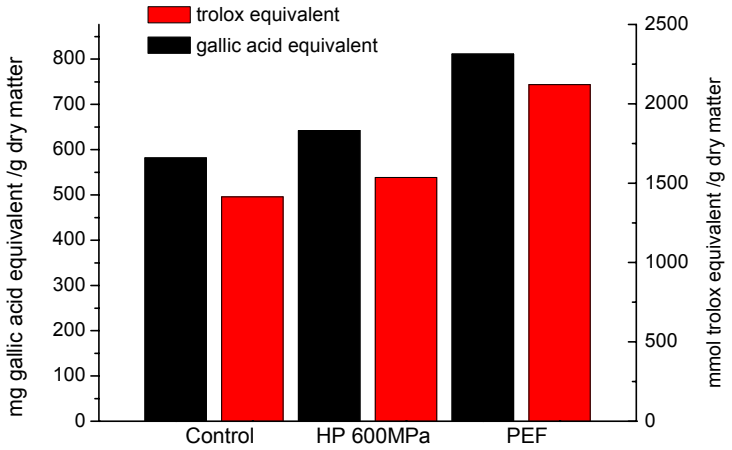



Figure 4.11: Comparison of the antioxidative capacity and total phenolic content in extracts of grape pomace after different pre-treatment methods.

## 4.1.3 Technical scale PEF-treatment of apple and other fruits

### 4.1.3.1 Processing of fresh apples

To scale up lab-scale experiments on PEF applicability for fruit juice production a pilot system with a production capacity of up to 5 t/h was realized. The system was used for technical scale tests during autumn 2005 and in spring 2006 to investigate PEF effect on freshly harvested and stored apples in cooperation with the University of Hohenheim, Germany and the University of Applied Sciences Geisenheim, Germany. A typical mixture of apple varieties used for industrial juice production has been obtained by Eckes-Granini, Nieder-Olm, Germany. Liquid solid separation has been performed using a Z23-3 decanter centrifuge or a HPL 200 horizontal press after a mash treatment with PEF or MA-Xpress pectolytic enzyme. The yield obtained after apple juice winning after different pretreatments is shown in Figure 4.12.

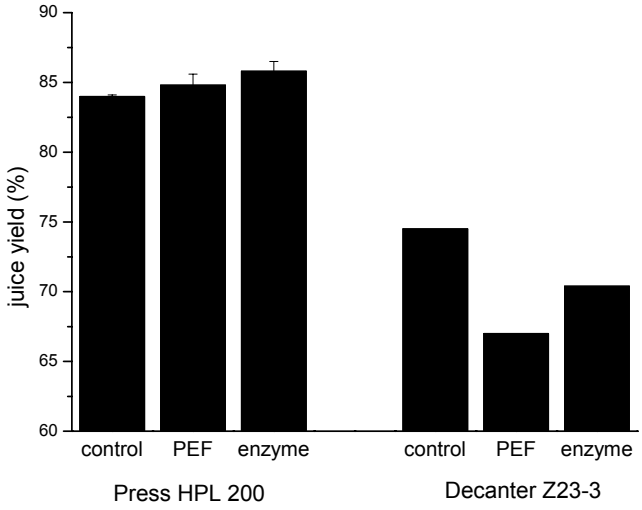



Figure 4.12: Comparison of juice yield in technical scale from typical industrial mixture of fresh apples after different pretreatments and liquid-solid separation techniques. PEF: 2 kV/cm, 10 kJ/kg

Using the press for liquid solid separation a slight trend towards an increase of juice yield after a PEF application can be observed. Treatment was performed with a press filling of 150 kg. No significant differences were found between control, enzymatic and PEF-treated mash after Tukey ($P > 0.05$). As freshly harvested fruits have been used for all samples good pressing results have been obtained. As expected the yield obtained using the decanter centrifuge is below the press yield, the lowest yield has been obtained for the PEF-treated sample. The experiment was started with separation of the PEF sample, so it can be assumed that for filling and adjusting of the system high losses occurred. Surprisingly also the enzyme treated sample showed a low yield, this effect might be caused by structural changes of mash towards a squashier structure and lower viscosity and related problems separability causing a need to improve decanter parameters settings. In addition to the final yield after pressing the impact of a PEF-treatment on pressing curves and juice flow was investigated. A pressing curve for control, enzyme and PEF-treated sample is shown in Figure 4.13. The experiments have been performed in duplicate, showing good agreement between replications. It is obvious that a PEF-treatment caused a decrease in juice flow in particular during beginning of the pressing. Whereas an enzymatic maceration allowed a juice yield of 75 % after 19 min, this yield was obtained after 25 and 34 min for control and PEF sample, respectively. After 50 min a yield of 85 % was obtained for the enzyme treated fruit mash, indicating the superior impact in comparison to control and PEF-treated mash. Though structural changes and PEF-induced cell disintegration of the apple mash were clearly visible by fast enzymatic browning, the impact on liquid-solid separability showed to be adverse. Structure disintegration and loss of mash compressibility might have caused a blockage of drainage channels as well as a loss of structural support necessary for liquid flow within the mash. Additionally the press design might play a role; a HPL 120 horizontal

hydraulic filter press with drainage elements implemented, the surface volume ratio of this press type is lower in comparison to a baling press. The effect of decreased juice flow will cause a drastic reduction in production capacity in comparison to an enzyme treated mash.

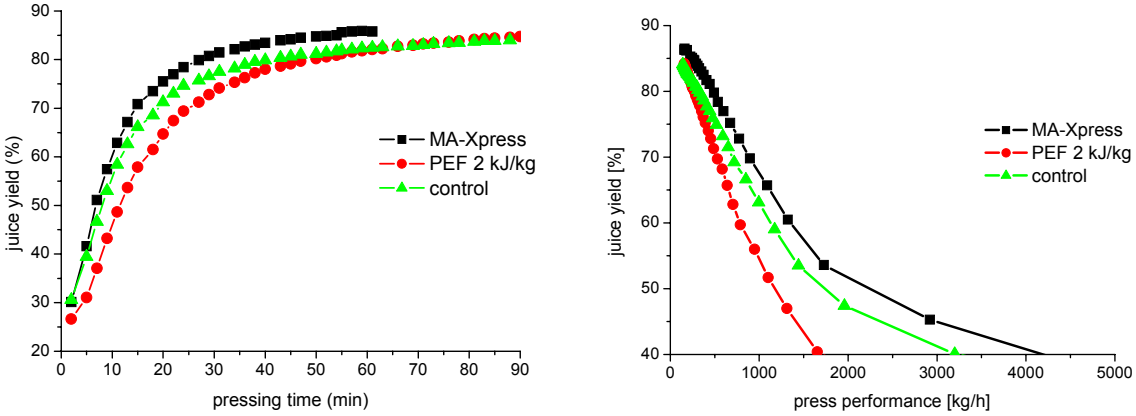



Figure 4.13: Left: Juice yield from industrial apple mixture dependent on press time and pre-treatment. Right: Performance-yield diagram with fresh apples (industrial mixture) after different mash treatments. PEF parameters: 3 kV/cm, 10 kJ/kg; enzyme: MA-Xpress, HPL 200 press.

The production capacity dependent on press yield is shown in Figure 4.13, right, indicating the decrease of production capacity of the filter press after a PEF-treatment. When a final yield of 75 % shall be obtained the press performance is 675, 520 and 380 kg/h for enzyme, untreated and PEF-treated mash, respectively. The impact of variation of mash particle size was investigated by using two different grinding machines, a Seepex BTM 5 pump with integrated macerator and a fruit mill (Amos Engineering, Heilbronn, Germany) both at a setting of 6 and 10 mm particle size. After a PEF-treatment at 1.5 kV/cm and 10 kJ/kg no impact of particle size was found (data not shown).

#### 4.1.3.2 Processing of stored apples

Subsequent experiments in March 2006 were performed using stored fruits (Jona Gold and Golden Delicious) from the 2005 harvesting season. Similar than for fresh fruits the juice yield during pressing with the HPL 200 has been monitored and is shown in Figure 4.14. In general the stored raw material showed decreased separability of juice when using a horizontal filter press; the maximum yield obtained with an untreated sample was 22 % after 80 min pressing time. A PEF-treatment at an energy input of 10 kJ/kg and a field strength of 10 kJ/kg resulted in a transition of solid particles into the juice and a blockage of drainage elements (data not shown), the pressing was cancelled. A lower intensity treatment at an energy input of 2 kJ/kg improved the separability, but still solid particles were found in the

juice, which had a highly viscous and mushy appearance. It is noteworthy that the press curve shows a close to linear shape over a wide range of pressing time, indicating the impact of a PEF-treatment on mash structure and mass transport.

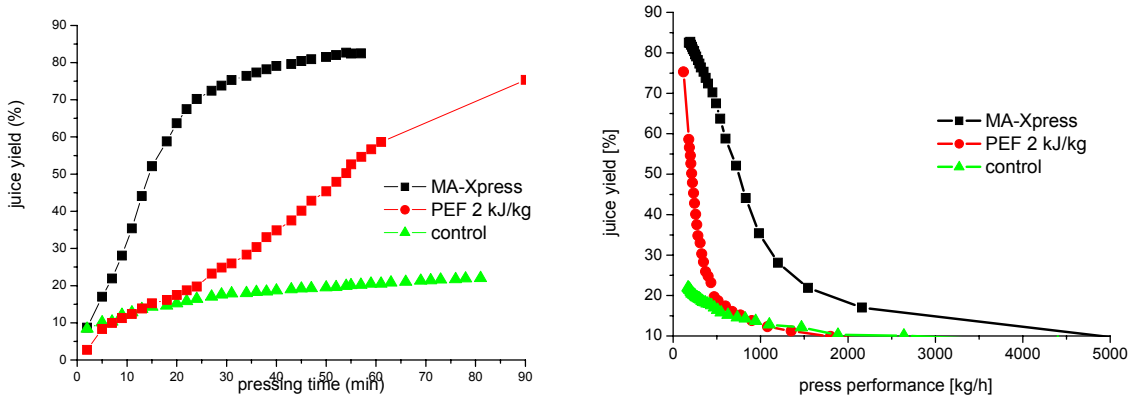



Figure 4.14: Left: Impact of PEF and enzyme treatment on press curve of stored apples (Jona Gold and Golden Delicious) in comparison to control. Right: Performance-yield diagram of a HPL 200 press with stored apples after different mash treatments. PEF-parameters: 3 kV/cm, 10 kJ/kg; enzyme: MA-Xpress.

After an enzymatic treatment a typical press curve was obtained, the maximum yield was 82.5 % after 60 min. The press performance is shown in Figure 4.14, right, for a final yield of 75 % the production capacity is 350 kg/h after an enzymatic maceration in comparison to 120 kg/h after a PEF-treatment. The disintegration of apple mash from stored apples was observed by fast browning and a mushy structure, but after enzyme treatment the filter elements showed to be less covered than after pressing of PEF-treated and untreated mash. Pectin depolymerization appeared to improve mash structure and to result in a lower mash viscosity. The impact of increasing mash particle size on separability was investigated by operating the BTM 5 pump without maceration unit. The apples were grinded by the mechanical stress within the screw pump only, average particle size was in a range of 2 cm, an automatic filling program was used for pressing, only filling a small amount of fresh mash into the press to avoid occurrence of juice inclusion in large mash volumes. Whereas for the control sample an improvement of juice flow and a final yield of 60 % was obtained after 80 min (see Figure 4.14), the liquid solid separability of PEF-treated mash remained poor, for control and after 2 kJ/kg a small amount of solids in the juice, after an energy input of 20 kJ/kg a mushy juice was observed.

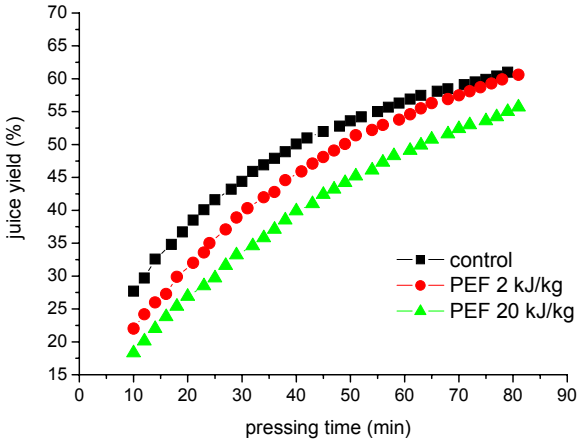



Figure 4.15: Impact of PEF-treatments with an energy input of 2 and 20 kJ/kg on press yield using a HPL 200 filter press for mash of Jona Gold and Golden delicious.

Application of a decanter centrifuge for juice winning from stored apples resulted in an increased transition of solids into the juice; final juice yield was increased dependent on treatment intensity (see Figure 4.16). Above a treatment intensity of 10 kJ/kg the juice contained too many solid particles and an additional separation would have been required. Similar results have been observed during treatment of olive mash in a cooperation with Westfalia Separator AG, Oelde, Germany (data not shown), but by modification of decanter settings the solids transition was prevented. An adjustment of decanter settings and/or modification of screw and weir might help to improve separability of apple mash. It was reported by Guenther (2006) that during pilot scale tests using a PEF equipment of KEA Tec, Karlsruhe, Germany an adaptation of parameter settings was required but subsequently a plain juice was obtained.

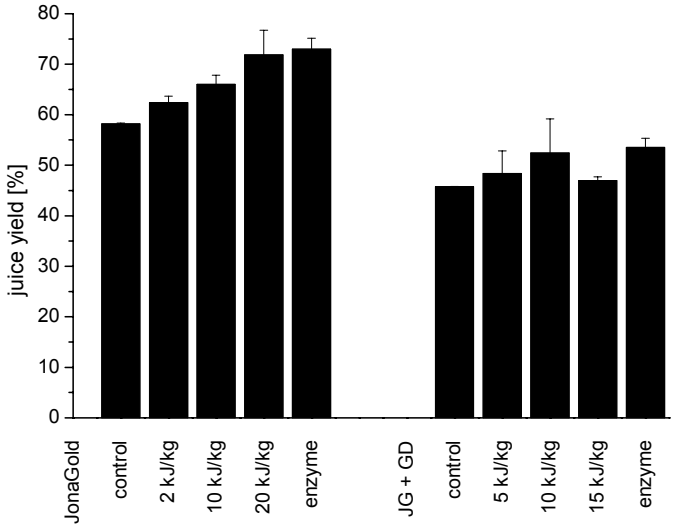



Figure 4.16: Impact of PEF-treatment at different intensities on juice yield using a decanter centrifuge. PEF-treatment at 2 kV/cm, Jona Gold (JG) and Golden Delicious (GD) were used, juice yield determined gravimetrically, including an eventual transition of solids to juice.

#### 4.1.3.3 Comparison to other press types

As the effect of mushy juice has not been found during lab scale experiments using a basket press the design of the filter press might have an impact on liquid-solid separability. Further experiments to evaluate separability of PEF-treated apple mash were performed using fresh apples and a Hollmann baling press with a lot size of 50 kg. The PEF-treatments were performed in batch to be able to investigate different processing parameters without requiring start up, operation, shut down and cleaning of the continuous equipment for all setups. For comparison between batch and continuous treatment the treatment at a field strength of 2 kV/cm and an energy input of 6 kJ/kg was also performed continuously, four lots of 50 kg each have been pressed. The impact of different PEF-treatment intensities on juice yield from Royal Gala and Jona Gold in a baling press in comparison to untreated and enzyme treated sample is shown in Figure 4.17. No difference was found comparing juice yield from batch and continuously treated apple mashes.

Press curves have been monitored, after 2 min of driving the piston against the bale without hydraulic pressure shown, subsequently every 2 min the pressure was increased by an increment of 4 bar. After 12 min the pressing was stopped, the yield of each pressing step was determined gravimetrically. The press curve for Jona Gold is shown in Figure 4.18, indicating that using other press types might provide a potential to utilize mash structural changes after a PEF-treatment in a beneficial way.

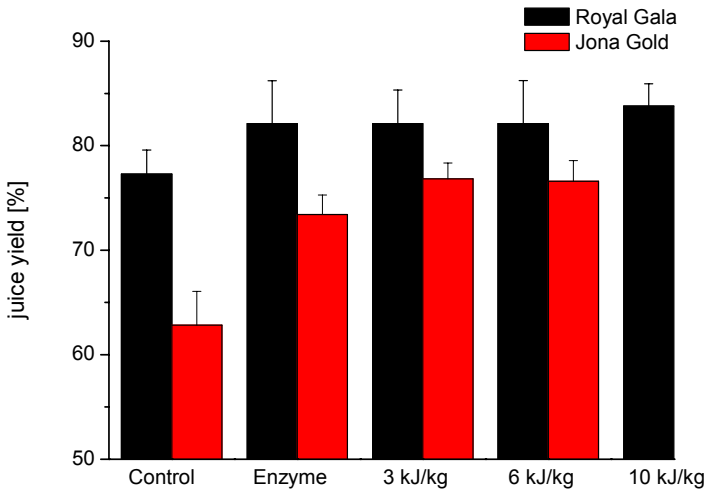



Figure 4.17: Juice yield obtained in baling press from two apple varieties after different PEF-treatment at 2 kV/cm and different specific energy input in comparison to enzyme treatment and untreated sample. 6kJ/kg: Average of continuous and batch treatments at same energy input.

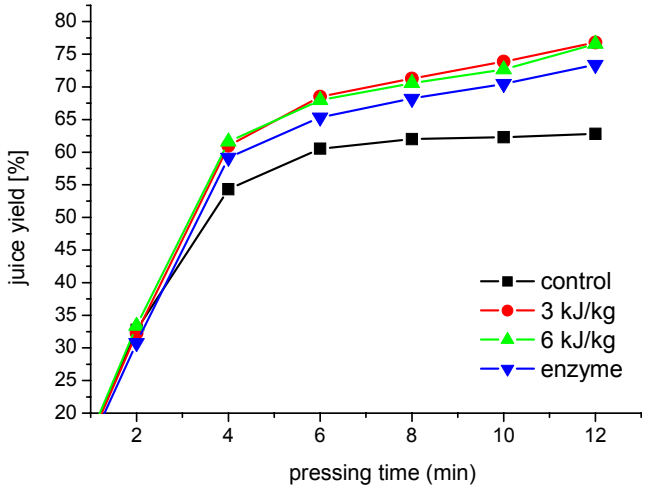



Figure 4.18: Press curve of Jona Gold mash after different pretreatments using a baling press. Pressure was increased from 0 to 20 bar in increments of 4 bar, during initial phase until 2 min the piston was driven in a way no hydraulic was monitored.

A baling press has been selected as the mash is divided into several lots, during the experiments 5 layers of mash packed in pressing cloth have been used. In comparison to a filter press a higher surface volume ratio is obtained. Even if a baling press is operable in batch mode only these findings are relevant for selection of appropriate liquid-solid separation techniques after a PEF-treatment. The application of belt presses could provide a highly promising and continuously operated alternative to application of filter presses. The potential of influencing tissue structure and integrity has been shown by this work, further experiments should concentrate on improvement and adaptation of separation techniques on structural changes of fruit mashes after a PEF-treatment.

#### 4.1.3.4 Impact of technical scale juice recovery on apple juice quality

Contrary to previous experiments in lab scale some juice quality parameters showed an impact of a PEF-treatment during technical scale tests. Total acids of decanter (4.1 g/l for control, 4.2 and 4.6 for PEF and enzyme treatment) and press juices (4.6, 4.9 and 5.1 g/l, respectively) were increased, along with a slight decrease in pH value and an increase of glucose, fructose and saccharose content for decanter juices. Phenolic compounds (Figure 4.19) showed an increase after PEF-treatment, which might be related to improved release in comparison to control sample and shorter processing time in comparison to enzyme treatment. In general the juice quality parameters were in accordance with the European legislation. The analysis of the juices produced in March 2006 using stored apples is not finished at present, but basic quality parameters showed equivalence to untreated samples.

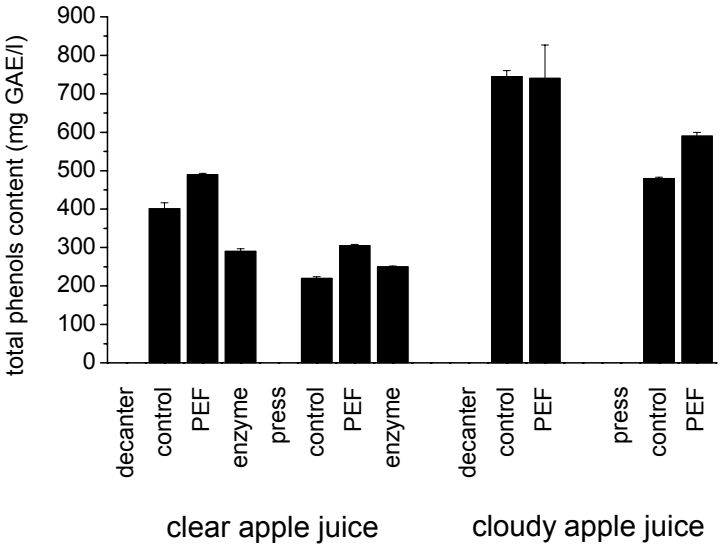



Figure 4.19: Total phenol content in apple juices after different mash treatments as gallic acid equivalent. PEF-treatment at 3 kV/cm, 15 kJ/kg, liquid solid separation by decanter centrifuge or horizontal filter press application. Enzyme: MA Xpress, Erbslöh, Geisenheim, Germany.

It was shown that, similar to lab scale experiments no degradation of apple pectin occurred during or after a PEF-treatment, indicating the potential to improve fruit juice production sustainability. Whereas by an enzymatic treatment a pectin depolymerization and deesterification reduces the quality and applicability of apple pectin as a gelatinization agent no detrimental effect of a PEF-treatment was found. These findings allow a utilization of pomace for pectin extraction while achieving a similar juice yield than after an enzymatic treatment. For production of cloudy apple juices an enzyme application can not performed, as turbidity stability would be degraded by pectin hydrolysis. During processing of cloudy apple juice an increase of yield can be expected while maintaining pectin and turbidity properties.

#### 4.1.3.5 Technical scale carrot juice recovery

In the previous sections the impact of a PEF-treatment on apple mash has been described, it was observed that the resulting, mushy structure can cause difficulties regarding liquid-solid separation. Structural properties of apple tissue might be a cause for that effect, loss of turgor after PEF-treatment is associated to a loss of supportive function of cells to maintain juice flow in the compressed mash. A treatment of carrot mash was performed to compare the effect of structural properties of different fruits. A comparison between an untreated sample, different PEF-treatment intensities and a Supraton®-homogenizer-treated mash is

shown in Figure 4.20. A temperature increase up to 80°C was required to allow pumping of carrot mash and to improve juice separation.

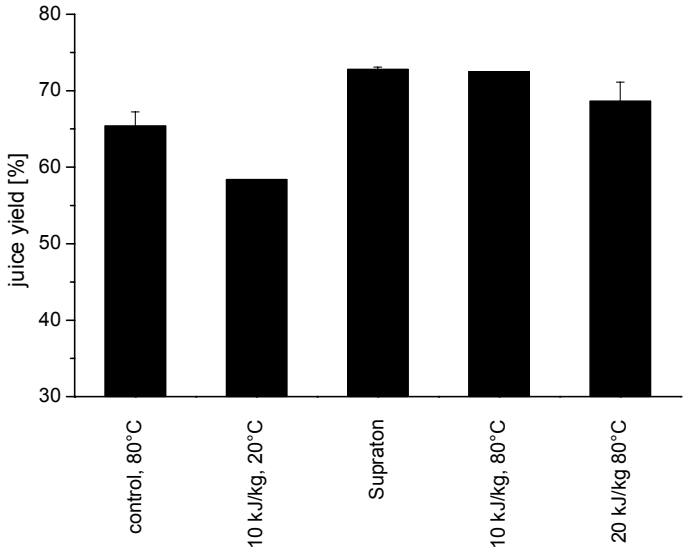



Figure 4.20: Carrot juice yield using a decanter centrifuge after different pretreatments in comparison to untreated control and Supraton®-homogenizer. PEF-treatment at 2 kV/cm. To increase mash and juice viscosity to allow pumping and separation a temperature increase up to 80°C was necessary.

After a PEF-treatment at 10 kJ/kg specific energy input and a temperature increase to 80°C a similar juice yield than after a Supraton®-treatment was obtained. In comparison to an untreated control (also heated to 80°C) the juice yield was increased by approx. 7 %. For carrot tissue the solids transition found for treatment of apple mash was not observed, indicating that separability of different raw material after a PEF-treatment is dependent on structural properties.

### 4.1.4 PEF-treatment for potato drying enhancement

In the course of this work PEF-treatments of potatoes have been performed to investigate the potential to improve drying as well as to induce structural changes in the tissue. The removal of water to preserve fruit and vegetable products or to facilitate handling and transport accounts for a significant amount of energy costs and time requirements of food processing. Membrane semi-permeability is often a limiting factor during drying. After removal of free surface water, the drying rate is mainly determined by water diffusivity from the sample core to its surface. An electropermeabilization is associated with a loss of semipermeability barrier, and can be used to enhance diffusion coefficients within fruit or vegetable tissues facilitating mass transport within biological tissue. As shown for apple and

potato tissue in 4.1.1, tissue disintegration can be obtained with an energy input as low as 10 kJ/kg. The impact of a PEF-treatment on drying of slices of potato (Bintje) was investigated, a drying curve of blanched potato samples after a treatment at 1.5 kV/cm and 60 pulses is shown in Figure 4.21, exemplarily.

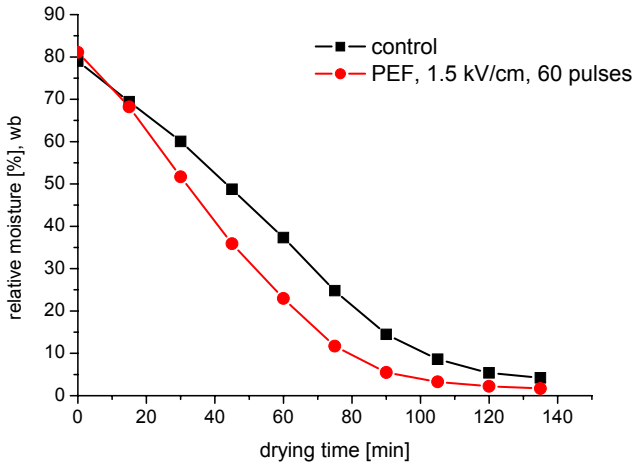



Figure 4.21: Relative moisture content (weight balance) of potato slices of 5 mm thickness during convective air drying at 80 °C air temperature, air velocity 1 m/s. A PEF-treatment was performed after sample blanching at 85 °C, 5 min, applying 60 pulses of 1.5 kV/cm.

It can be seen that a reduction of drying time to obtain 10 % relative moisture of approx. 20 % can be achieved after a PEF-treatment, similar as reported by Angersbach and Knorr (1997). The energy required for evaporation of water is, dependent on temperature and pressure in the range of 2.5 – 2.7 MJ/kg, but total energy input required for conventional drying is in the range of 4 – 6 MJ per kg of removed water dependent on thermal efficiency of the drying system. Dependent on type of dryer, slow heat and mass transfer within the product and losses on heating side as well as to surroundings cause low drying efficiencies in a range of 40 – 70%. The low energy input required for a PEF-treatment of plant or animal tissue (10 kJ/kg) causes estimated costs of operation 0,30 €/t for treatment of potato (see section 4.5). It is evident that by reduction of drying time there is a potential to reduce the total energy input and operation costs for product drying.

A fast drying of potato surface could help to minimize the amount of enzymatic browning; the potential to avoid blanching or to replace thermal enzyme inactivation by citric and ascorbic acid was investigated. A drying without blanching resulted in dark products due to polyphenoloxidase activity; this effect was even more pronounced after a PEF-treatment (see Figure 4.22). A 30 s dipping in ascorbic acid solution at a pH of 5 and 4 could not prevent browning. Immersion in a solution of pH 3 and 2 resulted in yellow potato slices, but products where covered with a layer of crystalline acid. When a PEF-treatment was applied a color change of the samples towards red was observed. This effect is based on release of

intracellular enzymes after a PEF-treatment and their diffusion to the surface and oxygen contact after loss of membrane barrier function. A combined application of PEF and ascorbic acid dipping can therefore not be utilized to produce dried potato slices without color changes. Similar results have been found after citric acid dipping. A PEF-pretreatment prior to drying will have to be applied with tubers or slices immersed in water as electric energy transfer medium, it is suggested to perform a treatment after blanching in blanching water. The impact of a treatment of slices and whole tubers was compared, as expected due to the differences in surface/volume ratio a treatment of slices resulted in a loss of soluble solids in the treatment media in a range of 2 to 4 %.


Figure 4.22: Impact of different treatments on dried potato slices color. From left to right: control, blanched; unblanched sample, unblanched, dipped into ascorbic acid solution pH 3, unblanched, dipped during PEF at 1 kV/cm, 2 kJ/kg, unblanched, dipped during PEF at 2 kV/cm 10 kJ/kg.

### 4.1.5 Impact of PEF on plant tissue structure

A PEF-treatment of biological tissue can not only be used to induce tissue disintegration to enhance mass transfer processes, but as reported for apple tissue in section 4.1.3 structural properties can be changed significantly. During pressing of apple a tissue softening and loss of compressibility showed to have a detrimental effect or at least to require an adjustment of separation technique used, but it is obvious that a change of textural properties can also be utilized during production and processing of various biological products. Exemplarily the impact on textural properties of potato tissue has been investigated. A force displacement curve of potato samples after a PEF-treatment in comparison to untreated samples is shown in Figure 4.23. Tissue softening of apple, potato and carrot tissue has also been described by Fincan and Dejmek (2003) and Lebovka *et al.* (2004), a reduction of elasticity modulus was reported. Approx. 50 % steady state cutting force reduction and improvement of cut quality have been described for sugar beet (Kraus 2003), which is in good agreement to own results.

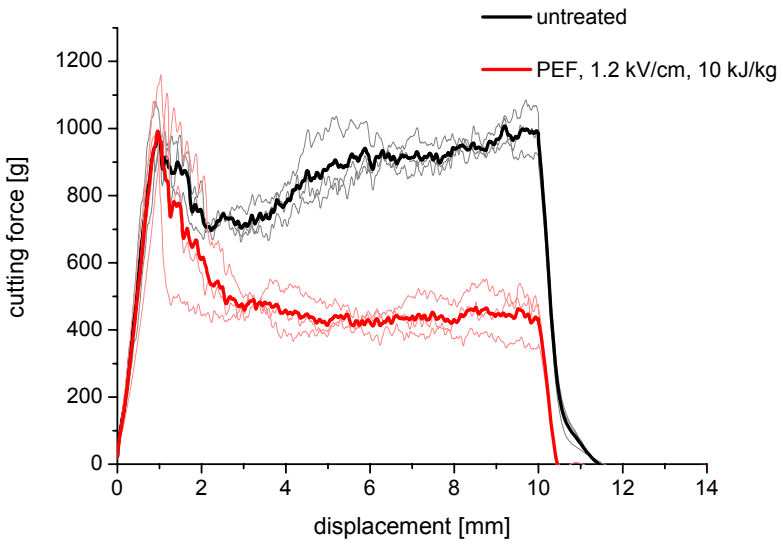



Figure 4.23: Impact of a PEF-treatment on textural properties of potato tissue, three samples for untreated (black) and PEF-treated (red) samples and average (bold lines) are shown.

The tissue softening effect of PEF, based on cell membrane electropermeabilization and loss of turgor can be utilized to reduce the energy required for cutting of plant material. With a continuous, short time and low energy (~10 kJ/kg) PEF-treatment of potato tissue, a reduction of grinding energy similar to that of thermal or enzymatic treatment can be achieved at lower energy input (see section 4.5) processing times. In addition a PEF pre-treatment therefore appears to be applicable to influence cutting behavior. Loss of turgor pressure and elastic modulus will change cutting properties of the tissue. Dependent on process requirements and parameters a cell membrane permeabilization and loss of turgor can be utilized for softening and tissue modification and replace or enhance conventional processing techniques.

## 4.1.6 PEF-treatment of meat and fish products

### 4.1.6.1 Impact of a PEF-treatment on meat tissue integrity

The impact of a PEF-application on mass processes in meat and meat products was investigated. In accordance to fruit and vegetable products it was assumed that a permeabilization of meat tissue can be utilized to enhance drying and/or brining processes. As impedance analysis did not deliver reproducible results for determination of meat tissue integrity the applicability of texture and conductivity measurements was evaluated to identify impact of processing parameters on meat structure and to be used for treatment parameter optimization. The applicability of a meat conductometer (Mathäus, Germany) to determine

tissue disintegration after PEF and tumbling application was evaluated using pork fillet and pork shoulder. A conductivity measurement only showed good results for meat without brine injection. The treatment chamber (G) with a volume of up to 100 l and a pulse modulator were designed to allow treatment of large meat pieces or sample sizes to minimize the impact of deviations in raw material properties.

In Figure 4.24 the impact of different PEF-treatments on increase in conductivity of meat samples with regard to the respective control sample is shown. The red columns show the impact of increasing treatment intensity, a trend towards higher increase in conductivity was found. The black columns represent treatment of samples with same energy input of 10 kJ/kg at different combinations of electric field strength and pulse number. Similar than described for apple tissue above, increase in tissue conductivity was at a similar level when same energy input was applied, indicating the major impact of specific energy input. On the other hand the high variability of meat raw material can be seen on these results.

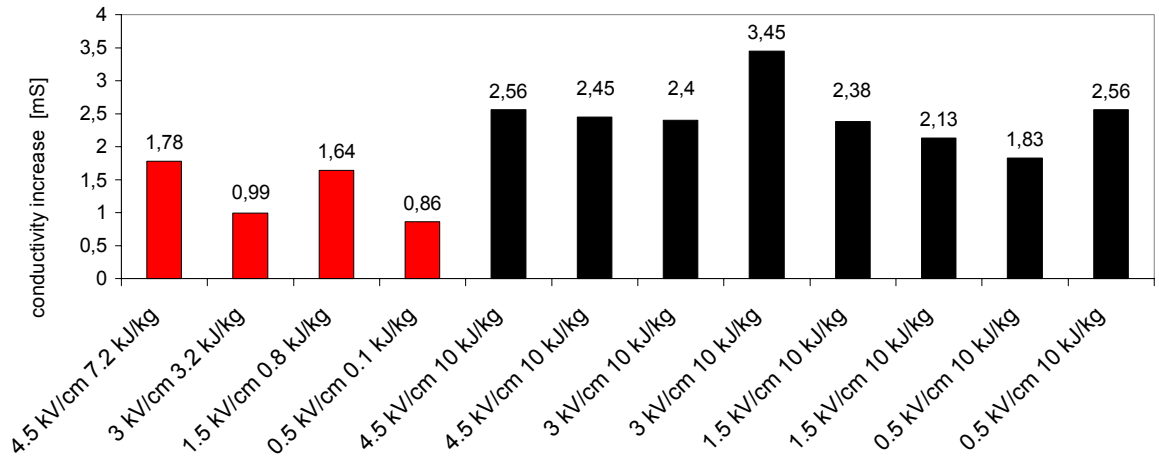



Figure 4.24: Increase of meat conductivity after a PEF-treatment at different treatment intensities. Red columns: treatment at constant pulse number (n=100) and increasing field strength, black columns: treatment at different field strength, pulse number has been adjusted to achieve an energy input of 10 kJ/kg for all samples. Pulse numbers applied: 0.5 kV/cm: n= 11111; 1.5 kV/cm n= 1809; 3.0 kV/cm n= 319; 4.5 kV/cm n= 139. Columns show average of three measurements for one sample, two samples per treatment.

After PEF-treatment the samples have been cooked and drip loss has been investigated to evaluate correlation of conductivity measurement to final product properties. The relative weight of pork shoulder samples after cooking to 64°C core temperature is shown in Figure 4.25.

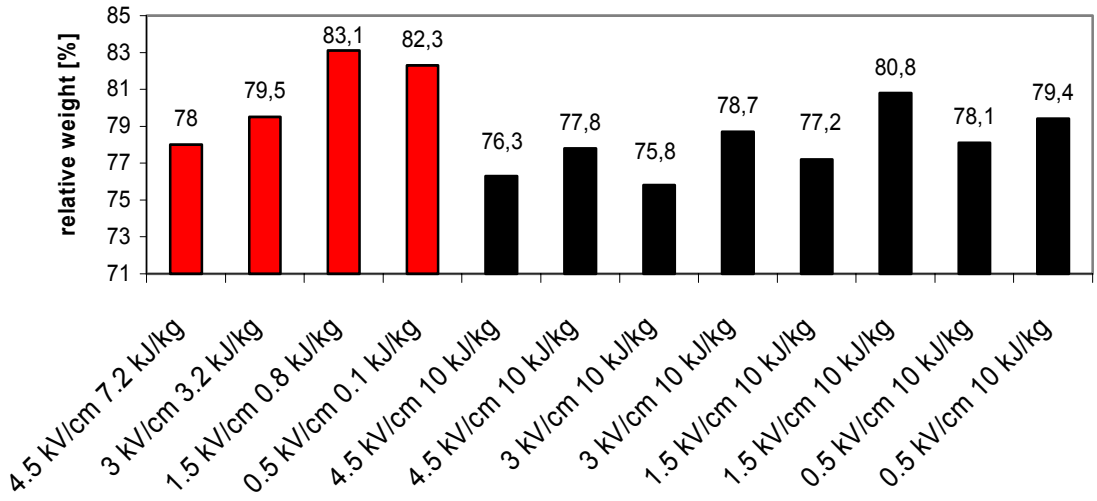



Figure 4.25. Impact of PEF-treatment at different intensity on weight loss of pork shoulder after cooking to 64°C core temperature. Red columns: treatment at constant pulse number (n=100) and increasing field strength, black columns: treatment at different field strength, pulse number has been adjusted to achieve an energy input of 10 kJ/kg for all samples. Pulse numbers applied: 0.5 kV/cm: n= 11111; 1.5 kV/cm n= 1809; 3.0 kV/cm n= 319; 4.5 kV/cm n= 139. Columns show average of three measurements.

A good correlation between conductivity measurement and weight loss during cooking can be found, increasing treatment intensity is increasing cell permeabilization, shown by higher tissue conductivity. Higher tissue conductivity resulted in increased drip loss. In general the drip loss was high as no injection of brine has been performed.

Subsequently the impact of tumbling on conductivity has been investigated to compare the effect on both treatments on meat tissue. As represented in Figure 4.26 for four pork shoulder pieces an increasing trend can be found for conductivity after a tumbling, dependent on tumbling intensity. Each sample was removed after 1000 TR and conductivity determined, no brine was injected as then no differences in conductivity would have been found due to high salt content. After tumbling the pieces were cooked, a trend of correlation between weight loss and conductivity was found. These results indicate that the mechanical stress during tumbling has an impact on sample conductivity similar than a PEF-treatment, though the time required is in different orders of magnitude. A PEF-treatment therefore might provide a potential to reduce tumbling time during cooked ham processing.

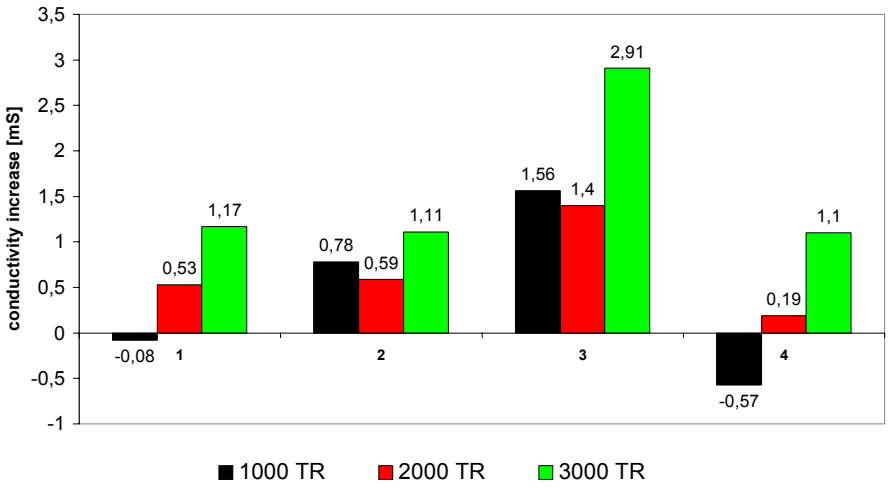



Figure 4.26: Impact of tumbling on conductivity increase of pork shoulder samples in comparison to untreated control. 1000 Tumble rounds (TR) corresponded to a tumbling time of 1 h.

### 4.1.6.2 Impact of a PEF-treatment on meat drying

The impact of a PEF-treatment on drying of pork shoulder is shown in Figure 4.27. Hand salting of 5 % fresh weight as used for production of traditional raw ham or injection of 10 % saturated salt brine was applied after PEF-application. Subsequent drying was performed using a climatic cabinet set to 8°C air temperature and a rel. humidity of 95 %.

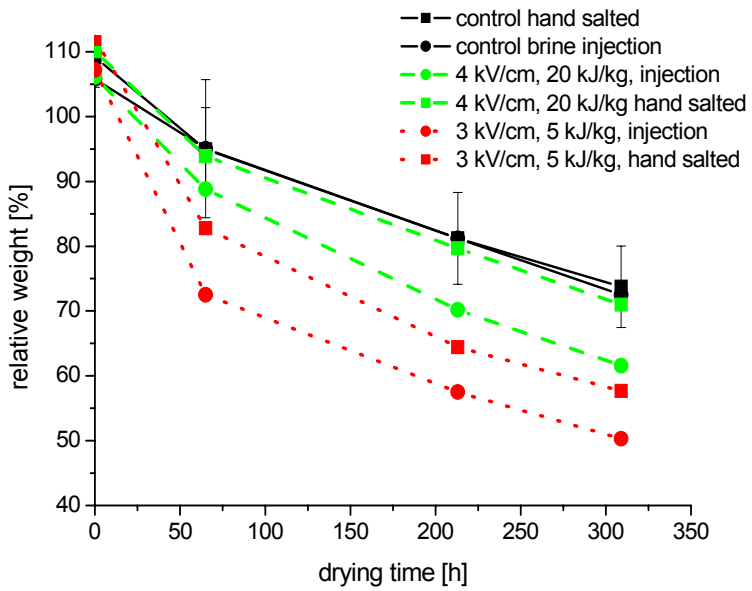



Figure 4.27: Drying of pork shoulder after hand salting (approx. 10 % of weight on surface) or saturated brine (approx. 8 %) injection after a PEF-treatment at different intensity in comparison to untreated control. Drying at 8°C, 95 % rel. humidity.

