Process performance analysis of pulsed electric field (PEF) food applications

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Kurzfassung

Die vorliegende Arbeit beschäftigt sich mit der Prozessanalyse der Anwendung gepulster elektrischer Felder (engl. pulsed electric fields PEF) zur Behandlung von Lebensmitteln. Der Schwerpunkt liegt dabei auf den zwei wesentlichen Anwendungsfeldern, der nicht-thermischen Inaktivierung von Mikroorganismen sowie dem Zellaufschluss von pflanzlichen Rohmaterialien zur Verbesserung von Stofftransportprozessen. Ausgehend von einer Strukturierung der Prozessanalyse in mikro-, meso- und makro-skalige Effekte und unter Berücksichtigung der Wechselwirkungen zwischen Prozess und Produkt werden zunächst Phänomene auf Zellebene erfasst. Das Auftreten sublethaler Schädigungen bei der Inaktivierung von Mikroorganismen mittels PEF sowie die Bedeutung von Schutzeffekten verschiedener Inhaltsstoffe der komplexen Lebensmittelmatrix wurden dabei als Kernpunkte zur Bewertung und Optimierung des Inaktivierungseffektes untersucht. Die Analyse der physiologischen Fitness von Mikroorganismen in Abhängigkeit der Behandlungsparameter und Matrixeffekte erfolgte sowohl hinsichtlich struktureller als auch funktioneller Zelleigenschaften mittels Durchflusszytometrie und selektiver Platten-Kultivierungsverfahren. Neben dem Nachweis reiner elektrischer Feldeffekte auf Mikroorganismen wurde die Prozessanalyse um die Berücksichtigung von thermischen Nebeneffekten erweitert. Es erfolgte eine Charakterisierung von elektrischer Feldverteilung, Strömungsverhältnissen und Temperaturverteilung in der Behandlungszelle mit Hilfe numerischer Simulation. Lokal auftretende, hohe Temperaturen konnten als wesentliche Ursache für die Inaktivierung thermisch empfindlicher Enzyme identifiziert werden. Nach einer experimentellen Validierung konnte durch gezielte Anpassung des Behandlungszellen-Design eine Reduzierung thermischer Effekte und ein Erhalt thermisch sensitiver Enzyme erreicht werden. Die gezielte Anwendung thermischer Effekte und die synergistische Wirkung von Temperatur und PEF wurden für eine komplexe Hochspannungsimpuls-Pasteurisierungsanlage analysiert. Es wurde ein Modell zur Differenzierung und Quantifizierung elektrischer Feldeffekte und thermischer Effekte bzgl. der Inaktivierung von Mikroorganismen und Enzymen entwickelt. Aspekte der Verfahrensintegration sind am Beispiel der Anwendung gepulster elektrischer Felder zum Aufschluss von Frucht- und Gemüsemaischen und der nachfolgenden Entsaftung verdeutlicht. Auch hier wurden zunächst im mikro- und meso-skaligen Bereich sowohl elektrische Feldeffekte auf die Pflanzenzelle als auch die Beeinflussung der Feldwirkung durch die Rohstoff-Materialeigenschaften analysiert. Darauf aufbauend konnte eine gezielte Variation von Prozess- und Anlagenparametern vor- und nachgeschalteter Prozessstufen der Zerkleinerung und fest-flüssig Trennung durchgeführt und durch die Untersuchungen zur Abstimmung relevanter Wechselwirkungen die Grundlage für eine Prozessintegration im industriellen Maßstab gelegt werden.

Abstract

The thesis covers the process performance analysis of the application of pulsed electric fields (PEF) for the treatment of foodstuffs. Emphasis is put on the two main fields of application, the non-thermal inactivation of microorganisms and the cell disintegration of plant raw materials for the improvement of mass transfer processes. A systematic process analysis was performed based on effects related to the micro-, meso- and macroscale and considering the interactions between process and product. Phenomena taking place on a cellular level such as the occurrence of sublethal injuries and the protective effect of food constituents during the inactivation of microorganisms by PEF were under investigation in order to evaluate and optimize the inactivation effectiveness. The analysis of the physiological fitness of microbial cells depending on treatment parameters and matrix effects was performed by flow cytometry and selective media plating technique considering structural and functional cell properties. In addition to the consideration of electric field effects, the process analysis was extended by the consideration of thermal side effects. A characterization of the electric field distribution, the flow characteristics as well as the temperature distribution inside the treatment chamber was performed using numerical simulation. Local high temperatures were identified as the main reason for the inactivation of heat sensitive enzymes. An experimental validation was performed followed by the targeted adjustment of the treatment chamber design in order to reduce unwanted thermal effects and improve the retention of heat sensitive compounds. The use of thermal effects and the synergism between temperature and electric field effects was analyzed for a complex pulsed electric field pasteurization unit. A model for the differentiation and quantification of electric field and thermal effects regarding their contribution to the inactivation of microorganisms and enzymes was developed. Aspects related to the process integration were investigated for the PEF cell disintegration of fruit and vegetable mashes and the subsequent de-juicing process. The impact of the electric field on plant cells as well as the effect of raw material properties on the process performance were analyzed on a micro- and mesoscale level. Subsequently, a targeted variation of process- and equipment parameters of connected processing steps such as milling and solid-liquid separation and the adjustment of relevant interactions was performed in order to provide the basis for the process integration in industrial scale.

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1. Introduction

According to Harvey (1986) there are three fundamental categories of attributes indispensable for the viability of any technical system: functionality, performance and efficiency. However, in some cases systems are already fully designed and functionally tested before an attempt is made to determine its performance characteristics (Ferrari, 1986). Re-design of the system and re-evaluation of former process results is usually the consequence.

The technical system of interest in the present study is the pulsed electric field (PEF) technology and its use in the food industry. PEF treatment involves the application of short pulses of high voltage in order to disrupt biological cells in the food material. The concept was established and reported for the first time more than 50 years ago (Doevenspeck, 1960; Gossling, 1960). However, besides some sporadic industrial applications conducted in the following decades it was just recently, that the technology was introduced at an increasing number as large scale commercial application (Toepfl, 2011).

The term ‘Process Performance Analysis’ in its classical meaning is related to quality management and statistical process control and may be applied to a wide range of processes including production and manufacturing lines, software development or even organizational and management processes. Integrating the process performance analysis assures efficient system design and allows a more accurate description of the systems behavior (Oakland, 2008).

Process performance in the meaning used in the present thesis is understood as the capability of a process to meet previously set requirements or to fulfill defined process goals. The approach is very similar to the ‘Process Performance Analysis’ as such. However, it is not the aim of the presented research work to apply the methodology of statistical process control to a single PEF process. Rather, the conceptual idea is used as a tool for the systematic process analysis in order to reveal discrepancies between the process goal and the actual process outcome. As a result, an improvement of the process performance is achieved by this analysis and by the subsequent adjustment of relevant process parameters.

To date, pulsed electric field technology application in the food industry encompasses the following two key processing goals:

A. Non-thermal inactivation of microorganisms
B. Cell disintegration for mass transfer enhancement

From a process performance point of view, the following four simple questions need to be answered in order to evaluate the capability of the PEF application to fulfill the above mentioned processing goals:

1. Is the level of microbial inactivation sufficient?
2. Is the process non-thermal, or do thermal effects occur?
3. What is the level of cell disintegration achieved by PEF?
4. Is the cell disintegration converted into the desired mass transfer enhancement?