It can be seen that drying rate was enhanced dependent on treatment intensity and salting procedure and a reduction of drying time was achieved. The shortest drying times were achieved when brine injection and a treatment at 3 kV/cm, 5 kJ/kg was used. Control samples have been performed in triplicate to minimize the impact of variability of raw material properties. An application of hand salting in combination with PEF caused formation of a dry, denatured meat surface, in particular found after a treatment at 4 kV/cm and 20 kJ/kg, which might have inhibited mass transfer from the centre to the surface. This pronounced effect might also have been related to the drying conditions applied. To avoid formation of a dry crust during the initial drying phase the relative humidity should have been increased, but for the lab scale tests performed the maximum humidity of the climatic cabinet of 95 % relative was limiting. Lower electric field strength than 3 kV/cm was not investigated as required number of pulses for a sufficient energy input would have been too high due to the large treatment chamber volume. In case of exterior salt application two mass transport processes will occur, a diffusion of water out of the tissue as well as salt diffusion into the tissue due to the concentration gradient applied. A pretreatment by PEF application could be utilized to improve uptake of salt and pickling salt during production of hand salted products. To investigate the larger scale feasibility experiments have been performed in cooperation with Abraham Schinken, Edewecht, Germany. Approx. 50 whole pork haunches have been subjected to a PEF-treatment at 3 kV/cm and 5 kJ/kg, subsequently applying pickling salt to the surface for production of Serrano-type ham. The results indicated an enhanced diffusion of salt and nitrite into the haunches after storage time of a few weeks.

In addition to drying of meat pieces a large amount of minced meat is processed for raw sausages such as Salami in industrial scale. Minced meat or sausage meat provides the advantage to be pumpable through a tube and therefore through a continuous treatment chamber. In cooperation with Frankenförder Forschungsgesellschaft, Luckenwalde, Germany the impact of a PEF-treatment of minced meat on lactic acid fermentation for raw sausage production was investigated. After a continuous treatment at 2 kV/cm and an energy input of 10 kJ/kg within a parallel plate chamber the time required to achieve a pH drop to a value of 5 was reduced by 30 %. A PEF-application could be performed prior to a filling of sausage meat into a casing, but on the other hand a salt content of up to 5 % is present at this stage. To allow a treatment of highly conductive sausage meat a colinear treatment chamber with a diameter of 60 mm has been realized. Pilot scale test at Kemper Fleischwaren, Nortrup, Germany revealed a decrease of curing time required from 11 to 9 days.

### 4.1.6.3 Meat brining and pickling after a PEF-treatment

The impact of a PEF-treatment on marination and brining was investigated. During production of cooked ham commonly up to 25 % of fresh weight pickling brine is injected to introduce nitrite as well as salt and spices and to improve yield after cooking. After injection, which is performed using needles with a diameter in a range of 2 to 4 mm and a distance of 20 to 30 mm a tumbling is applied to improve brine distribution within the tissue and to achieve a mechanical disintegration.

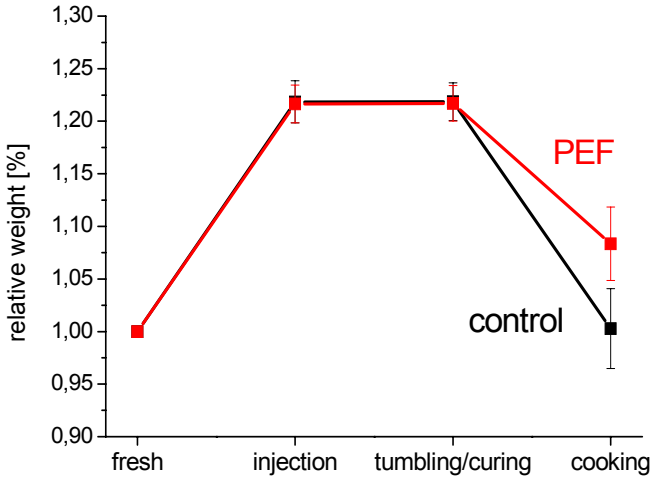



Figure 4.28: Relative weight development during production of cooked ham in relation to fresh weight. Injection: 22 % brine with 1.5 % phosphate addition, 2 h tumbling and 4 h curing, cooking up to 64°C core temperature.

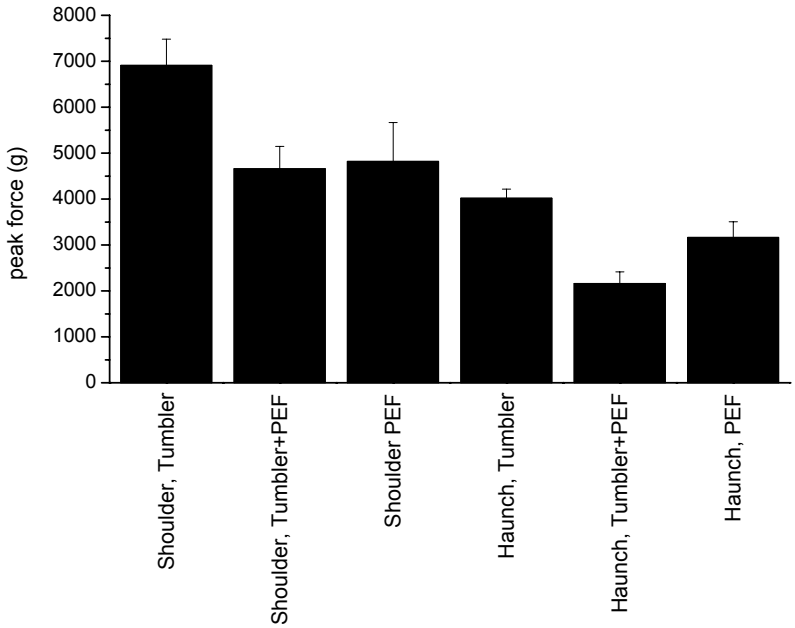



Figure 4.29: Textural properties of pork haunch and shoulder after tumbling, a PEF-treatment and a combination of both, samples were cooked to 64°C core temperature, analysis was performed after cooling to ambient temperature.

During tumbling the brine is spread within meat pieces by kneading and a partial protein denaturation occurs, improving water binding capacity of the protein matrix during cooking. The impact of PEF on mass transport and microdiffusion of brine was investigated dependent on treatment intensity, tumbling time and addition of different water binding agents. In Figure 4.28 the relative weight development of PEF (2 kV/cm, 10 kJ/kg) and control samples during cooked ham production is shown. After an injection of 22 % brine with 1.5 % phosphate, a 2 h tumbling and 4 h curing a cooking up to 64°C in a Ratiomat steam oven was applied. After cooling the drip loss during cooking was determined gravimetrically. Drip loss was reduced from almost 22 % to 12.7 % for the PEF-treated samples; in comparison to native weight an increase was achieved. Textural analysis (see Figure 4.29) revealed a decrease of maximum force after a PEF-treatment in combination with 2 h tumbling for pork haunch and shoulder, indicating a soft and tender product structure.

To investigate underlying mechanisms REM micrographs have been performed at the German Institute of Food Technology (DIL e.V.), Quakenbrück, Germany. The microstructure of ham samples is shown in Figure 4.30, indicating an enhanced protein swelling after a PEF-treatment. Facilitating brine and phosphate microdiffusion might cause a better distribution in the tissue and formation of protein-water interactions on a cellular level. Improved access of phosphates to protein filaments and intracellular structures after a PEF-treatment could cause an inclusion of free water after brine injection.

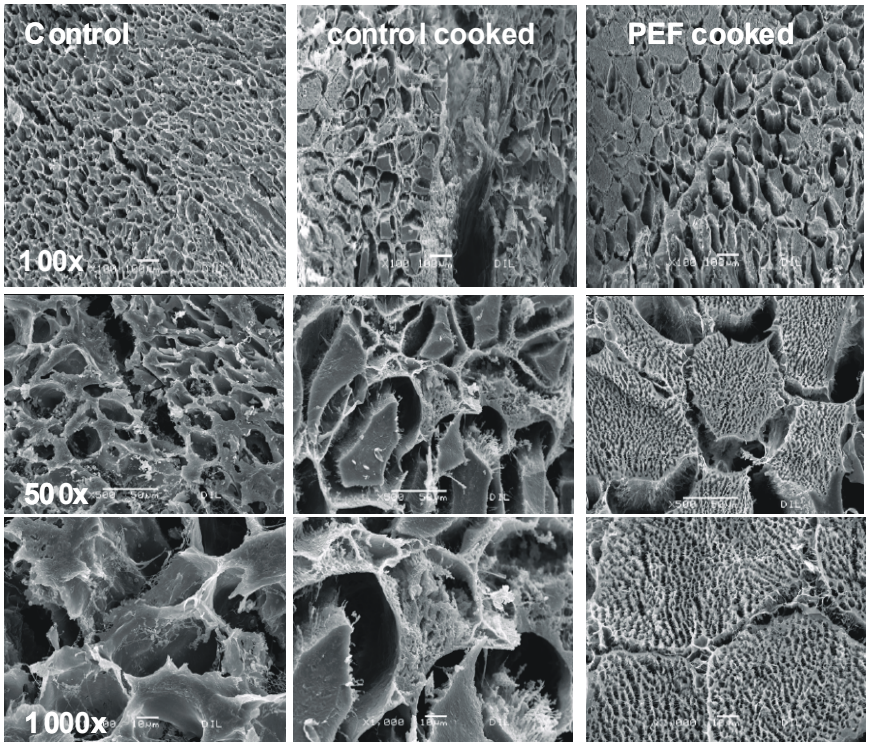



Figure 4.30: REM micrographs of ham samples prior and after cooking and a PEF-treatment at 2 kV/cm, 10 kJ/kg.

PEF application without addition of water binding agents showed to have an opposite effect, an increase of drip loss during cooking was observed, similar than reported for meat drying experiments (Figure 4.31).

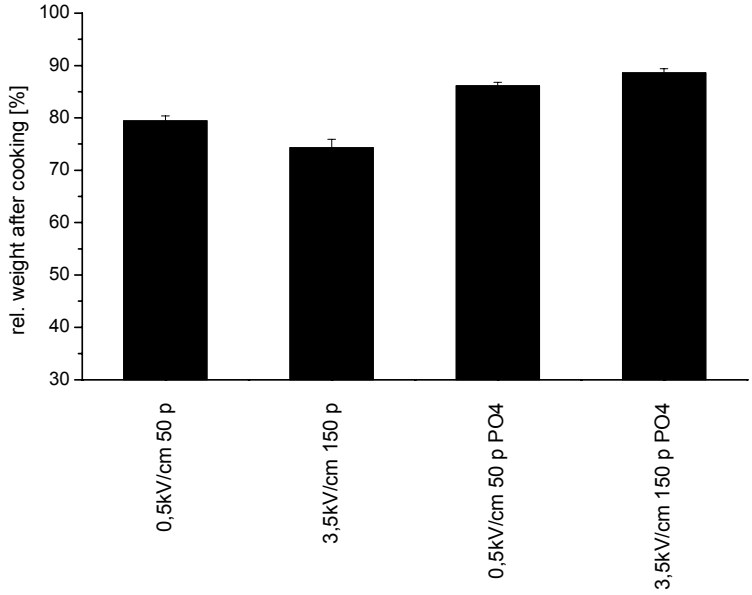



Figure 4.31: Impact of PEF-treatment intensity and 1.5 % phosphate (PO4) addition on drip loss during cooking of pork shoulder; injection of 25 % brine, tumbling for 2 h, cooking to 64°C core temperature.

Increasing treatment intensity (pulse number and electric field) strength without addition of phosphate causes an increase in drip loss during cooking, whereas addition of 1.5 % phosphate to the brine results in an improved water binding capacity at higher PEF intensity. These results indicate that after a PEF-treatment the tissue is disintegrated, but the PEF-treatment itself does not enhance water binding within meat tissue. An application of phosphate and PEF showed synergetic effects. The lowest weight loss during cooking was found when first the water binding capacity of the meat tissue was enhanced by injection of a concentrated phosphate solution and 10 min tumbling for dispersion and a subsequent injection of brine (data not shown). Though providing an additional increase of 1 to 2 % weight, this would have required a two step injection and tumbling process which appeared not to be feasible in industrial scale.

To investigate the impact of mechanical stress and protein disintegration during tumbling the tumbling time was reduced from 2 h (2500 rounds) to 30 min (500 rounds), results are shown in Figure 4.32. A tumbling time of 30 min showed not to be sufficient to achieve a good water binding capacity. This effect is probably based on insufficient mechanical stress and kneading of the pieces, necessary to achieve an even distribution of brine and partial protein disintegration. In comparison to untreated samples the tumbling time could be reduced from

4 to 2 h to achieve a similar weight yield (data not shown). A PEF-treatment appears to be applicable to reduce tumbling time requirements during cooked ham production, an advantage in terms of production time and costs. On the other hand it can be concluded that a PEF-treatment can not totally replace a mechanical tumbling, a minimum tumbling time of 2 h was required to achieve good results for pork shoulders.

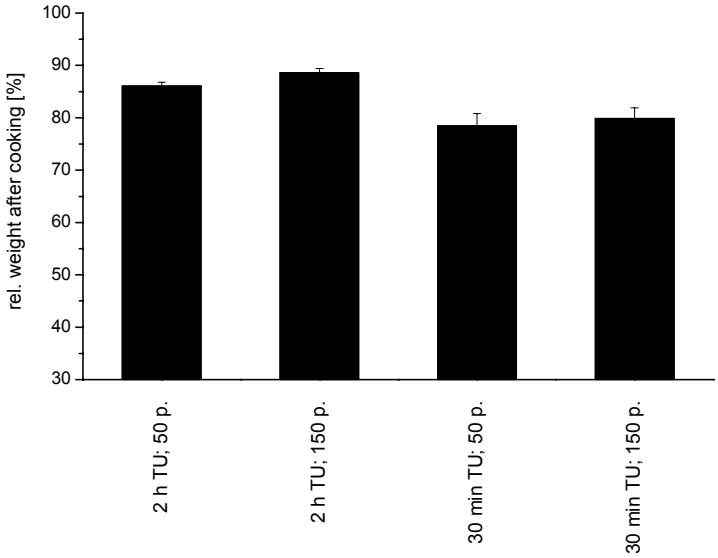



Figure 4.32: Impact of tumbling time and PEF intensity on drip loss during cooking of pork shoulder, PEF-treatment at 3.5 kV/cm, brine with 1.5 % phosphate, cooking to 64°C core temperature.

The impact of addition of soy protein and two types of carragenaan was investigated in cooperation with Hahn Stabilisierungstechnik, Lübeck, Germany, results are represented in Figure 4.33. Whereas an addition of soy protein showed a very small trend of weight increase only, addition of carragenaan (I) had a synergetic effect on water binding capacity. For soy different tumbling times were used, again a 30 min tumbling showed to be not sufficient. Differences in efficiency of different additives might be based on their molecular structure, as this may influence their diffusivity in the tissue and accessibility to protein fibrilles and their gelatinization properties such as gelatinization temperature. Additives have been selected by Hahn, detailed composition remained confidential, but it is indicated that a PEF-treatment can be utilized to improve water diffusivity and mobility within meat tissue. If the tissue has sufficient water binding capacity, achieved by good meat quality or water binding agents, synergetic effects have been found.

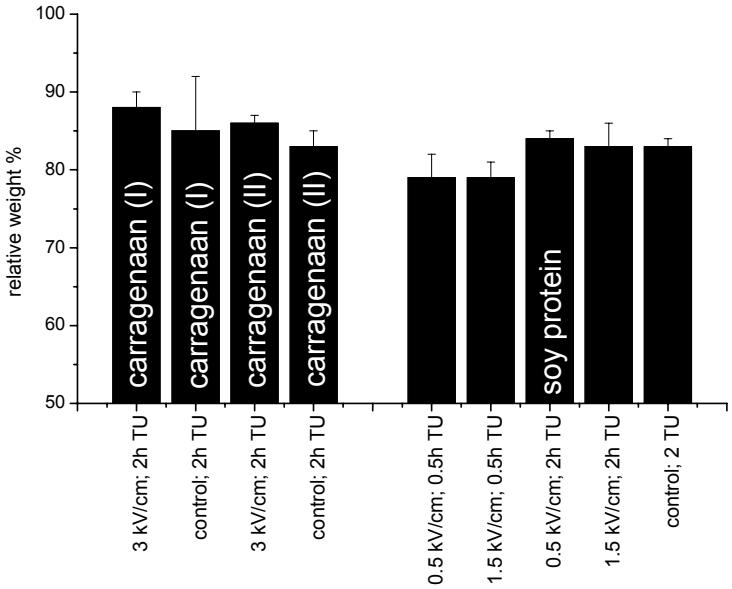



Figure 4.33: Impact of soy protein and carragenaan (I and II) addition on weight loss of pork shoulder after PEF-treatment at 0.5 and 3.5 kV/cm, 1 and 8 kJ/kg, respectively. Cooking to core temperature of 64°C.

A treatment of duck and beef meat revealed a tenderization effect of PEF and a subsequent storage for curing for 12 h, reported by a test panel of five persons of Rühle. Meat ageing is mainly based on activity of endogenous, proteloytic enzymes (Herrera-Medez *et al.* 2006), which may be released after a PEF application. Release of enzymes was also found for treatment of plant tissue, which shows fast enzymatic browning after an electropermeabilization. Commonly an ageing of up to 14 days is required for beef meat tenderization. It was shown that a PEF-treatment can be used to enhance mass transfer in meat products; similar than for plant tissue the energy input was a good intensity parameter if a threshold electric field strength was exceeded. Possible applications are improvement of drying, marination and brining as well as potentially in meat curing. At present an industrial prototype for treatment of meat pieces as well as a design for sausage meat is under realization in cooperation with Rühle, Grafenhausen, Germany.

#### 4.1.6.4 PEF-treatment of fish and seafood

A PEF-treatment of Pollock fillets, frozen cod loins, fresh cod fillets, frozen haddock loins, Iceland cyprine and common whelk was performed to evaluate PEF impact on different fish and seafood products. For cyprine and whelk a tissue softening was aimed for, as these species have a tough structure which diminishes their usability as seafood. After a PEF-treatment at 2 kV/cm, 90 pulses a 10 % salt brine injection was performed using a IR 8

injection machine or manually, using 20 ml syringes until an amount of 10 % brine was injected. After a storage at 4°C for 4 h fish samples were packed in vacuum bags and cooked in a water bath at 95°C for 20 min. In Figure 4.34 the drip loss for fresh and frozen cod and frozen haddock fillets is shown. For all samples a reduction of water loss was found, but the effect was less pronounced as for meat samples.

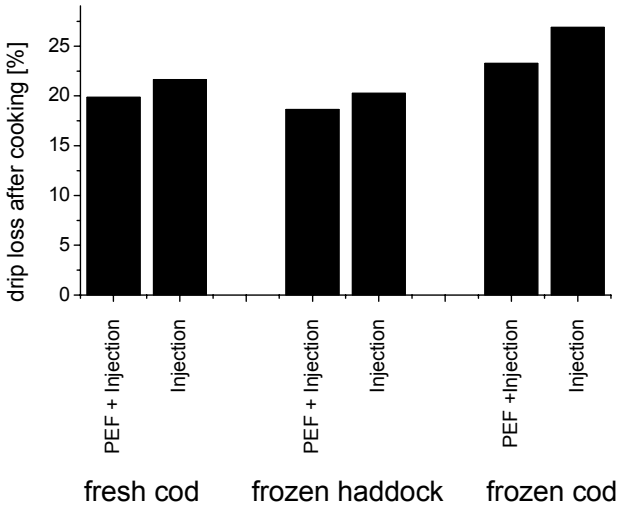



Figure 4.34: Water loss after cooking for fresh and frozen cod fillets and frozen haddock samples.

The impact of a PEF-treatment on fish muscle microstructure was investigated by REM micrographs, as shown in Figure 4.35. After a PEF-treatment a trend towards a more porous tissue structure was found. Differences in meat and fish muscle structure and less supportive tissue of fish muscles appeared to cause lower water binding of fish fillets in comparison to meat tissue, but the application of water binding agents such as phosphate could reduce water loss during cooking. The selected cooking temperature of 95°C was very high; at lower temperatures maybe a better water holding could have been obtained. A PEF-treatment of scallops (common whelk) and Iceland cyprine did not show an effect on product tenderness, the variation within raw material showed to be higher than differences between treated and untreated samples. Future work should concentrate on processing parameter optimization and adaptation to fish tissue.

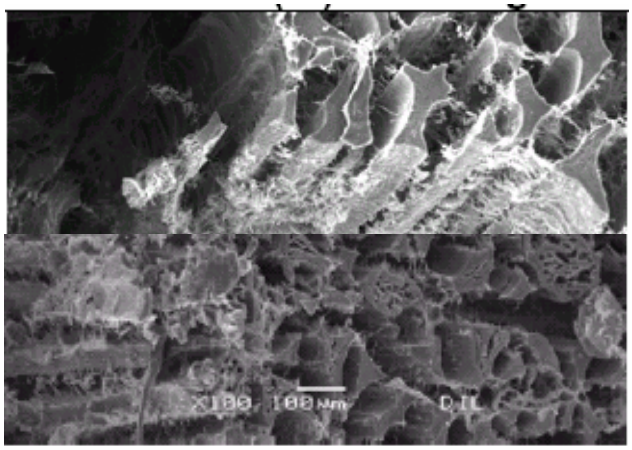


Figure 4.35: Longitudinal section of cod muscle after manual brine injection and cooking (top) and after PEF-treatment, brine injection and cooking (bottom). PEF-treatment at 2 kV/cm, 90 pulses.

## 4.2 Microbial Inactivation by PEF

### 4.2.1 Permeabilization of Liposomes as a model system

For basic mechanistic studies a model system be desirable, to avoid variability of biological membranes and their resistance against an electropermeabilization. The applicability of artificial phospholipid bilayers as a model system was investigated. Egg phosphatidylcholin vesicles, stained with carboxyfluorescine (cF), were producing using a LiposoFast (Avestin, Mannheim, Germany) handheld extruder and subjected to a PEF-treatment using the micro pulse modulator TUB 1 and 400 µl batch cuvettes. Determination of cF and PI staining after a treatment was performed by flow cytometric analysis. Density plots of cF-stained liposomes are shown in Figure 4.36.

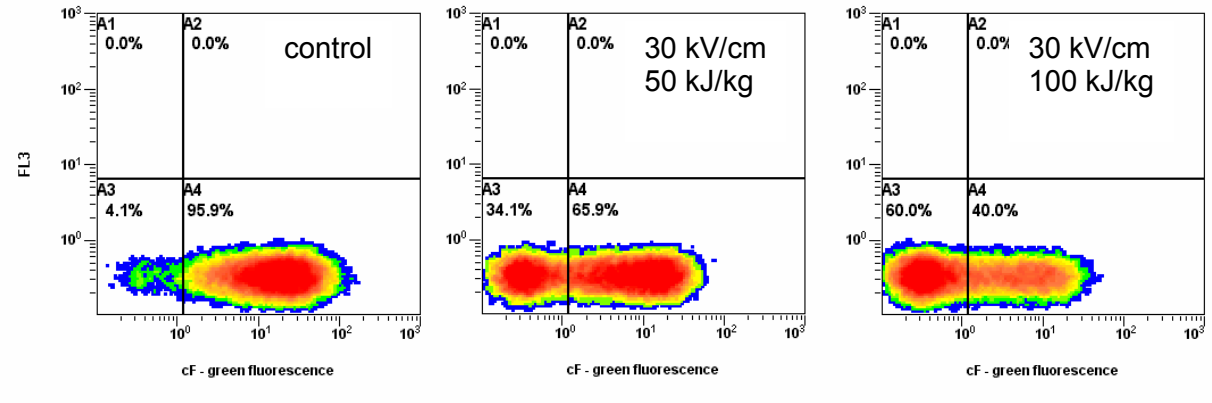



Figure 4.36: Density plot of flow cytometric analysis of cF-release from egg phosphatidylcholin (EPC) vesicles after a PEF-treatment in comparison to untreated control.

The impact of PEF on vesicle size, dye release and uptake was investigated dependent on electric field strength, energy input and pulse geometry and compared to microbial

inactivation after a treatment. A slight trend towards an increase in particle size due to electrofusion was found after a treatment, but no significant differences were found (data not shown). The release of cF from liposomes dependent on electric field strength applied is shown in Figure 4.37 at a constant pulse number of 200 pulses (left) and a constant energy input of 50 and 100 kJ/kg (right). To achieve a constant energy input while increasing field strength the pulse number was reduced, whereas when applying a constant pulse number of 200 resulting energy input increased exponential.

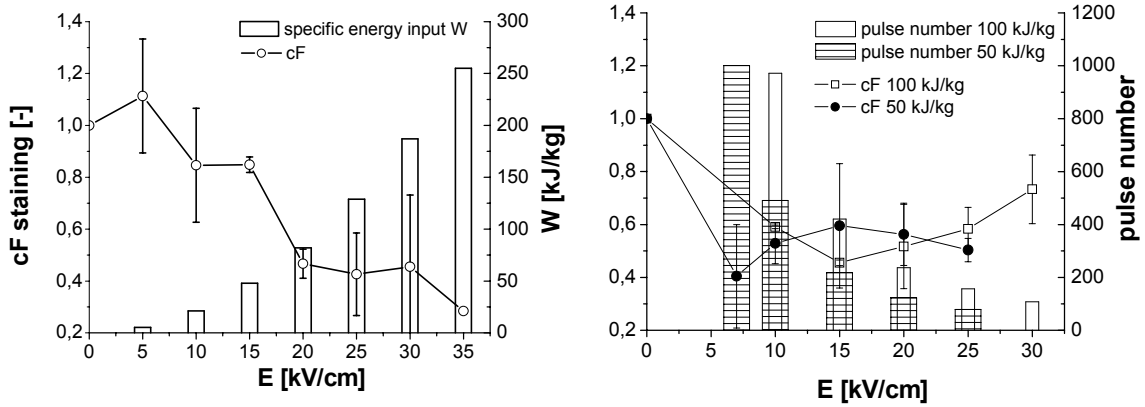



Figure 4.37: Impact of a PEF-treatment at different electric field strength on release of cF from EPC vesicles. Left: constant pulse number of 200, right: constant energy input of 50 and 100 kJ/kg applied. The columns show the resulting energy input and pulse number applied, respectively.

Though the small diameter of the vesicles (1 µm) no occurrence of a critical field strength was found. Increase of electric field strength and energy input resulted in higher release of dye from the vesicles (Figure 4.37, left), but when a constant energy input was applied the impact of increasing electric field was not as pronounced. Taking into account the large standard error the electric field applied appears to have a minor impact in comparison to energy input. At high field strengths only a small number of pulses was applied, this might explain the increasing trend in cF content observed at higher field strength levels.

The impact of a PEF-treatment on uptake of propidium iodide into *L. innocua* is shown in Figure 4.38 in comparison to cF-release from microbes and liposomes. For *L. innocua* a field strength of 35 kV/cm was required to achieve a permeabilization of cells and to allow significant cF-release and PI-uptake. Below 30 kV/cm a slight uptake of PI was found, but cells showed to be cultivable after a treatment at same intensity but without addition of (toxic) PI. A PI staining of liposomes was not possible as the dye is fluorescent after binding to DNA, only. These results are in good agreement with literature (Heinz *et al.* 2002; Fleischman *et al.* 2004; Aronsson *et al.* 2005) and own data regarding inactivation of *Listeria* strains. *Listeria* has been reported to be among the most resistant strains against a PEF-treatment, due to its cell size in a range of 0.625 x 0.25 µm (Figure 4.51) and has been

chosen for comparison to liposomes with an average diameter of 1 µm. The impact of different liposome diameters (400, 600, 800 and 1000 nm) has been investigated, but no significant differences in dye release or diameter after a PEF-treatment have been found (data not shown). Production of larger vesicles than 1 µm was not possible as polycarbonate membranes with larger pore sizes were not available.

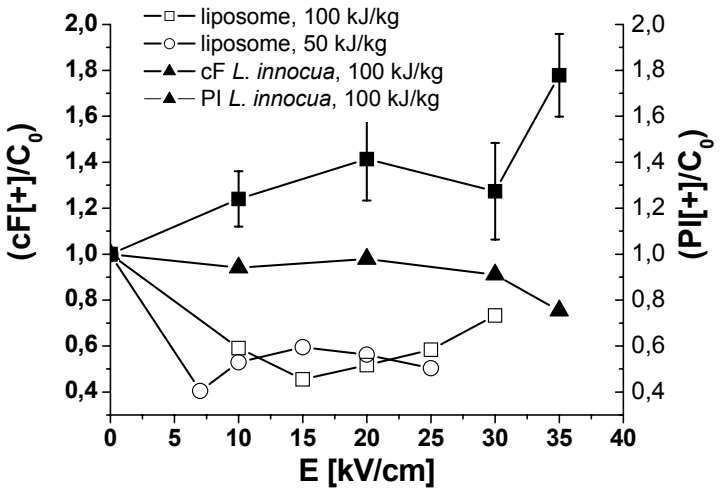



Figure 4.38: Release of cF after PEF-treatment of Liposomes and *L. innocua* dependent on electric field strength at a specific energy input of 50 and 100 kJ/kg and uptake of PI into *L. innocua*.

It was shown that for liposomes as well as for microbial cells a release of cF can be achieved after a PEF-treatment, whereas for liposomes no critical field strength was observed, for *Listeria* a field strength of 35 kV/cm was required for cF release. In Figure 4.39 a comparison of cF release and microbial inactivation determined as cfu by plate count is represented for *L. innocua* and *L. rhamnosus*, indicating a correlation between flow cytometric and inactivation data in the range of investigation.

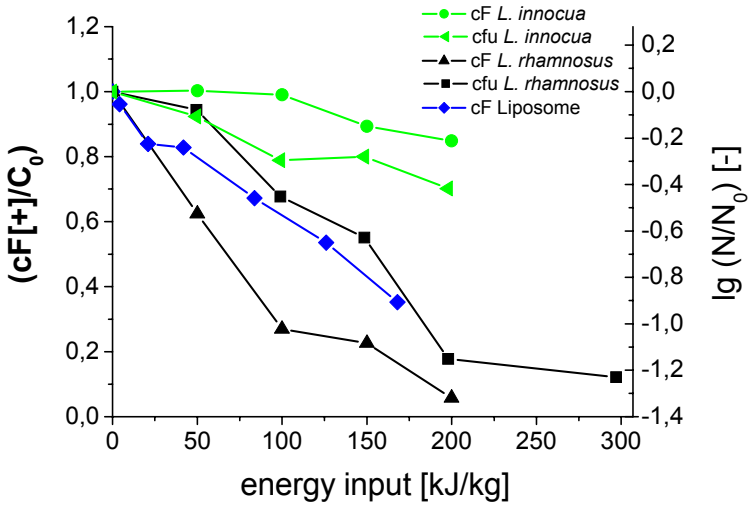



Figure 4.39: Release of cF from *L. innocua, L. rhamnosus* and liposomes after a PEF-treatment at 35 kV/cm in comparison to microbial inactivation determined as cfu.

## 4.2.2 Impact of pulse rise time and width on vesicle permeabilization and microbial inactivation

### 4.2.2.1 Impact of rise time and pulse geometry of exponential decay pulses

The impact of electric field strength and specific energy input during a PEF-treatment was shown in the previous sections. In addition the influence of pulse rise time and pulse geometry on microbial inactivation was investigated for exponential pulses, for rectangular pulses the effect of pulse width was evaluated. For exponential decay pulses the energy delivered per pulse is determined by the energy stored in the capacitor bank and the ratio between treatment chamber and protective resistance, mainly. Peak voltage and peak field strength can be influenced by the charging voltage, which is also influencing the stored energy. Pulse parameters for exponential decay pulses can not be selected independently. The pulse width is determined by the stored energy, the resistance and the inductance of the discharge circuit, pulse rise time is dependent on inductivity of the capacitors and the discharge circuit.

A micro PEF-system was developed to vary pulse geometry of exponential pulses by variation of storage capacity, discharge circuit inductivity, ratio of protective and treatment chamber resistance in small increments to maintain energy input per pulse constant while changing a pulse parameter. A Software was programmed based on TestPoint (CEC Equipment, Billerica, USA) to predict pulse shape and to determine the required setup of the system to produce a pulse with defined rise and fall time.

The capacity of the energy storage was set between 6.8 and 27.2 nF in steps of 2.3 nF, protective resistor was variable between 2.7 and 10 Ohm, treatment chamber resistance was variable by changing media conductivity only. By introducing coils into the discharge circuit the inductivity was variable. The initial inductivity of the circuit has been determined as 1.08 µH by comparison of measured pulse shape and simulated pulse shapes using the TestPoint code. Equipment and media conductivity parameters were fixed, and inductivity was varied until curve shapes overlapped. Introducing inductive elements the inductivity was increased to 2.8, 5.2, 10.4 and 14.3 µH. The resulting pulse shapes are shown in Figure 4.40. The rise time to 95% peak voltage was determined by an oscilloscope as 55, 115, 177, 295 and 373 ns, respectively. Measured values were in good accordance to simulated wave shapes, a maximum deviation of ± 5 % rise time was found. As seen on the pulse waveforms by introducing additional inductivity the peak voltage changed from 5 to 3.92 kV, causing a drop of field strength from 25 to19.6 kV/cm, whereas the energy delivered per pulse was 0.32 ±0.01 J for all waveforms, determined using a TDS430 (Tektronix, Beaverton, USA) oscilloscope and integration of current and voltage curves. In a second experiment the charging voltage was adjusted to maintain constant peak field strength. The charging voltage

was increased from 5.6 to 5.9, 6.3 and 7 kV, respectively. To maintain pulse energy nearly constant at a level of 0.32 ±0.01 J the storage capacity was reduced from 20.4 to 18.1, and 15.9 nF. A further decrease of capacity was not possible, as a minimum RC combination for system damping needed to be maintained. For the pulse at 10.4 µH and voltage adaptation an energy input of 0.38 J was obtained, in this case no reduction was possible. The rise time to 95% peak voltage of the adapted pulses was 125, 180 and 290 ns, respectively. Due to voltage adaptation the rise time decreased in comparison to the first waveform series.

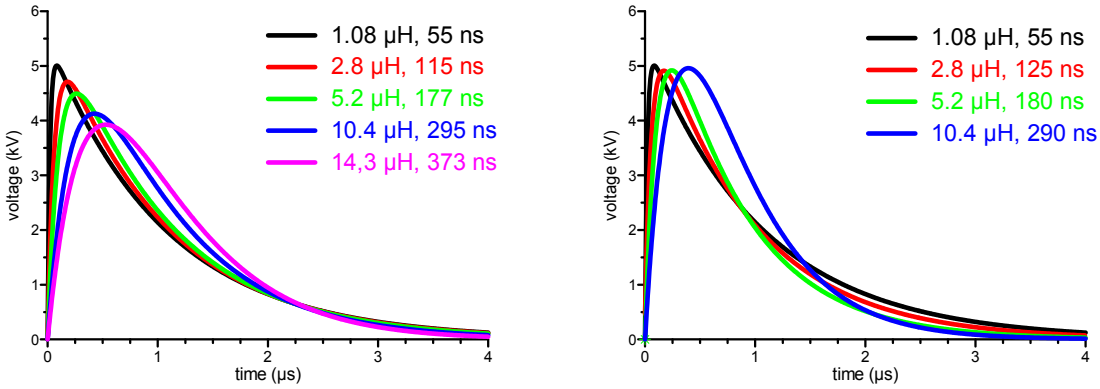



Figure 4.40: Left: Simulated waveforms using a TestPoint code. Charging voltage: 5.6 kV, capacity 20.4 nF, $R_{prot}$ 2.7 Ohm, $R_{TC}$ 50 Ohm, inductivity was increased from 1.08 to 14.3 µH, the energy input per pulse was 0.32 ±0.01 J, determined by storage capacity. Right: Charging voltage was adapted to maintain peak voltage of 5 kV, storage capacity was reduced to 18.1 (2.8 µH), and 15.9 nF (5.2 and 10.4 µH) achieve similar energy input of 0.32 ±0.01 J per pulse, except blue line at 10.4 µH, where an energy input of 0.38 J occurred (see text).

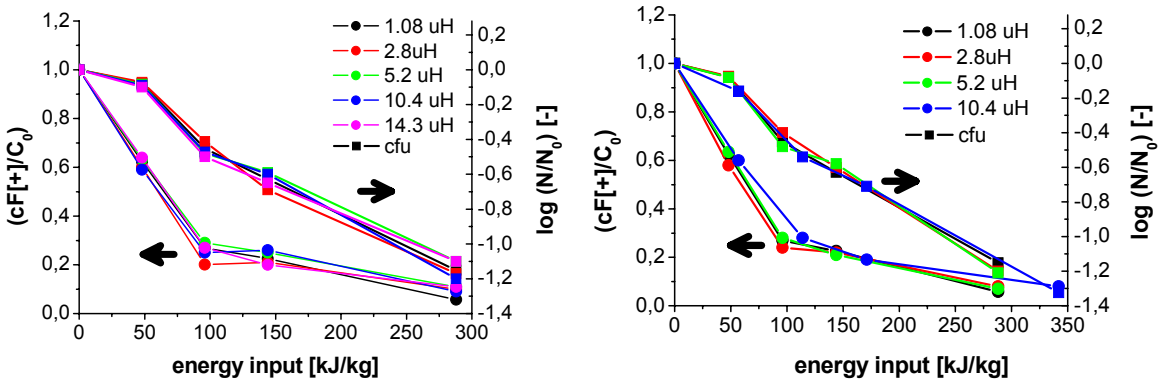



Figure 4.41: Release of cF from liposomes and inactivation of *L. rhamnosus* after PEF application with different pulse rise time dependent on energy input. Pulse shapes as represented in Figure 4.40. Data points are averages of two experiments.

The impact of these pulse waveforms on inactivation of *L. rhamnosus* and permeabilization of liposomes was investigated, applying 60, 120, 180 and 360 pulses to 400 µl sample in the

batch micro-cuvette. The average energy input was 48, 94, 144 and 288 kJ/kg for all PEF applications except the waveform at 10.4 µH of the second series (blue curve, right diagrams of Figure 4.40 and Figure 4.41), where 57, 114, 171 and 342 kJ/kg have been obtained.
As represented in Figure 4.41 no significant influence of pulse rise time was found. These results are in good agreement with Kotnik *et al.* (2003), investigating the impact of a rise time between 2 and 100 µs during application of 1 ms rectangular pulses.
It can be concluded that for microbial inactivation and liposome permeabilization the specific energy input has a major impact on processing efficiency. A reduction of electric field strength from 25 to 19.6 kV/cm did not show an effect when energy input was constant. It is assumed that, similar as reported for plant tissue (4.1.1) and *E. coli* in apple juice (4.2.8) at a field strength below a certain threshold level an impact of field strength on energy efficiency might be found.
The range of pulse rise times achievable was limited to a minimum determined by the inductivity of the components and a maximum to retain circuit damping to avoid current reversal at the switch. Pulse rise time did not show an impact in a range of 55 to 373 ns, also no impact of pulse wave shape or pulse decay time was found for the curves applied. Even if the variability of the parameters was limited due to coupling between different processing parameters these results suggest that the impact of pulse rise time was overestimated up to present. From a technical point of view these findings facilitate the design and realization of suitable pulse modulators, as shorter rise times require design of low inductivity discharge circuits and cause higher switching stress.

#### 4.2.2.2 Impact of pulse width of rectangular pulses

For rectangular pulses the impact of 3, 5 and 8 µs pulse width was investigated using the ScandiNova (I) pulse modulator and a colinear lab scale treatment chamber with a diameter of 6 mm (D). Pulses at a field strength of 35 kV/cm were applied on *E. coli* in apple juice at a total energy input in a range from 30 to 100 kJ/kg, the flow rate was 5 l/h. Pulse energy was determined using a Tektronix TDS430 with integrated math functions, the integral of voltage and current waveform was determined. When using apple juice with a conductivity of 0.8 mS/cm the treatment chamber resistance was 840 Ohm. A peak voltage of 20.6 kV was applied to achieve an average field strength of 35 kV/cm in the colinear chamber, the resulting peak current was 24.5 A. The energy per pulse was measured as 1.73, 2.83 and 4.41 J at 3, 5 and 8 µs. In Figure 4.42 the inactivation of *E. coli* using rectangular pulses with different pulse width is shown. No significant impact of pulse width was found, but a slight trend towards enhanced inactivation using higher pulse widths can be seen. This result is in

accordance to findings of Wouters (2001). The results show that pulse width has a minor impact on PEF efficacy, variable pulse width is commonly related to extra investment costs. Pulse width therefore could be fixed to achieve a pulse modulator setup with low investment costs to a value where a good flexibility of processing possibilities is obtained. A comparison of energy efficiency of exponential decay and rectangular pulses has been performed using apple juice in continuous experiments; results are shown in section 4.2.8.

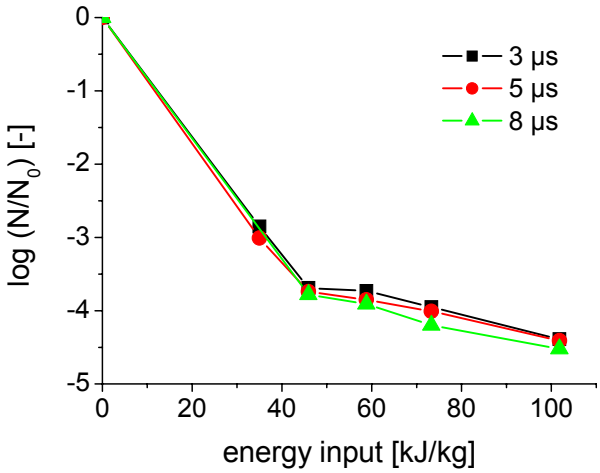



Figure 4.42: Impact of pulse width of 3, 5 and 8 µs of rectangular pulses at a field strength of 35 kV/cm on inactivation of *E. coli* in apple juice. Flow rate: 5 l/h, 30°C inlet temperature.

### 4.2.3 Flow cytometric analysis of membrane permeabilization

After PEF-treatment of *L. rhamnosus* a flow cytometric analysis was performed, using propidium iodide and carboxyfluorescine dyes. Density plots after a treatment at 15, 25 and 35 kV/cm and 50 pulses are shown in

Figure 4.43, left column. It was observed that increasing electric field strength the amount of PI-stained cells is increasing whereas cF-fluorescence is decreasing. PI is a membrane-impermeant, nucleotide-binding probe supposed not to penetrate cells with intact membranes. Following loss of membrane integrity after a PEF-treatment PI diffuses into and stains the cells. The second probe, cFDA, is used primarily for the evaluation of cellular enzymatic activity. It is a lipophilic, non-fluorescent precursor that readily diffuses across intact cell membranes. In the intracellular compartment it undergoes hydrolysis of diacetate groups by unspecific esterases into a polar, membrane-impermeant fluorescent compound carboxyfluorescine (cF). It is important to notice that though esterase activity the cells only remain putrescent if their membranes are intact and probes are unable to diffuse out, thus for cells to be associated as viable, this probe requires both active intracellular enzymes and

intact membranes (Hoefel *et al.* 2003). In addition the efflux of cF upon glucose addition can be used as an indicator of metabolic performance of the cell.

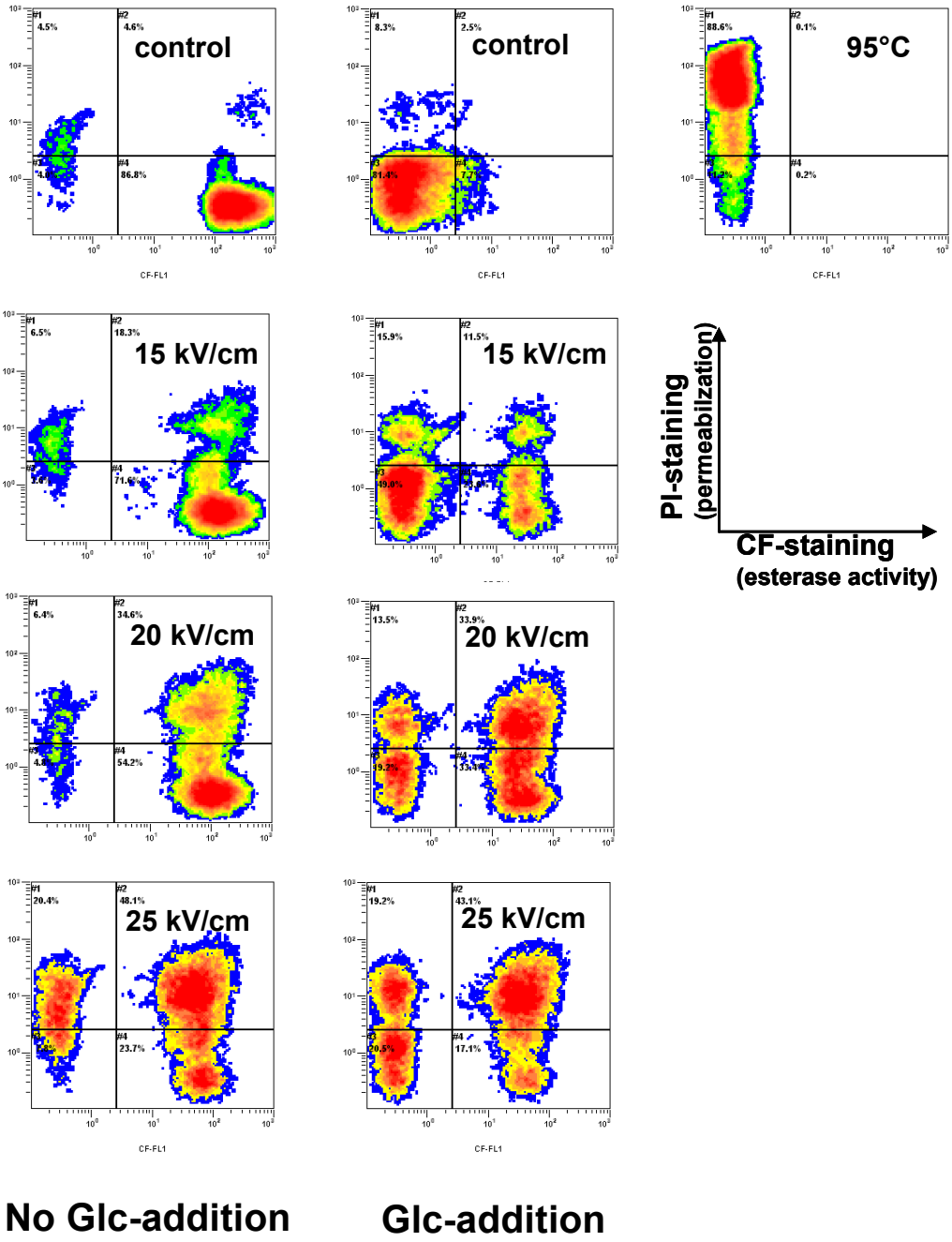



Figure 4.43: Flow cytometric analysis of impact of a PEF-treatment on *L. rhamnosus* at 15, 20 and 25 kV/cm and 50 pulses in comparison to an untreated control and thermal (95°C, 15 min) treatment. A density plot of fluorescence properties after staining with propidium iodide (PI) and carboxyflourescine (cF) is shown in the left column. In the medium column glucose was added to determine activity of active transport mechanisms as cF extrusion capability. Different gates are indicated by notations #1-#4.

After a treatment at 15 kV/cm PI was able to intrude into cells, indicating cell membrane damage, whereas residual esterase activity remained similar than for the control sample. A treatment at 10 kV/cm caused no PI uptake, even if pulse numbers up to 200 pulses were

applied (data not shown). Increasing field strength (and as a constant pulse number was applied also) energy input the degree of PI staining increased. At 15 kV/cm only a minor impact on esterase activity was monitored without glucose addition. Endogenous esterase appears not be inactivated by a PEF-treatment, on the other hand it is indicated that though membrane permeabilization shown by PI uptake the cells are able to retain cF. Above 25 kV/cm further increase of PI and a decrease in cF-fluorescence was shown. The specific energy input was 18, 32 and 50 kJ/kg at 15, 20 and 25 kV/cm, respectively. All treatments have been performed at ambient temperature; a thermal inactivation of esterase can be excluded, as the dissipated electrical energy could have caused a maximum temperature increase of 11°C. Using a fibre optic temperature sensor a maximum increase of 4°C was observed, as a major part of delivered energy was transferred to the electrode material. It is assumed that the apparent decrease of esterase activity is based on a release of cF after achieving a sufficient level of permeabilization; this assumption was supported by the observation of fluorescence of the cell suspension media after treatment.

After 20 min incubation with glucose the ability to extrude intracellular accumulated cF could be determined, results are shown in Figure 4.43. Control samples were able to fully extrude accumulated cF. After a treatment at 15 kV/cm the major proportion of the cells still showed that capability. A comparison to a sample incubated for 20 min without addition of glucose showed that the decrease of cF-fluorescence was indeed based on active extrusion instead of a release after membrane permeabilization. Whereas at 20 kV/cm a slightly reduced, at 25 kV/cm almost no extrusion was found, indicating the loss of active transport mechanism. This effect might be related to the loss of membrane semipermeability and formation of a chemical and electrochemical equilibrium with the surrounding, inhibiting transport mechanisms. On the other hand it is noteworthy that the samples incubated for 20 min showed a decrease in PI staining, which might be based on an at least partly resealing of cells. No increase of amount of cultivable cells was found, indicating that this effect is not necessarily related to an active repair and cell vitality but presumably to a reorganization of the phospholipid bilayer structure. Further investigations regarding the impact of moment of staining, prior or after treatment will be discussed in 4.2.4.

In Figure 4.44 an overview of the impact of field strength and pulse number on membrane permeabilization of *L. rhamnosus* is given. A number of 20, 50 and 100 pulses has been applied at 15 and 20 kV/cm and 20 and 50 pulses at 25 kV/cm. Addition of glucose and 20 min incubation were performed to monitor cF extrusion capability.

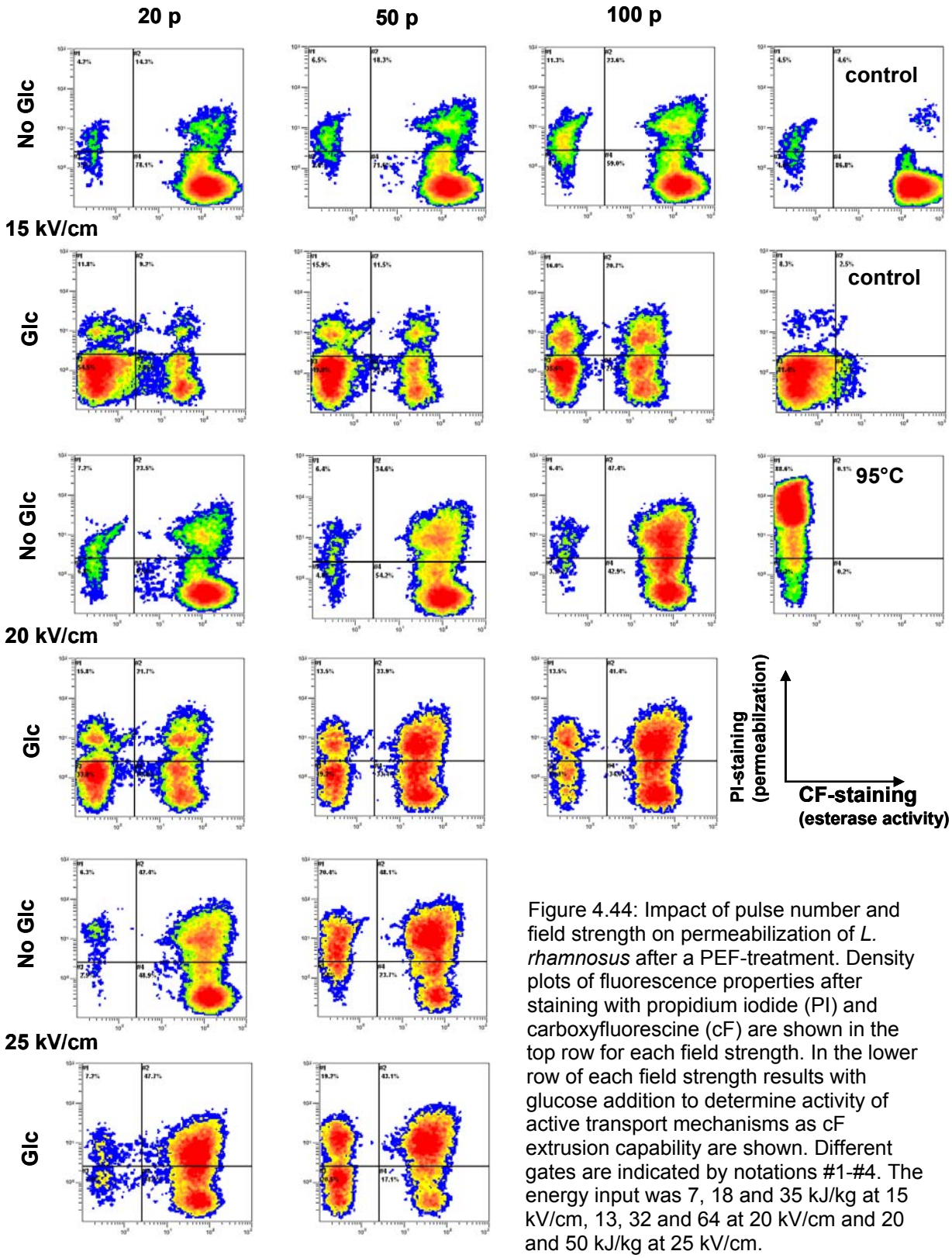



Figure 4.44: Impact of pulse number and field strength on permeabilization of *L. rhamnosus* after a PEF-treatment. Density plots of fluorescence properties after staining with propidium iodide (PI) and carboxyfluorescine (cF) are shown in the top row for each field strength. In the lower row of each field strength results with glucose addition to determine activity of active transport mechanisms as cF extrusion capability are shown. Different gates are indicated by notations #1-#4. The energy input was 7, 18 and 35 kJ/kg at 15 kV/cm, 13, 32 and 64 at 20 kV/cm and 20 and 50 kJ/kg at 25 kV/cm.

At all field strength levels an increase of pulse number resulted in an increase of permeabilization, at higher field strength the PI staining was increased. Comparing results of treatments with a similar energy input in a range of 18 - 20 kJ/kg (15 kV/cm, 50 pulses and 25 kV/cm, 20 pulses) or 32 – 35 kJ/kg (15 kV/cm, 100 pulses and 20 kV/cm, 50 pulses) it can be seen that a higher permeabilization was obtained for similar energy input at a higher field strength. Higher field strength was not applicable within the batch micro-cuvettes.

At a low treatment level of 15 kV/cm and 20 pulses PI staining and a fraction of cells not able to extrude cF can be found already, applying 50 and 100 pulses cellular damage increased. A plate count revealed no microbial inactivation at this low treatment level. After a 20 kV/cm treatment significant permeabilization occurs, when applying 100 pulses the major part of the cells did not show cF-extrusion capability. At 25 kV/cm application of 20 pulses was sufficient to almost completely inhibit the extrusion of cF, whereas an amount of 48 % of cells showed PI-staining, only. Increasing pulse number to 50 a higher level of permeabilization was observed by increase of PI staining. Also a decrease of esterase activity was shown by a decrease of amount of cF-stained cells, but surprisingly an extrusion of cF was noticed after 20 min incubation with glucose for approx. 10 % of cells in this sample. A comparison to a sample without glucose but 20 min delay before measurement revealed that glucose addition was not required to achieve cF-release, indicating that the release observed occurs due to membrane permeabilization and free diffusion instead of active transport.

Microbial inactivation determined by plate count is shown in Figure 4.45. In general the inactivation rate obtained was very low in comparison to inactivation after continuous PEF-treatments (see 4.2.7, 4.2.8 and 4.2.9). An energy input of several hundreds of kJ/kg was required to achieve an inactivation above 1 log-cycle. This is on the one hand based on quasi-isothermal or at least lower temperature conditions in contrast to adiabatic conditions in continuous flow, on the other hand inhomogeneous field distribution in boundary regions might inhibit achieving higher inactivation rates.

Comparing permeabilization in a range of 15 to 25 kV/cm and up to 100 pulses determined by flow cytometry or plate counting shown in Figure 4.45 the superior applicability of a flow cytometer for determination of inactivation mechanisms and to investigate membrane permeabilization with a low detection level is obvious. On the other hand a correlation to microbial inactivation of several log-cycles dependent on processing parameters appears virtually impossibly. These findings also indicate that a permeabilization resulting in a PI uptake or release of cF does not necessarily cause cell death, as after most of the treatment intensities shown in Figure 4.44 no microbial inactivation was found. PI and cF staining of microbes treated in a continuous treatment chamber at treatment intensities resulting in 3 to 6 log-cycles inactivation showed PI staining and no CF staining for almost all cells (see 4.2.8).

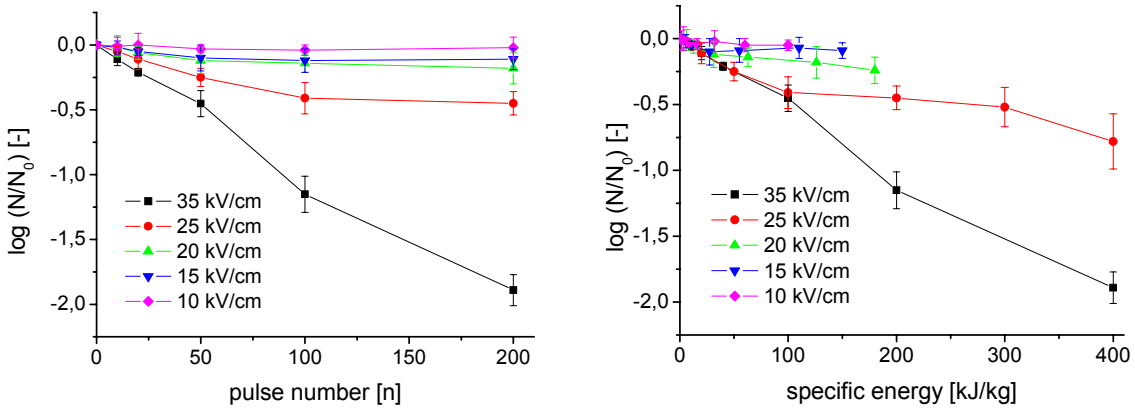



Figure 4.45: Inactivation of *L. rhamnosus* at different field strength dependent on pulse number (left) and energy input (right). Samples of 400 µl have been treated in a batch cuvette at 20°C, temperature increase was monitored using a fibre optic sensor and never exceeded 20°C. Pulse frequency was 2 Hz.

### 4.2.4 Occurrence of resealing and sublethal damage

Reversible permeabilization is widely used in bioengineering to introduce foreign DNA into plant or microbial cells. In contrast to microbial inactivation in foods cell viability needs to be maintained by carefully selecting processing parameters for this application. An overview of electropermeabilization devices available for genetic engineering was published by Puc *et al.* (2004). Gene transfusion requires that a non-lethal permeabilization of microbial cells can be obtained, as also indicated by results presented in the previous section. Literature data concerning the occurrence of sublethal damage is contradictory, after treatment in media with different pH sublethal damage has been described when plating microbial cells on stress growth medium (Yaqub *et al.* 2004; Garcia *et al.* 2005), whereas other research groups identified a PEF application as an all-or nothing effect (Simpson *et al.* 1999; Russel *et al.* 2000; Wuytack *et al.* 2003).

The occurrence of membrane resealing was investigated by flow cytometry and variation of the moment of dye addition. PI was used, which is not permeable through intact cell membranes, staining was applied prior, instantaneously after a PEF-treatment of *E. carotovorum* and after up to 60 min. The micro batch system was used for these experiments to allow a previous addition of PI. In Figure 4.46 results are shown. It is indicated that when PI-addition before treatment was applied a higher percentage of cells showed staining in comparison to a staining after treatment. At 7.5 kV a permeabilization of *Erwinia* was achieved, but showed to be reversible up to a number of 100 pulses. When applying up to 200 pulses a portion of cells showed irreversible membrane permeabilization 10 min after treatment.

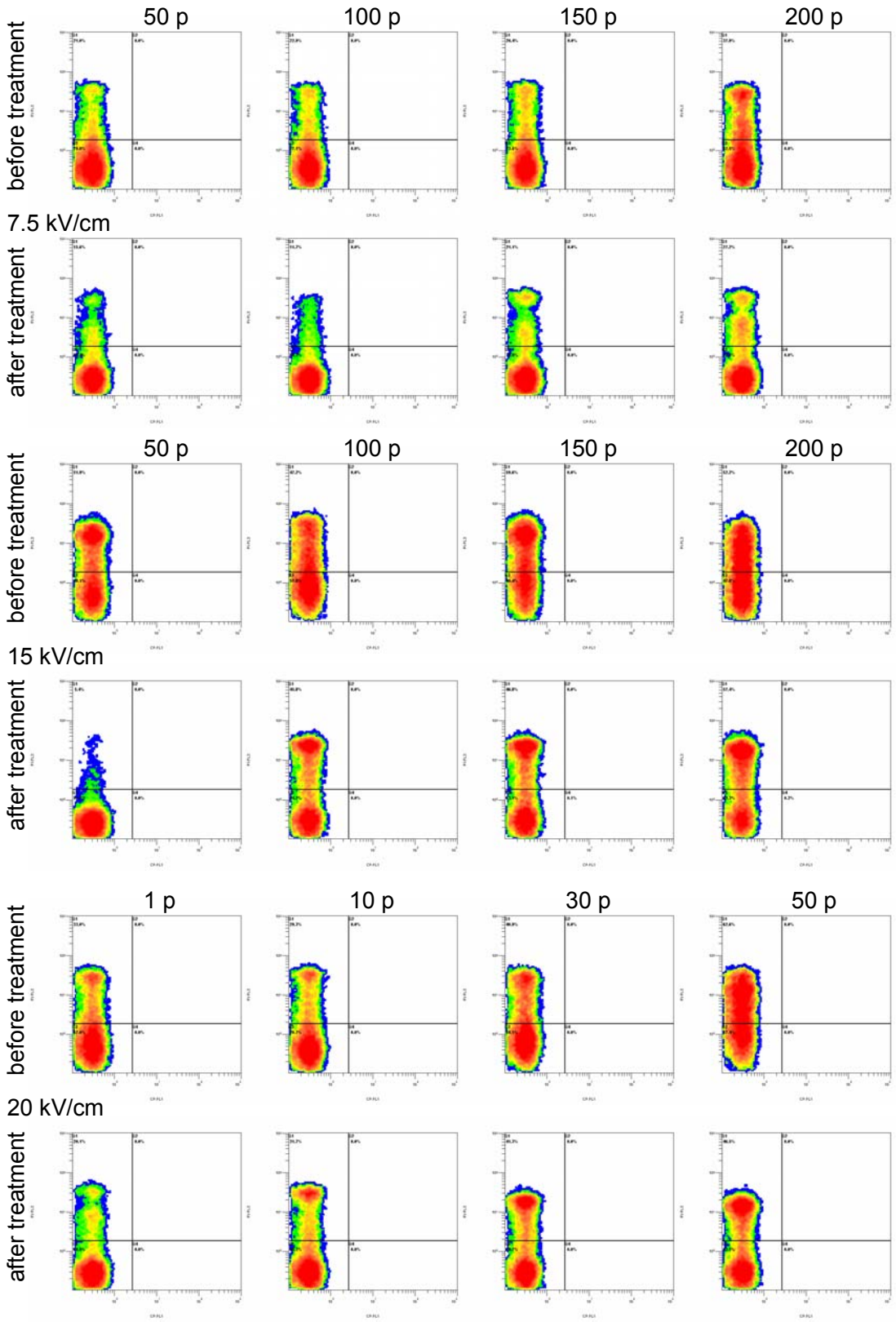



Figure 4.46: Impact of time of PI-staining and a PEF-treatment of *Erwinia carotovorum* at a field strength of 7.5, 15 and 20 kV/cm and different pulse numbers. Staining was applied 5 min prior or 10 min after the PEF-treatment in batch micro-cuvettes.

Increasing electric field strength to 15 kV/cm a permeabilization after 50 pulses and prior PI-addition was observed, which was not found when PI was added 5 min after treatment,

indicating a resealing or membrane reorientation within this timescale. Applying higher pulse numbers the amount of cells recovering is reduced, but a bimodal distribution can be observed in the density plots for a staining after PEF. In contrast a prior stating resulted in a unimodal distribution. If PI is present during a PEF-treatment it can penetrate through reversibly formed pores with short life time as well as irreversible pores, after a treatment cell membranes appear to be intact or permebealized, the intermediate state of medium PI fluorescence was found for prior application was not observed. This effect was also found at a field strength of 20 kV/cm, but lower treatment intensity was required to achieve membrane permeabilization. After a treatment with 1 pulse approx. 33 % of cell showed PI staining, this amount was reduced to 20 % when PI was added after treatment. After application of 30 kV/cm and 10 p also a partial membrane resealing was found, the occurrence of a bimodal distribution when PI was applied after a PEF-treatment is shown in Figure 4.47, exemplarily.

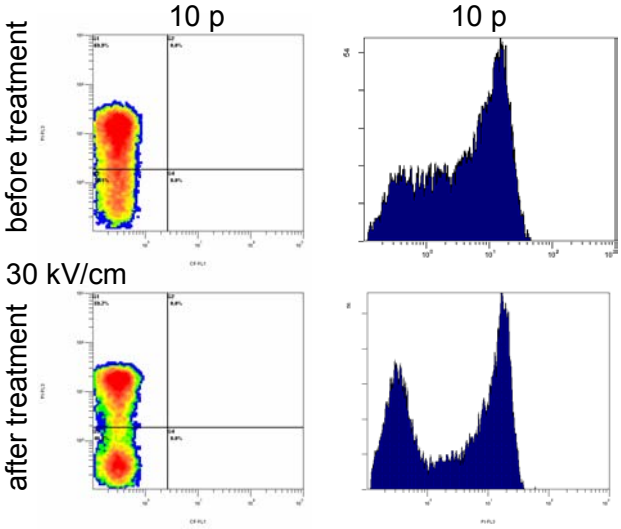



Figure 4.47: Density plot (left) and distribution of PI-intensity (right) for *E. carotovorum* after a PEF-treatment at 30 kV/cm and 10 pulses. PI staining was applied 5 min before or 5 min after treatment.

The impact of time of PI-addition to the sample prior or up to 30 min after treatment is shown in Figure 4.48. A difference between dye addition prior and 1 min after treatment can be seen; a prior addition resulted in a higher amount of permebealized cells in comparison to an addition after treatment, indicating a membrane resealing. Increasing the time of addition of up to 60 min an increasing permeability of cells after a PEF-treatment was observed, after 5 min a typical bimodal distribution was found, 15 to 60 min after the treatment membrane integrity of *Erwinia carotovorum* appeared to be almost totally lost.

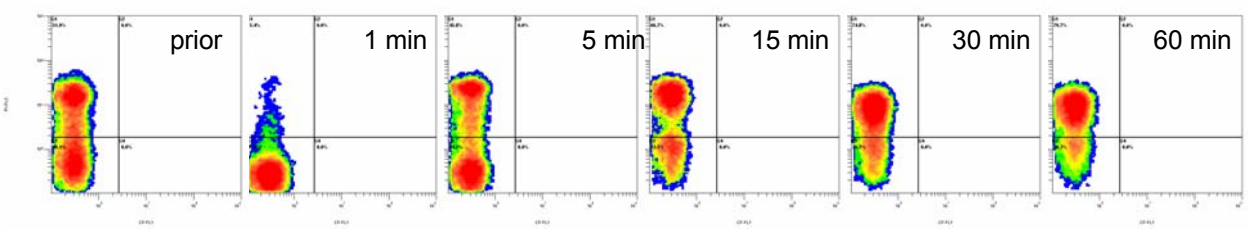



Figure 4.48: Impact of time of PI addition prior or after a PEF-treatment of *Erwinia carotovorum*. PEF-treatment in micro cuvettes, 15 kV/cm, 50 pulses.

Occurrence of sublethal damage as reported for *Lactobacillus* by Garcia *et al.* (2005), which was expected after the results presented above was investigated by plating treated samples on stress growth medium. Batch treatments of *Erwinia carotovorum* and a batch and continuous treatment *of L. rhamnosus* were performed, using the micro pulse modulator TUB 1 and batch cuvettes or the rectangular pulse modulator TUB 8 and a 6 mm colinear treatment chamber. For *Erwinia* a field strength of 9, 19 and 25 kV/cm was used, plating was performed on Standard Nutrient-I-Agar and on stress medium with addition of 3 % salt (Figure 4.49). A treatment at 9 kV/cm did not show significant inactivation but a slight increase of growth rates. Applying higher electric field strength an inactivation of up to 2 log-cycles was found. An inhibited growth on stress medium was found applying 9 and 19 kV/cm, but at 19 kV/cm a one log difference was found, corresponding to approx. 9 % of sublethally damaged cells. In comparison to results obtained by flow cytometry and in literature a smaller portion than expected showed a growth inhibition in presence of NaCl. Garcia *et al*. (2005) reported a portion of up to 4 log-cycles of sublethally damaged cells for *Listeria, Salmonella* and *E. coli*, an extent which was not found in our studies. The concentration of 3 % was determined as maximum concentration without an impact on growth rate in preliminary experiments.

For *Lactobacillus* (Figure 4.50) no sublethal damage was found in batch experiments (data not shown), but after continuous application of pulses at 15 kV/cm and a treatment temperature of 30 °C an inhibited growth on stress medium (3 % NaCl) was observed. Approx. one log difference was found. At higher electric field strength or initial treatment temperature no difference between standard and stress growth medium was found.

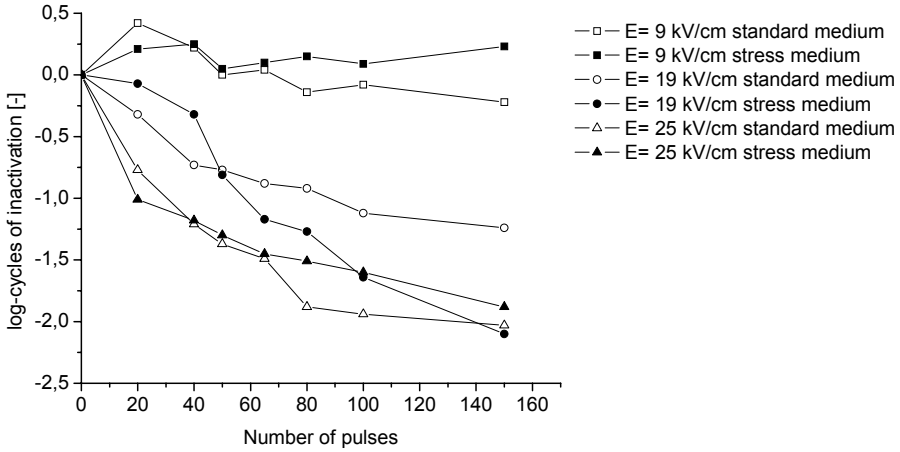



Figure 4.49: Impact of a PEF-treatment on *Erwinia carotovorum* in Ringer solution when plating on standard nutrient and stress media with addition of 3 % NaCl.

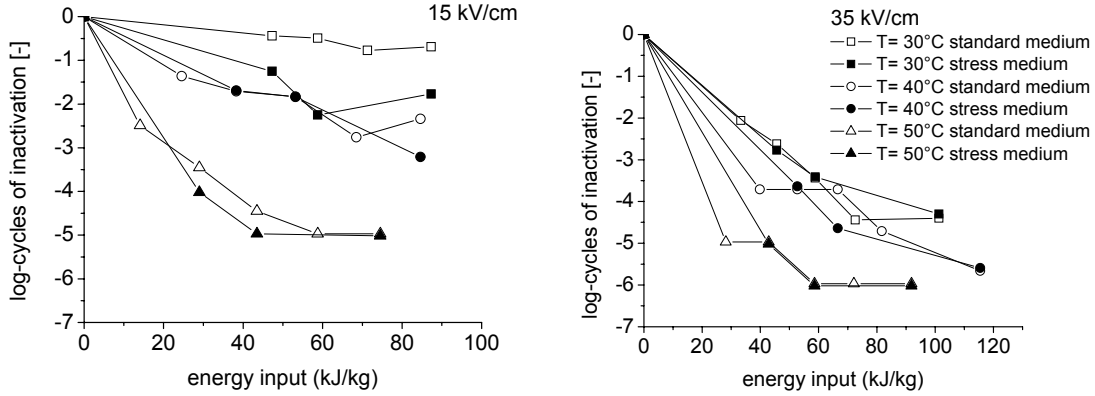



Figure 4.50: Plate counts of *Lactobacillus rhamnosus* in apple juice after a PEF-treatment at 15 (left) and 35 kV/cm and different initial treatment temperatures. Flow rate: 5 l/h, rectangular pulses, plating on MRS-agar and MRS-agar with 3 % NaCl-addition.

## 4.2.5 Impact of cell size and orientation

Equation 1 and Equation 2 have been used to theoretically predict the external electrical field $E_{crit}$ required to induce a transmembrane potential $\Delta\varphi_M$ = 1 V which in many publications is regarded to be the precondition for irreversible membrane breakdown and cell death in response to single pulse treatment. Exemplary, three microorganisms have been chosen which are considerably different in shape and in size, characteristical dimensions have been taken from Bergey (1986). The three semi-axes ($A_1$, $A_2$ and $A_3$) define the geometry of the equivalent ellipsoidal bodies used to calculate the shape factor f(A). $A_F$ denotes the length of the semi-axis in direction of the external field E. In Figure 4.51, top right, this is shown schematically for two-dimensional situations. The diagram on the left side in Figure 4.51 shows the fraction of cells in populations of microorganisms randomly distributed in

orientations which did not reach the critical membrane field strength $E_{crit}$ and, hence, will not be electroporated. Above the orientation of cells the natural size variation between different cells of a strain has to be taken into account.

It is evident that larger cells are more susceptible to electrical fields. Yeast cells of *Saccharomyces cerevisiae* are affected already at ca. 2 kV/cm if the longer semi-axis $A_1$ is directed in parallel to the external electrical field E. However, a field strength higher than 2 kV/cm is needed to affect also those cells having less favorable orientations. For yeast it is evident that most of the organisms are hit when the external field applied is in excess of 4 kV/cm.

In contrast, smaller cells like *Listeria innocua* require 15 kV/cm in minimum and theoretically more than 35 kV/cm to bring about extensive microbial inactivation. Most of the other bacteria relevant for food preservation are located between the curves of *Saccharomyces* and *Listeria*. For *E. coli* for example 15 kV/cm are predicted to be sufficient to lethally damage most of the organisms in a bacterial suspension exposed to PEF.

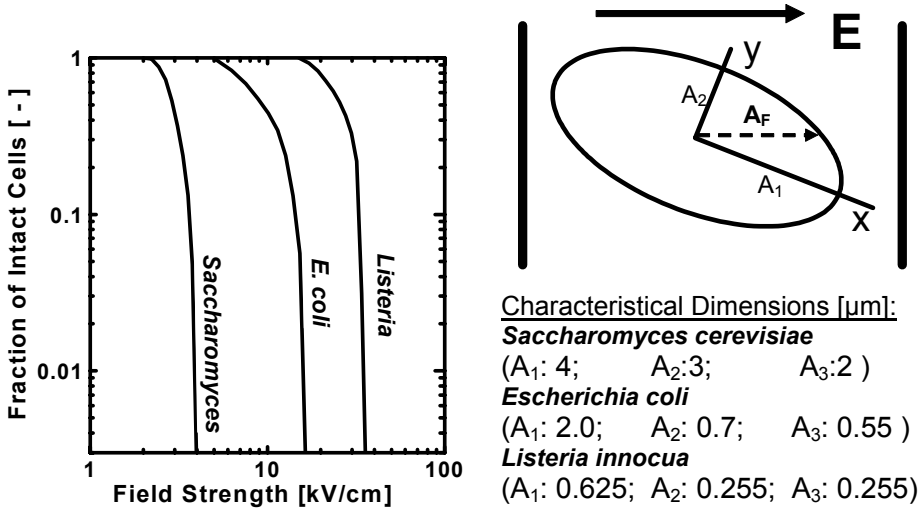



Figure 4.51: Impact of orientation of ellipsoidal microorganisms relative to the electrical field E. At a cell specific threshold level the field strength inside the cell membrane exceeds a threshold level $E_{crit}$. Those cells are electroporated which have their longer semi-axis in parallel to E. Other orientations require field strengths in excess of $E_{crit}$. By Equation 1 and Equation 2 the required external field strength has been calculated for all spacial orientations. Three organisms, different in geometry have been chosen as examples (Characteristical dimensions from Bergey (1986)). The chart on the left shows the fraction of cells which have an orientation which does not cause electroporation in response to the given external field strength.

### 4.2.6 Observation of bubble formation and pH-changes during treatment of microbial cells

During treatment of microbes within the microscopic treatment chamber (B) connected to the micro pulse modulator (TUB1) the formation of gas bubbles at the high voltage electrode (anode) was observed (Figure 4.52). The amount of bubble formation in this static treatment chamber was dependent on pulse number applied. The field strength applied was 10 kV/cm at a peak voltage of 500 V, the energy input per pulse was 0.85 mJ corresponding to a specific energy input of 2.8 kJ/kg. The total energy input applied was 2.8, 5.6, 8.4, 28, 56 and 84 kJ/kg after application of 1, 2, 3, 10, 20 and 30 pulses, respectively. Increasing treatment intensity arcing occurred, as a large portion of the chamber was filled with gas. Due to the small treatment chamber volume and application of a cover slip the bubbles generated showed higher lifetime than within treatment cuvettes or continuous treatment chambers.

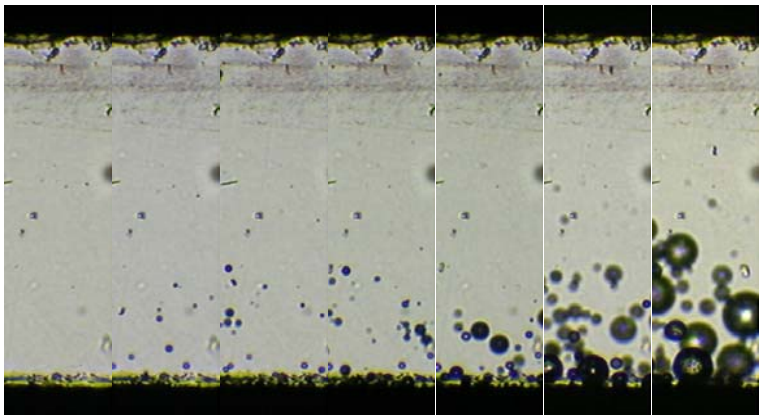


Figure 4.52: Formation of gas bubbles by electrolysis during a PEF-treatment, observed in a microscopic cell at 400 fold enlargement. Bottom electrode: Anode, top electrode: cathode. PEF-treatment at 10 kV/cm before and after application of 1, 2, 3, 10, 20 and 30 pulses (left to right).

During treatment of *E. coli* the bubble formation as well as an alignment and orientation of the cells in the electric field was observed, during PEF application pearl chains of microbes were formed (Figure 4.53). When increasing treatment intensity arcing across large bubbles was observed along with a shock wave, separating the chains. The orientation of cells observed might occur in static treatment chambers only, during continuous operation the media shear flow will prevent alignment by electrophoretic forces.

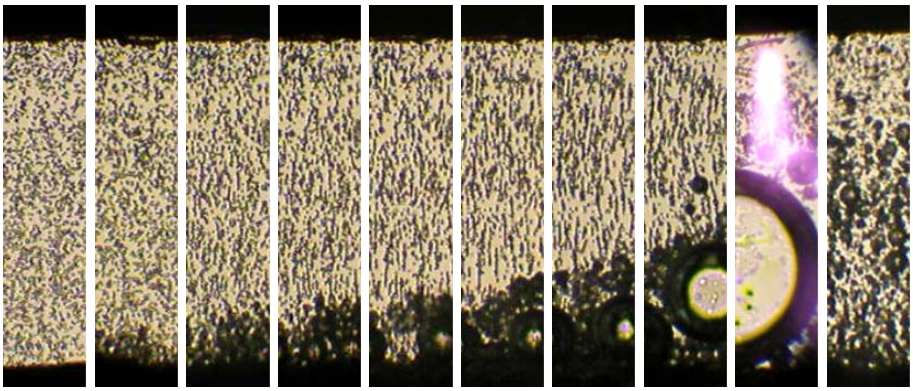


Figure 4.53: Microscopic observation of treatment of *E. coli* at 10 kV/cm and a pulse number of 0, 10, 20, 30, 40. 50, 80 and 100 pulses (left to right), corresponding to an energy input of 0, 28, 56, 84, 112, 140, 224 and 280 kJ/kg. after application of 250 pulses arcing occurred, pearl chains formed were destroyed by the shock wave generated. 400-fold enlargement.

The impact of a PEF-treatment on media pH (Ringer solution adjusted to 2 mS/cm) was investigated by staining with neutral red. A color change at the cathode was observed during a PEF-treatment, indicating a pH increase due to electrochemical reactions. A pH of approx. 9 was obtained. After mixing using a pipette this change showed to be reversible, this is in good agreement with results presented in 4.1.2.2 and 4.2.8, as a pH change during treatment of fruit juices or other products was not observed after a treatment. On the other hand local pH-changes will influence microbial or enzymatic resistance against PEF or thermal treatments and might for example cause partial enzyme inactivation as discussed in 4.2.9.3.

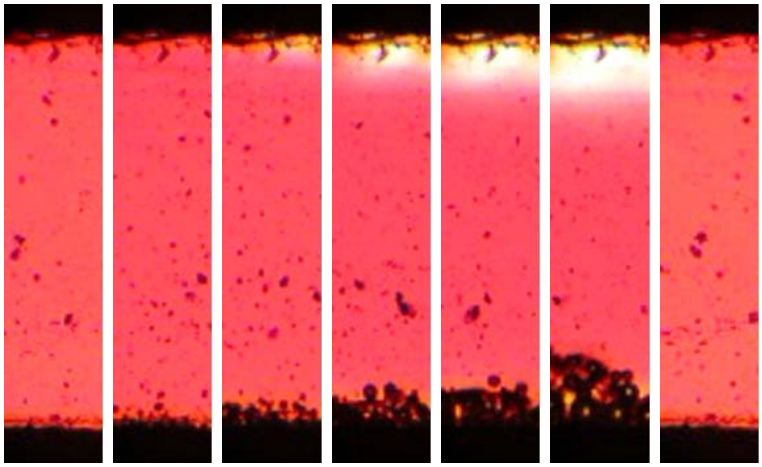


Figure 4.54: PEF-treatment of Ringer solution adjusted to an electrical conductivity of 2 mS/cm at 10 kV/cm, stained with neutral red, a specific energy input 0, 28, 56, 84, 112, 140 kJ/kg and after mixing with a pipette. Top electrode: cathode.

### 4.2.7 Inactivation of different microbial strains in model solutions

In Figure 4.55 the inactivation of four microbial strains is plotted in dependent on specific energy input, exemplarily, emphasizing the differences in PEF resistance between different microorganisms. It can be seen that consistent to the mathematic modeling (see Figure 4.51) the smallest organism, *Listeria innocua* has a higher resistivity than organisms with higher cell size like *E. coli* or *Bacillus megaterium*. Above that the cell membrane constitution has an important influence on the stability of the membrane. The tendency that gram positive bacteria are more resistant than gram negative species has frequently been reported (Hülsheger *et al.* 1983; Vega-Mercado *et al.* 1996; Qin *et al.* 1997; Wouters *et al.* 1999), for a detailed evaluation microbial strains with similar cell size and geometry should be compared.