The research work documented in the present thesis aimed at the analysis and optimization of PEF processing concepts including the processing equipment as well as the related process parameters.

The work was motivated mainly by three aspects:

i) Experimental results showed a lower level of microbial inactivation in complex matrices in comparison to simple buffer systems. Protective effects of food constituents were reported for other food preservation technologies but limited information was available for PEF processing. In addition, inactivation as such does not occur as an all-or-nothing effect. Intermediate levels in the physiological and functional state of a microbial cell may occur depending on the product matrix and the processing conditions. Both effects are of high relevance from a food safety point of view and did require further investigation.

ii) Results reported in literature regarding the PEF inactivation of enzymes were controversial. A theory on the inactivation of microorganisms by PEF due to membrane electroporation was available and widely accepted but the interpretation of results regarding the enzyme inactivation by PEF was not satisfying. Although PEF is considered as a non-thermal processing technology, it was assumed that thermal effects may play a role. The quantification of these thermal effects during PEF processing was required as well as design improvements for their control.

iii) PEF processing is intended to replace or complement existing food processing technologies. Most of the research activities focused on the PEF process as such in order to obtain a fundamental understanding of the related aspects. However, the technology is now introduced as commercial industrial scale application and most of the conventional processes are complex. Thus, their replacement or complementation introduces an additional level of complexity since the PEF process requires not only its technical implementation but also its integration considering connected processing steps.

An important focus of the thesis is on the bidirectional interactions of the PEF application and the treated product. It will be shown that PEF may affect food compounds during the pasteurization of liquid foods on the one hand but that food compounds may also affect the PEF performance and microbial inactivation efficiency on the other hand. In the same way, an interaction between solid products treated by PEF for cell disintegration will be discussed. The tissue properties are affecting the cell disintegration efficiency of a PEF treatment but at the same time, the PEF treatment is modifying the raw material structure in a way that will affect subsequent processing steps and product characteristics. The comprehensive and systematic consideration of these interactions occurring in a different way for each particular field of application is the prerequisite for a successful use of the PEF technology in order to replace or complement existing traditional food processing technologies.

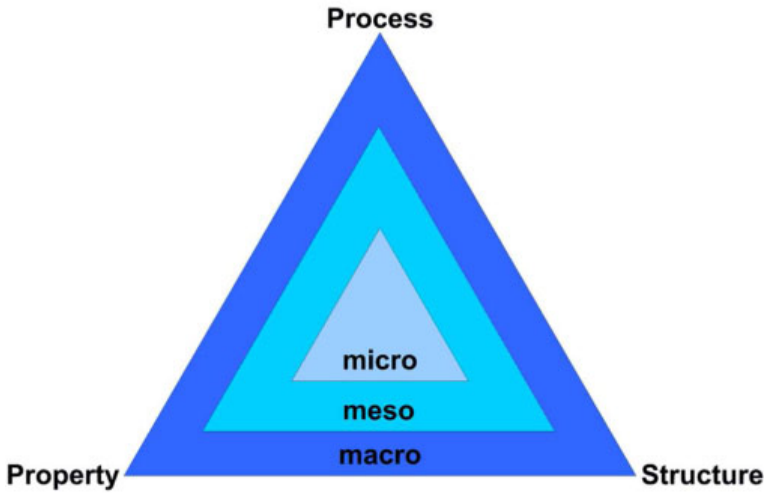
Although the technology of pulsed electric fields (PEF) follows general principles, various differences exist between the different application purposes and between the different research groups or equipment manufacturers involved in this topic. Different ways of the generation of PEF, different pulse characteristics and, which is probably the most important aspect with regard to the comparability of research data, differently designed treatment chambers are in use. Hence, another important focus of the thesis is to contribute to the establishment and development of standards for the proper design, performance and analysis of PEF experiments. This includes the description of the equipment and the process as well as the selection and documentation of relevant processing parameters not only related to the PEF treatment itself but also considering connected processing steps as well as particularities of the food matrix.

In order to systematically evaluate the aforementioned aspects, a performance analysis should consider the multi-scale nature of a PEF process. Figuratively, phenomena are allocated to dimensions defined as microscale, mesoscale and macroscale. For the aspects covered in the present thesis, Fig. 1 illustrates the different levels that are suggested for analysis. Adapting the S-PRO^2-scheme suggested by Windhab (2008), phenomena can be further clustered into process (PRO) related, structure (S) related or property (PRO) related.

The table in Fig. 1 gives some examples for the different scale levels related to PEF processing. Phenomena taking place on a cellular level such as the inactivation of microorganisms or permeabilization of plant cells as well as protective effects or the modification of food compounds are considered as the microscale. The mechanism of electroporation can also be described in the microscale, where a process induced accumulation of charges at the cell membrane takes place leading to a property change by increasing the transmembrane potential. The result is a structure modification, namely the pore formation in the membrane called electroporation.

Phenomena related to the PEF treatment chamber and its performance such as electric field, flow velocity or temperature distribution are considered as the mesoscale. The mesoscale is also applied to mass transfer processes taking place in plant raw materials such as the juice recovery from a fruit mash considering mash structure and particle size distribution still as mesoscale parameters. Structural changes in the microscale such as the pore formation lead to property changes in the mesoscale, e.g. tissue softening. The relevance of this change in property is related to cutting processes where the structure of the cutting surface will be affected depending on tissue properties.

The macroscale as defined in the present context involves design and integration aspects of the PEF process considering a whole processing concept as well as connected processing steps. Hence, the definition of property and structure is now more connected to the processing line rather than to the product itself. Structure may be related to the physical implementation of the PEF system in an existing processing line whereas the property may describe the capability of the different processing steps to interact and to be synchronized.



	Property	Process	Structure
micro	Transmembrane-potential	Charge accumulation by external electric field	Membrane pore
meso	Tissue softening	Cutting	Cutting surface
macro	Interaction ← capability	Process design and integration	→ Physical integration

Fig. 1: Matrix including the hierarchic structure scheme for the multi-scale nature of PEF processing and examples for phenomena related to the process, the structure and the property.

In addition to the different scales related to the size, the concept also applies to the timescale which is another important aspect in dynamic processes. Whereas the electroporation takes place in the microsecond range and may be temporary or permanent, affected processes

such as extraction or drying have much higher time requirements. Hence, categories such as ultra-short time, short time and long time can be defined in analogy to the micro, meso and macroscale.

Regardless of the position of a specific aspect within the matrix shown in Fig. 1, a general approach is required and was used in the presented studies in order to perform a stepwise analysis of the underlying interactions. The procedure is illustrated in Fig. 2.