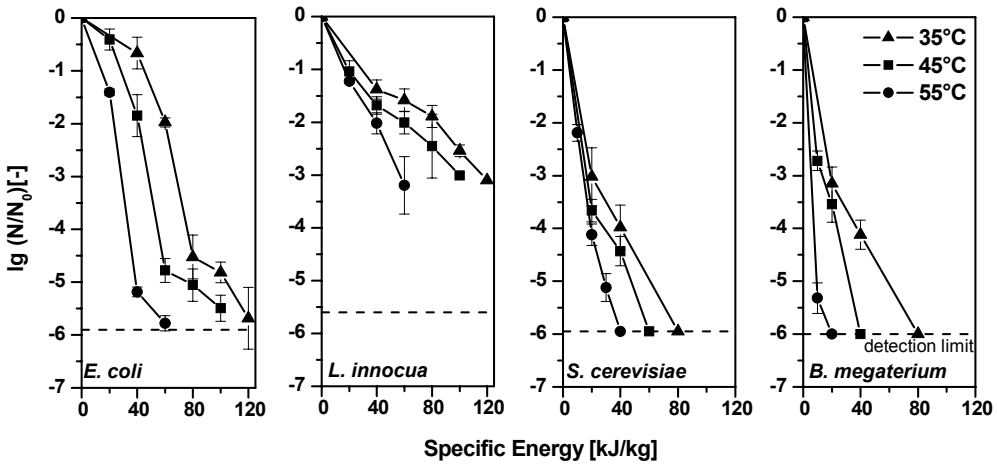



Figure 4.55: Inactivation of *E. coli, Listeria innocua, Saccharomyces cerevisae* and *Bacillus megaterium* in Ringer solution with an electrical conductivity of 1.25 mS/cm after PEF-treatment at a field strength of 20 kV/cm dependent on initial temperature and electric energy input. The flow rate was 5 kg/h.

Whereas *Listeria innocua* showed a close to linear relation between energy input and inactivation rate, for *E. coli* a sigmoid curve has been obtained. This curve shape is contrary to other studies conducted at a maximum field strength of up to 40 kV/cm. During these experiments, using a treatment chamber with co-linear electrode configuration with a gap of 6 mm the maximum electric field strength was limited to 20 kV/cm. An inhomogeneous distribution of the treatment intensity in the co-linear treatment chamber may have caused this effect, which was found at low treatment intensities only, where low impulse frequencies were applied. This may possibly result in under-processing of volume elements with a short residence time in the treatment zone. For inactivation of *Bacillus megaterium* and *Saccharomyces cerevisiae* a very low specific energy input in the range of 10 and 30 kJ/kg is required for a 5 log-cycle reduction at 55 °C initial treatment temperature. Further increase of

energy input led, due to synergetic effects of PEF and heat, to an inactivation below detection limit for these PEF-sensitive organisms. Temperatures higher than ambient and repetitive pulsing may have led to a reduction of the required transmembrane potential below 1 V, resulting in a higher inactivation of *Listeria innocua* than predicted in Figure 4.51.

## 4.2.8 Microbial inactivation in fruit juice

### 4.2.8.1 Impact of treatment temperature

The applicability of a PEF-treatment for preservation of fruit juices was investigated, using TUB lab scale exponential decay pulse modulators at 16 and 24 kV peak voltage and an average power of 800 J/s in combination with lab scale parallel plate and colinear treatment chambers C and D. The effect of the initial treatment temperature from 35 to 70°C on inactivation of *E. coli* cells after a PEF-treatment in apple juice is shown in Figure 4.56. A significant, close to linear relation of the reduction in survivor count to specific energy input was observed; a higher energy input resulted in higher inactivation rates. Increasing initial treatment temperature led to a further improvement of treatment efficiency, i.e. a reduction of 3 log-cycles can be obtained with a specific energy input of 60 kJ/kg at 35°C, at 65°C less than 5 kJ/kg electrical energy input are needed, as mainly thermal effects cause microbial inactivation. This linear behavior was found for apple juice, whereas for other treatment media such as milk also non-linear kinetics have been observed for *E. coli*. In addition to sugar content one aspect influencing susceptibility against a PEF-application will be the medium pH, which was in a range of 3.4 to 3.7 for the apple juices used.

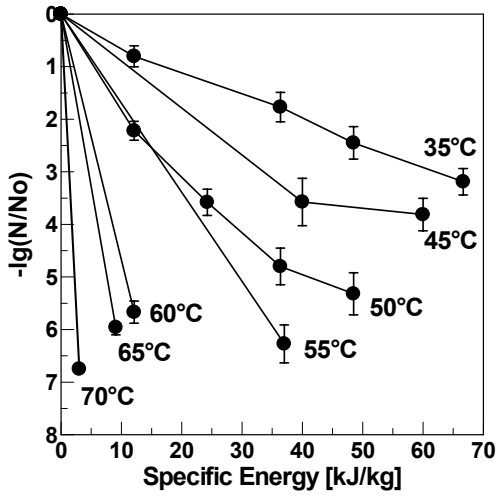



Figure 4.56: Inactivation of *E. coli* in apple juice in relation to specific energy input at different treatment temperatures. Electric field strength: 36 kV/cm, flow rate 5 kg/h. Results are means based on data from two experiments, standard deviations are shown by error bars.

Temperature has a significant influence on membrane properties, as a temperature related phase transition of the phospholipids from gel to liquid-crystalline structure occurs, which affects the stability of the cell membrane (Stanley 1991). Jayaram *et al.* (1993) proposed that this phase transition and the associated reduction of bilayer thickness lead to a permeabilization at lower field strength when using higher temperature. Therefore, at a higher temperature a given log-reduction can be achieved with less specific energy input resulting in a strong decrease of energy required for the preservation of liquid food material with pulsed electric fields. From a processing point of view this behavior may be exploited by splitting the total required energy input into (recoverable) thermal energy which makes the microbes more susceptible to PEF and electrical pulse energy which brings about the electroporation.

#### 4.2.8.2 Comparison of product thermal load for thermal, PEF and combined treatment

A comparison between a sole heat and a combined PEF-treatment of *E. coli* in apple juice in the lab-scale PEF system at different temperatures and energy levels in relation to the maximum temperature after treatment is shown in Figure 4.57. When using low initial temperatures high specific energy input is necessary to achieve sufficient inactivation rates. Since the electric energy is dissipated into the liquid media it leads to a temperature increase during the treatment. To obtain a 5 log-cycle inactivation at a temperature of 45°C an energy input of 100 kJ/kg is required, resulting in a temperature increase up to 71°C. Using higher initial treatment temperatures, for example 65°C, due to synergetic effects of PEF and temperature an inactivation of 6 log-cycles can be achieved with an energy input of less than 10 kJ/kg and a temperature increase of approximately 2.5°C. Therefore a lower maximum temperature of only 67.5°C is reached. The high energy input of a PEF-treatment at low temperatures results in high costs of operation. By using a combination of heat and PEF-treatment the energy consumption and the maximum temperature can be reduced. Compared to sole heat treatment a given inactivation can be obtained at lower temperatures, resulting in a lower thermal load.

In Figure 4.57, right, the temperature-time-profile of a suggested industrial continuous PEF-treatment of apple juice is compared to a commonly used high temperature-short time treatment at 85°C, 30 s. The PEF system is similar to the lab-scale system, consisting of a pump, treatment chamber, but the electric energy dissipated into the treated media is recovered in heat exchanger to preheat the untreated. The initial treatment temperature is 55°C; a specific energy input of 40 kJ/kg is applied. For simplification the temperature changes in the heat exchanger were assumed to be linear. During this treatment, resulting in

a 7.2 log-cycle inactivation, a maximum temperature of 66°C occurs. Due to short residence times at high temperatures and fast cooling after treatment the thermal load of the media is very low. By calculating the C-value and the PU number the thermal load can be estimated. For this combined PEF-treatment a C-value of $6.5 \cdot 10^{-3}$ and a PU value of $2.23 \cdot 10^{-3}$ are obtained, whereas for the HTST-treatment these are $6 \cdot 10^{-2}$ and 0.45, respectively. Combining PEF-treatment with heat treatment an inactivation of vegetative cells can be achieved with a very low thermal load. This should result in a improved preservation of quality and fresh-like character of the juice.

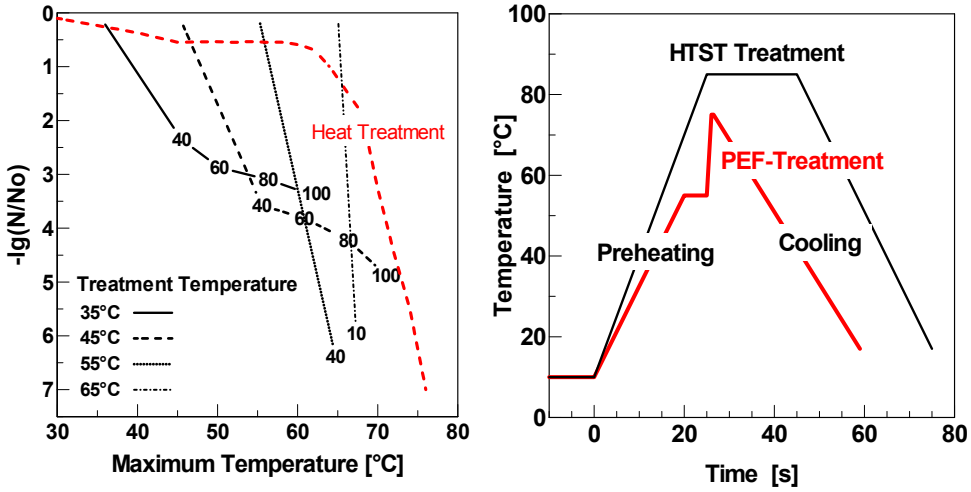



Figure 4.57: Left: Comparison of inactivation of *E. coli* in apple juice after combined PEF-treatment at 34 kV/cm with four different treatment temperatures with sole heat treatment in a lab-scale system in relation to achieved maximum temperature. The numbers at the curves represent the specific energy input in kJ/kg. The Flow rate was 3 kg/h, the total residence time 57 s, the red line shows the inactivation after heat treatment. Right: Temperature-time-profile of a suggested PEF-treatment of apple juice with an initial treatment temperature of 55°C, and a specific energy input of 40 kJ/kg compared to a HTST treatment 85°C, 30 s

### 4.2.8.3 Modeling of impact of different processing parameters

In order to optimize PEF-treatment the relationship between the main processing parameters field strength and treatment temperature was investigated. Based on the log linear shape of the microbial reduction as a function of total specific energy input w an empirical mathematical model shown in Equation 12 has been derived from a series of three experiments by curve fitting using the Software Table Curve 3D (Systat Software, Richmond, USA). By variation of one of the parameters electric field strength (18 - 42 kV/cm), the initial treatment temperature (35 - 70°C) and the specific energy input (5 - 100 kJ/kg) the functional relationship between the inactivation constant k(E,T) and the electric field strength E and the initial temperature $T_{in}$ was established (Equation 12/

Table 2). The correlation coefficient $R^2$ was equal to 0.975. According to Equation 13 the required specific energy input $w_{specific}$ for a given reduction in log survivor count can be estimated.

Table 2: Empirical model parameters

| Parameter | Value |
|---|---|
| $k_1$ | -4.30049 |
| $k_2$ | 0.00201 |
| $k_3$ | 1.00790 |
| $k_4$ | 0.00309 |
| $k_5$ | $-3.45 \cdot 10^{-5}$ |
| $k_6$ | $-4.89 \cdot 10^{-9}$ |
| $k_7$ | -0.23995 |
| $k_8$ | $4.95 \cdot 10^{-6}$ |

$$k(E, T_{in}) = e^{\frac{k_1 + k_2 \cdot E + k_3 \cdot \ln(T_{in})}{1 + k_4 \cdot E + k_5 \cdot E^2 + k_6 \cdot E^3 + k_7 \cdot \ln(T_{in}) + k_8 \cdot (\ln(T_{in}))^2}}$$

Equation 12

$$\lg \frac{N}{N_0} = -k(E, T_{in}) \cdot w_{specific}$$

Equation 13

In Figure 5 the calculated specific energy input to obtain a given inactivation of 7 log-cycles of *E. coli* in apple juice is shown in relation to temperature (a) and field strength (b) at different levels of field strength and temperature. It can be seen that field strength and temperature have a significant influence on the treatment efficiency. By raising field strength and/or temperature the required energy consumption can be reduced. When using low treatment temperatures a higher field strength produces a drastic reduction in energy consumption, but as also described in previous sections, when exceeding a certain threshold value in a range of 40 kV/cm no further impact of electric field strength is found and treatment efficiency seams to be dependent on energy input only. At higher treatment temperatures this threshold value appears to be reduced due to superposition with thermal effects on cell membrane constitution. The specific energy input necessary to obtain different reductions in log-survivor-counts of *E. coli* is shown in Figure 5 c.

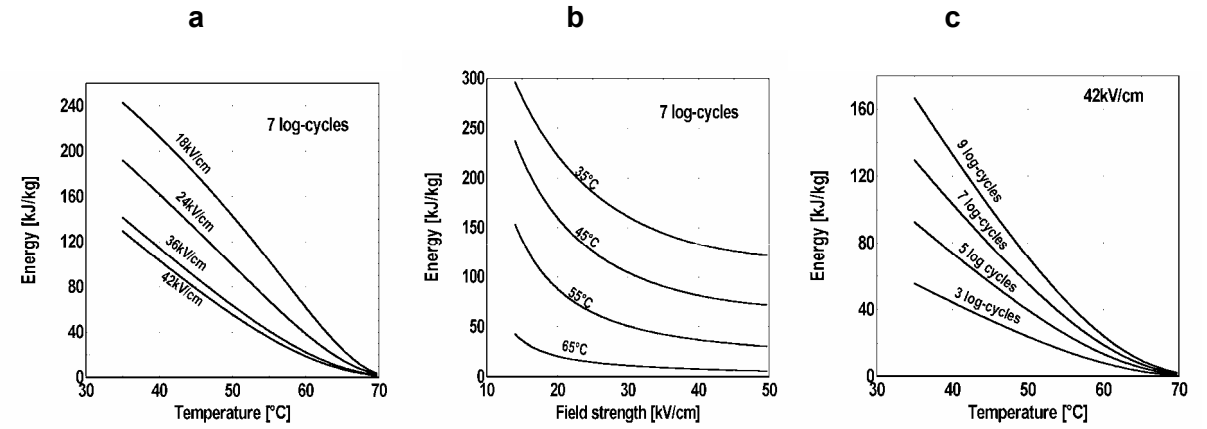



Figure 4.58: Calculated specific energy consumption for a reduction of *E. coli* in apple juice of 7 log-cycles at different electric field strengths and temperatures as function of treatment temperature (a)

and field strength (b). Energy consumption to obtain inactivation 3, 5, 7 and 9 log-cycles at a field strength of 42 kV/cm as function of treatment temperature (c).

#### 4.2.8.4 Comparison of inactivation efficiency of exponential decay and rectangular pulses

The energy efficiency of rectangular and exponential decay pulses has been analyzed by De Haan and Willcock (2002), it was concluded that 38 % efficiency can never be exceeded using an RC circuit for generation of exponential decay pulses, whereas application of rectangular pulses will cause an energy efficiency of up to 100 %. These assumptions where based on a minimum threshold voltage to be exceeded to supply energy at a useful level. For capacitor discharge a charging voltage of 1.6 to 3 times higher than the threshold voltage was suggested, below the threshold value no lethal effect was assumed. To investigate and evaluate this theoretical analysis experiments have been performed, comparing inactivation of *E. coli* in apple juice by exponential decay and rectangular pulses at a field strength of 15, 25 and 35 kV/cm and three treatment temperatures dependent on energy input. The peak field strength of exponential decay pulses and the maximum of square pulses were set to this value by adaptation of charging voltage. The exponential decay pulse modulator (D) and the rectangular pulse modulator (I) were used in connection with the colinear treatment chamber with a diameter of 6 mm. Pulse width was 3 µs for rectangular pulses and approx. 10 to 50 µs for exponential decay pulses, dependent on charging voltage applied, the capacity was 27.2 nF. The energy per pulse was determined using the TDS430 oscilloscope (Tektronix, Beaverton, USA); the desired specific energy input was achieved by variation of pulse repetition.

No clear impact of pulse waveform was found, application of both waveforms resulted in a similar extent of microbial inactivation when similar amount of energy was delivered to the medium. Even at a low field strength level where a low ratio between peak pulse voltage and critical or threshold voltage occurs no impact of pulse waveform was found. For exponential decay pulses a voltage above the threshold voltage is applied for very short time, whereas it was assumed that the energy delivered during the pulse decay is dissipated without inactivation effect (De Haan and Willcock 2002). In our experiments this effect was not found, if only 38 % efficiency would be obtained for exponential decay pulses an approximately 3 times higher energy requirement should have resulted. Form this point of view the short peak voltage exposure time appears to be sufficient for pore induction, increase of pore size and pore stabilization might also occur during current flow at lower voltage level. The charging time required to achieve polarization of cell membranes has been investigated for a variety of microbes in different media (Heinz *et al.* 2002), to achieve a sufficient transmembrane potential a time between 10 and 500 ns has been reported, *E. coli* showed a membrane

charging time of approx. 150 ns, exemplarily. Pore formation can therefore be expected to occur during already during rise time of exponential decay or rectangular pulses. The pulse rise time to 35 kV was 160 ns for exponential decay and 257 ns for rectangular pulses. As generation of rectangular pulses in general is associated with higher complexity of the pulse modulator typology and higher costs of investment these results are of high importance for selection of a reasonable pulse modulator design.

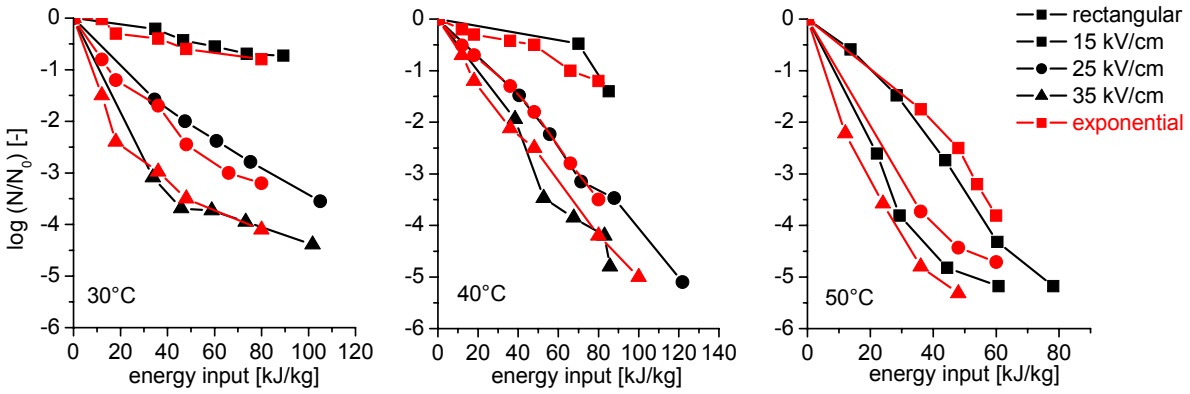



Figure 4.59: Comparison of inactivation of *E. coli* in apple juice after application of exponential decay (red curves) and rectangular pulses at different electric field strength and temperature levels. Pulse width: 3 µs for rectangular, 10 to 50 µs for exponential decay, dependent on charging voltage. Repetition rate was adapted to achieve selected specific energy input.

#### 4.2.8.5 Enthalpy balance and energetic optimization

An enthalpy diagram of the suggested PEF process for apple juice using heat recovery is shown in Figure 4.60. A specific energy input of 40 kJ/kg and an initial treatment temperature of 55°C are used. To preheat the juice to a temperature $T_{in,2}$ of 55°C the enthalpy of the treated product is utilized in a heat exchanger, therefore the product is cooled to a temperature of 17°C, at which no thermal damage occurs. The heat loss in the heat exchanger was estimated for 5%.

After a start-up phase the pasteurization process can be operated by the input of electrical energy only. The input of 40 kJ/kg is sufficient to cover the heat loss in the plant; no additional energy input is needed for heating and cooling. A PEF-treatment at lower treatment temperature does not provide a possibility to recover the dissipated electric energy, since no juice has to be preheated. Higher necessary input of electric energy to obtain sufficient inactivation and the need to cool the treated product lead to unacceptable costs of operation for industrial applications.

Optimum process parameters can be identified when using a field strength above 40 kV/cm and a temperature at which the energy consumption for the pulsed electric field is sufficient

to cover the heat loss in the plant. The dissipated electrical energy, resulting in a temperature increase of the product can be recovered and no additional energy is required to cool the product after treatment. As shown in Table 3 due to very low residence times, lower treatment temperature and cooling after treatment the thermal load of the product is very low even when using higher treatment temperatures in the range of 55°C. The Cook-Value is reduced by one, the PU value by several orders of magnitude compared to the sole heat treatment. It can be seen that if a low treatment temperature with a high energy input is used the temperature after treatment is higher than 30°C and the dissipated energy has to be removed from the product.

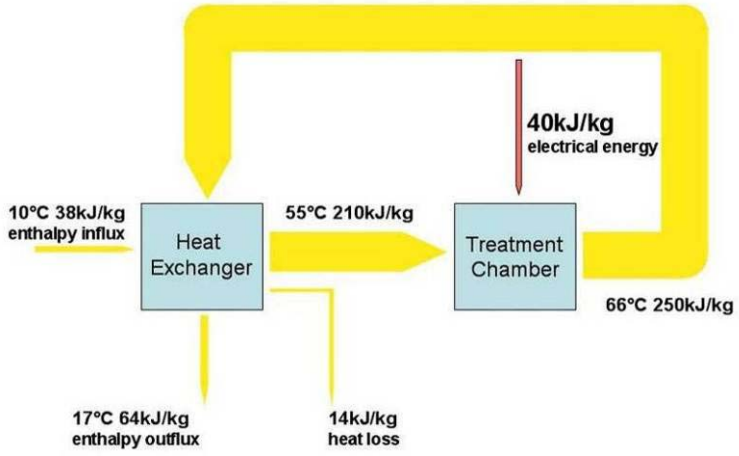



Figure 4.60: Enthalpy diagram of a suggested PEF-treatment system for apple juice with an initial temperature of 55°C and a specific energy input of 40 kJ/kg. As specific heat capacity 3.8 kJ/(kg·K) was used, the heat loss in the heat exchanger was estimated for 5%.

Table 3: Maximum temperature, outlet temperature, heat loss, cook value (C-value) and pasteurization units (PU) during PEF-treatment of apple juice at different combinations of the process parameters treatment temperature and specific energy input.

| $T_{in}$ (°C) | $w_{specific}$ (kJ/kg) | $T_{max}$ (°C) | $_{out}$ (°C) | $h_{loss}$ (kJ/kg) | C-value | PU |
|---|---|---|---|---|---|---|
| 35 | 40 | 45.5 | 17.8 | 10.6 | $1.57 \cdot 10^{-3}$ | $2.82 \cdot 10^{-5}$ |
| 35 | 60 | 50.8 | 22.8 | 11.6 | $2.00 \cdot 10^{-3}$ | $7.01 \cdot 10^{-5}$ |
| 35 | 80 | 56.1 | 27.8 | 12.6 | $2.64 \cdot 10^{-3}$ | $2.15 \cdot 10^{-4}$ |
| 35 | 100 | 61.3 | 32.8 | 13.6 | $3.56 \cdot 10^{-3}$ | $6.54 \cdot 10^{-4}$ |
| | | | | | | |
| 45 | 40 | 55.5 | 17.5 | 12.5 | $3.09 \cdot 10^{-3}$ | $2.33 \cdot 10^{-4}$ |
| 45 | 60 | 60.8 | 22.3 | 13.5 | $4.01 \cdot 10^{-3}$ | $6.38 \cdot 10^{-4}$ |
| 45 | 80 | 66.1 | 27.3 | 14.5 | $5.36 \cdot 10^{-3}$ | $1.99 \cdot 10^{-3}$ |
| 45 | 100 | 71.3 | 32.3 | 15.5 | $7.36 \cdot 10^{-3}$ | $6.06 \cdot 10^{-3}$ |
| | | | | | | |
| 55 | 20 | 60.3 | 11.8 | 13.4 | $5.12 \cdot 10^{-3}$ | $1.00 \cdot 10^{-3}$ |
| 55 | 40 | 65.5 | 16.8 | 14.4 | $6.42 \cdot 10^{-3}$ | $2.23 \cdot 10^{-3}$ |
| 55 | 60 | 70.8 | 21.8 | 15.4 | $8.40 \cdot 10^{-3}$ | $6.24 \cdot 10^{-3}$ |
| | | | | | | |
| 65 | 20 | 70.3 | 11.3 | 15.3 | $1.10 \cdot 10^{-2}$ | $7.17 \cdot 10^{-3}$ |
| 65 | 30 | 72.9 | 13.8 | 15.8 | $1.20 \cdot 10^{-2}$ | $9.64 \cdot 10^{-3}$ |
| 65 | 40 | 75.5 | 16.3 | 16.3 | $1.40 \cdot 10^{-2}$ | $1.40 \cdot 10^{-2}$ |

In experiments performing a PEF-treatment of apple juice under sterile conditions at a temperature of 55°C and an energy input of 70 kJ/kg a total inactivation from an initial count of $1.7 \cdot 10^6$ cfu/ml *S. cerevisae* and $2.1 \cdot 10^7$ cfu/ml *E. coli* was obtained.
In other experiments using additional microbial strains such as *L. rhamnosus*, *Rh. rubra, L. innocua, S. cerevisae* and *A. niger* in apple, orange, white and red grape juice it was shown that application of PEF at elevated treatment temperature and lower energy input provides a possibility for inactivation of a wide spectrum of microorganisms (data not shown).

#### 4.2.8.6 Impact on juice quality

In this study the focus was put on energetic optimization, since for most experiments juices made of fruit concentrate were used which already obtained a very high thermal load, higher fresh-like character and improvements in sensory quality have not been expected. In 2005 a first commercial application of PEF for fruit juice preservation has been achieved in a production scale of 200 l/h (Clark 2006). Products are distributed in a chilled supply chain by a fruit juice cooperative (Genesis, Eugene, USA) and promoted to be made of organic food and having a fresh like flavor. In addition to microbial inactivation residual enzyme activity will determine the shelf life of fruit juices if no chilled distribution is used. The effect of PEF-treatment on enzymes is discussed contrary in literature and seams to be dependent on specific enzyme as well as processing conditions applied. Own work discussed in 4.2.9.3 has shown a very limited impact of PEF on milk lactoperoxidase, but an inactivation was found when a combined treatment of mild heat and PEF was applied. It can be assumed that a treatment at elevated treatment temperature might also allow an at least partly inactivation of enzymes in fruit juices, as reported by Schuten *et al.* (2004) for orange juice. To rule out the possibility that electrolytic effects have a detrimental effect on the product taste orange juices treated with a combined PEF-heat process and a conventional heat treatment were compared. No significant differences between the conventional and PEF-treated juices have been found with in a triangle test with a panel of 20 persons (data not shown). The impact of a PEF-treatment on juice quality has been investigated by different research groups (Yeom *et al.* 2000; Zárate-Rodriguez and Ortega-Rivas 2000; Ayhan *et al.* 2002; Evrendilek *et al.* 2004; Cserhalmi *et al.* 2006), no apparent changes in physical or chemical properties caused by electric field exposure have been found in apple juice and cider, cranberry and orange juice as well as raw milk or green pea soup. In comparison to heat treated samples a higher amount of retained vitamin C concentration was found in apple juice (Barbosa-Cánovas *et al.* 1998) and orange juice (Yeom *et al.* 2000) along with a loss of 5 to 9 % of flavor compounds in comparison to up to 25 % loss after a thermal treatment and a lower browning index during

storage at 4°C. In a study investigating PEF-impact on four citrus juice varieties no real difference was found for °Brix, pH, conductivity, viscosity as well as nonenzymatic browning index and formation of hydroxymethylfurfurol after a treatment at 28 kV/cm and 50 pulses (Cserhalmi *et al.* 2006). The energy input is not given, but can be estimated to be in a range of 68 kJ/kg for grapefruit, 66 kJ/kg for lemon and orange juice and 83 kJ/kg for tangerine juice for the pulse parameters mentioned. Processing intensity was in a similar range than required for microbial inactivation, so these results underline the minor impact of a PEF application on juice quality in contrast to a conventional heat treatment.

## 4.2.9 Preservation of milk by PEF application

### 4.2.9.1 Microbial inactivation

Experiments have been performed to evaluate the impact of product properties and to investigate the applicability of a PEF-treatment for milk preservation. The impact of an electric energy input in a range of 30 to 206 kJ/kg was investigated at a field strength of 21.6 and 32.5 kV/cm using a colinear lab scale treatment chamber (D) and the exponential decay pulse modulator (D), modified by replacement of the solid state switch with a spark gap at 16.6 or at 25 kV breakdown voltage. Pulse repetition rate was varied between 10 and 70 Hz, the energy delivered per pulse was 4.15 J at 21.6 kV/cm field strength. The impact of initial treatment temperature was investigated in a range of 10 to 40°C. The inactivation of *E. coli Ps. flourescens* and *Lb. rhamnosus* in milk is shown in Figure 4.61.

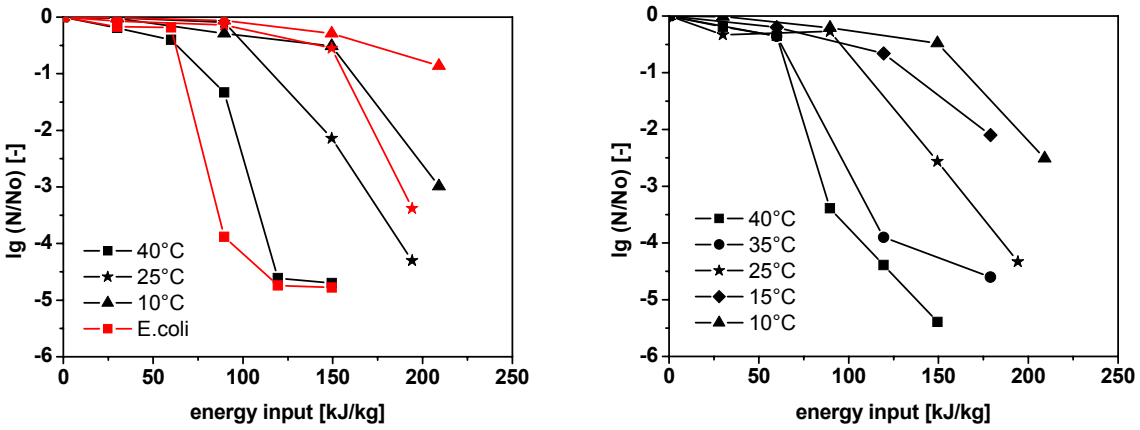



Figure 4.61: Left: Inactivation of *Ps. fluorescens* and *E. coli* (red curves) in milk dependent on specific energy input and treatment temperature. Right: Inactivation of *Lb. rhamnosus* in milk at different initial treatment temperatures. Field strength: 21.6 kV/cm, flow rate 5 l/h.

The synergetic effect of mild heat appeared to be very pronounced for these three microbial strains, at a temperature of 10°C an energy input above 100 kJ/kg is required to achieve a significant inactivation. At a temperature of 25°C treatment efficacy was improved, higher inactivation rates were found at an initial temperature of 40°C. The tailing effect observed for *E. coli* and *Ps. flourescens* at a temperature of 40°C is presumably based on the experimental design, as for each temperature setting the experiment was started at the lowest energy input. Between parameter variation and sampling a delay of 3 min was hold, but a contamination of subsequent cooling coils occurred during low intensity settings was not completely washed out during this time. The initial count was $7.3 \cdot 10^6$ cfu/ml. Similar than for fruit juice preservation a combined application of PEF and mild heat is suggested, as the energy required can then be split into a recoverable, thermal part and an electrical energy input. Increasing electric field strength from 21.6 to 32.5 kV/cm enhanced microbial inactivation at low energy input levels, as exemplarily shown for *Lb. rhamnosus* in Figure 4.62. During performance of this experiments a further increase of electric field strength was not possible, as due to malfunction of a solid state semiconductor switch spark gaps with a given breakdown voltage needed to be used.

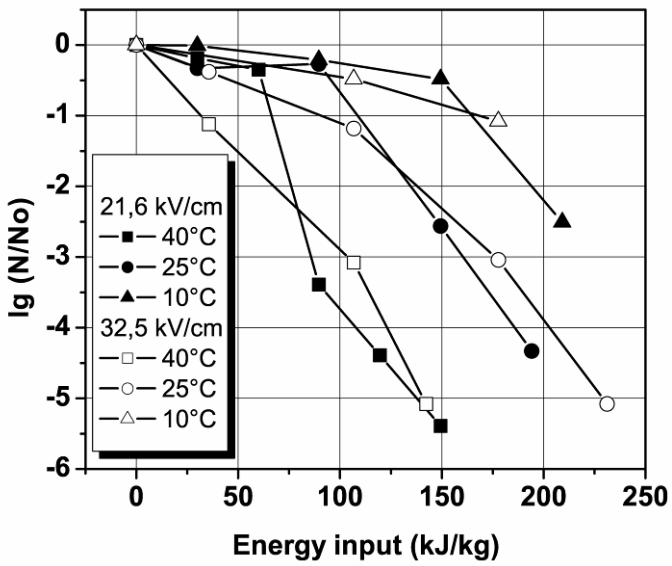



Figure 4.62: Impact of increase of electric field strength from 21.6 to 32.5 kV/cm on inactivation of *Lb. rhamnosus* in milk at different treatment temperatures.

In cooperation with the Federal Institute of Risk Assessment (BfR), Berlin, Germany the applicability of a PEF-treatment to achieve an inactivation of pathogenic *Listeria monocytogenes* in full fat and skimmed raw milk was studied. The effect of PEF at 23 kV/cm at treatment temperatures of 35, 45 and 55°C is shown in Figure 4.63. A trend of higher efficiency if a treatment at lower fat content was found at 35°C, but in general no significant impact was found. The results indicate that also this pathogenic strain can be inactivated by

a PEF-treatment of milk and the potential to improve safety of raw milk products. As indicated in the following section only a minor impact on enzymes and presumably proteins is found, this effect could be of interest for improvement of consumer safety during production of raw milk-type cheese products, where *Listeria* is commonly causing problems.

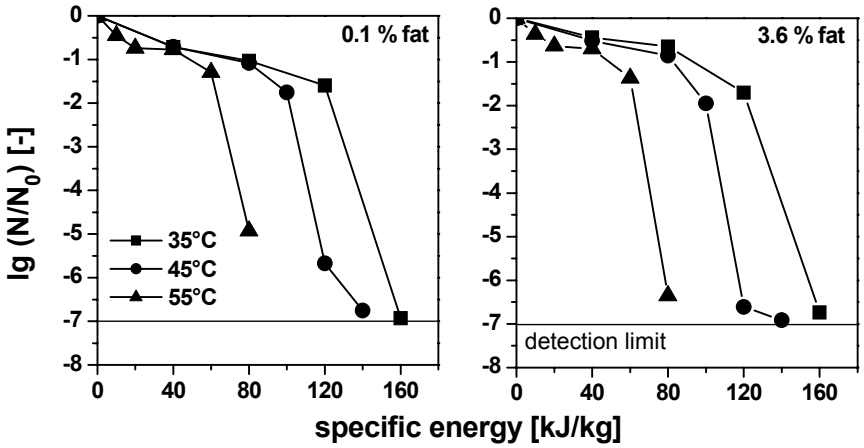



Figure 4.63: Inactivation of *Listeria monocytogenes* in raw milk (3.6 % fat) and skimmed raw milk (0.1 % fat) at different initial temperatures. Field strength: 23 kV/cm, flow rate 5 l/h.

### 4.2.9.2 Impact of fat globules

The impact of milk fat content from 0.3 to 10 % on treatment efficacy was investigated using skimmed milk and addition of cream. When modeling electric field distribution in milk in the surrounding of a fat globule with a diameter of 1.5 µm and a dielectric constant of 2 a concentration of equipotential lines within the globules was observed (Figure 4.64). Microbes in the boundary area of a fat globule might be exposed to an electric field lower than assumed, causing a reduction of membrane potential induced. For three examples (A, B, and C) the impact on transmembrane potential induced has been calculated and is shown in Figure 4.64, right. A membrane thickness of 5 nm and a dielectric constant of 2 were assumed. The induced transmembrane potential corresponds to the voltage drop as indicated for curve A, exemplarily. It was observed that dependent on orientation a different transmembrane potential is induced. For a microbe attached to the fat globule (C) no transmembrane potential was induced at the left pole, the right pole showed a decrease of potential induced. In this case PEF-efficiency is expected to be reduced drastically.

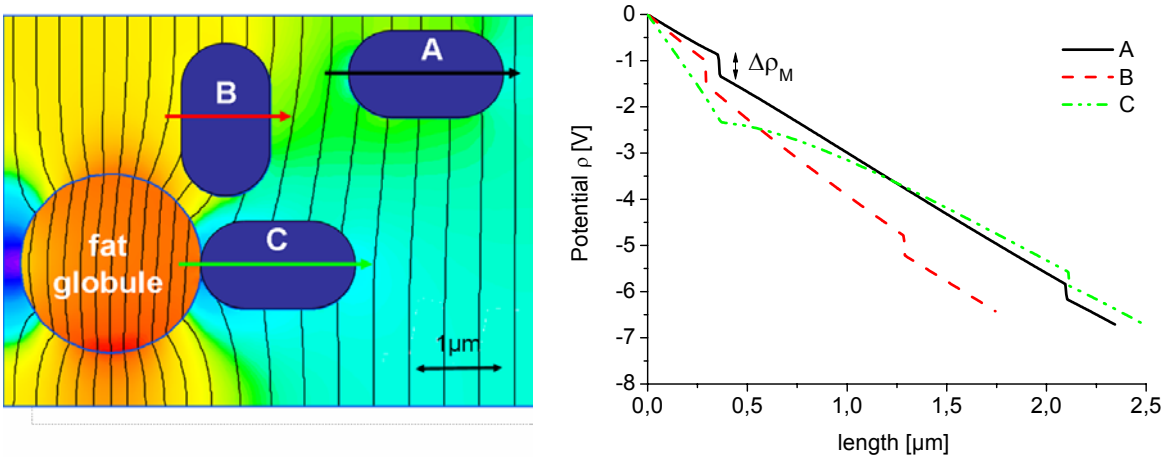



Figure 4.64: Left: Electric field distribution in an aqueous medium containing a fat globule with a dielectric constant of 2 (1.5 µm diameter) and rod-shaped microorganisms (1 x 2 µm). The QuickField FEM code was used, an electric field of 33 kV/cm was applied. Right: Voltage drop across A, B and C; a membrane thickness of 5 nm and a dielectric constant of 2 was assumed, the dielectric constant of the aqueous media was 80. The induced transmembrane potential can be observed at the voltage drop across the membrane, as indicated as $\Delta\rho_M$ for curve A, exemplarily.

The Inactivation of *Lb. rhamnosus* and *Ps. flourescens* is shown in Figure 4.65. No pronounced effect of fat content was found, for *Lb. rhamnosus* a trend towards decrease of inactivation efficacy at higher fat content was assumed but not confirmed. By modeling of electric field distribution in presence of fat globules (section 4.4) or air bubbles (Góngora-Nieto *et al.* 2003) an adverse effect on inactivation results was expected. Increase of fat content was performed by addition of cream, but as an inoculation of the emulsion with microbes was performed it can be assumed that these were located in the aqueous phase mainly. A microbial growth in raw milk at different fat content instead of inoculation or a subsequent homogenization would presumably have shown a higher effect of presence of fat globules.

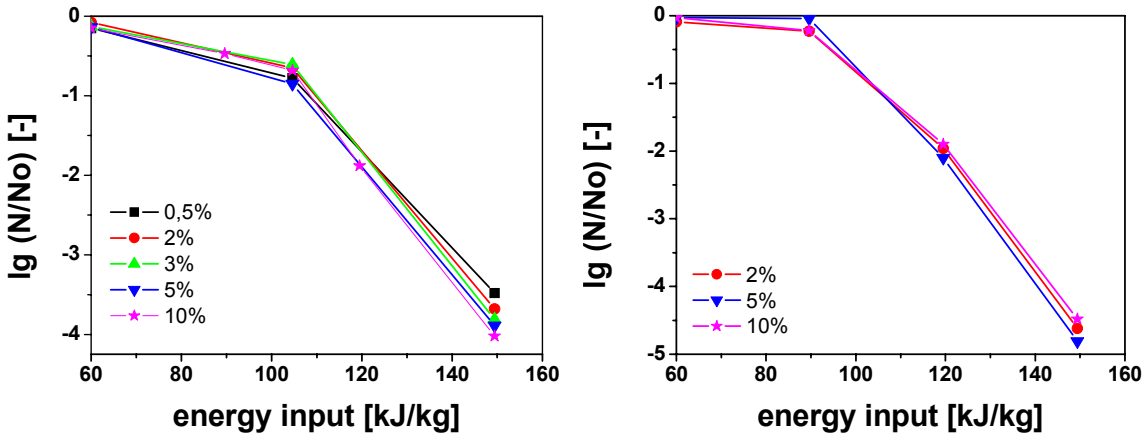



Figure 4.65: Inactivation of *Lb. rhamnosus* (left) and *Ps. flourescens* (right) in milk with different fat content at a field strength of 21.6 kV/cm.

In addition to fat content the influence of initial count number was investigated. No impact of initial count of $6.2 \cdot 10^4$, $1.1 \cdot 10^6$ and $8.9 \cdot 10^6$ for *Lb. rhamnosus* and $1.3 \cdot 10^5$, $2.0 \cdot 10^6$ and $1.8 \cdot 10^7$ for *Ps. fluorescens* on microbial inactivation in milk was found (data not shown).

#### 4.2.9.3 Enzyme inactivation

The inactivation of lactoperoxidase in milk by exposure to pulsed electric fields was investigated and compared to thermal inactivation. Glass capillaries have been used to determine thermal inactivation kinetics at 60, 70, 75, 80 and 85°C and short residence times in a scale of seconds. Capillaries were used to achieve minimum time requirements for heating and cooling of samples. In Figure 4.66 the inactivation results for 60, 70, 80 and 85°C are shown.

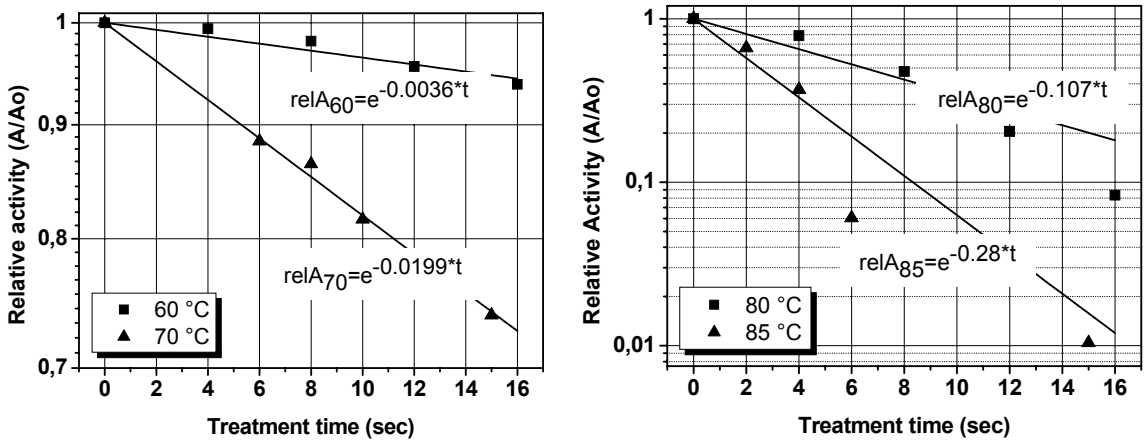



Figure 4.66: Thermal inactivation of lactoperoxidase in raw milk at different temperatures and residence time determined using a glass capillary method.

Linear regression at each temperature level was performed to determine inactivation constant k(T), as shown in Figure 4.67, left. Using the dependency of k on temperature, Equation 14 is obtained, describing relative enzyme activity A dependent on temperature T and treatment time t as shown in Figure 4.67, right.

$$relA = e^{-\left(e^{-16.48+0.18 \cdot T}\right) t}$$

Equation 14

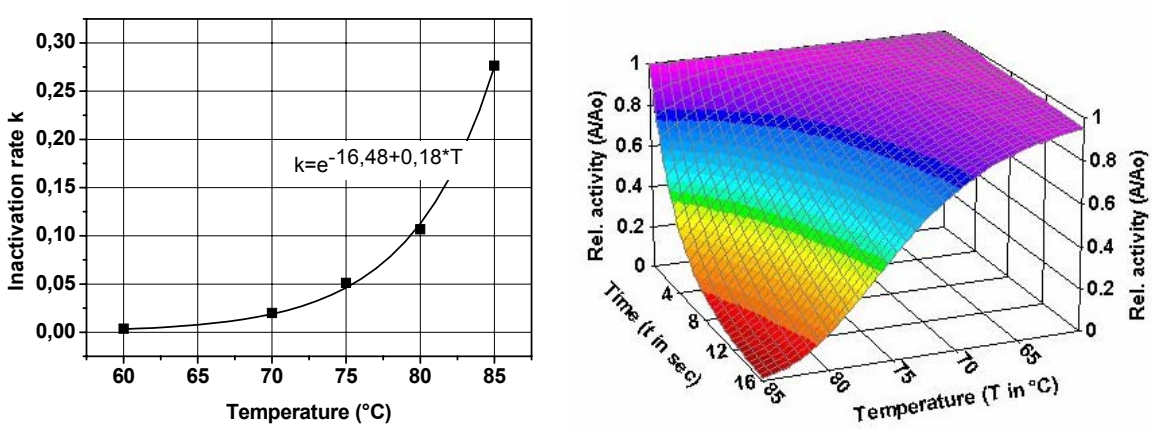



Figure 4.67: Inactivation rate constant k of thermal lactoperoxidase inactivation in milk dependent on temperature (left). Inactivation dependent on temperature-time conditions after Equation 14.

Based on these results the thermal inactivation of lactoperoxidase during a PEF-treatment has been calculated. An experimental determination of thermal impact following the temperature-time profile of a PEF-treatment showed to be not feasible, as the instantaneous and direct temperature increase by electric energy input could not be realized by indirect heat transfer within the lab scale system.

For determination of the temperature-time profile of a treatment with an initial temperature of 25°C the temperature increase during PEF exposure was assumed to occur within the 0.8 s residence time in the treatment chamber, the energy input was 4.1 J per pulse, resulting in a temperature increase of 0.9°C per pulse. Subsequently the product was cooled in a cooling coil submerged in ice-water; temperature decrease was calculated based on a classical heat transfer approach (calculation not shown). The resulting temperature-time profile for a treatment at 25°C initial temperature, an energy input of 149 kJ/kg and subsequent cooling is shown in Figure 4.68 (left).

Enzyme inactivation is dependent on temperature-time conditions applied during the treatment and can be estimated by integration of this temperature-time-profile and coupling with inactivation constant k(T) (Equation 15).

$$relA(T,t) = e^{\int_0^t -\kappa(T)dt}$$

Equation 15

The inactivation of lactoperoxidase during this temperature-time-profile is shown in Figure 4.68, right.

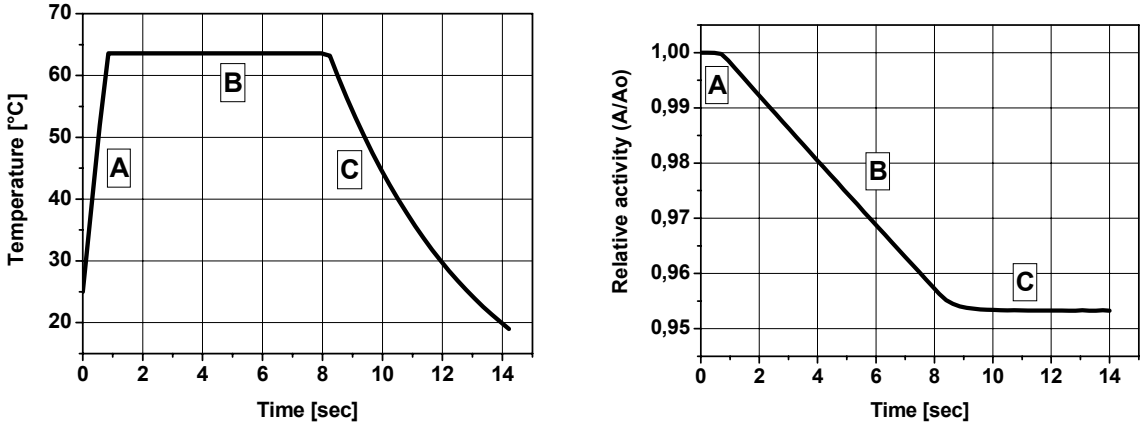



Figure 4.68: Left: Temperature time-profile of a PEF-treatment at 25°C initial temperature and an energy input of 149 kJ/kg. The residence time in the treatment chamber was 0.8 s (A), the product passed a pipe (B) with 7 s residence time to cooling coil submerged in ice water (C). The total residence time was 14 s. Right: Relative activity of lactoperoxidase in milk determined by integration of thermal effect during the temperature-time regime applied without a PEF impact on enzyme activity.

During temperature increase due to electric energy dissipation (A) enzyme inactivation is increasing, during the transfer of the product to the cooling coil (B) a constant temperature and inactivation rate causes a linear decrease of enzyme activity until temperature decrease in the cooling coil (C). It is obvious that mainly the time between energy dissipation and subsequent cooling of the product determines the extent of thermal damage of lactoperoxidase; it is suggested to maintain the tube length in between this sections as short as possible. This finding can be transferred as well to other product constituents degradation based on a first order kinetic. A residual activity of 95.4 % of lactoperoxidase in milk was expected when subjecting milk to the temperature-time-profile used, exemplarily. A MathCad (MathSoft, Cambdridge, USA) tool was programmed to be able to investigate the impact of different profiles on residual enzyme activities in comparison to results obtained after a PEF-treatment.

Lactoperoxidase inactivation after a PEF-treatment at different flow rates is shown in Figure 4.69 (left) dependent on specific energy input and maximum outlet temperature after treatment. A comparison to the modeled, solely thermal effect caused by the temperature-time-profile applied is shown in the chart right. In comparison to the modeled thermal effect an approx 5 to 10 % additional inactivation was found after a PEF-treatment at low treatment intensities, whereas at higher intensities the inactivation effect appeared to be based on thermal effects mainly. These findings are in good agreement with the results published by van Loey *et al.* (2001), who showed that enzyme inactivation in model and real food systems could be explained by thermal effects. Inactivation mechanisms of enzymes after PEF application have been discussed in literature (Castro *et al.* 2001; Bendicho *et al.* 2002;

Bendicho *et al.* 2003; Yang *et al.* 2004; Bendicho *et al.* 2005; Elez-Martínez *et al.* 2005; Elez-Martínez *et al.* 2006), several effects of electric fields on enzymes have been proposed, including conformational changes of protein structure, local thermal and pH effects as well as formation of radicals or other electrochemical reactions. Electroporation is related to cell (or artificial) membrane structures only, but different other impact factors might cause enzyme inactivation. Subjecting ions or charged particles to an electric field electrophoretic effects might cause conformational changes or the release of central ions from complex protein structures. Changes of pH (see 4.2.6) or local differences in temperature increase as well as the release of metallic particles of the electrodes might play an additional role. As a conclusion, for lactoperoxidase it can be stated that only minor inactivation was found after a PEF-treatment at low intensity, whereas at higher intensities a thermal inactivation of this enzyme was found. During storage tests of PEF-treated raw milk it was shown that the antimicrobial activity of the milk lactoperoxidase system was retained after application of pulses of 20.6 kV/cm and an energy input of 149 kJ/kg. A delay in growth of coliform strains, *Lactobacillus* and *Pseudomonas* was found, indicating that no detrimental effect on lactoperoxidase activity was caused by the PEF application (data not shown).

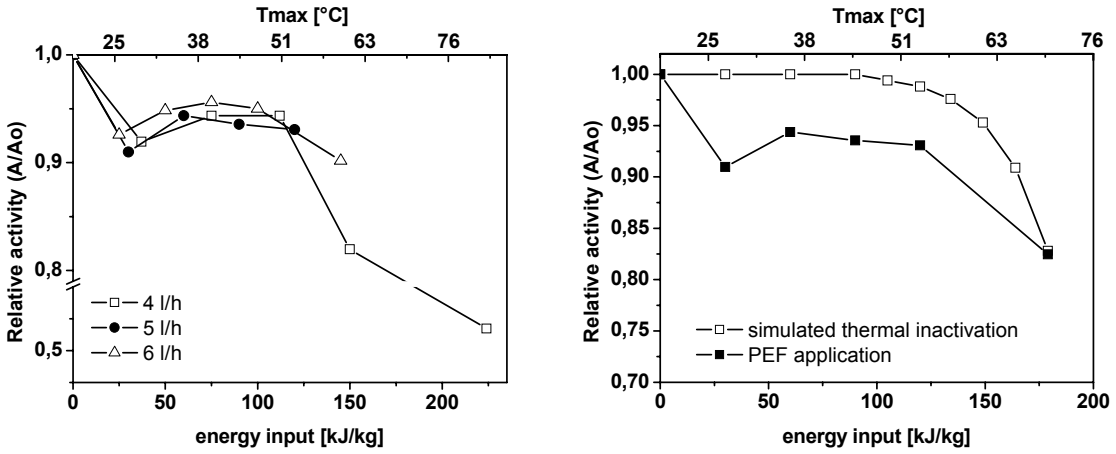



Figure 4.69: Inactivation of lactoperoxidase in milk by PEF application dependent on energy input and flow rate applied. Field strength 20.6 kV/cm, initial temperature 25°C (left). Right: comparison of simulated thermal inactivation effect with inactivation after a PEF-treatment at a flow rate of 5 l/h.

The minor effect of PEF on enzymes could therefore be used as a benefit, as a microbial inactivation could be obtained without a detrimental effect on enzymes and presumably other protein structures. As described previously for apple juice in general only minor impact on physical or chemical properties of liquid media have been found, indicating the techniques potential for liquid media decontamination while maintaining product quality. Membrane structures are directly affected by a PEF-treatment, whereas in contrast to application of thermal energy or high pressure an only limited impact on other food constituents was found. A reduction of maximum temperature during milk preservation and the short residence time

of a PEF-treatment presumably will cause less detrimental effects on milk and whey proteins and could allow the production of raw milk-like cheese products even after microbial decontamination. A combined application of mild heat and PEF for milk preservation could also provide a possibility to operate at lower maximum temperature than during conventional processing, a factor of high interest with regard to servicing times of heat exchangers. Reduction of biofouling and associated efforts for cleaning of heat exchanger plates (Beuf *et al.* 2004) could provide a potential to increase operation times and cost reduction.

### 4.2.10 Production and preservation of microalgae extracts

Different varieties of macro- and microalgae are sources of vitamins, pigments, proteins as well as antioxidative and bioactive substances (Stolz and Obermayer 2005). *Chlorella vulgaris*, a protozoic green algae was shown to posses a high content of proteins and minerals and to have a positive influence on collagen synthesis. *Spirulina platensis* contains a high amount of proteins and the Vitamins E, B1, B2, B3, B6, B12 and H as well as carotenoids. Algae extracts are commonly produced by hot water extraction, but a PEF-treatment could provide a potential towards a gentler downstream processing. The extractability of proteins, chlorophyll and carotenoids as well as protease activity of extracts from *Spirulina* and *Chlorella* after a PEF-treatment has been investigated in cooperation with IGV, Potsdam, Germany. An increase of 27, 809 and 525 % has been found for protein, chlorophyll and carotenoids content in chlorella extract after a treatment at 15 kV/cm and a specific energy input of 100 kJ/kg, respectively (see

Table 4). Antioxidative activity of the extract was increased by almost 100 %. In mice cell studies higher growth stimulation has been found in comparison to a conventional extract (data not shown). It is noteworthy, as the characteristic diameter of *Chlorella* cells is in the range of a few µm only, microalgae disintegration by PEF requires a comparable high treatment intensity in contrast to plant or animal cells. In addition to cell disintegration also a microbial decontamination is obtained at these processing intensity. A treatment with the parameters given was shown to result in a microbial decontamination of previously inoculated extracts of 7.1, 6.0, and 4.1 log-cycles of *Escherichia coli*, *Bacillus subtilis* and *Candida utilis*, respectively. The inactivation kinetics of *E. coli* and *Candida utilis* in *Spirulina* and *Chlorella* extract is shown in Figure 4.70.

Table 4: Increase of extraction of intracellular compounds from *Chlorella* and *Spirulina* microalgae after a PEF-treatment at 15 kV/cm and 100 kJ/kg. dm: dry matter, BM: ball mill.

| Component | *Chl.* dm BM | *Chl*. dm PEF BM | Yield increase% | *Spir*. dm BM | *Spir.* dm PEF BM | Yield increase % |
|---|---|---|---|---|---|---|
| Protein g/100g | 5,48 | 6,98 | + 27 | 33,68 | 38,12 | +13 |
| Chlorophyll g/100g | 0,011 | 0,1 | + 809 | 0,17 | 0,26 | + 52,9 |
| Carotenoids g/100g | 0,008 | 0,05 | + 525 | 0,044 | 0,11 | + 150 |
| Protease Unit/100g | 204,7 | 707,2 | + 245,5 | 864,2 | 812,5 | 94 |

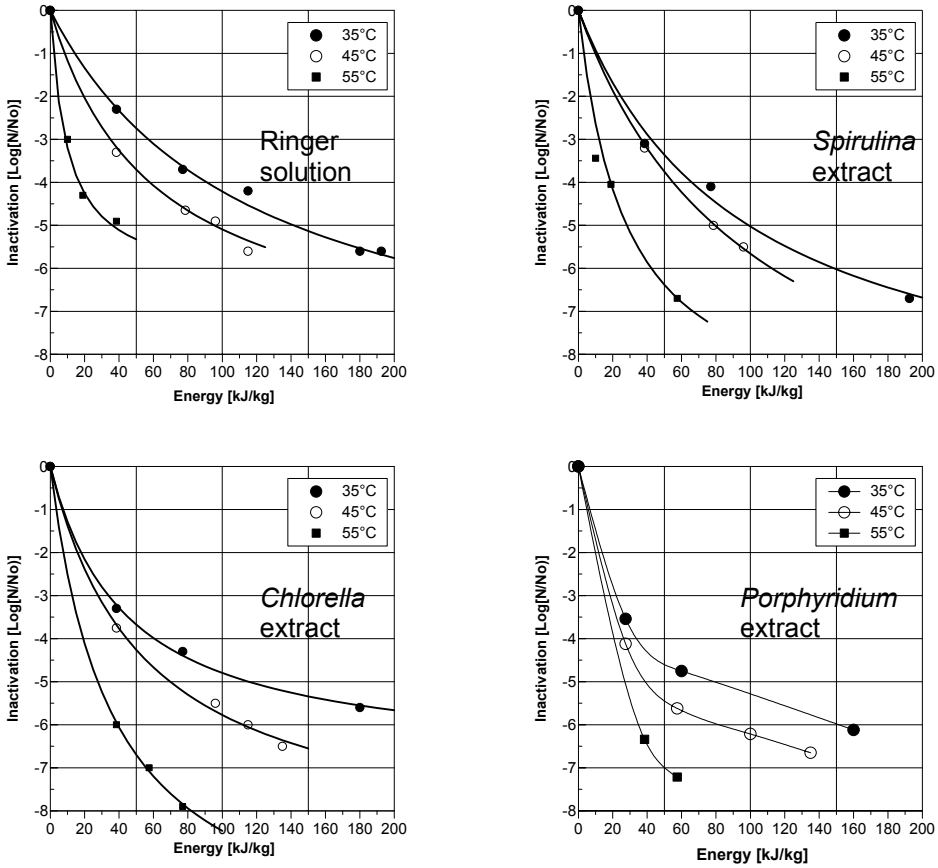



Figure 4.70: Inactivation *of E. coli* in Ringer solution*, Spirulina, Chlorella* and *Porphyridium* extract after a PEF-treatment at 18 kV/cm at different initial temperatures.

In contrast to microalgae multicellular macroalgae such as Kelp (*Laminaria digitata)* with larger cell diameters in a range of 100 µm can be electroporated with processing parameters similar to that of plant cells. The extractability of growth hormone formulations from Kelp after a PEF-treatment has been investigated by Heinz and Klonowski, indicating an improved yield after pressing (unpublished data). The expressible moisture of seaweed, a very tough material rich of alginate, was increased from 2 up to 6.5 % after a PEF-treatment (Hafsteinsson *et al.* 2000).

### 4.2.11 Electrode erosion and electrochemical reactions

As electrode material stainless steel is commonly used, but problems with electrolysis, formation of deposits, electrode corrosion and transfer of particles into the treated media have been reported (Caplot and Cote 1999; Góngora-Nieto *et al.* 2003; Morren *et al.* 2003; Mastwijk 2004). To avoid electrochemical reactions also other materials like platin or metal oxides (Bushnell *et al.* 1996) or polymer coatings have been suggested (Qin *et al.* 1997). Faradaic reactions taking place at the interface electrode|media may result in partial electrolysis of the treated media as well as in electrode corrosion. An overview of possible electrochemical reactions is shown in Table 5.

Table 5: Possible electrochemical reactions at the electrode|media interface

| Anode | Cathode |
|---|---|
| $2\ H_20 \rightarrow H^+ + OH^-$ | $2\ H^+ + 2\ e^- \rightarrow H_2\ (g)$ |
| $2\ HO^o \rightarrow H_2O_2^-$ | |
| $4\ OH^- \rightarrow O_2\ (g) + 2\ H_2O + 4e$ | |
| $2\ Cl^-\ (aq) \rightarrow Cl_2\ (g) + 2e^-$ | |
| $Fe\ (s) \rightarrow Fe^{2+}\ (aq) + 2e^-$ | |
| oxidation | reduction |

Transferring electrical energy leads to formation of charged double layers at the electrode surface. Using steady conditions, as used for electrolysis, these layers remain charged during the whole process, acting as capacitance. The transferred current flows via the Faradaic impedances in parallel to the double layer capacitance leading to Faradaic redox reactions at the interface (Amatore *et al.* 1998). To transfer a high amount of energy avoiding Faradaic processes it is sufficient that the potential drop across each double layer capacitor remains smaller than the threshold voltage above which significant electrochemical reactions occur. Under such circumstances the current flow would be purely capacitive, avoiding oxidative and reductive reactions at the electrode interfaces. To minimize the extent of this reaction to a tolerated maximum level the treatment chamber has to be submitted to short pulses, so that only a small portion of the applied potential builds up across the two double layer capacitors. The application of short pulses to avoid electrochemical reactions and electrode erosion has been investigated by Morren *et al.* (2003). Dependent on the electrochemical properties, mainly the double layer capacity of its material, an electrode can withstand a pulse with a certain current density and an impulse length without significant damage. Stainless steel has a very low double layer capacity (35 µF/cm$^2$) compared to graphite (260 µF/cm$^2$), resulting in a maximum pulse width avoiding electrochemical reactions as low as 0.5 µs with current densities of 200 A/cm$^2$ (Caplot and Cote 1999),

dependent on energy per single pulse. Electrode erosion became obvious when operating the PEF systems in long-term trials during sludge treatment (see 4.2.11). In Figure 4.71 the electrode erosion of three central anodes of the colinear treatment chamber with a diameter of 6 mm is shown after an operation time of 21 days.

Figure 4.71: Electrodes of colinear treatment chamber with an inner diameter of 6 mm after 21 days continuous operation time at 20 kV/cm, 100 kJ/kg and a flow rate of 5 kg/h. Stainless steel, graphite and copper-beryllium (left to right).

The materials used were stainless steel, graphite and copper-beryllium. It can be seen that for all materials electrode erosion occurred. The erosion showed a linear dependency on time of operation and energy input. As shown in Figure 4.72 the amount of stainless steel electrode erosion did not change when changing the storage capacity and adapting the pulse repetition to achieve a similar energy input. When using a spark gap instead of a thyristor switch a significant reduction of electrode erosion was found.

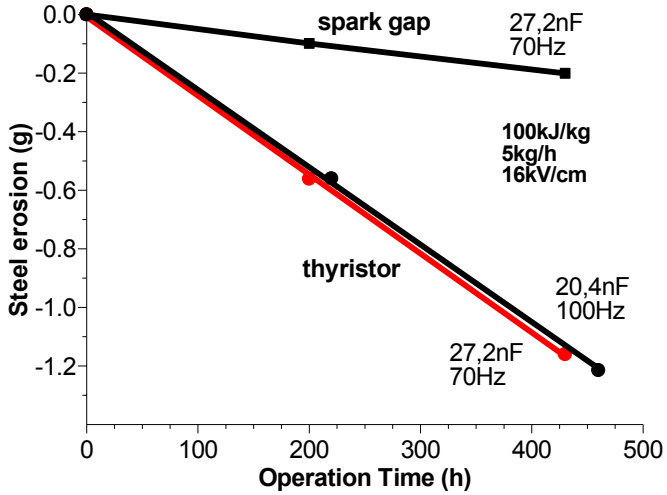



Figure 4.72: Erosion of stainless steel electrodes after a PEF-treatment at 20 kV/cm and 100 kJ/kg specific energy input, using at thyristor or a spark gap for pulse switching, For thyristor application two different storage capacities were used, pulse repetition was adapted to achieve similar energy input.

Using a thyristor for stainless steel a loss of 2.7 mg ± 0.2 mg per hour of operation was found, corresponding to an average release of 0.54 mg/kg of product treated.
Subjecting water and orange juice to a treatment at 30 kV/cm and approx 53 kJ/kg a similar amount of release was reported for exponential pulses using solid state switches, whereas after application of rectangular pulses a decrease of about 10 to 30 fold of release of metal particles was reported (Roodenburg *et al.* 2005, 2005b). The pulse modulator topology was based on a decoupling of pulse generation and treatment chamber and pulse modulator, avoiding leakage current as occurring for application of a direct switching based on a thyristor setup as used for this study. A leakage current of a few mA, typically 2 to 5 mA was found for our thyristor setups and might have caused electrolysis and electrode erosion also in between high voltage pulses. In comparison to other food operations such as grinding or homogenization, where up to 5 mg/kg of product might be released, erosion was 10 fold lower in our case and in accordance to European food legislation and the AIJN code of practice. For fruit a juice a maximum iron content of 5 mg/kg dissolved iron is mentioned in the AIJN code of practice. For copper beryllium a slightly lower erosion of 1.9 mg/h of operation was found, but an increased microbial inactivation was observed after release of toxic metal particles (data not shown).
Replacing the thyristor with a spark gap leakage current can be avoided; the erosion was reduced to 0.26 mg/hour of operation, corresponding to a metal release of 0.052 mg/kg of product. For graphite a (presumably) mechanical and thermal erosion was found, approximately 0.8 mg were released per hour of operation. As spark gap lifetime showed to be limited during long-term trials a replacement of solid states switches against a spark gap can not be suggested, but these findings indicate the necessity to avoid leakage current and associated electrolysis. Using an IGBT-switch or introduction of a transformer for could provide alternatives to avoid presence of leakage current.
In Figure 4.73 a comparison between inactivation using a thyristor switch in combination with steel and graphite electrodes at an electric field strength of 16 kV/cm is shown at different initial treatment temperatures, indicating a significant increase in treatment efficiency when using graphite. This finding might be caused by an improved homogeneity of the electric field distribution in the treatment zone. Due to the higher double layer capacity of graphite the occurrence of Faradaic reactions at the electrode surface might be reduced (Amatore *et al.* 1998), possibly resulting in less electrolysis and bubble formation. Gas bubbles, formed by electrolysis, cavity effects or release of dissolved gasses caused by heating have lower dielectric breakdown strength than the liquid media; their presence will lead to perturbations of the electric field distribution. The lower dielectric permittivity of air causes a concentration of potential within the bubbles increasing the chance for a dielectric breakdown and arcing (Góngora-Nieto *et al.* 2003). Modeling the electric field distribution with bubbles present in

the treatment chamber it was shown that the field strength in the boundary region of a bubble is very low, possibly leading to under-processing, in particular between several bubbles. By using an electrode material with higher double layer capacity like graphite electrolytic effects should be reduced. Under-treatment in boundary regions of bubbles can be avoided, resulting in higher microbial inactivation.

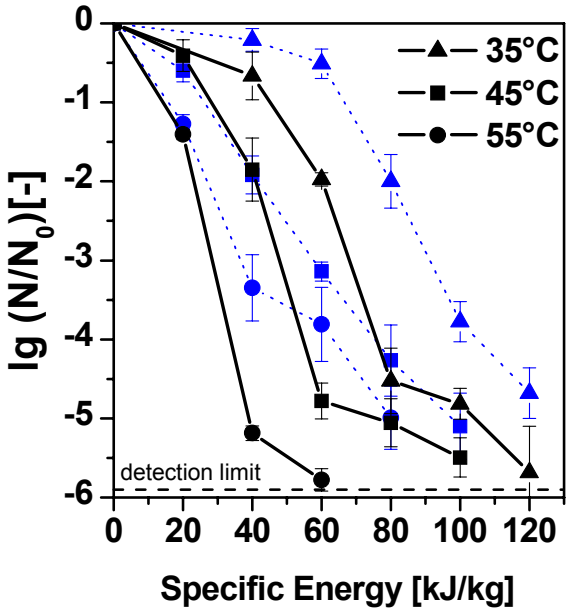



Figure 4.73: Comparison of inactivation of *E. coli* in Ringer solution at a field strength of 16 kV/cm at different initial treatment temperatures with graphite (—) or steel (···) anode. The flow rate was 5 kg/h.

Investigating the treated media at the outlet of the chamber an reduced amount of bubbles when using graphite instead of a steel anode could be confirmed, but still small bubbles (<< 1 mm) were found, which might also result from oversaturation of air due to heating by energy dissipation into the media. Local discharges and dielectric breakdown as well as perturbations of electric field homogeneity due to the presence of bubbles presumably have caused the lower microbial inactivation in case of the stainless steel anode. Arcing, as often observed in presence of a large single air bubble in the treatment chamber was not observed in both cases. Usage of graphite increased inactivation of *E. coli* more than one log-cycle as for example at 45°C and 60 kJ/kg. Application of electrodes with low amount of electrochemical reactions and pressurizing the treatment system to inhibit bubble formation as well as the application of higher electric field strengths should lead to further improvements in treatment efficiency. Bubbles can be avoided by degassing the treatment media, processing under pressure particularly in case of sparkling products and avoiding electrochemical effects at the electrode | media interface.

## 4.3 Waste and Processing Water Treatment

During biological waste water treatment the minimization of excess sludge production became, due to strengthened ecological and legislative measures, an issue of high importance. A disintegration of excess sludge and destruction of cells, organic matter consisting of a broad variety of different organisms, and subsequent release of intracellular material can be utilized to initiate biodegradation and autolysis of cells (Kopplow *et al.* 2004). After a treatment at 15 kV/cm a chemical oxygen demand (COD) release of up to 15 % was reported after an energy input of 360 kJ/l. Volatile suspended solids and gas production during anaerobic degradation were found to be improved by 8 and 19 % after a PEF energy input of 150 kJ/l in contrast to 445 kJ/l for a thermal treatment. Heat recovery from sludge is difficult; as viscosity is high and particles are present only tubular heat exchangers can be used and will require large exchange surface. Biofouling and deposits on the surface will cause short service times and require intense cleaning. Taking into account a maximum heat recovery in the range of 50 % the energy requirement for thermal treatment could be reduced to approx. 225 kJ/kg. The main advantages of a PEF application in contrast to conventional disintegration techniques such as mechanical rupture, ozone application, thermal or ultrasound treatment are short processing times and a direct and efficient permeabilization of cell membranes. PEF-treated sludge showed a reduction of biological activity and an increase in organic matter in the water fraction (Loeffler *et al.* 2001). The chemical oxygen demand of sludge filtrate was increased up to 25 % after a treatment at 26 kV/cm and an energy input of 800 kJ/kg, energy requirements were reduced to 250 kJ/kg when a temperature increase above 40°C was allowed. Release of organic material will improve digestion as well as subsequent dewatering.

A total reduction up to 53 % of volatile suspended solids and 45 % of total suspended solids in the excess sludge was achieved after a PEF-treatment at 15 kV/cm, 35°C and an energy input of 100 kJ/kg during a 2-month field tests at a waster water plant of Ondeo, Evry, France. During these experiments a flow of 5 l/h of sludge (3 % dry matter) was subjected to an electropermeabilization and returned to the aerated reactor with a volume of 200 l, corresponding to a stress frequency of 0.47 per day. The sludge retention time was 14 days. Running the PEF system 24 h a day and 7 days per week electrode erosion and wear of pulse modulator components could be observed. Due to electrode erosion the content of metallic particles in the sludge was increased (Table 6).

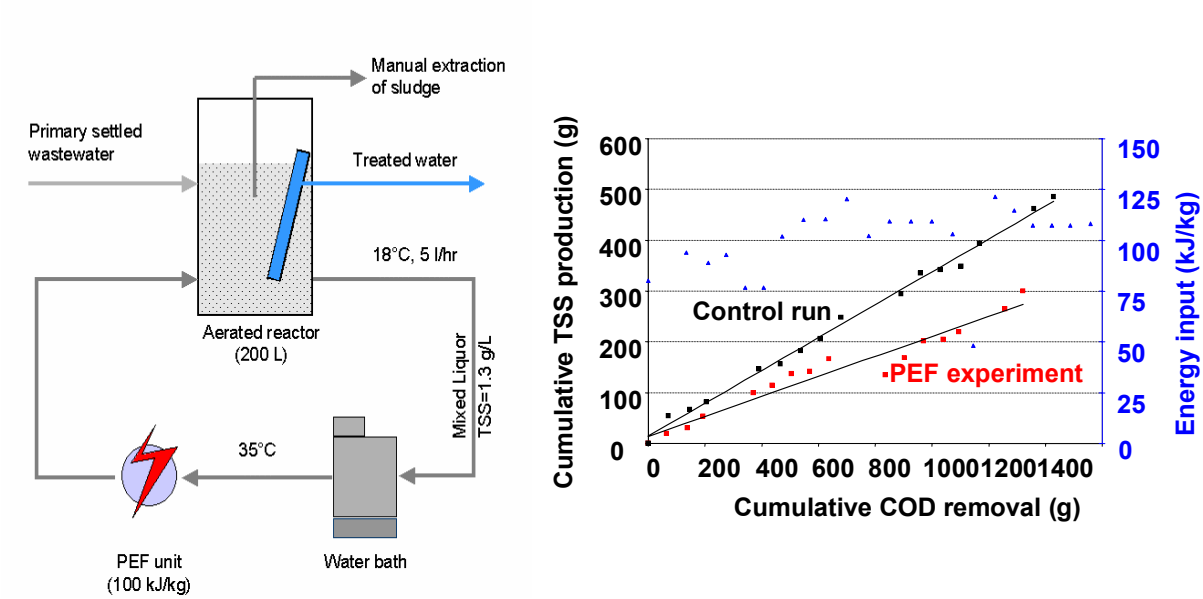



Figure 4.74: Schematic diagram of pilot wastewater treatment system with PEF application (left), cumulative total soluble solids (TSS) and energy input production during 2 month trial (right).

Table 6: Content of heavy metals in sludge after 14 days retention time (mg/kg TSS).

| | Fe | Mn | Mo | Zn | Ni | Cu | Cr |
|---|---|---|---|---|---|---|---|
| Control | 3870 | 267 | 7 | 443 | 47 | 503 | 343 |
| PEF | 7720 | 416 | 16 | 676 | 204 | 588 | 1136 |

It has been shown that quality of the treated water was maintained and sludge quality was still acceptable in spite of drastic reduction of excess sludge production. It is assumed that lysis-cryptic growth and an uncoupling of cellular metabolism, where the energy produced in catabolic reactions is driven towards maintenance and reparation functions, instead of being lead towards biomass production, caused the reduction of sludge production. The lysis-cryptic growth describes the cell growth resulting from cell lysis, an autochthonous substrate, which is reused in microbial metabolism and a portion of carbon is liberated as product of respiration and finally results in a reduced overall biomass production. Settling experiments indicated an improved agglomeration and sludge separation. In comparison to a PEF application for microbial inactivation in food products it has to be considered that the biocenose within the sludge is much more diverse; its impact on the broad variety of organisms has to be further studied. Additional research work will be required for optimization of PEF-treatment parameters as well as equipment design, but it was indicated that PEF application provides a potential to reduce the amount of excess sludge and can be utilized to improve anaerobic digestion. The impact of a PEF-treatment on aquatic nuisance species such as zebra mussels, hydrozoans or barnacles was reported (Schoenbach *et al.* 1996). After a treatment of tidal water at a field strength of 12 kV/cm biofouling was

prevented, indicating that a PEF-treatment can be utilized to protect lake or river water operated cooling systems from clogging due to biofouling. Abou-Ghazala and Schoenbach (2000) showed that even at treatment intensities as low as 1 kV/cm and an energy input in range of 16 kJ/kg up to 90 % of barnacles could be inactivated. A 100 % protection against fouling was obtained after a treatment at 6 kV/cm and an energy input of 560 kJ/kg. Energy efficiency was found to be increased when reducing pulse width of the rectangular pulses from 10 to 0.5 µs. Further research work will have to focus on determination of process parameter requirements and their optimization with regard to sludge reduction as well as energy efficiency.

## *4.4 Equipment Design Considerations*

During the course of this work a series of PEF equipments has been developed and realized (see 3.1 and 3.2). Experience obtained with lab scale designs proved to be helpful for improvement of performance and reliability of technical scale pulse modulators and treatment chambers. To select a treatment chamber and a pulse modulator the aim of the application and the production capacity will determine the peak voltage and the maximum average power. A small treatment chamber cross section and high flow velocity are favorable as lower peak voltage is required and commonly an increase of pulse repetition in a kHz range is feasible using solid state switches. Fruit mash or fruit, vegetable or meat pieces will require larger chamber sizes and adaptation of conveying systems. Increasing treatment chamber size requires increase of peak voltage and energy delivered per pulse. In the following sections design considerations for a PEF application for different products will be presented and their industrial feasibility will be discussed.

### 4.4.1 Treatment chamber design

Mainly three different treatment chamber types have been used for performance of PEF experiments, parallel plate, coaxial and colinear configurations of electrode (see Figure 2.10). The applicability of a particular design is determined by several crucial properties of the chamber. To achieve sufficient treatment intensity for all volume elements as well to prevent over-processing or arching the electric field should be free of local intensity peaks. A colinear configuration is producing a flow pattern which is desirable for food processing and cleaning in place. Relative to the electrodes, the inner diameter of the insulator should be slightly pinched in order to produce a more homogeneous electrical field (Lindgren 2001). The treatment chamber configuration determines the resistive load and therefore the

properties of the discharging circuit. The load resistance of a chamber is dependent on the conductivity of the treated media, resulting in limitations in the range of media conductivity to which the electric pulses can be applied. From an engineering point of view the load impedance needs to be matched to the pulse modulator used. An overview of electrical conductivity of certain liquid food products at different temperatures is typically in a range of 1 to 20 mS/cm, as shown in Figure 4.75

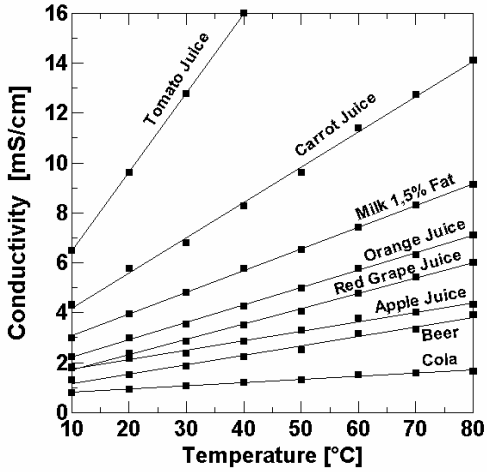



Figure 4.75: Electric conductivity κ(T) of different liquid food systems as function of the temperature.

For parallel plate electrode geometries the electrode area A is large in comparison to electrode gap d, the resistance R can be calculated by Equation 16 and is typically in a range of 5 to 50 Ohm.

$$R = \frac{d}{A} \cdot \frac{1}{\kappa(T)}$$

Equation 16

For coaxial treatment chambers a modification of Equation 16 can be used, but a similar range of load resistance is achieved, in addition the central electrode is influencing product flow pattern and cleanability of the electrode system. For a scale up to technical scale both designs provide a load resistance to small for most pulse modulator typologies, in general a load resistance above 100 Ohm is favorable. The resistance of the treatment chamber determines the voltage drop across it, if its resistance is similar to that of connectors and protective resistor a reduced peak voltage across the electrodes will result. Adaptation of the protective resistors to low resistance chambers might be difficult, as most of the available switching systems and in particular semiconductor switches require a maximum current limitation in case of short circuit. Usage of a treatment chamber with a high resistance such as a co-linear electrode configuration with a resistance in the range of several hundred Ohm

results in a more effective voltage division, a high electric field strength can be achieved even with highly conductive treatment media. Colinear treatment chambers with a diameter of 6, 10, 30 and 60 mm have been designed and realized for preservation of liquid food and treatment of fruit mashes or minced meat; electric field strength distribution and current flow have been modeled.

#### 4.4.1.1 Colinear chambers (6 and 10 mm) for treatment of pumpable fluids

A colinear design with an inner diameter of 6 mm was developed; one cylindrical central high voltage electrode made from stainless steel has two grounded counterparts at the inlet and outlet of the cuvette separated by insulating ceramics, producing a gap of 6 mm. Two treatment zones were formed by using cylindrical insulator segments made from Macor® or Acetal Delrin®. The electrode configuration of the colinear treatment chamber is presented schematically in Figure 4.76.

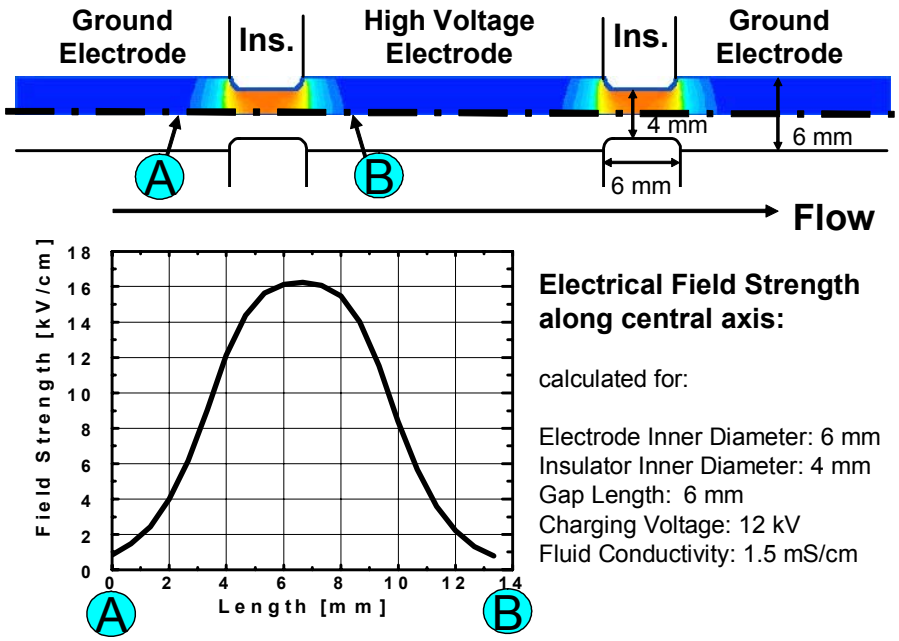



Figure 4.76: Features of 6 mm co-linear PEF-treatment chamber. Top: Sectional view of the electrode configuration and the resulting electrical field. The central high voltage electrode is separated from two grounded electrodes on either side by an electrical insulator. One example is given which shows the field strength along the central axis at the treatment zone A-B

For reason of symmetry only for the upper half of the treatment chamber is the field distribution shown. Depending on the position inside the treatment zone, the peak field strength can take different levels. For numerical simulation of the electric field strength in the treatment zone the Quick Field® FEM code (Tera Analysis Ltd, Denmark) has been used.