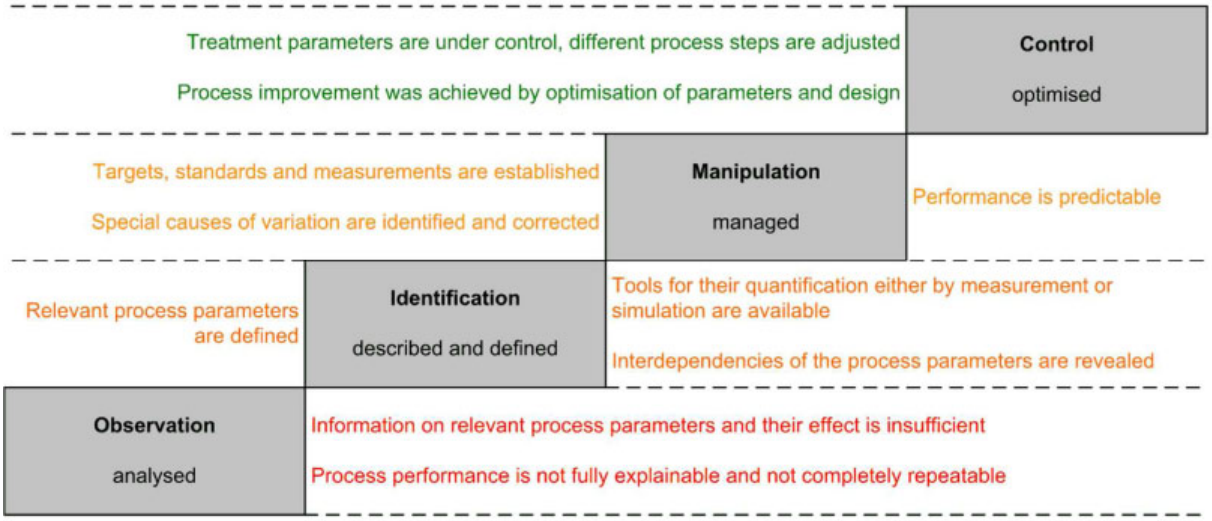


Fig. 2: Systematic approach suggested for a PEF process analysis and optimization. The model can be applied to different scales and levels of complexity as shown by the examples presented in the thesis.

An optimized process offers a maximum of process control with defined process standards, a set of measurements and key performance indicators. This is not only relevant for the improvement of emerging food processing technologies such as PEF but also for the re-design of traditional food processing practices. A better understanding of the process-structure-property relationships and the design of innovative, scalable and flexible food manufacturing techniques will allow the creation of tailor-made raw material properties, processing technologies and final food products as it will be shown by the examples presented in the thesis.

1.1. PEF – basic principles and application

Pulsed electric field technology can be used to induce non-thermal permeabilization of cell membranes. Depending on the treatment intensity (external electric field strength, number and duration of the electric pulses) and cell properties (size, shape, orientation, conductivity) the pore formation may be permanent or temporary (Zimmermann et al., 1974; Zimmermann et al., 1976; Angersbach et al., 2000).

The treatment consists of the application of very short electric pulses (1 – 100 µs) at electric field intensities in the range of 0.1 – 1 kV/cm (reversible permeabilization for stress induction in plant cells), 0.5 – 3 kV/cm (irreversible permeabilization of plant and animal tissue) and 15 – 40 kV/cm for the irreversible permeabilization of microbial cells. The aforementioned field intensities lead to the formation of a critical transmembrane potential, which is regarded to be the precondition for cell membrane breakdown and electroporation (Tsong, 1996).

The irreversible electroporation results in a loss of turgor, the leakage of cytoplasmic content and lysis (Rubinsky, 2010). Reversible permeabilization leads to the formation of conductive channels across the cell membrane but electrically insulating properties will recover within seconds (Glaser et al., 1988; Angersbach et al., 2000). The inactivation of microorganisms in liquid products or changes in the microstructure and texture of treated solid raw materials can be expected as a consequence of the irreversible permeabilization of cell membranes.

The following sections will give an overview of the PEF technology covering the generation of pulsed electric fields, their impact on biological cells and the resulting food industry applications. The consideration of these three steps: - generation – impact – application - is essential in order to develop suitable application concepts and to analyze and optimize existing PEF food applications.

Step 1: Particularities resulting from the different ways of the generation and technical application of PEF will determine the electric field characteristics including aspects such as the pulse shape or pulse duration as well as aspects such as the distribution of the electric field in a treatment chamber. Exactly defined electric field conditions are a prerequisite for the subsequent evaluation of the electric field impact on the biological cell.

Step 2: Direct and indirect methods are available in order to analyze the electric field effects on a cellular and tissue level. The degree of cell permeabilization needs to be quantified but also evaluated from a qualitative point of view (e.g. reversible or irreversible permeabilization).

Step 3: As a third step, an analysis is required in order to evaluate the extent to which these basic effects can be converted into tangible process outcomes leading to beneficial applications. For example, a specific level of cell membrane permeabilization of fruit mashes determined in step 2 does not necessarily result in an increased juice yield since other impact factors such as mash structure or the solid-liquid separation will affect the beneficial outcome of the cell disintegration. The same is true for the microbial inactivation by PEF. A specific level of pore formation detected in step 2 does not guarantee a permanent inactivation since the cells may recover under optimal conditions.

Hence, it is essential to take all three steps into account and to consider the underlying basic principles in order to develop a comprehensive understanding of complex process applications.

1.1.1. Generation of pulsed electric fields

Generally, high intensity electric pulses can be generated by the switched discharge of a suitable capacitor bank. The characteristics of the discharge circuit determine the shape of the time dependent potential in the treatment chamber where the product is exposed to the electric field (Barsotti et al., 1999). The geometry of the treatment chamber has a considerable effect on the electric field distribution and on the total resistance and therewith on the discharge circuit. Exponential decay pulses represent a complete discharge of the capacitance. The voltage decay depends on the capacitance of the capacitors and the resistance of the circuit (Ho et al., 2000). A rectangular shape of the pulse can be produced by using special switches, capable to interrupt the current at high potentials, or the implementation of a pulse forming network. If an additional capacitor is used together with a parallel switch, bipolar pulses can be obtained (Beveridge, 2002). The generated electrical pulses are applied to the food via a treatment chamber. The food in turn can be modelled as a resistor in parallel to a capacitor. The resistance is inversely proportional to the electrical conductivity of the food whereas the capacitance depends on the dielectric permittivity of the food. Due to the short pulse durations, the capacitor properties can be neglected. The electrical conductivity of the media together with the electrical resistance as affected by the electrode configuration determines the total resistance of the treatment chamber (Loeffler, 2006). The usage of a treatment chamber with a high resistance results in a more effective voltage division between the treatment chamber and other electrical resistances in the circuit and higher electric field strength in the treatment chamber can be achieved at the same power level. Further aspects regarding the treatment chamber performance and the

characteristics of the discharge circuit will be discussed in section 1.2 ‘Treatment chamber design’.

1.1.2. PEF impact on biological cells

The optimum design and characterization of critical process parameters for the application of PEF in the food industry requires the fundamental understanding of the phenomena related to the electroporation. Until now there has been no clear evidence on underlying mechanisms at a cellular level but the main effects have been described to be triggered by the electric field, the ionic punch-through (Coster, 1965; Coster 2009) and the dielectric breakdown of the membrane (Zimmermann, et al. 1974).

The exposure of the biological cell to an intense external electric field leads to the accumulation of ions on both sides of the electrically insulating phospholipid bi-layer membrane. An increased transmembrane potential is generated which imposes an electro-compression of the membrane. This compression can be equilibrated with elastic deformation to a certain extent but the thickness of the membrane is reduced. When a critical level of the trans-membrane potential, which depends on the compressibility, the permittivity and the initial thickness of the membrane, is exceeded, an electrical breakdown of the membrane occurs. The transmembrane potential induced by an external electric field depends on the intensity of the external electric field and the cell size, shape and composition of the membrane. The minimum field strength level of the external electric field which is required to increase the trans-membrane potential and to cause the pore formation is defined as critical electric field strength E_{crit} which depends on the before mentioned cell and membrane characteristics (Rubinsky, 2009). A further increase of the external electric field strength or the exposure time will increase the size and number of membrane pores and irreversible breakdown occurs which is associated with mechanical destruction of the cell membrane (Crowley, 1973; Zimmermann, 1974). Since the formation of the trans-membrane potential already takes place within the sub-microsecond level, the application of the high intensity electric field in the form of short pulses presents the advantage of minimizing the energy consumption (Barsotti et al., 1999).

As already discussed, the effect of an external electric field on the biological cell needs to be analyzed form a qualitative point of view, namely reversible and irreversible permeabilization, and from a quantitative point of view (degree of membrane permeabilization).

For **microbial cells**, different methods are available in order to evaluate their physiological fitness including structural as well as functional properties. Depending on the analytical method, different physiological fitness criteria can be analyzed as illustrated in Fig. 3.

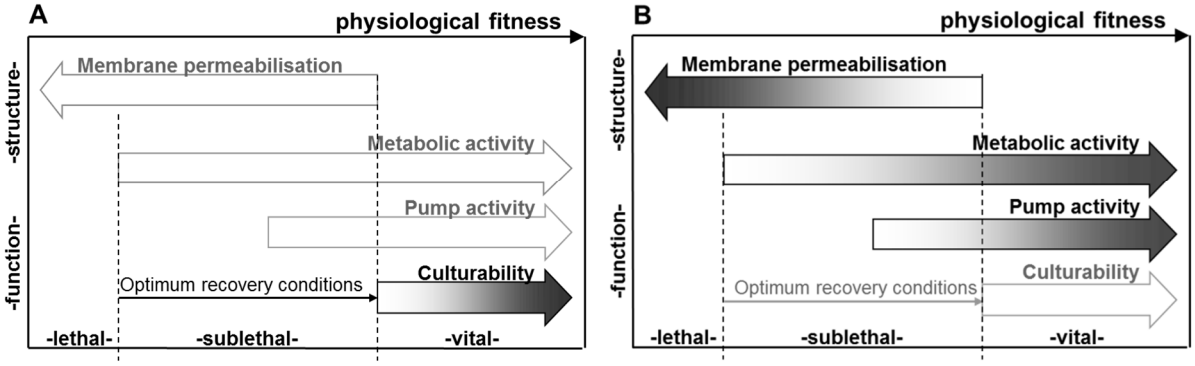


Fig. 3: Criteria related to the evaluation of the physiological fitness of cells (according to Bunthoff et al., 2002). A) Culturability used as an indicator for sublethal injuries by selective media plating technique under optimal and stress conditions. B) Membrane integrity, esterase metabolism and pump activity applied as indicators by flow cytometry analysis.

Again, the PEF process induced modification of the structure and related function is affecting the physiological fitness criteria. Although PEF is causing membrane electroporation thus affecting a structural component of the cell, functional characteristics such as metabolic activity or pump activity will be affected due to the loss of membrane semipermeability. As a consequence, the vitality of the cell is reduced leading to the formation of sublethally injured cell fractions that may regain their structural integrity and their culturability. Using the selective media plating technique in order to analyze the physiological state of a microbial cell, only the culturability criteria can be considered. Based on the evaluation of the growth behavior of the population on selective medium containing some stress conditions such as a higher salt content or a modified pH (Somolinos et al., 2008) in comparison to the growth on medium providing optimal recovery conditions, conclusions can be drawn regarding the cell damage and the occurrence of sublethal injuries. Sublethally injured cell populations will not grow on stress medium but on medium providing optimal recovery conditions. Reproductive growth is regarded as the highest level of physiological fitness. However, cells can enter a non-culturable state but still exhibit metabolic activity.

A more specific analytical method is provided by flow cytometry. Using this technique, a rapid determination of structural and functional cell characteristics can be performed simultaneously (Ueckert et al., 1995). Cells can be stained with fluorescent dyes that are used as indicators for specific cell vitality criteria. The microbial cells in suspension flow through a laser illuminated zone where they scatter light and emit fluorescence. Stains such as propidium iodide (PI) can be used in order to reveal pore formation in the membrane. This

compound is only introduced into the cell through membrane pores and starts its fluorescence after binding to the DNA. Other fluorogenic substrates such as cFDA (carboxyfluorescein-diacetate) diffuses across the intact cell membrane and is converted by esterases to carboxyfluorescin (cF) which is a membrane impermeant fluorescent compound that is detected by the flow cytometric analysis. Thus, an impression about the distribution of a variety of properties of interest among the cells in the microbial population can be gained (Shapiro, 2003).

For more complex **plant tissue**, several methods have been proposed to study and quantify the degree of membrane permeabilization and cell damage. The damage degree is defined as the ratio of damaged cells to the total number of cells. Microscopic analysis of PEF treated tissue stained with neutral red was applied successfully by Fincan et al. (2002). Regarding plant tissue as a semi-solid, multiphase, heterogeneous material, the authors emphasized the need to characterize the desired level of cell permeabilization in order to optimize PEF treatments and to elucidate effects, such as mass transport and mixing of the vacuole content, the cytoplasm and the liquid of the apoplastic space as well as their interaction with the cell wall. More complex staining methods using selective and fluorescent dyes have been used by Phoon et al. (2008) in combination with microscopic analysis. Measurement of cell membrane permeabilization of single cells or protoplasts using fluorescent dyes is also possible using flow cytometry (Shapiro, 2003) and it is gaining increasing interest for the evaluation of electric field effects on plant cell microstructure.

Since the membrane permeabilization is affecting the turgor of the cell, turgor measurements (Tomos, 2000) as well as measurement of texture characteristics, such as stress deformation and relaxation tests of complex tissue may be used in order to evaluate the degree of cell rupture and tissue damage (Lebovka et al., 2004).

Other simple methods to analyze the degree of cell rupture could entail the measurement of the release of plant pigments from permeabilized cells (Dörnenburg et al., 1993) or the release of ions into the cell containing media (Saulis et al., 2007). Knorr et al. (1998) used a method to quantify the liquid release of permeabilized cells by subjecting the tissue to a centrifugal force at which no liquid release occurred from non-damaged cells but from PEF treated cells. The estimation of the damage degree from the diffusion coefficient measurements in the PEF treated biological tissue was suggested by Jemai et al. (2002). It can be determined from solute extraction or convective drying experiments but diffusion techniques are indirect and invasive for biological objects and may affect the structure of the tissue.