For the central axis (between position A and B) the spacial distribution is plotted for the specified treatment conditions (Figure 4.76, bottom). The current flow across the cross section was simulated using the Quick field code, a current of 25 A per treatment zone was obtained for a voltage of 15 kV and a media conductivity of 2 mS/cm, corresponding to total resistance of 300 Ohm. The simulation results showed to be in good accordance to the treatment chamber behavior, except that the temperature dependency of media conductivity needed to be implemented. The conductivity of different food material was increased during the treatment due to energy dissipation. A linear increase of conductivity was found when increasing temperature, to predict the average product conductivity during treatment the use of average product temperature showed to provide good results. Use of this treatment chamber showed good inactivation results, no difference in energy efficiency was found for inactivation of *E. coli* in apple juice in comparison to experiments using a parallel plate chamber at same average field strength. In between both treatment zones mixing might occur and help to improve treatment intensity distribution. The maximum field strength achievable with this setup was 34 kV/cm, applying a peak voltage of 26 kV. Above that voltage level external arcing occurred across high voltage and ground connectors, as the setup was insulated by air. A reduction of electrode gap to 4 mm allowed to achieve a maximum field strength of 44 kV/cm, to further increase maximum field strength a design submerged in transformer oil is currently under realization.

#### 4.4.1.2 Colinear chambers (30 and 60 mm) for fruit mash and minced meat treatment

For treatment of up to 5 t/h of fruit or vegetable mash a chamber was designed. In comparison to liquid food treatment the cross section needed to be increased due to higher fluid viscosity and presence of particles. A design with a minimum cross section of 7.1 $cm^2$, a diameter of 30 mm was realized (see Figure 3.7), maintaining a similar aspect ratio of diameter and gap than for the 6 mm chamber. For reason of symmetry only one half of one treatment zone is shown. To improve field homogeneity the insulators where pinched in comparison to the electrodes inner diameter. The intensity distribution within one treatment zone was modeled with the Quick Field code previously described, using a typical conductivity of 0.8 mS/cm for apple mash. The current flow across the cross section of one treatment zone was 13.85 A, resulting in a total chamber resistance of 360 Ohm. It is shown that treatment homogeneity is improved by electrode pinching, peak electric field at hot spots is reduced from 5.8 to 5.3 kV/cm and the average electric field in the center is increased from 2.3 to 2.7 kV/cm. With an insulator diameter of 34 mm the chamber resistance was reduced to 260 Ohm. The radius selected for rounding the treatment chamber edges was 1 mm.

Further decrease of insulator inner diameter to 25 mm led to an improvement of field homogeneity but due to expected clogging a chamber with a diameter of 30 mm was realized.

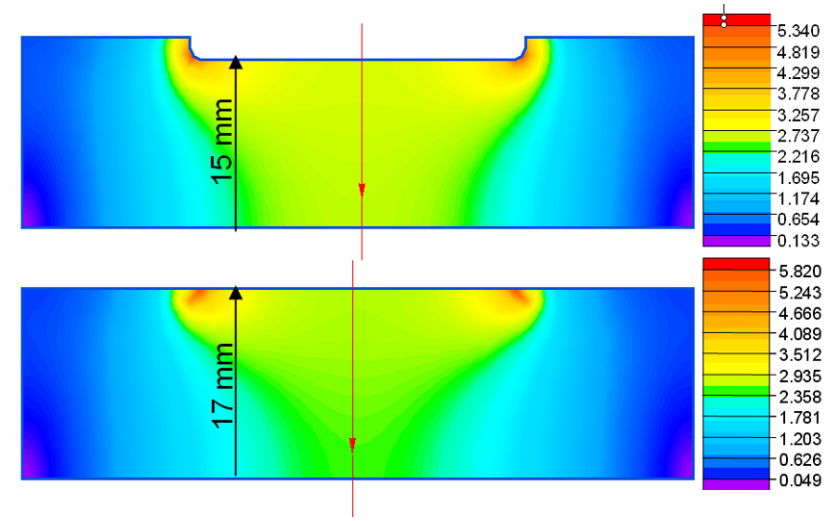



Figure 4.77: Electric field strength distribution (in kV/cm) in one treatment zone of a 30 mm colinear treatment chamber with (top) and without (bottom) pinching of insulator diameter. High voltage potential (left): 10 kV, ground potential (right): 0 V. Electrode inner diameter: 34 mm, insulator inner diameter 30 and 34 mm, electrode gap: 30 mm. Apple mash with a conductivity of 0.8 mS/cm was used as media. For reasons of symmetry only one half of the diameter is shown. The current flow across the surface (red line) was 13.85 and 19.10 A, resulting in a total resistance of 360 and 260 Ohm, respectively.

For treatment of minced meat and salted sausage meat a further increase of treatment chamber cross section was necessary. The conductivity of minced meat was 4 to 5 mS/cm, of salted meat for salami production up to 10 mS/cm were reached. A pinching of insulator inner diameter was not possible to avoid disturbance of product flow associated to clogging as well as separation of fat-meat emulsion. The field distribution within a 60 mm treatment chamber with an insulator diameter of 58 and 60 mm is shown in Figure 4.78. As only minor improvement of intensity distribution was obtained by reduction of the insulator diameter to 58 mm a chamber without pinching was realized and used for treatment of meat in field tests.

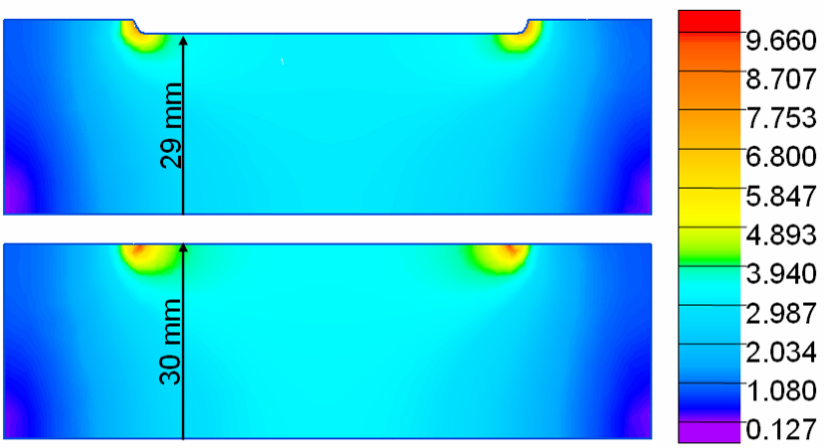



Figure 4.78: Electric field distribution (in kV/cm) in a treatment chamber with an insulator inner diameter of 58 (top) and 60 mm (bottom). High voltage potential (left): 25 kV, ground potential (right): 0 V. Electrode inner diameter: 60 mm, electrode gap: 60 mm. Sausage meat with a conductivity of 10 mS//cm was used as media. For reasons of symmetry only one half of the diameter is shown. The current flow across one treatment zone was 77.8 and 95.1 A, resulting in a total resistance of 160 and 130 Ohm, respectively. The electric field in the center was 3.0 and 3.2 kV/cm, respectively.

#### 4.4.1.3 Batch and continuous chambers for meat pieces

Treatment of meat pieces was performed using a batch treatment chamber (see 3.2). Dependent on amount and type of pieces treated the chamber was filled with water to provide a transferring media. As this procedure is undesired in meat processing due to possibility of cross contamination a direct treatment would be favorable. For shoulder pieces the feasibility of a direct application was shown, but irregular pieces such as haunches will require a submersion in a transfer medium. As an alternative needle electrodes and a modification of commercially available injection machines have been suggested. The field distribution for needle electrodes in meat pieces is shown in Figure 4.79. A voltage of 5 kV applied to needles with a diameter of 3 mm and a distance of 15 mm was simulated using a Quick Field FEM code. Field distribution appears to be very inhomogeneous, but treatment efficiency could be improved by application of several needle bars and stepwise meat piece transport after each treatment, similar as during injection. To investigate the feasibility of needle electrode application a prototype will have to be designed. Arching between needles not introduced into meat tissue could be prevented by disconnecting these needles using contactors reacting on pressure. The main advantage of needle application could be found in a reduction of electrode distance and peak voltage requirements for large meat samples.

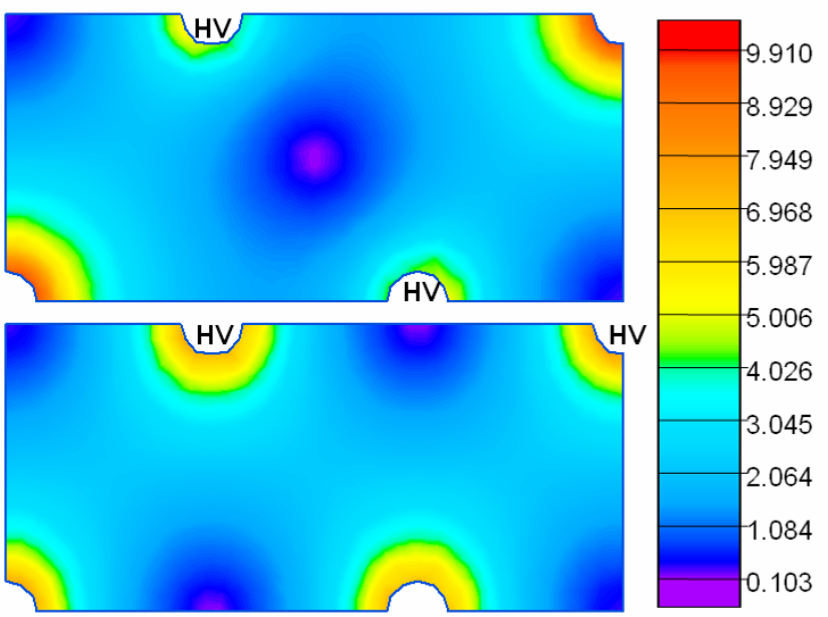



Figure 4.79: Electric field distribution in meat tissue with a conductivity of 5 mS/cm for needle electrodes with different alignment. HV-potential: 5 kV, 3 mm needle diameter and 15 mm distance. A Quick Field FEM code was used.

Other options for a continuous treatment to meat pieces could be the application of conveying belts, cartridges or pistons to transport slightly compressed meat pieces through electrode systems. A system applicable for the large variety of irregular meat pieces and products will require further development efforts, in particular when operator safety and hygienic design are taken into account. The development of a belt system to convey meat pieces through stainless steel electrode bars has been initiated in cooperation with Rühle, Grafenhausen, Germany and will provide experience regarding feasibility of continuous treatments without addition of water as a transferring medium. A design concept is shown in Figure 4.80. Another alternative to water usage could be application of a slight excess of salt brine to fill crevices or cavities. Brine will cause a very high media conductivity and require development of pulse modulators with high peak current capability. First estimations have resulted in a basic specification of 25 kV peak voltage and 25 kA peak current required for a treatment chamber with a gap of 15 cm, a width of 30 cm and an electrode area of 30 $cm^2$ at a conductivity of up to 10 mS/cm. For preliminary tests a spark gap pulse modulator has been developed (see 3.1), which proved to be reliable, but due to high peak current a fast derating of tungsten spark gap surfaces was observed. The design of a pulse modulator based on a solid state design would be feasible in general using a parallel setup of thyristor switches to share the current load.

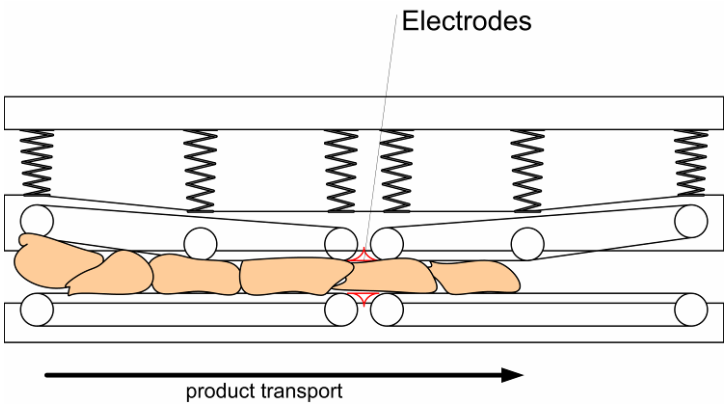



Figure 4.80: Design concept for a continuous treatment of meat pieces, consisting of a fixed and a movable conveyor belt to transport pieces through an electrode pair.

### 4.4.2 Pulse modulator design

The generation of pulsed power requires a fast, repetitive discharge of an energy storage into an electrical load matched to the pulse modulator. As energy storage mainly capacitors have been used, but in general also inductive energy storage would be possible (Bluhm 2006; Loeffler 2006). An overview of different pulse modulator typologies is shown in Figure 4.81. The simplest circuit is provided by an RC (resistance – capacitance) system (a), charging a capacitor with a high voltage power supply and discharging the stored energy across a high voltage switch into the product placed in a treatment chamber. Apart from one rectangular waveform pulse modulator RC-circuits have been used in the course of this work, based on thyristor and spark gap switches. Typical parameters of different switch types are shown in Table 7. Whereas all switch types have been used for PEF generation their main difference can be found in the maximum lifetime in terms of pulse number. At a repetition rate of 50 Hz the estimated operation hours are given. It is obvious that spark gap utilization will require a frequent servicing and switch replacement. By over-dimensioning the wear of a spark gap can be reduced, but still replacing will be required.

Generation of square wave pulses requires the use of an on/off switch such as gate turn off (GTO) thyristors, insulated gate bipolar transistors (IGBT), or symmetrical gate commutated thyristors (SGCT) or implication of a pulse forming network (PFN) consisting of inductive and capacitive elements. Main disadvantage in comparison to an RC-circuit is the requirement for adaptation of the modulator impedance to the load impedance to maintain rectangular waveform and avoid damping issues.

Table 7: Typical peak voltage, peak current, maximum pulse number and lifetime at a repetition of 50 Hz for different switch types.

| Switch type | $U_0$ | $I_{max}$ | $f_{max}$ | Max. pulse number | Lifetime at 50 Hz repetition |
|---|---|---|---|---|---|
| Ignitron | 20 kV | 100 kA | 1 – 50 Hz | $10^4$ | |
| Spark gap | 40 kV | 20 kA | 50 Hz | $10^6$ | 6 h |
| Thyrathron | 50 kV | 20 kA | 50 Hz | $10^9$ | 230 d |
| Tetrode | 20 kV | 10 kA | 20 Hz | $10^9$ | 230 d |
| Thyristor | 60 kV | 10 kA | 1 kHz | $10^{10}$ | 6 a |

Typical pulse requirements are a peak voltage of 10 to 60 kV, a repetition rate up to 1 kHz and an average power dependent on application and production capacity desired. Whereas for disintegration of plant or animal tissue 3 kW average power per ton of product will be sufficient, for preservation due to higher specific energy input approx. 30 kW per ton of product to be processed (100 kJ/kg) need to be installed.

For selection of a pulse modulator in a first step the desired peak voltage should be fixed by estimating the electrode gap and electric field strength required. Pulse modulators can also be designed suitable for various applications, but to limit switching stress it is generally recommended to reduce the electrode gap and treatment chamber volume as far as possible to allow operation at lower peak voltage and less energy per single pulse. Using solid state switches the repetition rate can be increased easier than the switching voltage to achieve sufficient average power. Solid state switches for capacitor discharge with a good reliability are limited to a range of 25 to 50 kV if no voltage multiplier or transformer is applied; the peak current typically is in a range of a few kA. Thyristor switches have proven to be applicable for a reliable and safe pulse modulator operation if several precautions and protective measures are taken. A current limiting resistor is required to prevent overcurrent in case of short circuit or arcing with a resistance in a range of 1 to 10 Ohm. A significant amount of up to 20 % of the energy might be dissipating at this protective resistance dependent on the ratio of protective and load resistance. Proper grounding and protection against electromagnetic interference between the switch trigger unit and the pulsed power is necessary. Cooling of protective resistors will be required; in general the load resistance should be increased as far as possible to reduce energy losses, as the minimum protective resistance is determined by the peak current of the switch used.

For treatment of liquid products a small treatment chamber cross section is feasible, but for treatment of fruit or vegetable mashes, particles, tubers or pieces the cross section will have to be increased. After selection of treatment chamber diameter and voltage the required pulse repetition and energy per pulse need to be selected dependent on product flow rate, residence time and specific energy input requirements.

Increasing peak voltage the availability of solid state switches with sufficient lifetime and reliability becomes more and more critical. For generation of pulses at higher peak voltage

pulse transformers can be used, as applied in the rectangular pulse modulator TUB 8 (I) with a ratio of 1:50. Low voltage level switching allows application of solid state switches with lower peak voltage, but to maintain pulse energy a higher current level is required. Typical IGBT switches are capable of switching 2 to 4 kA at a voltage level of 1.5 kV, to increase maximum current several switches can be operated in parallel, but still the peak current will be limited in comparison to a thyristor setup. The IGBT modulator (I) used has a peak output current of 200 A at 50 kV, the minimum load resistance is 250 Ohm in this case. The applicability of such modulator designs is therefore limited to applications for pumpable media, where a high resistive treatment chamber can be used. In general the introduction of a pulse transformer can be beneficial, as the occurrence of leakage currents, which can cause a significant proportion of electrode erosion, is avoided. In addition operator safety and modulator reliability can be improved by transformer introduction, as the stored energy is discharged into a well defined load and direct (leakage or undesired) currents will not be forwarded to the secondary circuit and the treatment chamber.

To supply pulsed power to treatment chambers with lower resistance in a range of 10 to 20 Ohm a combination of thyristor switches and a pulse transformer at transfer ratio of 1:10 to 1:20 could provide a good compromise. As an example a Dynex PT85QWx45 is suggested, with a peak voltage of 4 kA and a surge peak current of 37 kA. Using a primary voltage of 2 kV and a secondary of 40 kV a transformer ratio of 20 is required; the secondary and primary peak current level is 2 kA and 40 kA, respectively. For thyristor switches the peak current is less limiting, 40 kA can be obtained using a parallel setup of two switches. The current increase di/dt is limited to a range of 20 kA/µs for each switch module, requiring implementation of inductive elements to increase pulse rise time up to 1 µs. A further increase of voltage by increasing transformer ratio will cause higher primary currents and require more switch units to share the current load.

To generate pulsed power at voltage levels above 100 kV a voltage multiplying circuit such as a Marx generator can be used (Marx 1923), a setup of storage capacitors is charged in parallel, by discharge across spark gaps a series connection and voltage multiplication is obtained. Though peak voltages up to several hundred of kV can be obtained the repetition rate of such pulsed power generators is limited due to spark gap application and time for recovery. Erosion of spark gap material will cause a limited lifetime and require servicing. A design making use of gas conditioning to enhance spark gap recovery and to compensate spark gap erosion by adjusting gas pressure was patented (Kern 2005). The maximum repetition rate is 30 Hz at a peak voltage of ± 180 kV and a peak current of 9 kA, allowing a treatment at a load resistance of 20 Ohm. Lifetime of spark gaps was estimated to be up to 2 years. Marx Generator application could provide an alternative for treatment of large

products at low repetition rates, whereas at a lower field strength level using solid state switches is favorable.

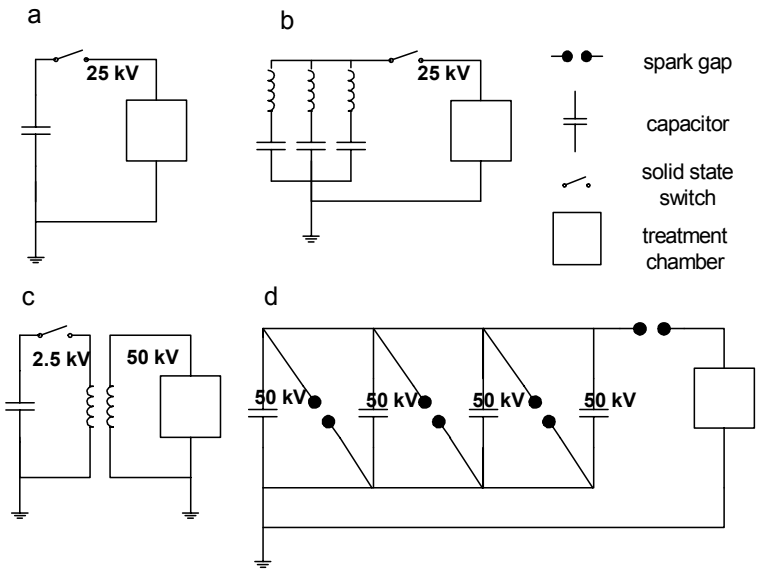



Figure 4.81: Overview of pulse modulator typologies for PEF applications, a) RC-circuit with direct pulse switching, b) pulse forming network, c) pulse transformer, d) Marx generator.

## 4.5 Industrial Feasibility and Cost and Efficiency Analysis

Within this section the treatment costs as well as the costs for investment will be estimated exemplarily for disintegration of fruit tissue for juice winning, sugar beet treatment and for pasteurization of liquid food. These applications have been discussed extensively in literature within the last decades, but still no industrial exploitation has been achieved. In principle the costs for other applications of PEF in food processing can be derived from the examples given, as the processing parameters required are similar for disintegration of other plant or animal cells or for pasteurization of other liquids, respectively. As an overview for production capacities up to 20 t/h the range of costs of investment for PEF application for disintegration of fruit mashes and fruit juices preservation is shown in Figure 4.82. The data is based on experience obtained during realization of lab and technical scale equipment as well as discussions and quotations from pulse modulator and component suppliers. Prices quoted from different suppliers had to be regarded as confidential, but an overview on expected investment costs is given. Dependent on supplier, pulse modulator typology and components as well as processing and product parameters a wide range of investment costs was obtained, in particular for microbial inactivation, where high treatment intensity is required.

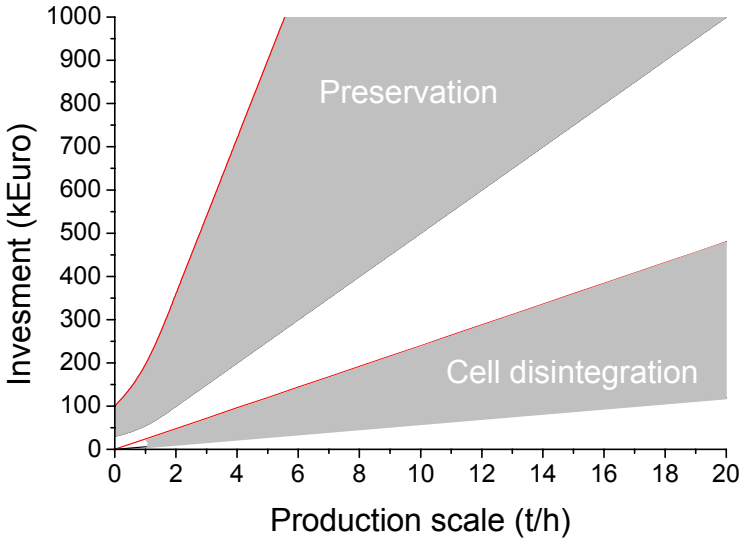



Figure 4.82: Estimated costs of investment for PEF application as cell disintegration and preservation technique in fruit juice production dependent on production capacity. Cost estimations are based on experience obtained during design of lab and technical scale systems and quotations of different pulsed power systems and component suppliers based on energy requirements of 1 to 3 kW/t for cell disintegration and 30 to 50 kW/t for preservation.

### 4.5.1 Fruit mash disintegration for juice winning

Considering the processing parameters reported, an electric field strength in the range of 1 – 2 kV/cm, exponential decay or rectangular pulses with a pulse width in range of µs and a total energy input of 10 – 20 kJ/kg the design parameters for an industrial scale system with a production capacity of 10 t/h of fruit mash can be determined. Based on a treatment chamber diameter of 50 mm a peak pulse voltage of 20 kV will be sufficient. To limit the impact of product conductivity, use of a co-linear treatment chamber is suggested, which provides a high load resistance. Two subsequent treatment zones are formed by a setup of three electrodes, consisting of a central high voltage electrode and two grounded counterparts. The average residence time within a treatment zone with a diameter of 50 mm and a gap of 50 mm will be $2.8 \cdot 10^{-2}$ s. To subject every volume element to an average number of 20 pulses (10 in each zone) a minimum repetition rate of 350 Hz will be required. At a flow rate of 10 t/h and an energy input of 10 kJ/kg an energy supply with an average output power of 30 kW is required. The price for such a unit can be estimated to be in the range of 150,000 €, from an engineering point of view a PEF system for this application is feasible as low peak voltage and pulse repetition rates are required. To achieve larger production capacities a setup of several units in parallel is favorable in contrast to an increase in treatment chamber diameter to avoid the necessity to increase the peak voltage required.

An energy input of 10 kJ/kg corresponds to an electric power consumption of approx. 3 kWh/t of product. Based on a price of 10 ct/kWh the pure electric energy costs for the PEF-treatment can be estimated as 0.30 €/t, adding 10 % overhead a total power of 0.33 €/t is assumed. For a conventional enzymatic maceration the treatment costs can be estimated at 7.50 €/t, where a significant amount is contributed by enzyme costs. A calculation of profitability is shown in

Table 8, taking into account the costs for investment as well as variable and maintenance costs.

Table 8: Estimation of total costs of a PEF cell disintegration in comparison to an enzymatic maceration for a production capacity of 10 t/h and an operation time of 1,875 h/a. Load voltage: 20 kV, average power 30 kW, estimated investment cost 150,000 €

| **Cost Type** | **Unit** | **Enzymatic Maceration** | **PEF** |
|---|---|---|---|
| production per a | t | 18,750 | 18,750 |
| investment | EUR | 37,500 | 150,000 |
| residual value. | EUR | - | - |
| replacement value | EUR | 45,000 | 175,000 |
| expenditure | EUR | 45,000 | 175,000 |
| depreciation range | years | 7 | 7 |
| Interest | % | 7 | 7 |
| depreciation | EUR/a | 6,000 | 22,000 |
| Interest | EUR/a | 3,150 | 12,250 |
| maintenance | EUR/a | 9,150 | 10,000 |
| fixed costs p.a. | EUR/a | 18,300 | 44,250 |
| variable costs p.a. | EUR/a | 140,625 | 6,188 |
| total costs p.a. | EUR/a | 158,925 | 50,438 |
| variable costs p.t | EUR/t | 7.5 | 0,33 |
| total costs p.t | EUR/t | 8.48 | 2.69 |
| | | | |
| Δ total costs p.a. | EUR/a | 108,487 | |
| Δ total costs p. t | EUR/t | 5.79 | |
| | | | |
| reflux time | years | 1.38 | |
| profitability | % | 119 | |

It is obvious that the high initial costs for installation of a PEF system can amortize within a very short period of time, as the treatment costs per ton are quite low. Even if under economic pressure the enzyme costs would decrease it is evident that there is a large span in range of 7 €/t between both techniques. This estimation is based on the assumption that the same juice yield is obtained after PEF or enzymatic pre-treatment, which is confirmed by literature data available as well as first reports from the first industrial installation in Germany (Günther, 2006, personal communication). Additional consumer benefit due to less detrimental impact on product quality is not included into this balance, same as the drastic reduction of processing time and a possible potential to extract native structure pectin from the pomace. From this point of view an application of PEF in fruit juice processing provides a

tremendous potential to reduce processing times and costs, as soon as a robust and reliable PEF system is available on the market. This comparison indicates the potential of the technique as well as the necessity to develop a turnkey-system for small or medium sized enterprises. For an application of PEF for disintegration of other cellular tissue or to improve drying or extraction, similar processing parameters (1 – 3 kV/cm, 10 kJ/kg) have been reported, the total costs for the PEF-treatment in the range of 2.69 € per ton of product therefore can be also expected for other applications with similar production capacity.

### 4.5.2 Treatment of sugar beet

Exemplarily for production scales above 100 t/h the industrial feasibility of PEF sugar processing will be discussed. Conventional procedures for production of sugar from beets involve an extraction at elevated temperatures (68 - 72°C) after carving the fruits into cossettes and for disintegration and destruction of cell membranes. The thermal denaturation as well as the hot water extraction require a significant amount of energy, as high as 175 kJ/kg of treated beet (Schultheiss *et al.* 2002). It has been reported that mechanically pressed, raw juice has a higher sugar concentration and contains less non-sugars, but juice yield remains unacceptable (Bliesener *et al.* 1991). A PEF-treatment of sugar beets prior to extraction could increase mass transfer rates and could allow to reduce extraction temperatures or to apply mechanical pressing. The applicability of a PEF pre-treatment prior to an extraction at ambient temperature has been investigated by Eshtiaghi and Knorr (2002). It was shown that after a PEF-treatment at 2.4 kV/cm and a pulse number of 60 similar cell disintegration than after a thermal treatment at 75°C for 15 min was obtained. A three step pressing at a pressure of 5 MPa and intermediate addition of water was suggested to achieve a high sucrose content juice after a short processing time of 30 min in comparison to up to 90 min for thermal extraction. The energy input required was 12 kJ/kg (Eshtiaghi and Knorr 1999). From an engineering point of view this high energy input could be significantly reduced by selection of appropriate processing parameters. Schultheiss *et al.* (2004) reported an increase of juice yield by a factor of 2.1 after a PEF-treatment in comparison to an untreated sample. In contrast to a thermal denaturation (175 kJ/kg) the energy input required was in the range of 2 – 3 kJ/kg, achieving similar juice yield. Raw juice quality was maintained or improved even when using low quality beet as raw material. El-Belghiti *et al.* (2005) developed a two-exponential kinetic model to describe diffusion during extraction of sugar. Optimum PEF processing parameters have been identified as 670 V/cm and a pulse number of 250. A comparison of quality in comparison to juice from thermally treated beet revealed superior juice quality. These results indicate the tremendous potential of PEF

application for sugar processing, in particular against the background of changing legislative situation in the European Community. The reform of the sugar sector in Europe (EU IP/04/915) will remove or at least reduce trade barriers as well as export subsidies, thus reduction of energy requirements and production costs will be inevitable to stabilize the sales of beet sugar in competition to cane products, and PEF could provide an alternative to conventional processing.

But considering the production scale of today's sugar refineries – an total average production of 104,644 t/d has been reported for the nine production sites of Südzucker AG (Kraus 2003) – it is obvious that the PEF equipment available at present is far away from reaching these capacity levels in the range of up to 500 t/h per refinery. To treat an amount of 500 t/h of sugar beet suspended in water with a packing density of 500 kg/m$^3$ an amount of 1,000 t/h has to be treated. Limiting the flow velocity below 1 m/s a cross section of 2,700 cm$^2$ is required, corresponding to treatment chamber diameters in range of 600 mm. It is evident that a peak voltage of above 200 kV will be required to operate at a field strength of 3 kV/cm when including losses in switching system and connections. The average power consumption required to achieve an energy input of 10 kJ/kg is 2.7 MW. It remains questionable if a power supply with these ratings and in particular a pulse modulator with these power ratings can be integrated into an existing refinery. To limit losses the power supply needs to be set as close as possible to the treatment chamber, but at same hand legal and constructional restrictions have to be accounted when operating at 200 kV. Even when separating the total flow to several lines with capacities of 100 t/h and limiting the diameter to 300 mm (below clogging might become a problem) a load voltage in the range 100 kV remains. Up to present pulsed power systems with these power ratings have not been successfully used for PEF application, in particular the pulse modulation has proven to be a challenging task. Dependent on the length of the treatment chamber a minimum pulse repetition rate will be required. To keep the load resistance high the electrode area should be as small as possible, resulting in shorter residence time and higher necessary repetition rate. To provide a pulse with a peak voltage in the range of 100 kV a Marx generator (Marx 1923) has to be applied, as only few switches can handle such parameters with any reliability (Kuthi *et al.* 2003). Prior to large industrial scale application of PEF as required for sugar processing the development of systems with production scale in a range of 10 – 20 t/h and their successful exploitation will be required to confirm the technical feasibility.

### 4.5.3 Cost estimation for beverage pasteurization

For an efficient pasteurization of liquid food a required peak electric field in the range of 30 - 40 kV/cm has been reported. Dependent on type of product, experimental setup, treatment chamber geometry and processing parameters such as pulse wave shape and processing temperature the specific energy input requirements are varying in a broad range from 50 up to several hundreds of kJ/kg in particular when enzyme inactivation was taken into consideration, up to 8,000 kJ/kg of treated product have been reported (Zhang *et al.* 1994; Zhang *et al.* 1995; Góngora-Nieto *et al.* 2003; Heinz *et al.* 2003; Aronsson *et al.* 2005; Evrendilek and Zhang 2005; Giner *et al.* 2005). Treatment intensity required, in comparison to disintegration of plant or animal tissue is much higher in terms of electric field strength as well as energy input. PEF systems for preservation in industrial scale are not on the market, but pilot scale equipment is available at Ohio State University (USA), Stork Food and Dairy Systems (The Netherlands), SIK (Sweden) and Berlin University of Technology (Germany), pulse modulators are commercially available from Diversified Technologies, Canada, or Saligus, Sweden exemplarily. Some limitations have to be kept in mind when considering preservation by PEF in comparison to a conventional thermal processing:

- the treatment is affecting vegetative microbes only, spores or viruses are not susceptible to electric fields at the parameters applied
- an aseptic filling subsequent to treatment will be required (in contrast to a high hydrostatic pressure or hot filling process)
- efficiency regarding enzyme inactivation remains unclear, in most cases chilled storage might be necessary.

A thermal preservation of fruit juice is commonly performed at a temperature level of 85°C and a holding time of 30 s (Pandur 1988), the temperature increase from 20 to 85°C will require an energy input of approx. 250 kJ/kg for apple juice. Taking into account heat recovery with a recovery rate of 90 – 95 % the average energy input required will be in the range of 20 kJ/kg for thermal processing. It is obvious from the energy requirements reported, that a PEF-treatment, even when making use of heat recovery to reduce the electric energy input required to 40 kJ/kg as shown by Heinz et al. (2003) will require a higher specific energy input, dependent on parameters applied up to 20-fold in comparison to thermal treatment. It has to be considered that use of electric power is commonly connected to higher costs than heat derived by fuel or gas. If a PEF-treatment shall be performed at ambient temperature, the electric energy input required will have to be removed by an active cooling system, causing additional costs of operation and investment. The costs for a treatment of apple juice at an energy input of 50 (data from Heinz et al., (2003)), applying heat recovery and with an energy input of 705 kJ/kg and active cooling (data from Evrendilek

and Zhang (2005)) will be estimated. An overview of these estimations can be found in Table 9.

Table 9: Estimation of investment costs for power supply and pulse modulator based on processing parameters from literature (Heinz *et al.* 2003; Evrendilek and Zhang 2005).

| | | Heinz *et al.* | Evrendilek *et al.* |
|---|---|---|---|
| Specific energy input | kJ/kg | 50.00 | 700.00 |
| Electric power consumption | kWh/t | 13.89 | 194.44 |
| Power price | Eur/kWh | 0.10 | 0.10 |
| Power costs | Eur/t | 1.39 | 19.44 |
| Flow rate at industrial scale | t/h | 10.00 | 10.00 |
| Average power required | kW | 138.89 | 1944.44 |
| Power supply cost estimation | MEur | 0.14 | 1.94 |
| Overhead for other components | | 2.00 | 2.00 |
| **Investment cost estimation** | **MEur** | **0.42** | **5.83** |

An energy input of 50 kJ/kg can be translated into an electric power consumption of 13 kWh/ton of product or, at a current price of 10 ct/kWh to 1.3 €/t of treated product. For treatment of a flow of 10 t/h at an energy input of 50 kJ/kg an average power of 150 kW is required. Operating at an energy input of 700 kJ/kg will result in a power consumption of 194 kWh/t, electric power costs of 19.4 €/t and require an average power of 2 MW. Based on the processing requirements discussed in 4.2.8 the costs of investment for such PEF systems can be estimated. For the power supply costs in the range of 0.5 to 1 k€/kW are commonly assumed, resulting in costs of 150 to 2000 k€. Including an overhead for pulse modulation, capacitors and control equipment in the range of twice the costs of the power supply the estimated total installation costs will be approx. 3 k€/kW or, € 0.42 and 5.8 million for the two examples, respectively.

Not many data is available concerning equipment costs of large scale PEF equipment, Braakman (2003) reported investment costs in range of € 2 million for a flow capacity of 5 t/h or € 4 for 10 t/h capacity, specific energy input is not given, but the cost range in good accordance to the previous estimation. As an industrial system is not available at present the costs can only be estimated based on data available from other pulsed power applications, or scaled up from pilot scale equipment. Diversified Technologies (Gaudreau *et al.* 2004) reported costs in the range of 150 to 200 k€ for pilot scale design with an average power of 20 kW and a peak voltage of 20 kV. The investment cost for this system are in the range of 7.5 k€/kW average output power. The costs of investment can be regarded as quite significant, apart from the fact that no system has been proven to operate at sufficient reliability and lifetime. It has to be kept in mind that for the application operating at ambient temperature an active cooling device has to be added, being able to remove 700 kJ/kg of thermal energy, or scaled up to 10 t/h a cooling capacity in range of 1.9 MJ/s.

Excepting doubts on technical feasibility of a pulse generator for an industrial scale system one can proceed with the investment cost estimations to set up a total cost balance including costs of operation and depreciation. An overview of the cost balance for the two variations of specific energy input can be found in

Table 10. Supposing a depreciation time of 7 years total production costs of 8.51 and 114 €/t of product are obtained for the two examples selected. Costs for oil derived thermal pasteurization can be estimated to be in the range of 0.20 €/t when applying heat recovery. Thermal pasteurization accounts, though requiring a significant amount of energy, only for a small amount of the total product costs. It can be easily seen that application of PEF will cause a significant increase from 0.20 to 8.51 or even up to 100 €/t of product when operating at ambient temperature and high electric energy input. Braakman (2003) reported extra cost estimations in a range of 0.01 to 0.02 €/kg or 10 to 20 €/t of product, which are in accordance with our estimations and values given by Clark (2006) for the first commercial PEF application. These extra costs will have to be justified by sufficient margins or consumer benefits and most probably inhibit an industrial application as long as equipment availability and reliability, consumer benefit as well as consumer acceptance of the technique are not proven in a production scale of tons per hour.

Table 10: Estimation of total costs of PEF preservation at two different specific energy inputs at a production capacity of 10 t/h and an operation time of 1875 h/a. Investment costs are based on estimations given in the text, cooling system to maintain treatment temperature is not included.

| | | specific energy input | |
|---|---|---|---|
| Cost Type | Unit | 50 kJ/kg | 700 kJ/kg |
| production per a | t | 18750 | 18750 |
| investment | EUR | 420,000 | 5,830,000 |
| residual value. | EUR | - | |
| replacement value | EUR | 504,000 | 6,996,000 |
| expenditure | EUR | 504,000 | 6,996,000 |
| depreciation range | years | 7 | 7 |
| interest | % | 7 | 7 |
| depreciation | EUR/a | 72,000 | 999,429 |
| interest | EUR/a | 35,280 | 489,720 |
| maintenance | EUR/a | 28,000 | 280,000 |
| fixed costs p.a. | EUR/a | 135,280 | 1,769,149 |
| variable costs p.a. | EUR/a | 24,375 | 363,750 |
| total costs p.a. | EUR/a | 159,655 | 2,132,899 |
| variable costs p.t | EUR/t | 1.3 | 19.4 |
| total costs p.t | EUR/t | 8.51 | 113.75 |

### 4.5.4 Cost efficiency of waste water treatment in comparison to conventional treatment

The average costs for sludge deposition in Germany are in the range of 50 €/t (Anonymous 2003), for incineration of mechanically dewatered sludge costs in the range of 300 to 750 € per ton of original dry matter have to be estimated (Halbach *et al.* 2003).

As a PEF-treatment for excess sludge reduction still needs to be optimized from technical and economic point of view and the results reported are obtained in lab- or pilot-scale the energy requirements can only be estimated at present. To improve treatment efficiency it is suggested to increase the dry matter of the sludge prior to treatment as at low solid concentration the main part of energy is dissipated into the liquid media without benefit. An increase of dry matter from 3 to 5 % could lead to a drastic increase of electrical energy utilization. A treatment of sludge with an energy input of 50 kJ/kg of liquid sludge with 5 % dry matter, a stress frequency of 0.4 and a sludge retention time of 14 days, will require an energy input of 5.6 MJ/kg or 1.55 MWh/t of original dry matter. Taking into account a charge of 0.10 € per kWh this results in energy costs of 155 €/t of dry matter, indicating that a PEF-treatment can be utilized as an effective and cost efficient alternative to reduce the amount of excess sludge from civil or industrial waste water prior to incineration.

## *4.6 Approach for a General Characterization of Requirements for Membrane Permeabilization of Biological Cells*

During the course of this work different biological cells and tissues have been treated by PEF. Comparing the requirements to achieve a sufficient cell disintegration of plant or animal tissue, to induce disintegration of microbes and protozoa in waste water and for decontamination of liquid food it is obvious that electric energy and field strength need to be selected dependent on target organism. When comparing the different applications it has to be kept in mind that dependent on the aim of the process the desired level of cell permeabilization can range from a few percent of reversibly permebealized cells for induction of stress response over 90 to 99 % for improvement of mass transfer in plant tissue and up to 5 to 8 log-cycles for a safe microbial decontamination.