Using the electrical conductivity response of plant tissue subjected to PEF, cell disintegration criteria can be defined based on impedance measurement (Knorr et al., 1998; Lebovka et al. 2001). This method is based on interfacial polarization effects of the Maxwell-Wagner type at the intact membrane interfaces. Measuring conductivity-frequency spectra (in the frequency range from 10^3 - 10^7 Hz) allows the quantification of the degree of membrane disintegration. Conductivity changes in the low frequency ranges are the result of irreversible membrane permeabilization. In the high frequency ranges, the conductivity value of intact cells and cells with ruptured cell membranes remains the same since within this frequency range the cell membrane does not present resistance to the electrical current measured.

The application of an acoustically derived determination of the degree of cell permeabilization caused by PEF was successfully applied by Grimi et al. (2010) and a relation between the acoustic disintegration index and the conductivity disintegration index was established. This non-destructive acoustic technique is especially applicable if fruits or vegetables are processed as whole unpeeled samples based on the modulus of elasticity which is proportional to the index of firmness of the plant tissue. Applying a series of gentle taps to the sample, it resonates at a frequency that depends on weight and firmness. The vibration of the sample is captured by acoustic measurements. An acoustic spectrum can be derived showing the amplitude-frequency correlation for the untreated or PEF treated sample and an acoustic disintegration index can be calculated by comparing the pulse response derived index of firmness of the samples.

Cell disintegration may also be achieved by other techniques with different mechanism of action. Thermal treatment causes the denaturation of membrane proteins as well as the expansion of the intracellular liquid resulting in the permeabilization of the cell. Cell disintegration as a result of freeze-thawing treatment is based on the destruction of the cell envelope by the formation of large ice crystals applying slow freezing rates. Mechanical cell disintegration by crushing or milling leads to a particle size reduction which results in turn in an increasing degree of cell disintegration when the particle size gets close or below the cell size. Since membrane structures are also affected by these physical means of cell disintegration, the aforementioned analytical techniques are appropriate. However, for mechanical cell disintegration the degree of membrane permeabilization is directly correlated to particle size reduction. For the cell disintegration by heating, freeze-thawing or by electroporation, which furthermore works at much lower energy consumption than the thermal disintegration technologies, the degree of cell disintegration can be controlled independently from the particle size reduction. De-linking of particle size and cell disintegration provides an additional degree of freedom in the control of the raw material

microstructure. This aspect will be discussed extensively in research paper IV included in this thesis.

The methods presented above are valuable tools in order to investigate the impact of PEF on biological cells and some of them already consider more complex structures such as multi-cellular systems or tissue. Complex structures are of particular interest for plant raw materials whereas for microorganisms, the single cell level is sufficient in most cases in order to evaluate occurring inactivation effects. However, no studies are available on the inactivation effectiveness of PEF regarding multi-cellular microbial structures such as chain-forming microorganisms or agglomerates. Protective effects of agglomerates due to the alteration of the electric field distribution were shown by Toepfl et al. (2007) using numerical simulation but further experimental studies are still required.

1.1.3. Applications of PEF in food processing

As discussed above, the electroporation of the cell membrane is the basic mechanism of action of the external electric field application. Pore formation increases the membrane permeability which results in the loss of cell content or intrusion of surrounding media as well as in loss of cell vitality used for the inactivation of microbial cells.

Low intensity treatment with controlled reversible permeabilization offers the potential for a sublethal stress induction on biological cells triggering a metabolic response resulting in the promotion of a defense mechanism by increased production of secondary metabolites (Dörnenburg et al., 1993; Bonnafous et al., 1999; Gomez-Galindo et al., 2009; Gomez-Galindo et al., 2008). It also offers the potential for the “infusion” of precursors or other desired constituents into cells as well as the recovery of metabolites from cells while maintaining their viability and productivity (Tryfona et al., 2008).

An irreversible perforation of the cell membrane reduces its barrier effect permanently and causes cell death which can be applied for plant and animal raw material disintegration (Angersbach et al., 1998; Toepfl et al., 2007) as well as for the non-thermal inactivation of microorganisms (Lelieveld et al., 2007).

The pore formation as such may have a direct impact on cell viability and may lead to the inactivation of microorganisms which is the key processing goal for the application of PEF as a non-thermal preservation method. However, in case of the PEF application for the disintegration of plant raw materials, the electroporation of the cell in a complex tissue is only a first step. Tangible potential applications result from the secondary effect of the pore

formation on tissue physical properties such as tissue softening relevant for subsequent cutting operations or the improvement of mass transfer processes relevant for subsequent processing steps such as extraction, juice recovery or drying. Hence, a correlation needs to be established between the pore formation on a cellular level (referred to as microscale in the introduction) and the modification of the microstructure with an effect on tissue physical properties (referred to as mesoscale in the introduction) which will then lead to the PEF food application as discussed below.

The irreversible rupture of plant membranes offers various applications to replace or support conventional thermal as well as enzymatic processes for cell disintegration. Irreversible permeabilization allows significant improvement of mass transfer especially for drying, expression, concentration and extraction resulting into higher product yields, shorter processing times and consequently reduced energy consumption. Processing concepts for the disintegration of fruit and vegetable mashes to enhance the juice yield will be presented in detail in research paper IV.

The irreversible electroporation of plant tissue results in the loss of membrane semipermeability and subsequently in a decrease of turgor pressure within the cell. Since this pressure acts as the plant cell supportive structure, a loss of turgor results in tissue softening. It is obvious, that a change of textural properties can be utilized during production and processing of various biological products. Tissue softening of apple, potato and carrot after PEF treatment has been described and a reduction of elasticity modulus were reported (Fincan et al., 2003; Lebovka et al. 2004). For sugar beet 50 % steady state cutting force reduction and improvement of cut quality could be observed (Kraus, 2003) going along with less abrasion of the cutting devices. A reduction of grinding energy of potato similar to that of thermal or enzymatic treatment can be achieved with a continuous, short time and low energy (~10 kJ/kg) PEF treatment. Cutting behaviour and properties of the cut surface are reported to be changed resulting for example in a lower fat uptake of French fries when processing PEF-pretreated potato (Janositz, 2005).

Extractability of intracellular pigments facilitated with PEF treatment showed to be a very efficient process for the recovery of these valuable components with beneficial antioxidant properties. PEF-induced cell permeabilization and the release of intracellular pigments (anthocyanins) from wine grapes were studied by Praporscic et al. (2007) and Puertolas et al. (2010).

Drying of solid food matrices strongly depends on diffusion and mass transfer through the cells and tissue. The presence of intact cell membranes in the food raw material limits mass transfer processes due to their barrier function. Hence, the application of an external electric

field, the induced rapid electrical breakdown, local structural changes of the cell membrane and the increase in permeability due to the appearance of pores was found to positively affect drying processes of porous plant materials (Ade-Omowaye et al., 2003).