A comparison of the different processing intensity requirements of PEF, MEF and ohmic heating in terms of electric field strength and specific energy input for different applications is shown in Figure 4.83. Whereas field strength is mainly influencing the investment costs the energy input applied is primarily determining the costs of operation. When increasing field strength the total costs to achieve a certain specific energy input are increased as can be

seen at the drop of the red curves of similar treatment costs. These lines show the estimated total costs including electric energy, investment and depreciation for a treatment. An energy input level of 200 – 250 kJ/kg typically is the limit where thermal effects are predominant if the initial temperature is 10 to 20°C and no cooling is applied during continuous processing under adiabatic conditions. For disintegration of plant or animal tissue the superior energy efficiency of a PEF-treatment in comparison to an MEF application can be seen. Energy requirements have been reported to be in a range of 2 to 20 kJ/kg in contrast to 20 to 40 kJ/kg for a MEF-treatment. For decontamination of liquid food dependent on processing conditions such as temperature, field strength or mode of operation often an energy input of 300 to 1000 kJ/kg was reported, in particular when maintaining treatment temperature at an ambient level. As a comparison, for a conventional thermal pasteurization of fruit juice an energy input of approx. 20 kJ/kg is sufficient due to heat recovery potential of up to 95 %.

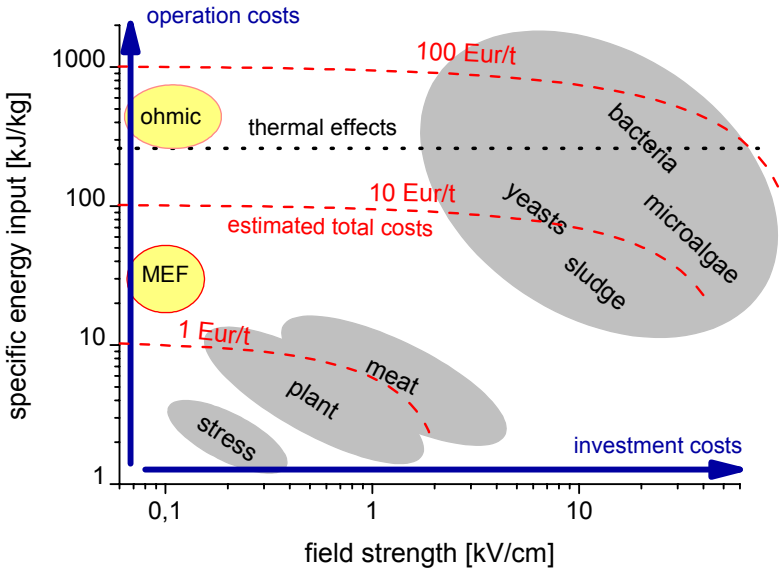



Figure 4.83: Overview of required processing intensity for PEF application to induce stress reactions, disintegration of plant or animal cells and microbial inactivation in comparison to a MEF treatment or ohmic heating. Exceeding an energy input of 250 kJ/kg predominantly thermal effects occur.

Though presenting an overview of energy required to achieve a certain processing aim this graph does not allow a comparison of energy requirements to achieve permeabilization of cells of different origin or size. It is noteworthy that energy input required for preservation was reported to be 10 to 1000 fold higher than for cell disintegration. As discussed in 4.2.5 the characteristical cell diameter appears to have a major impact on susceptibility against a PEF-treatment, as the transmembrane potential induced is dependent on electric field strength applied and the semi-axis in field direction (Equation 1). To perform a general approach on energy requirements for membrane permeabilization a level of 95 % efficiency was selected.

For plant tissue a 95 % disintegration index, for meat 95 % of the maximum increase in conductivity found, and for microbial cells a 1.3 log-cycle inactivation level was used. The membrane potential induced when exposing cells to an external electrical field was calculated by Equation 1 at a cos α of 1 and a characteristical, average cell diameter (Bergey 1986). As shown in Figure 4.84 a typical curve shape for all membrane types was found. Increasing the induced transmembrane potential (TMP) the energy efficiency is improved until a saturation level, which was obtained in a range of 10 to 15 V for most membrane types. It is noteworthy that energy requirements to achieve a similar level of permeabilization in plant and microbial cells show to be very similar when same TMP is induced.

For *E .coli* the impact of temperature on transmembrane voltage and energy requirement is shown, lower transmembrane potential and energy input are indicating an increased susceptibility at higher temperatures. Whereas for apple or potato tissue a transmembrane potential above 20 V can be easily obtained exceeding a field strength of 3 kV/cm for *E. coli* or other microbes more than 65 kV/cm are required. To obtain a TMP of 10 V in *L. monocytogenes* an external field strength above 100 kV/cm is required. In addition to maximum dielectric strength of the liquid media often the pulse modulator and the minimum treatment chamber diameter are limiting to achieve such high field strength. Data regarding energy efficiency of microbial inactivation at different field strength levels and in continuous operation is scarce, as many of the manuscripts published are based on batch experiments or consider total treatment time as intensity parameter and mention not enough details to calculate energy input applied. The results presented give an indication on dependence on energy requirements on transmembrane potential applied, but it has to be kept in mind that several simplifications have been made. Variations in size or orientation as well as distributions in resistivity and growth conditions have been neglected. Differences in TMP might therefore be based on membrane constitution or variations in resistance, cell size and orientation as well as experimental design. As inactivation curves in many papers showed a tailing no linear increase of energy input can be assumed to increase inactivation from 1.3 up to 5 or 7 log-cycles. The TMP has been calculated for an average, characteristical diameter of cells.

Nevertheless these results show that the energy input required to achieve a permeabilization of membranes of different origin appears to be in a similar range if a sufficient transmembrane voltage is induced (see right chart). In accordance to previous sections a critical TMP of approx. 1 V as well as a threshold value in a range of 10 to 15 V was found, above that a further increase of TMP (and external field strength) does not result in an improvement of energy efficiency. Zimmermann reported a minimum TMP of 1 V to be induced to achieve a pore induction, but further increase results in a drastic improvement of energy efficiency. Higher energy requirements for microbial inactivation in comparison to

tissue disintegration are not necessarily based on higher resistance of microbial phospholipid membranes against PEF, but might result from a higher level of desired permeabilization far above 95 % as well as lower energy efficiency due to technical limitations of external field strength applied. Future work should focus on providing data to evaluate energy efficiency of microbial membrane permeabilization and impact of processing or product parameters.

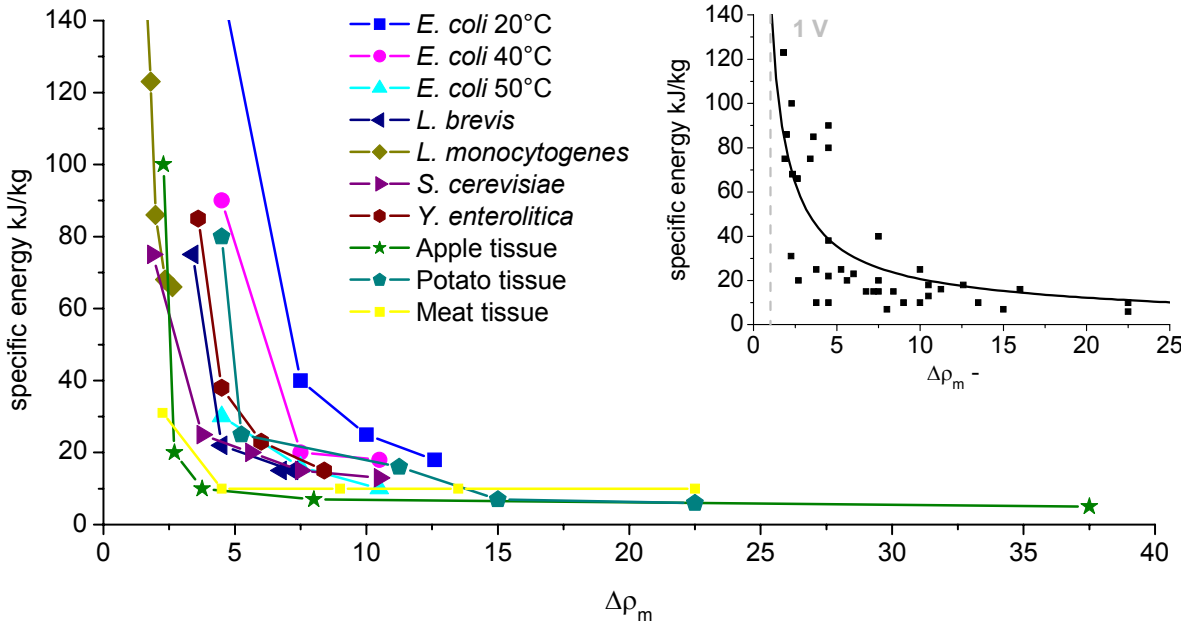



Figure 4.84: Energy requirement to achieve a 95 % tissue disintegration or 95 % microbial inactivation (1.3 log-cycles) for different biological cells and tissues. Characteristical dimensions and data used: *E. coli*: 4 µm (own data), *L. brevis* 3 µm (Grahl and Märkl 1996), *L. monocytogenes* 1.25µm (own data and (Alvarez *et al.* 2006), *S. cerevisiae* 5 µm (Zhang *et al.* 1994; Qin *et al.* 1995; Grahl and Märkl 1996), *Y. enterolitica* 2 µm (Heinz *et al.* 2002), apple tissue 80 µm (own data), meat tissue 60 µm (own data) and potato tissue 100 µm (Knorr and Angersbach 1998). In the right chart the black curve is showing an average for all samples.

# 5 Conclusions and Outlook

The applicability of a PEF-treatment has been investigated for various applications in food processing in the course of this work. It was shown that generally an electropermeabilization of artificial or biological cell membranes can be achieved. Whereas for biological cell membranes a critical field strength for pore induction was found this was not observed for artificial phospholipids bilayers. The degree of permeabilization was shown to be dependent on specific energy input for phospholipid vesicles, plant, animal and microbial membranes mainly, when a certain threshold field strength was exceeded. A comparison of PEF impact on liposomes and microbial cells revealed that artificial phospholipids show similarities in susceptibility to an electric field, but applicability as a model system to predict microbial inactivation was limited.

For plant tissue it was shown that an application of pulses of 0.3 kV/cm is sufficient to induce pore formation, but energy efficiency was improved by increasing electric field strength above 1 kV/cm. Above this level the specific energy input could describe processing intensity in a wide range of pulse parameters such as pulse width and energy per pulse applied. A comparison to application of moderate field alternating current, also resulting in tissue disintegration showed the superior energy efficiency of a pulsed electric field application for apple and potato tissue. Textural changes of plant tissue have been shown and related to a loss of turgor pressure, this effect might be used to improve cutting quality or to modify or standardize product structural properties.

The impact of a PEF-treatment on extractability of intracellular compounds such as anthocyanins from grapes and violet fleshed potatoes and apple and carrot juice yield was investigated. Apple juice yield was increased up to 7 % during lab scale experiments, whereas juice quality was equivalent to untreated samples. During technical scale treatments the effect of a PEF-treatment on apple mash structure was clearly visible and resulted in difficulties during juice separation using a horizontal press with implemented filter elements. Application of a baling press with different surface-volume ratio was favorable and resulted in an increase in juice yield of up to 6 % for royal gala apples. These results indicate that liquid-solid separation technique might need to be adapted to changes in mash structure after a PEF-treatment. For industrial scale use the application of belt presses is suggested. Also for decanter separation an impact of a PEF-treatment was found, an optimization of decanter settings and adaptation to the changed mash structure resulted in an improved juice yield in comparison to an untreated sample. A treatment of carrot juice was performed to investigate the impact of raw material and mash properties, after a PEF-treatment juice yield was increased in comparison to an untreated sample and the yield of an optimized Supraton®-treatment was achieved. Quality of apple and carrot juices obtained in technical scale

experiments was equivalent to control sample quality and in accordance to German and European food legislation and the AIJN code of practice. A treatment of potatoes showed an increase of mass transfer rates during convective air drying, which allows reducing drying times to achieve a final moisture of 5 % of about 20 %, providing a potential to reduce processing time and energy usage and to increase production capacity.

Besides disintegration of plant tissue a treatment of meat, meat products and fish was performed. Mass transfer could also be enhanced in meat, as shown by enhanced drying rates of raw ham and sausage products after a PEF-treatment. During production of cooked ham and pickled products a potential for improvement of water binding capacity was found. The impact of processing parameters and addition of water binding agents was investigated. Degree of meat tissue permeabilization was determined using a conductometer, similar than for plant material the energy input was shown to be the applicable as processing intensity parameter, though variability of raw material was higher than for plant tissue. Increasing treatment intensity was resulting in higher amount of cell disintegration and improved mass transfer rates. The impact of a PEF-treatment was compared to the impact of a mechanical treatment as used during tumbling of meat and related to drip loss during cooking. A PEF-treatment alone was shown to increase water loss, but when applying water binding agents such as phosphate or hydrocolloids a synergetic effect and an enhanced water holding was found. This effect was based on improvement of micro-diffusion of brine and additives within the tissue at a cellular level. Protein swelling after a PEF-treatment was shown by REM-micrographs. Increasing water binding could provide an economic as well as a consumer benefit, as in addition to weight increase product tenderness was shown to be improved. Release of endogenous enzymes in meat tissue might have caused a tenderization as observed during raw ham production and might as well provide a potential to reduce curing times for beef meat. A treatment of fish fillets resulted in a less pronounced effect on water binding, presumably due to different muscle structure and supportive tissue content of fish meat. At present an industrial prototype is developed, a design concept has been presented.

Membrane permeabilization mechanisms and the impact of electric field strength and energy input on microbial inactivation have been investigated by flow cytometry and application of two different dyes to determine metabolic activity and degree of membrane permeabilization. It was shown that the effect of a PEF-treatment is based on electropermeabilization, a decrease of intracellular enzyme activity was only observed after application of high treatment intensity and could also be related to a release of the enzyme or the reaction products. Incubation of treated cells with glucose was used to investigate their transport channel activity; it was shown that mass transfer was inhibited after a PEF-treatment at high intensity. Loss of cell membrane semipermeability and formation of a chemical and electrical equilibrium between cytoplasm and its surrounding might cause the loss of transport

mechanism activity. At lower treatment intensities the occurrence of membrane resealing was observed, sublethal damage was found after plating on stress growth medium at low electric field strength level for *E. carotovorum* and *L. rhamnosus*.

No impact of pulse rise time or pulse geometry on permeabilization of liposomes or microbial inactivation was found for exponential decay pulses, no significant impact of pulse width was found for rectangular pulses. Energy efficiency was similar for inactivation of *E. coli* in apple juice for both waveforms. Using a microscopic PEF-treatment chamber the formation of bubbles due to electrochemical reactions was observed along with changes of medium pH, which was shown to be reversible. The inactivation of a broad variety of microbes was shown in apple juice as well as in milk; the effect of a combination of PEF with mild heat was reported to be highly synergetic for both fluids. Modeling of impact of processing parameters for apple juice preservation was performed; indicating that an increase of electric field strength up to 40 kV/cm is enhancing energy efficiency, above this threshold the efficacy was mainly dependent on specific energy input. Increase of treatment temperature resulted in a drastic reduction of electric energy requirements and provided a potential to split the total energy input into a recoverable thermal and an electrical part. Making use of heat recovery the energy efficiency of a PEF processing can be improved. Product thermal load was evaluated, integrating temperature-time profiles, cook- and PU values have been determined as benchmarks. A combined application of mild heat and a low intensity PEF-treatment was shown to provide a potential to reduce maximum treatment temperature and residence time in comparison to a conventional thermal preservation of fruit juices. Inactivation of lactoperoxidase in milk after a PEF-treatment was compared to thermal inactivation effects; it was shown that a reduction of 5 – 10 % of activity occurred after a low intensity treatment, whereas increasing the energy input mainly thermal effects appeared to cause enzyme inactivation. When operating at low treatment temperature these findings allow to achieve a microbial inactivation while maintaining enzyme activity such as the bactericidal lactoperoxidase system. On the other hand retaining protein structures could be used for decontamination of milk for cheese production or for extraction of valuable substances of cells grown in a fermenter. Erosion of stainless steel electrodes was shown to be dependent on energy transferred across the electrode|media interface and pulse parameters. Leakage current of solid state switches proved to cause a large amount of electrolysis and electrode erosion, avoiding leakage current the amount of metal particles released remained far below legislative limits for tap water or fruit juices.

Apart from food processing a feasibility of PEF-application for waste and processing water was shown, a reduction of excess sludge formation was found after sludge disintegration, similar as after thermal or mechanical treatment. A treatment of microalgae extracts revealed

the applicability as a disintegration and preservation technique for cosmetic products and nutrient media.

General requirements for processing of different biological cells have been identified, a comparison of specific energy required to achieve a permeabilization of biological membranes of different origin revealed that exceeding a transmembrane potential of 1 V a permeabilization is induced. An increase of transmembrane potential improved energy efficiency for microbial, plant or animal cells. Above a potential of 10 V to 15 V the energy required to achieve a 95 % permeabilization or a 1.3 log-cycle inactivation appears to be in a range of 10 to 20 kJ/g for all biological membranes investigated. Dependent on characteristical size of biological cells a specific external electrical field needs to applied, whereas for apple or potato tissue 2 kV/cm are sufficient to induce this potential up to 100 kV/cm or above are required for small microbes such as *Listeria.*

Pilot and technical scale equipment for various applications has been designed; batch and continuous treatment chambers were realized and successfully used for field tests in cooperation with industrial partners. Based on experience obtained with this systems investment and operation costs for industrial equipment were estimated and compared to conventional processing techniques. It was shown that a disintegration of plant or animal tissue provides a tremendous economic potential in comparison to enzyme, thermal or mechanical treatment; a first industrial application has been reported in 2006 along with very promising experiences of by a German fruit juice producer. Preservation of liquid media by PEF was shown to cause operation costs in a range of 1 to 2 €-cents per liter, about 10 fold higher than for conventional thermal processing, but due to lower maximum temperature and residence time quality and consumer benefits can be found. A commercial application in a scale of 200 l/h was achieved for preservation of premium fruit juices in the USA in 2005, indicating the techniques potential to retain high quality of liquid foods. In addition different mechanisms of action in comparison to thermal processing allow the development of liquid decontamination techniques while possibly maintaining native protein structures.

Within this work the applicability of PEF was investigated from a micro-batch up to technical scale treatment. Basic data on impact of processing parameters has been acquired in lab-scale and was used to perform technical scale experiments, also investigating the PEF impact on subsequent processing such as tumbling, cooking or juice winning. A PEF application was shown to be feasible at industrial scale, as continuous treatment chambers can be scaled up within the limits technical parameters of pulse modulators. The technique can easily be implemented into existing production lines. An application in a scale larger than 100 t/h as used in sugar processing, exemplarily appears to be above the technical limited for design of pulse modulators at present. Fruit juice and fruit and vegetable processing for extraction and drying as well as treatment of meat have been identified as the most

promising fields of applications, since energy and time requirements for this applications have been shown to be superior to conventional processing. Technical scale equipment has been developed and successfully used in field tests. At present an industrial prototype for treatment of meat as well as for treatment of olives, grapes and fruit mashes is in realization.
Within the course of this work the applicability of the technique to enhance mass transfer, to modify product structures and develop new processes as well as to achieve microbial preservation was shown from research to an application level. Requirements and design considerations for PEF equipments have been presented to initiate and continue the development of pulse modulators and components to allow a transfer of the technique also to food preservation in a larger scale. Approximately 50 years after the first empirical reports of PEF application we will hopefully experience further commercial and industrial exploitation of this technique in a near future.

# Curriculum Vitae and List of Publications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### Biography

| | |
|---|---|
| Since 07/2002 | Ph.D. student at Berlin University of Technology, Prof. D. Knorr, Thesis: Pulsed Electric Fields (PEF) for Permeabilization of Cell Membranes in Food- and Bioprocessing – Applications, Process and Equipment Design and Cost Analysis. |
| 06/2002 | M.Eng. (Dipl.-Ing.) Food Technology, Berlin University of Technology Thesis: Improvement of lethal effect of Pulsed Electric Fields (PEF) for Microbial Inactivation by variation of pulse characteristics. |
| 1996-2002 | Studies of Food Technology at Berlin University of Technology |
| Date of birth | July, 25th, 1976 |

### Fields of interest

- Investigation of membrane permeabilization mechanisms and kinetics.
- Improvement of mass transfer processes in plant or animal tissue by PEF
- Impact of processing parameters on microbial inactivation by PEF
- Energy efficiency of PEF-treatment, modeling and optimization of field distribution
- Development of pulse modulation systems, treatment chamber design
- Flow cytometric analysis of electroporation of lipid bilayer vesicles and microbial membranes

## List of Publications:

### Book chapters

Toepfl, S., Heinz, V. and Knorr, D. (2006) History of pulsed electric field application. In: *Preservation of food by Pulsed Electric Fields* – Ed.: Lelieveld, H., Notermans, S., De Haan, S.W. (in press)

Toepfl, S., Heinz, V. and Knorr, D. (2006) Applications of pulsed electric field technology for the food industry: In: *Pulsed Electric Field Treatment of Foods*, Ed: Raso, J. and Heinz, V., p. 197-221, Elsevier, Oxford, UK.

Toepfl, S., Heinz, V. and Knorr, D. (2005) Overview of Pulsed Electric Field Processing of Foods. In: *Emerging Technologies for Food Processing*, Ed: Sun, D-W., p. 67-97, Elsevier, Oxford, UK.

### Publications

Toepfl, S., Mathys, A., Heinz, V. and Knorr, D. (2006) Potential of emerging technologies for energy efficient and environmentally friendly food processing. Food Reviews International (accepted, published in issue 4/2006)

Toepfl, S., Heinz, V. and Knorr, D. (2006) High Intensity Pulsed Electric Fields applied for Food Preservation. Chemical Engineering and Processing (in press)

Toepfl, S., and Knorr, D.: Pulsed electric fields as a pre-treatment technique in drying processes. Stewart Postharvesting Review (3)4, doi 10.2212/spr.2006.4.3

Heinz, V., Sitzmann, W. and Toepfl, S. (2006) Das Elsteril-Verfahren. Chemie Ingenieur Technik (submitted)

Lehmann, C., Biela, S, Toepfl, S., Jansen, G. and Voegel, R. Solanum scabrum (Garden Huckleberry) – a potential source of a coloring plant extract. Euyphytica (submitted)

Schilling, S., Alber, T., Toepfl, S., Neidhart, S., Knorr, D., Schieber, A., and Carle, R. (2006) Effects of Pulsed Electric Field Treatment of Apple Mash on Juice Yield and Quality Attributes of Apple Juices. Innovative Food Science and Emerging Technologies (submitted)

Corrales, M., Toepfl, S., Smittlein T, Butz, P., Knorr, D. and Tauscher, B. (2006) Recovery of anthocyanins and phenolics from Dornfelder grape pomace (*Vitis vinifera spp*.) with high-hydrostatic pressures. Comparison with other extraction methods. Trends in Food Science and Technology (submitted)

Toepfl, S., Jaeger, H., Heinz., V and Knorr, D. (2006) Neues Verfahren zur Haltbarmachung von Milch. Deutsche Molkerei Zeitung, 127:24-28

Toepfl, S., Heinz, V. and Knorr, D. (2005) PEF application in food production – development of a technical scale system Proceedings of: Intradfood 2005, Elsevier, London, 1387-1390. ISBN 84-9705-881-8.

Toepfl, S. and Knorr, D. (2005) The use of pulsed electric field (PEF) technology in food processing (applications in addition to microbial inactivation). International Review of Food Science and Technology, 109-111.

Guderjan, M., Toepfl, S., Angersbach, A. and D. Knorr (2005) Impact of pulsed electric field treatment on the recovery and quality of plant oils. Journal of Food Engineering (67)3, 281-287.

Toepfl, S., Heinz, V. and Knorr, D. (2004) Optimization of Pulsed Electric Field Treatment for Liquid Food Pasteurization. In: 2nd European Pulsed Power Symposium (EPPS) 2004. Ed.: International Society on Pulsed Power Applications, Shaker, Aachen. p. 68-72. ISBN 3-8322-3217-6.

Koners, U. Toepfl, S., Heinz, V., Camacho, P. Ginestet, P. and Knorr, D. (2004) Application of Pulsed Electric Field Treatment for sludge reduction on waste water treatment plants. In: 2nd European Pulsed Power Symposium (EPPS) 2004. Ed.: International Society on Pulsed Power Applications, Shaker, Aachen. p. 47-52. ISBN 3-8322-3217-6.

Toepfl, S., Dominkovics, M., Heinz, V., Bartelt, E. and Knorr, D. (2004) Pulsed Electric Field Application for Inactivation of *L. monocytogenes* in raw milk. In: Proceedings of the *5th World Congress Foodborne Infections and Intoxications*. June, 7 – 11th, Berlin, Germany

Heinz, V., Toepfl, S., and Knorr, D. (2003) Impact of Temperature on Lethality and Energy Efficiency of Apple Juice Pasteurization by Pulsed Electric Fields Treatment. Innovative Food Science and Emerging Technologies. (4) 167-175.

Heinz, V., Toepfl, S., and Knorr, D. (2002) Optimization of heat decontamination of liquid media using Pulsed electric fields, In: *Proceedings of the IEEE Pulsed Power Symposium 2002,* pp 21/1-21/6.

**Presentations**

Toepfl, S., Heinz, V. and Knorr, D. (2006) Meat drying enhancement by Pulsed Electric Fields. Presented at: IUFOST World Congress Food is Life, September 21st, Nantes, France.

Toepfl, S., Zunke, M., Heinz, V. and Knorr, D. (2006) Enhancement of Meat Processing by PEF appplication. Presented at: IFT-EFFOST Workshop on Applications of Novel Technologies in Food and Biotechnology, September, 11th, Cork, Ireland.

Toepfl, S., Zunke, M., Heinz, V. and Knorr, D. (2006) Pulsed Electric Field (PEF) Processing of Meat. Presented at: *Food Factory of the Future 3,* June, 8th, Gothenburg, Sweden.

Toepfl, S., Heinz, V. and Knorr, D. (2006) Developments of PEF Application for Liquid Food Processing. Presented at: *Effost Congress Processing Developments of Liquids*, April, 3rd, Cologne, Germany.

Toepfl, S., Heinz, V. and Knorr, D. (2006) Die Anwendung von Hochspannungsimpulsverfahren zur Konservierung von Lebensmitteln. Presented at: *Konservieren ohne Konservierungsstoffe*, 22.02.2006, München, Germany.

Toepfl, S. and Knorr, D. (2005) Anwendung von Hochspannungsimpulsen als Zellaufschlussverfahren bei der Apfelsaftproduktion. Presented at: *VDF Professorentreffen Hohenheim*, November, 3rd, Hohenheim, Germany.

Toepfl, S., Heinz, V. and Knorr, D. (2005) Anwendung gepulster elektrischer Felder als Zellaufschluss- und Konservierungsverfahren. Presented at: *GDL Kongress Lebensmitteltechn*ologie, Oktober 7th, Dresden, Germany.

Koehler, E., S. Toepfl, D. Knorr and O. Pulz (2005). Unconventional procedures for the production and stabilization of extracts with active agents. Presented at: 6th European Workshop Microalgal Biotechnology, May, 23rd, Potsdam, Germany.

Toepfl, S. and Knorr, D. (2005) Pulsed Electric Fields in Food Technology
-Applicability for Fruit Juice Production. Presented at: *European Symposium on Apple Processing*, March, 16th, 2005, Rennes, France.

Toepfl, S., Heinz, V. and Knorr, D. (2005) Die Anwendung gepulster elektrischer Felder in der Lebensmitteltechnologie am Beispiel der Fruchtsaftproduktion. Presented at: *VDI GVC Fachausschussitzung Lebensmittelverfahrenstechnik,* March, 8, 2005, Berlin, Germany.

Toepfl, S., Heinz, V. and Knorr, D. (2004) Die Anwendung von Hochspannungsimpulsverfahren und Ultraschall in der Lebensmitteltechnologie. Presented at: *Verband der deutschen Fruchtsaftindustrie (VdF) e.V.,Professorentreff.* November 3rd, Berlin, Germany.

Toepfl, S., Heinz, V. and Knorr, D. (2004) Optimization of Pulsed Electric Field Treatment for Liquid Food Pasteurization. Presented at: *2nd European Pulsed Power Symposium (EPPS) 2004*. September, 20-23, Hamburg, Germany.

Toepfl, S., Heinz, V. and Knorr, D. (2004) Die Anwendung von Hochspannungsimpulsverfahren in der Lebensmitteltechnologie. Presented at: *DFG – Senatskommission zur Beurteilung der gesundheitlichen Unbedenklichkeit von Lebensmitteln (SKLM).* April 22nd, Berlin, Germany.

Toepfl, S., Heinz, V. and Knorr, D. (2004) Optimization of Pulsed Electric Field Processing Parameters and Treatment Chamber Design. Presented at: *Safe Consortium Seminar: Novel Preservation technologies in relation to food safety.* January 23, Brussel, Belgium.

Toepfl, S., Heinz, V. and Knorr, D. (2003) Optimization of Pulsed Electric Field Application for Liquid Food Decontamination. Presented at: *IFT Workshop on Nonthermal Food Preservation.* September, 7th – 10th, Wageningen, The Netherlands.

Toepfl, S., Heinz, V. and Knorr, D. (2003) Pulsed Electric Field Processing in Improving Product Safety in Apple Juice. Presented at: *Safe Consortium Seminar: Newly Emerging Pathogens, including risk assessment and management.* April 25th, Brussel, Belgium.

**Poster presentations**

Toepfl, S., Heinz, V. and Knorr, D. (2006) Meat drying enhancement by Pulsed Electric Fields. Presented at: IUFOST World Congress Food is Life, September 21st, Nantes, France.

Toepfl, S., Jaeger, H. Heinz, V. and Knorr, D. (2006) Milk Preservation by PEF – Potential to utilize native antimicrobial activity of milk Lactoperoxidase system. Presented at: IUFOST World Congress Food is Life, September 21st, Nantes, France.

Janositz, A., Toepfl, S., and Knorr, D. (2006) Pulsed Electric Field Application for Reduction of Vegetable Grinding Energy. Presented at: IUFOST World Congress Food is Life, September 21st, Nantes, France.

Toepfl, S., Heinz, V. and Knorr, D. (2006) Pulsed Power Application in Food Processing
- Equipment Design and Cost Analysis. Presented at: Euro-Asian Pulsed Power Conference 2006, September, 18th, Chengdu, China.

Toepfl, S., Zunke, M., Heinz, V. and Knorr, D. (2006) Enhancement of Meat Processing by PEF appplication. Presented at: IFT-EFFOST Workshop on Applications of Novel Technologies in Food and Biotechnology, September, 11th, Cork, Ireland.

Janositz, A., Toepfl, S. and Knorr, D. (2006) Impact of Pulsed Electric Fields on membranes on a cellular level. Presented at: *Food Factory of the Future 3*, June, 8th, Gothenburg, Sweden.

C. Lehmann, Biela, C., Toepfl , S., Jansen, G. und R. Vögel (2006) Ist (Garden Huckleberry) zur Gewinnung von Lebensmittelfarbe geeignet? Presented at: *43rd Conference of Horticultural Science*, February 22nd – 24th, Potsdam, Germany.

Toepfl, S., Heinz, S. and Knorr, D. (2005) Improvement of mass transport in cellular tissue by PEF – applications, equipment design and cost estimation. Presented at: *Intradfood 2005*, October, 25 - 28th, Valencia, Spain.

Toepfl, S., Ruehle, C. and Heinz, V. (2005) PEF Fish and Meat Processing. Presented at: *Intradfood 2005*, October, 25 - 28th, Valencia, Spain.

Balasa, A., Toepfl, S. and Knorr, D. (2005) Impact of Pulsed Electric Field Treatment on extraction yield of total polyphenolics from grapes, Presented at: *78. FEI Jahrestagung*, September, 13 – 14th, Berlin, Germany.

Janositz, A., Toepfl, S. and Knorr, D. (2005), Mikroskopische Visualisierung von pflanzlichem Gewebe während der Behandlung mit Hochspannungsimpulsen, Presented at: *78. FEI Jahrestagung*, September, 13 – 14th, Berlin, Germany

Toepfl, S., Ruehle, C. and Heinz, V. (2005) PEF Fish and Meat Processing. Presented at: *Workshop of IFT Nonthermal Processing Division*, September 14 – 16th, Philadelphia. US

Toepfl, S., Heinz, V. and Knorr, D. (2005) Improvement of mass transport in cellular tissue by PEF – applications, equipment design and cost estimation. Presented at: *Workshop of IFT Nonthermal Processing Division*, September 14 – 16th, Philadelphia. US

Maslak, D., Heinz, V., Toepfl, S. and Knorr, D. Determining bacterial inactivation in response to simultaneous treatment with Pulsed Electric Fields and High Pressure by flow cytometric assessment. Presented at*: Workshop of IFT Nonthermal Processing Division*, September 14 – 16th, Philadelphia. US

Toepfl, S., Heinz, V. and Knorr, D. (2005) Pulsed Electric Field Application of Liquid Food. Presented at: *ISEKI-Foods Network Meeting*, May 26-28th, Faro, Portugal.

Toepfl, S., Heinz, V. and Knorr, D. (2005) Potential of High Hydrostatic Pressure and Pulsed Electric Fields in Bio-Processing. Presented at: *6th European Workshop of Microalgal Biotechnology*, May 23-25th, Potsdam, Germany.

Toepfl, S., Ananta, E., Heinz, V. and Knorr, D. (2004). Inactivation of *L. monocytogenes* in raw milk by high intensity pulsed electric fields. Presented at: *Food Innovations for an Expanding Europe 2004,* October 27 - 296th, Warszawa, Poland.

Toepfl, S., Ananta, E., Heinz, V. and Knorr, D. (2004. Inactivation of *L. monocytogenes* in raw milk by high intensity pulsed electric fields. Presented at: *FoodMicro 2004,* September, 12 - 16th, Portoroz, Slovenia.

Toepfl, S., Dominkovics, M., Heinz, V., Bartelt, E. and Knorr, D. (2004) Pulsed Electric Field Application for Inactivation of *L. monocytogenes* in raw milk. Presented at*: 5th World Congress Foodborne Infections and Intoxications*. June, 7 – 11th, Berlin, Germany

Toepfl, S., Heinz, V. and Knorr, D. (2004) Optimization of pulsed electric field treatment efficiency for fruit juice pasteurization. Presented at: *International Conference on Engineering and Foods (ICEF9)*. March, 7-11th, Montpellier, France.

# References

Abou-Ghazala, A. and Schoenbach, K. H. (2000). Biofouling Prevention with Pulsed Electric Fields. IEEE Transactions on Plasma Science 28(1): 115-121.

Abram, F., Smelt, J. P. P. M., Bos, R. and Wouters, P. C. (2003). Modelling and optimization of inactivation of Lactobacillus plantarum by pulsed electric field treatment. Journal of Applied Microbiology 94: 571-579.

Ade-Omowaye, B. I. O., Angersbach, A., Taiwo, K. A. and Knorr, D. (2001). Use of pulsed electric field treatment to improve dehydration characteristics of plant based foods. Trends in Food Science and Technology 12: 285-295.

Ade-Omowaye, B. I. O., Rastogi, N. K., Angersbach, A. and Knorr, D. (2001). Effects of high hydrostatic pressure or high intensity electrical field pulse pre-treatment on dehydration characteristics of red paprika. Innovative Food Science and Emerging Technologies 2: 1-7.

Allen, M. and Soike, K. (1966). Sterilization by electrohydraulic treatment. Science 154: 155-157.

Allen, M. and Soike, K. (1967). Disinfection by electrohydraulic treatment. Science 156: 524-525.

Alvarez, I., Condon, S. and Raso, J. (2006). Microbial inactivation by pulsed electric fields. *Pulsed Electric Field Technology for the Food Industry. Fundamentals and Applications.* J. Raso and V. Heinz. Berlin, Springer: 95-127.

Alvarez, I., Pagan, R., Condon, S. and Raso, J. (2003). The influence of process parameters for the inactivation of *L. monocytogenes* by pulsed electric fields. International Journal of Food Microbiology 87: 87-95.

Alvarez, I., Raso, J., Palop, A. and Sala, F.J. (2000). Influence of factors on the inactivation of *Salmonella Senftemberg* by pulsed electric fields. International Journal of Food Microbiology 55: 143-146.

Amatore, C., Berthou, M. and Hébert, S. (1998). Fundamental Principles of electrochemical ohmic heating of solutions. Journal of Electroanalytical Chemistry(457): 191-203.

Angersbach, A., Heinz, V. and Knorr, D. (1997). Elektrische Leitfähigkeit als Maß des Zellaufschlussgrades von zellularen Materialien durch Verarbeitungsprozesse. Lebensmittelverfahrenstechnik 42: 195-200.

Angersbach, A., Heinz, V. and Knorr, D. (1999). Electrophysiological model of intact and processed plant tissues: cell disintegration criteria. Biotechnol.Prog. 15: 753-762.

Angersbach, A., Heinz, V. and Knorr, D. (2000). Effects of pulsed electric fields on cell membranes in real food systems. Innovative Food Science and Emerging Technologies 1: 135-149.

Angersbach, A. and Knorr, D. (1997). Anwendung elektrischer Hochspannungsimpulse als Vorbehandlungsverfahren zur Beeinflussung der Trocknungscharakteristika und Rehydrationseigenschaften von Kartoffelwürfeln. Nahrung 41: 194-200.

Anonymous (1995). Kalt erwischt. Wirtschaftswoche 12: 119-123.

Anonymous (1998). Outline of the Japanese Research and Development Association for Application of Electronic Technology in Food Industry., The Japanese Research and Development Association for Application of Electronic Technology in Food Industry.

Anonymous (2003). Umfrage zur Klärschlamm-Entsorgung
in Baden-Württemberg. Herzebrock-Clarholz, Dienstleistungsgesellschaft des Gemeindetags Baden-Würtemberg.

Anonymous (2006). Verfahren zur besseren und schonenden Freisetzung wertgebender Inhaltsstoffe aus Weinbeeren, ein daraus gewonnener Most sowie daraus erzeugter Wein und eine Einrichtung zur Durchführung der Elektroporation.German Patent, DE 10 2004 013 762 A1.

Arevalo, P., Ngadi, M. O., Bazhal, M. and Raghavan, V. G. S. (2004). Impact of Pulsed Electric Fields on the Dehydration and Physical Properties of Apple and Potato Slices. Drying Technology 22(5): 1233-1246.

Aronsson, K., Rönner, U. and Borch, E. (2005). Inactivation of *Escherichia coli, Listeria innocua* and *Saccharomyces cerevisiae* in relation to membrane permeabilization and subsequent leakage of intracellular compounds due to pulsed electric field processing. International Journal of Food Microbiology 99: 19-32.

Ayhan, Z., Zhang, Q. H. and Min, D. B. (2002). Effects of pulsed electric field processing and storage on the quality and stability of single-strength orange juice. Journal of Food Protection 65(10): 1623-1627.

Balasa, A., Toepfl, S. and Knorr, D. (2006). Pulsed Electric Field treatment of grapes. *Food Factory of the Future 3*, Gothenburg, Sweden.

Barbosa-Cánovas, G. V., Góngora-Nieto, M. M., Pothakamury, U. R. and Swanson, B. G. (1999). Preservation of foods with pulsed electric fields. San Diego, Academic Press.

Barbosa-Cánovas, G. V., Pothakamury, U. R., Palou, E. and Swanson, B. G. (1998). Nonthermal preservation of foods. New York, Marcel Dekker.

Barsotti, L., Merle, P. and Cheftel, J. C. (1999). Food processing by pulsed electric fields: 2. Biological effects. Food Reviews International 15(2): 181-213.

Bazhal, M., Lebovka, N. I. and Vorobiev, E. (2001). Pulsed electric field treatment of apple tissue during compression for juice extraction. Journal of Food Engineering 50: 129-139.

Bazhal, M., Ngadi, M. O. and Raghavan, V. G. S. (2003). Influence of pulsed electroplasmolysis on the porous structure of apple tissue. Biosystems Engineering 86(1): 51-57.

Bazhal, M. and Vorobiev, E. (2000). Electrical Treatment of apple cossettes for intensifying juice pressing. Journal of the Science of Food and Agriculture 80: 1668-1674.

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. Journal of Hygiene 24: 123-137.

Bendicho, S., Barbosa-Cánovas, G. V. and Martin, O. (2002). Milk processing by high intensity pulsed electric fields. Trends in Food Science and Technology 13(195-204).

Bendicho, S., Barbosa-Cánovas, G. V. and Martín, O. (2003). Reduction of Protease Activity in Simulated Milk Ultrafiltrate by Continuous Flow High Intensity Pulsed Electric Field Treatments. Journal of Food Science 68(3): 952-957.

Bendicho, S., Estela, C., Giner, J., Barbosa-Cánovas, G. V. and Martin, O. (2002). Effects of high intensity pulsed electric field and thermal treatments on a lipase from *Pseudomonas fluorescens*. Journal of Dairy Science 85: 19-27.

Bendicho, S., Marsellles-Fontanet, A. R., Barbosa-Cánovas, G. V. and Martin-Belloso, O. (2005). High Intensity pulsed electric fields and heat treatments applied to a protease from *Bacillus subtilis*. A comparison of multiple systems. Journal of Food Engineering 69: 317-323.

Bergey (1986). Manual of Systematic Bacteriology. Baltimore, Williams and Wilkins.

Beuf, M., Rizzo, G., Leuliet, J. C., Müller-Steinhagen, H., Yiantsios, S., Karabelas, A. and Benezech, T. (2004). Fouling and cleaning of modified stainless steel plate heat exchangers processing milk products. *ECI Conference on Heat Exchanger Fouling and Cleaning: Fundamentals and Applications*, Santa Fe, USA.

Bliesener, K.-M., Miehe, D. and Buchholz, K. (1991). Verfahrensentwicklungen zur Schnitzelentwässerung. Zuckerindustrie 116: 978-986.

Bluhm, H. J. (2006). Pulsed Power Systems. Berlin, Germany, Springer.

Bouzrara, H. and Vorobiev, E. (2000). Beet Juice Extraction by pressing and pulsed electric fields. International Sugar Journal 102(1216): 194-200.

Boyko, N. I., Tur, A. N., Evdoshenko, L. S., Zarochentsev, A. I. and Ivanov, V. M. (1998). A high-voltage pulse generator with mean power up to 50kW for the treatment of foodstuffs. Instruments and Experimental Techniques 41(2): 111-117.

Braakman, L. (2003). Breakthrough in Pasteurisation - Pulsed Electric Fields. Food Engineering and Ingredients: 34-38.