Pulsed electric field treatment can be an attractive alternative to traditional thermal pasteurization since it combines gentle food preservation with short treatment times, continuous operation as well as ease of implementation into existing product flow. Microbial inactivation of vegetative cells via PEF offers pasteurization with low energy input, selective inactivation of microorganisms depending on cell size or shape (Toepfl, et al. 2007) as well as retention of bioactive heat sensitive food compounds while inactivating pathogenic microorganisms and increasing product shelf live and safety (Guerrero-Beltrán, et al. 2010, Jaeger et al., 2009; Sui et al., 2010). The potential to achieve reduction of microbes in various food products like fruit or vegetable juices (Evrendilek et al., 2000; Heinz et al., 2003; Lechner et al, 2004; McDonald et al., 2000; Min et al., 2003; Molinari et al., 2004), model beer (Ulmer et al., 2002) or milk (Bendicho et al., 2002; Toepfl, et al. 2006) could be shown.

Various other applications exist regarding the cell disintegration of plant and animal raw materials and specific processing concepts have been developed for the non-thermal pasteurisation of liquid products by PEF. A detailed overview can be obtained from Raso et al. (2007), Lelieveld et al. (2007) or Vorobiev et al. (2008).

1.2. Treatment chamber design

The treatment chamber is the key component of the PEF system for the direct application of the electric field in contact with the treatment media. Its design should consider the uniformity of the electric field distribution in combination with the flow characteristics in continuous applications as well as the extent of the temperature increase. These aspects related to design optimization concepts are presented in detail in research paper II. The following section aims at giving a general introduction in treatment chamber design and performance criteria.

Since PEF processing in an industrial scale will be preferably applied for a continuous product flow, the efficiency of the treatment strongly depends on the design of the flow-through treatment chamber which is basically composed of two electrodes and an insulating body. For pumpable media, such as fruit juices, milk or fruit mashes, treatment can be performed continuously in a tubular treatment chamber consisting of cylindrical electrodes. Whole fruits or pieces can be submerged in water in a flume and a dry PEF treatment with direct contact between the product and the electrodes is desirable for meat pieces in order to

prevent cross contamination. First industrial large scale applications have been realized for the disintegration of plant raw materials such as sugar beet and fruit mashes (Mueller et al., 2007; Bluhm et al., 2009).

Various treatment chamber designs such as parallel plates, coaxial cylinders or co-linear configurations have been used for PEF processing and some modifications of these basic designs have been proposed (Alkhafaji et al., 2007; Huang et al., 2009; Jaeger et al. 2009). A comprehensive overview of different treatment chamber configurations can be found in Barbosa-Cánovas et al. (1999), Lelieveld et al. (2007) and Huang et al. (2009).

An adequate shape of the electrodes and the insulator is a prerequisite for an optimal electric field distribution and will reduce dielectric breakdown effects of the food, since local high electric field strength levels can be avoided e.g., by providing a round-edged insulator geometry (Dunn et al., 1987). Dielectric breakdowns of the food are undesired since they cause arcing, which leads to the destruction of the food, damage on the electrode and insulator surface, as well as an explosion of the treatment chamber due to the pressure increase. Electric field homogeneity and the avoidance of low field intensities are not only desirable from the microbial inactivation point of view, but also from the fact that energy dissipation and power consumption will take place in low field regions without contributing to the microbial inactivation.

Parallel plates are the simplest in design and produce the most uniform distribution of the electric field (Jeyamkondan et al., 1999), but their lower electrical resistance is limiting the application possibilities. The geometry consists of a rectangular duct of insulating material with two limited electrodes on opposite sides. Only the electrode length and distance determine the electric field distribution.

In a co-axial treatment chamber, the food is placed between two cylinders, one internal cylinder used as high voltage electrode and an external cylinder as ground electrode. The electric field within this treatment chamber is not homogeneous, because it is distributed in descendant order, from the central cylinder towards the external cylinder. One of the major advantages of this type of chambers is that peak values of local electric field strength are minimized or eliminated. The liquid food is flowing through the thin gap between the two cylinders so that the application of this chamber is restricted to liquid foods with only small particles. Furthermore, the effective area of the electrodes is very large causing a high current flow and a low resistance of the treatment chamber.

The co-field (or co-linear) treatment chamber is one of the most utilized to operate in continuous systems. A flow pattern suitable for food processing can be achieved with the co-linear design. A hollow high voltage and grounded electrode with a circular inner hole are

kept on a defined distance by an insulating spacer. The product is pumped through the drilling forming the electrical load of the high voltage discharge circuit. The electric field strength is not homogeneous and depends strongly on the insulator geometry that is placed between the two electrodes (Meneses et al., 2011). By modification of the insulator geometry, it is possible to obtain different electric field strength distributions, which can be simulated with numerical software (Gerlach et al., 2008).

To achieve sufficient treatment intensity for all volume elements as well as to prevent over-processing or arcing, the electric field should be free of local peak values. Relative to the electrodes, the inner diameter of the insulator should be slightly pinched in order to produce a more homogeneous electrical field (Toepfl et al., 2007). Co-field chambers with a small electrode surface area and a large electrode gap will provide a high load resistance.

Another important aspect for the electrode design and the numerical simulation of occurring electric field and thermal effects is the possibility to cool the electrodes in order to control the temperature. Examples of batch treatment chambers with temperature control used for kinetic inactivation studies are given by Qin et al. (1994), Bazhal et al. (2006) and Saldana et al. (2010).

The treatment chamber design has a major impact on the performance of a PEF treatment and affects various other process parameters as shown in Fig. 4. The interdependency of the most relevant process parameters will be discussed below in order to provide a basis for the evaluation of the treatment intensity and to provide a framework for experimental design considerations.