Bushnell, A. H., Clark, R. W., Dunn, J. E. and Lloyd, S. W. (1996). Process for reducing levels of microorganisms in pumpable food products using a high pulsed voltage system.US Patent, 5,514,391.

Caplot, M. and Cote, G. (1999). Les technologies nécessaires aux machines de traitment par Champs Electriques Pulsés. *Formation Technologique en Agro-Alimentaire: La conservation de demain*, Bordeaux Pessac, France.

Castro, A. J., Swanson, B. G., Barbosa-Cánovas, G. V. and Zhang, Q. H. (2001). Pulsed electric field modification of milk alkaline phosphatase activity. *Electric Fields in Food Processing*. G. V. Barbosa-Cánovas and Q. H. Zhang. Lancaster, PA, Technomic: 65-82.

Chalermchat, Y. (2005). Effects of Pulsed Electric Fields on Plant Tissue. Department of Food Technology, Engineering and Nutrition. Lund, Sweden, Lund University.

Chang, D. C., Chassy, B. M., Saunders, J. A. and Sower, A. E. (1992). Guide to electroporation and electrofusion. San Diego, Academic Press.

Chernomordik, L. V. (1992). Electropores in lipid bilayers and cell membranes. *Guide to Electroporation and Electrofusion*. D. C. Chang, B. M. Chassy, J. A. Saunders and A. E. Sowers. San Diego, Academic Press.

Chernomordik, L. V., Sukharev, S. I., Popov, S. V., Pastushenko, V. F., Sokirko, A. V., Abidor, I. G. and Chizmadzhev, Y. A. (1987). The electrical breakdown of cell and lipid membranes: The similarities of phenomenologies. Biochimica Biophysica Acta(902): 360-373.

Clark, P. (2006). Pulsed Electric Field Processing. Food Technology 60: 66-67.

Coster, H. G. L. (1965). A quantitive analysis of the voltage-current relationships of fixed charge membranes and the associated property of "punch-through". Biophysical Journal. 5: 669-686.

Cousin, J.-F. (2003). Process for treating vegetables and fruits before cooking.US Patent, 20030165608.

Crowley, J. M. (1973). Electrical breakdown of bimolecular lipid membranes as an electromechanical instability. Biophysical Journal. 13: 711-724.

Cserhalmi, Z., Sass-Kiss, A., Tóth-Markus, M. and Lechner, N. (2006). Study of pulsed electric field treated citrus juices. Innovative Food Science and Emerging Technologies 7: 49-54.

Cserhalmi, Z., Vidacs, I., Beczner, J. and Czukor, B. (2002). Inactivation of *Saccharomyces cerevisiae* and *Bacillus cereus* by pulsed electric field technology. Innovative Food Science and Emerging Technologies 3(1): 41-45.

De Haan, S. W. H. and Willcock, P. R. (2002). Comparison of the energy performance of pulse generation circuits for PEF. Innovative Food Science and Emerging Technologies(3): 349-356.

Dimitrov, D. S. (1984). Electric field-induced breakdown of lipid bilayers and cell membranes: a thin viscoelstic film model. The Journal of Membrane Biology 78: 53-60.

Doevenspeck, H. (1960). Verfahren und Vorrichtung zur Gewinnung der einzelnen Phasen aus dispersen Systemen.German Patent, DE 1237541.

Doevenspeck, H. (1961). Influencing cells and cell walls by electrostatic impulses. Fleischwirtschaft 13(12): 968-987.

Doevenspeck, H. (1963). Elektro-Impuls-Verfahren zur Anregung des Stoffwechsels von Mikroorganismen. Minden: 3.

Doevenspeck, H. (1975). Beobachtungen der Auswirkungen von Kondensatorentladungen an animalischen und vegetabilen Rohstoffen sowie bei der Behandlung von Mikroorganismen und molekularen Strukturen. Research Report. Minden.

Dörnenburg, H. and Knorr, D. (1998). Monitoring the impact of high pressure processing on the biosynthesis of plant metabolites using plant cell cultures. Trends in Food Science and Technology 9: 355-361.

Dunn, J. E. and Pearlman, J. S. (1987). Methods and apparatus for extending the shelf life of fluid food products.United States, US 4695472.

Edebo, L. and Selin, I. (1968). The effect of the pressure shock wave and some electrical quantities in the microbicidal effect of transient electric arcs in aqueous systems. Journal of General Microbiology 50: 253-259.

El-Belghiti, K., Rabhi, Z. and Vorobiev, E. (2005). Kinetic model of sugar diffusion from sugar beet tissue treated by pulsed electric field. Journal of the Science of Food and Agriculture 85: 213-218.

Elez-Martínez, P., Aguilo-Aguayo, I. and Martín-Belloso, O. (2006). Inactivation of orange juice peroxidase by high-intensity pulsed electric fields as influeced by processing parameters. Journal of the Science of Food and Agriculture 86: 71-81.

Elez-Martínez, P., Suarez-Recio, M. and Martín-Belloso, O. (2005). Modelling the reduction of pectin methyl esterase activity in orange juice by high intensity pulsed electric fields. Journal of Food Engineering: in press.

Eshtiaghi, M. N. and Knorr, D. (1999). Process for treatment of sugar beet.European Patent, EP 99923708.

Eshtiaghi, M. N. and Knorr, D. (2000). Anwendung elektrischer Hochspannungsimpulse zum Zellaufschluss bei der Saftgewinnung am Beispiel von Weintrauben. LVT 45: 23-27.

Eshtiaghi, M. N. and Knorr, D. (2002). High electric field pulse treatment: potential for sugar beet processing. Journal of Food Engineering 52: 265-272.

Evrendilek, G. A., Jin, Z. T., Ruhlman, K. T., Qiu, X., Zhang, Q. H. and Richter, E. R. (2000). Microbial safety and shelf-life of apple juice and cider processed by bench and pilot scale PEF systems. Innovative Food Science and Emerging Technologies 1: 77-86.

Evrendilek, G. A., Li, S., Dantzer, W. Q. and Zhang, Q. H. (2004). Pulsed Electric Field processing of beer: microbial, sensory and quality analyses. Journal of Food Science 96(8): 228-232.

Evrendilek, G. A. and Zhang, Q. H. (2003). Effects of pH, temperature, and pre-pulsed electric field treatment on pulsed electric field and heat inactivation of *Escherichia coli* O157:H7. Journal of Food Protection 66(5): 755-759.

Evrendilek, G. A. and Zhang, Q. H. (2005). Effect of pulse polarity and pulse delaying time on pulsed electric fields-induces pasteurization of *E. coli* O157:H7. Journal of Food Engineering 68: 271-276.

FDA (2003). Genesis Warning Letter. Bothell, USA.

Fetterman, J. C. (1928). The electrical conductivity method of processing milk. Agricultural Engineering 9(4): 107-108.

Fiala, A., Wouters, P. C., van den Bosch, E. and Creyghton, Y. L. M. (2001). Coupled electrical-fluid model of pulsed electric field treatment in a model food system. Innovative Food Science and Energing Technologies 2: 229-238.

Fincan, M. and Dejmek, P. (2003). Effect of osmotic pretreatment and pulsed electric field on the viscoleastic properties of potato tissue. Journal of Food Engineering 59: 169-175.

Fincan, M., DeVito, F., Dejmek, P. (2004). Pulsed electric field treatment for solid-liquid extraction of red beetroot pigment. Journal of Food Engineering 64: 381-388.

Flaumenbaum, B. L. (1949). Electrical treatment of fruits and vegetables before juice extraction. Trudy OTIKP 3: 15-20.

Flaumenbaum, B. L. (1968). Anwendung der Elektroplasmolyse bei der Herstellung von Fruchtsäften. Flüssiges Obst 35: 19-22.

Fleischman, G., Ravishankar, S. and V.M., B. (2004). The inactivation of *Listeria monocytogenes* by pulsed electric field (PEF) treatment in a static chamber. Food Microbiology 21: 91-95.

Fox, M. B., Esveld, D. C., Valero, A., Luttge, R., Mastwijk, H. C., Bartels, P. V., Van den Berg, A. and Boom, R. M. (2006). Electroporation of cell in microfluidic devices: a review. Analytical and Bioanalytical Chemistry 385: 474-485.

Fruengel, F. (1960). Method and device for electrically sterilizing and cleaning milking machines or the like.US Patent, 2931947.

Garcia, D., Gómez, N., Manas, P., Condon, S., Raso, J. and Pagan, R. (2005). Occurence of sublethal injury after pulsed electric fields depending on the microorganism, the treatment medium pH and the intensity of the treatment investigated. Journal of Applied Microbiology 99(1): 94-104.

Gaskova, D., Sigler, K., Janderova, B. and Plasek, J. (1996). Effect of high-voltage electric pulses on yeast cells: factors influencing the killing efficiency,. Bioelectrochemistry Bioenergetics 39: 195-202.

Gaudreau, M. P. J., Hawkey, T., Petry, J. and Kempkes, M. (2004). Design considerations for pulsed electric field processing. *2nd European Pulsed Power Symposium*, Hamburg, Shaker, Aachen.

Gaudreau, M. P. J., Hawkey, T., Petry, J. and Kempkes, M. A. (2001). A solid state pulsed power system for food processing. *24th Pulses Power Plasma Science Conference*, Las Vegas, USA.

Getchell, B. E. (1935). Electric pasteurization of milk. Agricultural Engineering 16(10): 408-410.

Geulen, M., Teichgräber, P., Knorr, D., Grahl, T. and Sitzmann, W. (1992). Elektroimpulsbehandlung von Karottenmaische. *Symposium aktueller Stand und Trends der Lebensmitteltechnologie*, Hohenheim, Germany.

Gilliland, S. E. and Speck, M. L. (1967a). Inactivation of microorganisms by electrohydraulic shock. Applied Microbiology 15(5): 1031-1037.

Gilliland, S. E. and Speck, M. L. (1967b). Mechanism of the bactericidal action produced by electrohydraulic shock. Applied Microbiology 15(5): 1038-1044.

Giner, J., Bailo, E., Gimeno, V. and Martin-Belloso, O. (2005). Models in a Bayesian framework for inactivation of pectinesterase in a commercial enzyme formulation by pulsed electric fields. European Food Research Technology 221: 255-264.

Glaser, R. W., Leikin, S. L., Chernomordik, L. V. and Pastushenko, V. F. a. S., A.I. (1998). Reversible electrical breakdown of lipid bilayers: Formation and evolution of pores. Biochimica Biophysica Acta(940): 275-287.

Góngora-Nieto, M. M., Pedrow, P. D., Swanson, B. G. and Barbosa-Cánovas, G. V. (2003). Energy analysis of liquid whole egg pasteurized by pulsed electric fields. Journal of Food Engineering 57: 209-216.

Góngora-Nieto, M. M., Pedrow, P. D., Swanson, B. G. and Barbosa-Cánovas, G. V. (2003). Impact of air bubbles in a dielectric liquid when subjected to high electric field strengths. Innovative Food Science and Emerging Technologies 4: 57-67.

Góngora-Nieto, M. M., Sepúlveda, D. R., Pedrow, P., Barbosa-Cánovas, G. V. and Swanson, B. G. (2002). Food Processing by Pulsed Electric Fields: Treatment Delivery, Inactivation Level and Regulatory Aspects. Lebensmittel-Wissenschaft und Technologie. 35: 375-388.

Gossling, B. S. (1960). Artificial Mutation of micro-organisms by electrical shock.United Kingdom, UK 845743.

Grahl, T. (1994). Abtöten von Mikroorganismen mit Hilfe elektrischer Hochspannungsimpulse. Hamburg, TU Hamburg-Harburg, Germany.

Grahl, T. and Märkl, H. (1996). Killing of microorganisms by pulsed electric fields. Applied Microbiology and Biotechnology 45: 148-157.

Guderjan, M., Toepfl, S., Angersbach, A. and Knorr, D. (2005). Impact of pulsed electric field treatment on the recovery and quality of plant oils. Journal of Food Engineering 67(3): 281-287.

Guenther, U. (2006). Application of Electroporation for treatment of apple mash in indsutrial scale. Personal communication.

Hafsteinsson, H., Gudmundsson, M., Arnarson, G. Ö., Jónsson, Á. and Siguroardottir, M. S. (2000). High electric field pulses: food safety; quality; and critical parameters.: Technological Institute of Iceland (IceTec).

Halbach, U., Eichhorn, U., Hallbach, M. and Behlke, A. (2003). Abwasserentsorgung in Brandenburg. U. u. R. d. L. B. Ministerium für Landwirtschaft. Potsdam, Ministerium für Landwirtschaft, Umweltschutz und Raumordnung des Landes Brandenburg: 61.

Harrison, S. L., Barbosa-Cánovas, G. V. and Swanson, B. G. (1997). Saccharomyces cerevisae structural changes induced by pulsed electric field treatment. Lebensmittel-Wissenschaft und Technologie. 30: 236-240.

Heinz, V., Alvarez, I., Angersbach, A. and Knorr, D. (2002). Preservation of liquid foods by high intensity pulsed electric fields-basic concepts for process design. Trends in Food Science and Technology 12: 103-111.

Heinz, V. and Knorr, D. (2000). Effect of pH, ethanol addition and high hydrostatic pressure on the inactivation of *Bacillus subtilis* by pulsed electric fields. Innovative Food Science and Emerging Technologies 1: 151-159.

Heinz, V., Phillips, S. T., Zenker, M. and Knorr, D. (1999). Inactivation of *Bacillus subtilis* by high intensity pulsed electric fields under close to isothermal conditions. Food Biotechnology 13(2): 155-168.

Heinz, V., Toepfl, S. and Knorr, D. (2003). Impact of temperature on lethality and energy efficiency of apple juice pasteurization by pulsed electric fields treatment. Innovative Food Science and Emerging Technologies 4(2): 167-175.

Herrera-Medez, C. H., Becila, S., Boudjellal, A. and Oualo, A. (2006). Meat ageing, reconsideration of the current concept. Trends in Food Science and Technology 17: 394-405.

Ho, S. and Mittal, G. S. (2000). High voltage pulsed electrical field for liquid food pasteurization. Food Reviews International 16(4): 395-434.

Ho, S. Y. and Mittal, G. S. (1996). Electroporation of cell membranes: a review. Critical Reviews in Biotechnology 16(4): 349-362.

Hodgins, A. M., Mittal, G. S. and Griffiths, M. W. (2002). Pasteurization of Fresh Orange Juice Using Low-Energy Pulsed Electrical Field. Journal of Food Science 67(6): 2294-2299.

Hoefel, D., Grooby, W. L., Monis, P. T., Andrews, S. and Saint, C. P. (2003). A comparative study of carboxyfluorescein diacetate and carboxyfluorescein diacetate succinimidyl ester as indicators of bacterial activity. Journal of Microbiolical Methods 52: 379–388.

Hülsheger, H. and Niemann, E. G. (1980). Lethal effects of high voltage pulses on *E. coli* K12. Radiation and Environmental Biophysics 18: 281-288.

Hülsheger, H., Potel, J. and Niemann, E. G. (1981). Killing of bacteria with electric pulses of high field strength. Radiation and Environmental Biophysics 20: 53-65.

Hülsheger, H., Potel, J. and Niemann, E. G. (1983). Electric field effects on bacteria and yeast cells. Radiation and Environmental Biophysics 22(149-162).

Jacob, H. E., Förster, W. and Berg, H. (1981). Microbiological Implications of electric field effects. II. Inactivation of yeast cells and repair of their envelope. Zeitschrift für Allgemeine Mikrobiologie 21(3): 225-233.

Jayaram, S., Castle, G. S. P. and Magaritis, A. (1993). The effect of high field DC pulse and liquid medium conductivity on survivability of *L. brevis*. Applied Microbiology and Biotechnology. 40: 117-122.

Jemai, A. B. and Vorobiev, E. (2002). Effect of moderate electric field pulses on the diffusion coefficient of soluble substances from apple slices. International Journal of Food Science and Technology 37: 73-86.

Jeyamkondan, S., Jayas, D. S. and Holley, R. A. (1999). Pulsed electric field processing of foods: a review. Journal of Food Protection 62(9): 1088-1096.

Kern, M. (2005). Bipolarer Marxgenerator mit Schalterturm, direkter Triggerung und Gaskonditionierung für industriellen Dauerbetrieb.German Patent, DE 10 2004 001 782 A1.

Kern, M. (2006). Installation of an industrial scale PEF equipment. Personal communication

Kinosita, K. and Tsong, T. Y. (1997). Voltage induced pore formation and hemolysis of human erythrocyte membranes. Biochim. Biophys. Acta(471): 227-242.

Knorr, D. and Angersbach, A. (1998). Impact of high-intensity electric field pulses on plant membrane permeabilization. Trends in Food Science and Technology 9: 185-191.

Knorr, D., Angersbach, A., Eshtiaghi, M., Heinz, V. and Lee, D.-U. (2001). Processing concepts based on high intensity electric field pulses. Trends in Food Science and Technology 12: 129-135.

Knorr, D., Geulen, M., Grahl, T. and Sitzmann, W. (1994). Food application of high electric field pulses. Trends in Food Science and Technology 5: 71-75.

Kopplow, O., Barjenbruch, M. and Heinz, V. (2004). Sludge pre-treatment with pulsed electric fields. Water Science and Technology 49(10): 123-129.

Kotnik, T., Pucihar, G., Rebersek, M., Miklavcic, D. and Mir, L. M. (2003). Role of pulse shape in cell membrane electropermeabilization. Biochimica Biophysica Acta(1614): 193-200.

Kraus, W. (2003). The 2002 beet campaign - VDZ Zweigverein Süd. Zuckerindustrie 128(5): 344-354.

Kraus, W. (2004). Reports on the 2003 campaign - VDZ Zweigverein Süd. Zuckerindustrie 129(5): 349-363.

Krupp (1988). Fischverarbeitung nach dem ELCRACK Prozess. Krupp Maschinentechnik. GmbH. Hamburg-Harburg, Germany.

Kuthi, A., Alde, R., Gundersen, M. and Neuber, A. (2003). Marx generator using pseudospark switches. *Pulsed Power Conferenc 2003, Digest of Technical Papers*, Dallas, USA, June 15$^{th}$ -18$^{th}$.

Lado, B. H. and Yousef, A. E. (2003). Selection and Identification of a *Listeria monocytogenes* target strain for pulsed electric field process optimization. Applied and Environmental Microbiology 69: 2223-2229.

Lebovka, N. I., Bazhal, M. I. and Vorobiev, E. (2002). Estimation of characteristic damage time of food materials in pulsed electric fields. Journal of Food Engineering 54: 337-346.

Lebovka, N. I., Praporscic, I., Ghnimi, S. and Vorobiev, E. (2005). Does electroporation occur during ohmic heating of food? Journal of Food Science 70(5): E308-311.

Lebovka, N. I., Praporscic, I. and Vorobiev, E. (2004). Combined treatment of apples by pulsed electric fields and by heating at moderate temperature. Journal of Food Engineering 65: 211-217.

Lebovka, N. I., Praporscic, I. and Vorobiev, E. (2004). Effect of moderate thermal and pulsed electric field treatments on textural properties of carrots, potatoes and apples. Innovative Food Science and Emerging Technologies 5(1): 9-16.

Lebovka, N. I., Shynkaryk, N. V. and Vorobiev, E. (2006). Pulsed electric field enhanced drying of potato tissue. Journal of Food Engineering: in press.

Lebovka, N. I. and Vorobiev, E. (2004). On the origin of the deviation from the first order kinetics in inactivation of microbial cells by pulses electric fields. International Journal of Food Microbiology 91: 83-89.

Lechner, N. and Cserhalmi, Z. (2004). Pulsed electric field (PEF) processing effects on physical and chemical properties of vegetable juices. *Safe Consortium Seminar: Novel Preservation technologies in relation to food safety.*, Brussel, Belgium.

Li, S. Q., Zhang, Q. H., Tony, Z. J., Turek, E. J. and Lau, M. H. (2005). Elimination of Lactobacillus plantarum and achievement of shelf stable model salad dressing by pilot scale pulsed electric fields combined with mild heat. Innovative Food Science and Emerging Technologies 6: 125-133.

Lindgren, M. (2001). Pulsed electric field food treatment and low frequency bioelectromagnetics. Electromagnetics Department. Gotenburg, Sweden, Chalmers University of Technology.

Lindgren, M. (2002). Arrangement and method for treatment of a pumpable substance.European Patent, EP 1 455 606 A1.

Lindgren, M., Aronsson, K., Galt, S. and Ohlsson, T. (2002). Simulation of the temperature increase in pulsed electric field (PEF) continuous flow treatment chambers. Innovative Food Science and Emerging Technologies 3: 233-245.

Loeffler, M. (2006). Generation and Application of High Intensity Pulsed Electric Fields. *Pulsed Electric Field Technology for the Food Industry. Fundamentals and Applications.* J. Raso and V. Heinz. Berlin, Springer: 27-72.

Loeffler, M., Schmidt, W., Schuhmann, R., Röttering, A., Neumann, J. and Dreesen, C. (2001). Treatment of sewage sludge with pulsed electric fields. *International Conference on Pulsed Power Applications*, Gelsenkirchen, Germany.

Lubicki, P. and Jayaram, S. (1997). High voltage pulse application for the destruction of gram-negative bacterium *Yersinia enterocolitica*. Bioelectrochemistry Bioenergetics 43: 135-141.

Mankovski, J. a. K., M. (2000). A review of short pulse generator technology. IEEE Transactions on Plasma Science 28(1): 102-108.

Martín-Belloso, O., Vega-Mercado, H., Qin, B. L., Chang, F. J., Barbosa-Cánovas, G. V. and Swanson, B. G. (1997). Inactivation of *Escherichia coli* suspended in liquid egg using pulsed electric fields. Journal of Food Processing and Preservation. 21(193-203).

Marx, E. (1923). Verfahren zur Schlagprüfung von Isolatoren und anderen elektrischen Vorrichtungen.Germany, German Patent, 455933.

Mastwijk, H. (2004). Recent Developments in Pulsed Electrical Field Treatment in Relation to Food Safety. *Safe Consortium Seminar: Novel Preservation technologies in relation to food safety*, Brussels, Belgium.

McDonald, C. J., Lloyd, S. W., Vitale, M. A., Petersson, K. and Innings, F. (2000). Effect of pulsed electric fields on microorganisms in orange juice using electric field strengths of 30 and 50 kV/cm. Journal of Food Science 65(6): 984-989.

McLellan, M. R., Kime, R. L. and Lind, K. R. (1991). Electroplasmolysis and other treatments to improve apple juice yield. Journal of Science of Food and Agriculture 57(2): 303-306.

Mermelstein, N. H. (1998). Interest in Pulsed Electric Field Processing Increases. Food Technology 52(1): 81-82.

Mertens, B. and Knorr, D. (1992). Developments of nonthermal processes for food preservation. Food Technology 46(5): 124-133.

Mizuno, A. and Hori, Y. (1988). Destruction of living cells by pulsed high-voltage application. IEEE Trans. Ind. Appl. 24(3): 387-394.

Mohan, N., Undeland, T. N. and Robbins, W. P. (1995). Power Electronics; Converters and Design. New York, Wiley.

Molinari, P., Pilosof, A. M. R. and Jagus, R. J. (2004). Effect of growth phase and inoculum size on the inactivation of *S. cerevisiae* in fruit juices by pulsed electric fields. Food Research International 37(8): 793-798.

Morren, J., Roodenburg, B. and de Haan, S. W. H. (2003). Electrochemical reactions and electrode corrosion in pulsed electric field (PEF) treatment chambers. Innovative Food Science and Emerging Technologies 4(3): 285-295.

Moses, B. D. (1938). Electric Pasteurization of Milk. Agricultural Engineering 19(12): 525-526.

Neumann, E. and Rosenheck, K. (1972). Permeability changes induced by electric impulses in vesicular membranes. J. Membrane Biol. 10: 279-290.

Neumann, E., Toensing, K., Kakorin, P., Budde, P. and Frey, J. (1998). Mechanism of electroporative dye uptake by mouse B cells. Biophysical Journal. 74: 98-108.

Ngadi, M. O., Bazhal, M. and Raghavan, V. G. S. (2003). Engineering Aspects of Pulsed Electroplasmolysis of Vegetable Tissues. Agricultural Engineering International 5(2): 1-10.

Nöldechem, A. (1993). Neues Elektroschock-Verfahren zur Konservierung flüssiger Lebensmittel. ZFL 44(9): 476-483.

Ohlson, T. (1980). Optimal temperatures for sterilization of flat containers. Journal of Food Science 45: 848-852/859.

Palaniappan, S., Richter, E. R. and Sastry, S. K. (1990). Effects of electricity on microorganisms: A review. Journal of Food Processing and Preservation. 14(393-414).

Pandur, A. (1988). Haltbarmachung von Fruchtsaftgetränken. Getränkeindustrie 42(2): 96-102.

Pareilleux, A. and Sicard, N. (1970). Lethal Effects of Electric Current on *Escherichia coli*. Applied Microbiology 19(3): 421-424.

Pavlin, M., Pavselj, N. and Miklavcic, D. (2002). Dependence of induced transmembrane potential on cell density, arrangement and cell position inside a cell system. IEEE Transactions on Biomedical Engineering 49(6): 605-612.

Peleg, M. (1995). A model of microbial survival after exposure to pulsed electic fields. Journal of Science of Food and Agriculture 67: 93-99.

Peleg, M. and Cole, M. B. (1998). Reinterpretation of microbial survival curves. Critical Reviews in Food Science 38(5): 353-380.

Pothakamury, U. R. (1995). Preservation of Foods by nonthermal processes. Pullman, USA, Washington State University.

Praporscic, I., Lebovka, N. I., Ghnimi, S. and Vorobiev, E. (2006). Ohmically heated, enhanced expression of juice from apple and potato tissues. Biosystems Engineering 93(2): 199-204.

Praporscic, I., Lebovka, N. I., Vorobiev, E. and Mietton-Peuchot, M. (2006). Pulsed electric field enhanced expression and juice quality of white grapes. Separation and Purification Technology: doi:10.1016/j.seppur.2006.06.007.

Prasanna, G. L. and Panda, T. (1997). Electroporation: basic principles, practical considerations and applications in molecular biology. Bioprocess Engineering 16: 261-264.

Prochownick, L. and Spaeth, F. (1890). Über die keimtötende Wirkung des galvanischen Stroms. Deutsche Medizinische Wochenschrift 26: 564-565.

Puc, M., Corovic, S., Flisar, K., Petkovsek, M., Nastran, J. and Miklavcic, D. (2004). Techniques of signal generation for electropermeabilization. Survey of electropermeabilization devices. Bioelectrochemistry 64: 113-124.

Qin, B.-L., Chang, F.-J., Barbosa-Canovas, G. and Swanson, B. G. (1995). Nonthermal inactivation of *Saccharomyces cerevisiae* in apple juice using pulsed electric fields. Lebensmittel-Wissenschaft und Technologie 28: 564-568.

Qin, B.-L., Zhang, Q., Barbosa-Cánovas, G. V., Swanson, B. G. and Pedrow, P. D. (1994). Inactivation of microorganisms by pulsed electric fields of different voltage waveforms. IEEE Transactions on Dielectrics and Electrical Insulation 1(6): 1047-1057.

Qin, B. L., Barbosa-Cánovas, G. V., Pedroe, P. D., Olsen, R. G., Swanson, B. G. and Zhang, Q. (1997). Continuous flow electrical treatment of flowable food products.US Patent,

Qui, X., Sharma, S., Tuhela, L., Jia, M. and Zhang, Q. H. (1998). An integrated PEF pilot plant for continuous nonthermal pasteurization of fresh orange juice. Transactions ASAE 41(4): 1069-1074.

Raso, J., Calderón, M. L., Góngora, M., Barbosa-Cánovas, G. V. and Swanson, B. G. (1998). Inactivation of mold ascospores and conidiospores suspended in fruit juices by pulsed electric fields. Lebensmittel-Wissenschaft und Technologie. 31: 668-672.

Rastogi, N. K., Eshtiaghi, M. N. and Knorr, D. (1999). Accelerated mass transfer during osmotic dehydration of high intensity electrical field pulse pretreated carrots. Journal of Food Science 64(6): 1020-1023.

Reina, L. D., Jin, Z. T., Zhang, Q. H. and Yousef, A. E. (1998). Inactivation of *Listeria monocytogenes* in milk by pulsed electric field. Journal of Food Protection 61(1203-1206).

Reitler, W. (1990). Conductive heating of foods. Munich, Germany, Technical University of Munich.

Reyns, K. M., Diels, A. M. and Michiels, C. W. (2004). Generation of bactericidal and mutagenic components by pulsed electric field treatment. International Journal of Food Microbiology 93: 165-173.

Reznick, D. (1996). Ohmic heating of fluid foods. Food Technology(5): 250-251.

Rodrigo, D., Barbosa-Cánovas, G. V., Martínez, A. and Rodrigo, M. (2003). Weibull distribution Function Based on an Empirical Mathematical Model for Inactivation of *Escherichia coli* by Pulsed Electric Fields. Journal of Food Protection 66(6): 1007-10012.

Rogers, H. J., Perkins, H. R. and Ward, J. B. (1980). Microbial Cell Walls and Membranes. London, Chapman&Hall.

Roodenburg, B., Morren, J., Berg, H. E. and de Haan, S. W. H. (2005). Metal release in a stainless steel pulsed electric field system. Part I. Effect of different pulse shapes; theory and experimental method. Innovative Food Science and Emerging Technologies 6(3): 327-336.

Roodenburg, B., Morren, J., Berg, H. E. and de Haan, S. W. H. (2005b). Metal release in a stainless steel pulsed electric field system. Part II. The treatment of orange juice; related to legislation and treatment chamber lifetime. Innovative Food Science and Emerging Technologies 6(3): 337-345.

Roodenburg, B., Morren, J., de Haan, S. W. H. and Berg, H. E. (2003). Corrosion Experiments in a PEF-treatment Chamber with stainless steel electrodes. *Workshop Nonthermal Food Preservation*, Wageningen, The Netherlands.

Rosenberg, B., Van Camp, L. and Krigas, T. (1965). Inhibition of cell division in *Escherichia coli* by electrolysis products from a platinum electrode. Nature 205: 698-699.

Russel, N. J., Colley, M., Simpson, R. K., Trivett, A. J. and Evans, R. I. (2000). Mechanism of action of pulsed high electric field (PHEF) on the membranes of food-poisoning bacteria is an 'all-or-nothing' effect. International Journal of Food Microbiology 55: 133-136.

Sale, A. J. and Hamilton, W. A. (1967). Effect of high electric fields on micro-organisms. I. Killing of bacteria and yeast. II. Mechanism of action of the lethal effect. Biochimica Biophysica Acta 148: 781-800.

Sale, A. J. H. and Hamilton, W. A. (1968). Effects of High Electric Fields on Microorganisms. III. Lysis of Erythrocytes and Protoplasts. Biochimica Biophysica Acta 163: 37-43.

Schilling, S., Alber, T., Toepfl, S., Neidhart, S.,, Knorr D., Schieber, A. and Carle, R. (2006). Effects of Pulsed Electric Field Treatment of Apple Mash on Juice Yield and Quality Attributes of Apple Juices. Innovative Food Science and Emerging Technologies: submitted.

Schoenbach, K. H., Alden, R. W. and Fox, T. J. (1996). Effect of pulsed electric fields on aquatic nuisance species. *Sixth International Zebra Mussel and other Aquatic Nuisance Species Conference*, Chelsea, Michigan, Ann Arbor Press, Inc.

Schoenbach, K. H., Peterkin, F. E., Alden, R. W. I. and Beebe, S. J. (1997). The effect of pulsed electric on biological cells: experiments and applications. IEEE Transactions on Plasma Science. 25(2): 284-292.

Schultheiss, C., Bluhm, H. J., Mayer, H. G., Kern, M., Michelberger, T. and Witte, G. (2002). Processing of sugar beets with pulsed electric fields. IEEE Transactions on Plasma Science 30(4): 1547-1551.

Schultheiss, C., Bluhm, H. J., Sack, M. and Kern, M. (2004). Principle of electroporation and development of industrial devices. Zuckerindustrie 129(1): 40-44.

Schuten, H., Gulfo-van Beusekom, K., Pol, I., Mastwijk, H. and Bartels, P. (2004). Enzymatic stability of PEF processed orange juice. *Safe Consortium Seminar: Novel Preservation technologies in relation to food safety.*, Brussels, Belgium.

Selma, M. V., Salmerón, M. C., Valero, M. and Fernández, P. S. (2004). Control of *Lactobacillus plantarum* and *Escherichia coli* by pulsed electric fields in MRS Broth, nutrient broth and carrot-apple juice. Food Microbiology 21: 519-525.

Sensoy, I. and Sastry, S. K. (2004). Extraction Using Moderate Electric Fields. Journal of Food Science 69(1): 7-13.

Shapton, D. A., Lovelock, D. W. and Laurita-Longo, R. (1971). The evaluation of sterilization and pasteurization processes from temperature measurements in °C. Journal o. Applied Bacteriology 34: 491-500.

Simpson, R. K., Whittington, R., Earnshaw, R. G. and Russel, N. J. (1999). Pulsed high electric field causes ´all or nothing´ membrane damage *in Listeria monocytogenes* and *Salmonella typhimurium*, but membrane H+-ATPase is not a primary target. International Journal of Food Microbiology 48: 1-10.

Sitzmann, W. (1987). Verarbeitung tierischer Rohstoffe. *GVC Fachausschuss Lebensmittelverfahrenstechnik*, Munich, Germany.

Sitzmann, W. (1995). High voltage pulse techniques. *New methods for food preservation*. G. W. Gould. London, Graham & Hill.

Sitzmann, W. (2006). Technologieentwicklung der Elektroporation. Personal communication.

Sitzmann, W. and Münch, E.-W. (1987). Verarbeitung tierischer Rohstoffe. Fat Science Technology 9: 368.

Sitzmann, W. and Münch, E.-W. (1988). ELCRACK® und ELSTERIL® - Zwei neue Verfahren zur gezielten Beeinflussung von Phasengrenzflächen. *GVC Fachausschuss*, Brussel, Belgium.

Sitzmann, W. and Münch, E.-W. (1989). Elektrische Verfahren zur Keimabtötung. Die Ernährungsindustrie 6: 54-58.

Sitzmann, W. and Münch, E. W. (1988). Das ELCRACK® Verfahren: Ein neues Verfahren zur Verarbeitung tierischer Rohstoffe. Die Fleischmehlindustrie 40(2): 22-28.

Stanley, D. W. (1991). Biological membrane deterioration and associated quality losses in food tissues. *Critical Reviews in Food Science and Nutrition*. F. M. Clydesdale. New York, CRC Press.

Stolz, P. and Obermayer, B. (2005). Manufacturing Microalgae for Skin Care. Cosmetics and Toiletries 120(3): 99-106.

Sugar, I. P. and Neumann, E. (1984). Stochastic model for electric field-induced membrane pores electroporation. Biophysical Chemistry 19: 211-225.

Taiwo, K. A., Angersbach, A. and Knorr, D. (2002). Influence of high intensity electric field pulses and osmotic dehydration on the rehydration characteristics of apple slices at different temperatures. Journal of Food Engineering 52: 185-192.

Tedjo, W., Taiwo, K. A., Eshtiaghi, M. N. and Knorr, D. (2002). Comparison of pretreatment methods on water and solid diffusion kinetcs of osmotically dehydrated mangos. Journal of Food Engineering 53: 133-142.

Teissie, J., Golzio, M. and Rols, M. P. (2005). Mechanisms of cell membrane electropermeabolization: A minireview of our present (lack of ?) knowledge. Biochimica Biophysica Acta 1724: 270-280.

Toepfl, S., Heinz, V. and Knorr, D. (2003). Optimization of Pulsed Electric Field Application for Liquid Food Decontamination. *IFT Workshop on Nonthermal Food Preservation*, Wageningen, The Netherlands.

Toepfl, S., Heinz, V. and Knorr, D. (2006). High intensity Pulsed Electric Fields applied for food preservation. Chemical Engineering and Processing.: in press.

Toepfl, S. and Knorr, D. (2006). Pulsed electric fields as a pretreatment in drying processes. Stewart Postharvesting Reviews (3)4, doi 10.2212/spr.2006.4.3.

Tomov, T. and Tsoneva, I., (2000). Are the stainless steel electrodes inert? Bioelectrochemistry 51: 207-209.

Tracy, R. L. (1932). Lethal effect of alternating currents on yeast cells. Journal of Bacteriology 24: 423-438.

Tsong, T. Y. (1990). Review: On electroporation of cell membranes and some related phenomena. Bioelectrochemistry Bioenergetics 24(271-295).

Tsong, T. Y. (1991). Electroporation of cell membranes. Biophysical Journal. 60: 297-306.

Ulmer, H. M., Heinz, V., Gaenzle, M. G., Knorr, D. and Vogel, R. F. (2002). Effects of pulsed electric fields on inactivation and metabolic activity of *Lactobacillus plantarum* in model beer. Journal of Applied Microbiology 93(2): 326-335.

Unal, R., Kim, J.-G. and Yousef, A. E. (2001). Inactivation of *Escherichia coli* O157:H7, *Listeria monocytogenes,* and *Lactobacillus leichmannii* by combinations of ozone and pulsed electric field. Journal of Food Protection 64: 777-782.

Valic, B., Golzio, M., Pavlin, M., Schatz, A., Faurie, C., Gabriel, B., Teissie, J., Rols, M. P. and Miklavcic, D. (2003). Effect of electric field induced transmembrane potential on spheroidal cells: theory and experiment. European Biophysical Journal 32: 519-528.

Van Loey, A., Verachtert, B. and Hendrickx, M. (2001). Effects of high electric field pulses on enzymes. Trends in Food Science and Technology 12: 94-102.

Vega-Mercado, H., Pothakamury, U. R., Chang, F.-J., Barbosa-Cánovas, G. V. and Swanson, B. G. (1996). Inactivation of *Escherichia coli* by combining pH, ionic strength and pulsed electric field hurdles. Food Research International 29(2): 117-121.

Weaver, J. C. (2000). Electroporation of cells and tissues. IEEE Transactions on Plasma Science 28(1): 24-33.

Weaver, J. C. and Powell, K. T. (1989). Theory of electroporation. *Electroporation and electrofusion in cell biology*. E. Neumann, A. E. Sowers and C. Jordan. New York, Plenum Press: 111-126.

Wenske, K. (2005). Application of Thyratrons for a technical scale pulse modulator. Berlin: personal communication.

Wouters, P. C. (2001). Pulsed Electric Field Inactivation of Microorganisms. Berlin, Berlin University of Technology.

Wouters, P. C., Alvarez, I., Angersbach, A. and Knorr, D. (2001). Critical factors determining inactivation kinetics by pulsed electric field food processing. Food ScienceandTechnology 12: 112-121.

Wouters, P. C., Dutreux, N., Smelt, J. P. P. M. and Lelieveld, H. L. M. (1999). Effects of pulsed electric fields on inactivation kinetics of *Listeria innocua*. Applied and Environmental Microbiology 65(12): 5364-5371.

Wouters, P. C. and Smelt, J. P. P. M. (1997). Inactivation of microorganisms with pulsed electric fields: Potential for food preservation. Food Biotechnology 11: 193-229.

Wuytack, E., Phuong, L. D. T., Aersten, A., Reyns, K. M. F., Marquenie, D., De Ketelaere, B., Masschalck, B., Van Opstal, I., Diels, A. M. J. and Michiels, C. W. (2003). Comparison of Sublethal Injury Induced in *Salmonella enterica* serovar Typhimurium by Heat and by Different Nonthermal Treatments. Journal of Food Protection 66(1): 31-37.

Yang, R. J., Li, S. Q. and Zhang, Q. H. (2004). Effects of pulsed electric fields on the activity of enzymes in aqueous solution. Journal of Food Science 69(4): 241-248.

Yaqub, S., Anderson, J. G., MacGregor, S. J. and Rowan, N. J. (2004). Use of a fluorescent viability stain to assess lethal and sublethal injury in food-borne bacteria exposed to high-intensity pulsed electric fields. Letters in Applied Microbiology 39: 246-251.

Yeom, H. W., Streaker, C. B., Zhang, Q. H. and Min, D. B. (2000). Effects of Pulsed Electric Fields on the Quality of Orange Juice and Comparison with Heat Pasteurization. Journal of Agricultural Food Chemistry 48: 4597-4605.

Zárate-Rodriguez, E. and Ortega-Rivas, E. (2000). Quality changes in apple juice as related to nonthermal processing. Journal of Food Quality 23: 337-349.

Zhang, Q., Barbosa-Cánovas, G. V. and Swanson, B. G. (1995). Engineering aspects of pulsed electric field pasteurization. Journal of Food Engineering 25: 261-281.

Zhang, Q., Monsalve-González, A., 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. Journal of Food Process Engineering 17: 469-478.

Zhang, Q., Qin, B. L., Barbosa-Cánovas, G. V. and Swanson, B. G. (1995). Inactivation of *Escherichia coli* for food pasteurization by high-strength pulsed electric fields. Journal of Food Processing and Preservation. 19: 103-118.

Zimmermann, U. (1982). Electric field-mediated fusion and related electrical phenomena. Biochimica et Biophysica Acta 694: 227-277.

Zimmermann, U. (1996). The effect of high intensity electric field pulses on eucaryotic cell membranes: fundamentals and applications. *Electromanipulation of cells*. U. Zimmermann and G. A. Neil. Boca Raton, CRC Press: 1-106.

Zimmermann, U. and Neil, G. A. (1996). Electromanipulation of cellls. Boca Raton, Florida, US, CRC Press.

Zimmermann, U., Pilwat, G., Beckers, F. and Riemann, F. (1976). Effects of external electrical fields on cell membranes. Bioelectrochemistry Bioenergetics 3: 58-83.

Zimmermann, U., Pilwat, G. and Riemann, F. (1974). Dielectric breakdown in cell membranes. Biophysical Journal. 14: 881-899.

Zimmermann, U., Schulz, J. and Pilwat, G. (1973). Transcellular ion flow in *E. coli* and electrical sizing of bacteria. Biophysical Journal. 13: 1005.