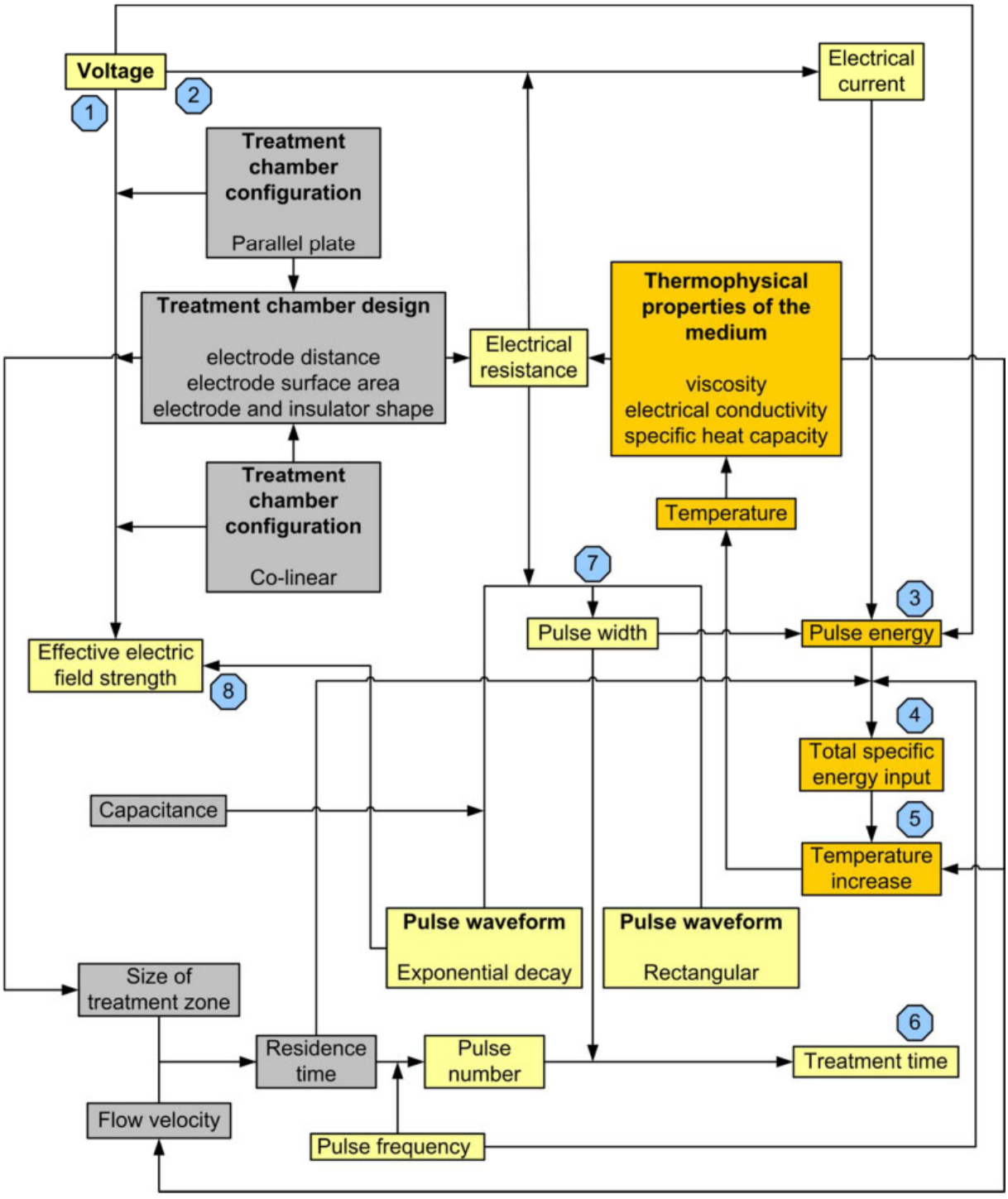


Fig. 4: Schematic drawing of the various interdependencies of PEF processing parameters related to the pulse generation system, the treatment chamber as well as to the treatment medium. Considered are a parallel plate and co-linear treatment chamber as well as exponential decay and rectangular pulses exemplarily. Numbers indicated in the drawing will be referred to in the text below.

re 1) The application of a voltage between the electrodes will generate an electric field. The electric field strength is a key parameter in order to describe the treatment intensity since it will affect the electroporation efficiency (see section 1.1.2. ‘PEF impact on biological cells’). Besides on the voltage, the electric field strength depends on the treatment chamber

configuration and design. For a parallel plate electrode configuration, the electric field will be homogeneous and the field strength can be calculated depending on the electrode distance (equation 1). For a co-linear electrode configuration, an average electric field strength needs to be estimated since the electric field is not homogeneous. Numerical simulation procedures are applied in order to determine a treatment chamber specific factor for the conversion of the applied voltage into the resulting average electric field strength according to Meneses et al. (2010) as shown by equation (2). Another method used by Gerlach (2008) is based on the consideration of distinct volume elements and the calculation of an average field strength resulting from the respective local field strength (equation 3).

$E = \frac{U}{d}$	E U d	electric field strength [kV/cm] voltage [kV] electrode distance [cm]	(1)
$E_{aveg} = g \cdot U$	E_{aveg} g	average electric field strength [kV/cm] conversion factor [cm^{-1}]	(2)
$E_{aveg} = \frac{1}{V_{treat}} \cdot \sum_{i=1}^{N} E_i \cdot \delta V_i$ $V_{treat} = \sum_{i=1}^{N} \delta V_i$	V_{treat} δV_i E_i	total volume in the treatment zone [cm^3] volume element [cm^3] electric field strength in a volume element [kV/cm]	(3)

re 2) The applied voltage will result in an electric current flow due to the electrical conductive media placed between the electrodes according to equation (4). The electrical resistance of the treatment chamber, which depends on dimensional properties as well as on the electrical conductivity of the treatment media, will affect the current level. The electrical resistance of a treatment chamber in turn can be determined experimentally by measurement of the voltage and current (equation 4), it can be determined using numerical simulations (Knoerzer et al., 2011) or, for a parallel plate configuration, it can be calculated based on equation (5). Since the media specific electrical resistance is temperature dependent, the resistance of the treatment chamber and the resulting current will also be affected by the product temperature in addition to the impact of the treatment chamber design characteristics.

$I = \frac{U}{R}$	I U R	electrical current [A] voltage [V] resistance [Ω]	(4)
$R = \rho \cdot \frac{d}{A}$	ρ d A	specific electrical resistance of the medium electrode distance (m) effective cross sectional area for the current flow [m^2]	(5)

re 3) Electrical energy is applied to the treatment media and the amount of energy delivered per single pulse (pulse energy) can be calculated based on the voltage and electrical current

(equation 6) with simplifications for rectangular pulses (equation 7) or for exponential decay pulses for which a calculation can be performed based on the energy discharge of the capacitor bank by equation (8).

$W_{pulse} = \int U(t) \cdot I(t) \cdot dt$	W_{pulse} U I	pulse energy [J] voltage [V] current [A]	(6)
$W_{pulse} = U \cdot I \cdot \tau$	τ	pulse width [s]	(7)
$W_{pulse} = \frac{1}{2} \cdot U^2 \cdot C$	C	capacitance [F]	(8)

re 4) Depending on the number of pulses applied to an amount of product, a total specific energy input is calculated according to equation (9) and can be interpreted as a dose parameter with regard to the treatment intensity. For continuous flow-through PEF application, the number of pulses applied to a given amount of product depends on the residence time of the product in the treatment chamber and the pulse frequency. The total specific energy input is regarded as another key parameter in order to describe the treatment intensity. It allows an estimation of the temperature increase occurring during the treatment.

$W_{spec} = \frac{f}{\dot{m}} \cdot W_{pulse}$	W_{spec} W_{pulse} f $\dot{m}$	total specific energy input [kJ/kg] pulse energy [kJ] frequency [s^{-1}] mass flow rate [kg s^{-1}]	(9)

re 5) The dissipation of the electrical energy delivered to the product will cause a temperature increase due to Joule heating depending on the specific heat capacity of the product. This increase in temperature will have an impact on the temperature dependent thermophysical properties of the product. This in turn will affect related parameters of the discharge circuit such as the electrical resistance due to changes in the electrical conductivity of the product or it will affect the flow velocity due to changes in product viscosity. Hence, a cycle of various interdependencies is initiated (Fiala et al., 2001; Lindgren et al., 2002; van den Bosch et al., 2002; Gerlach et al., 2008; Jaeger et al., 2009). The Joule heating and the temperature change can be described as a function of the electric field strength in conductive media according to equation (10). The resulting temperature increase can be estimated based on the total specific energy input according to equation (11) assuming that a complete dissipation of electrical energy takes place.

$Q = \sigma \cdot E^2$	Q σ E	electrical energy [J] electrical conductivity [S m^{-1}] electric field strength [kV cm^{-1}]	(10)
$\Delta T = \frac{W_{spec}}{c_p}$	ΔT W_{spec} c_p	temperature increase [K] total specific energy input specific heat capacity [kJ kg^{-1} K^{-1}]	(11)

re 6) The total treatment time corresponds to the exposure time of the media to the electric field while passing through the treatment zone. Depending on the length of the treatment zone and the flow velocity (equation 12) or depending on the flow rate and the volume of the treatment zone (equation 13), a specific residence time of the product in the treatment zone can be calculated. During this residence time, a definite number of pulses will be applied depending on the pulse frequency (equation 14). A total treatment time can be defined based on the pulse width of each single pulse and the number of pulses (equation 15). The total treatment time is regarded as another key parameter for the description of the treatment intensity since it correlates directly to the electric field exposure time. However, it hast to be stated that the treatment time distribution within the treatment chamber is not homogeneous due to the flow velocity and residence time distribution (Jaeger et al., 2009).

$t_{res} = \frac{L}{u}$	t_{res} L u	residence time [s] length of the treatment zone [m] flow velocity [m s^{-1}]	(12)
$t_{res} = \frac{m}{\dot{m}}$	m $\dot{m}$	mass of product in the treatment zone [g] mass flow rate [g s^{-1}]	(13)
$n = f \cdot t_{res}$	n f	number of pulses [-] pulse frequency [s^{-1}]	(14)
$t_{treat} = n \cdot \tau$	t_{treat} τ	treatment time [s] pulse width [s]	(15)

re 7) Pulses with a rectangular pulse shape can be controlled in their duration by an electrical switch with on/off-function. For exponential decay pulses, the voltage decrease (equation 16) and the pulse width depend on the electrical resistance of the discharge circuit as well as on the capacitance of the capacitor bank used for the generation. Hence, a different pulse width will result due to changes of the media electrical conductivity that may occur due to temperature changes as exemplified by Meneses et al. (2011). The pulse width of an exponential decay electrical pulse is determined at a point where the voltage has decreased to around 37 % of its initial maximum value and can be calculated by equation (17) according to Barsotti et al. (1999) and Ho et al. (2000).

$V(t) = V_0 \cdot e^{-\frac{t}{R \cdot C}}$	V V_0 t R C	voltage [V] initial maximum voltage [V] time [s] resitance [Ω] capacitance [F]	(16)
$\tau = R \cdot C$	τ	pulse width [s]	(17)

re 8) Whereas a rectangular pulse maintains the defined voltage and the corresponding electric field strength for a large part of the pulse width, the voltage and electric field strength is decreasing exponentially for exponential decay pulses after the maximum value was

reached (see equation 16). Hence, the term effective electric field strength is introduced and refers to an electric field strength above the critical electric field strength required for electroporation. This critical electric field strength depends on the characteristics of the particular biological cell (Grahl et al., 1996; Toepfl et al., 2007).

The above discussed relations of the different parameters illustrate the complex interdependencies. In addition, as indicated already, most of the parameters are not homogeneously distributed. This is the case for the electric field strength as well as for the flow velocity and the related residence time and will affect the energy input as well as the temperature distribution.

All of the above discussed aspects concern the microscale and mesoscale of the PEF application as presented in the introduction section. They refer to the process itself as well as to the properties of the treatment media. The presented overview only includes parameters directly related to the phenomena in the treatment chamber. Taking into account the need of establishing a standard experimental procedure for the performance of PEF treatments, these parameters are essential in order to provide a detailed insight in PEF related processing criteria. It is obvious, that it is not possible to change one single parameter without affecting related treatment conditions. Hence, a systematic study of the impact of one single parameter by its variation while keeping all other parameters at a fixed level is impossible.

Therefore, as a first step in the experimental design of the PEF treatment, it is necessary to choose the most suitable key parameter to describe the treatment intensity from parameters such as the electric field strength, the treatment time or the total specific energy input. As a second step, using the schematic shown in Fig. 4, it will be possible to evaluate the interdependencies of these with other parameters. These interdependencies need to be taken into account when interpreting obtained process results. A variation of the electric field strength may have caused a change in the total specific energy input or in the temperature increase as well, which then has to be taken into account as additional impact factor. Changes in the treatment chamber design or changes in the electrical circuit for the generation of the electric pulses, such as a modification of the capacitance or the resistance, provide a possibility to control the treatment parameters. Increasing the electric field strength by increasing the applied voltage will lead to increased pulse energy values. This can be compensated by reducing the pulse width for rectangular pulses according to equation (7) or by decreasing the capacitance of the circuit in case of exponential decay pulses according to equation (8). In both cases, a variation of the treatment time (equation 15 and 17) will result as a consequence of this adjustment. This example shows that calculations need to be performed using the above given equations in order to develop a suitable experimental

procedure for each specific case of investigation which can only be a compromise regarding the selection of treatment parameters to be kept constant.

In addition to the investigation of treatment parameters, the comparison of treatment chambers with different design characteristics can be of interest. However, using the same initial treatment parameter settings will not allow an adequate establishment of comparable treatment conditions. Even when using the same treatment chamber configuration, deviations in the dimension will have a major impact on electrical resistance and electric field strength and on the parameters indirectly linked to the design such as the residence and treatment time or the total specific energy input. In this case, indirect measures are required in order to achieve comparable treatment intensities. A concept could entail the definition of a similar level of cell disintegration or microbial inactivation to be achieved by the different treatment systems. Based on such a comparable process result, the process parameters can be analyzed and compared in terms of the required treatment intensity or even in terms of the required energy consumption. A major research need can be defined regarding this aspect since up to now, no suitable concepts for the process evaluation including indicators for a process performance analysis are available.

1.3. Thermal effects

Although the PEF treatment is a non-thermal food processing technology, there is a significant temperature increase during high intensity PEF treatment, as applied for pasteurization purposes due to Joule heating. Many authors have described the temperature distribution in a PEF treatment chamber and reported the occurrence of high local temperatures due to inhomogeneous field distribution of the electrical field, limited flow velocity and recirculation of the liquid (Fiala et al., 2001; Lindgren et al., 2002; van den Bosch et al., 2002; Gerlach et al., 2008; Jaeger et al., 2009). Numerical simulations using computational fluid dynamics gain growing interest for this purpose since experimental measurement of the related parameters is not possible in most cases due to small dimensions of the treatment chamber as well as the interference of the measuring device with the product flow and electric field. Apart from the overall liquid temperature measured at the outlet of the treatment chamber, treatment inhomogeneity and the occurrence of temperature peaks within the treatment chamber have to be considered as thermal impact factors. This is of particular importance when discussing PEF effects on functionality of heat sensitive compounds, such as proteins, or when conducting kinetic inactivation studies. Additional thermal effects may occur in case of application of high total energy inputs,

insufficient temperature control or unfavorable treatment chamber design. This aspect will be discussed in detail in research paper II.

On the other hand, the application of PEF in combination with mild heat seems to be a promising technique for a gentle, multi hurdle preservation process. The synergism between temperature and PEF membrane electroporation can be used in order to improve the inactivation efficiency. A number of studies have shown this synergetic effect between PEF and heat treatment at non-lethal processing temperatures. The phospholipid bilayer structure of the cell membrane changes from a gel-like to a liquid crystalline state when increasing the temperature and the increased membrane fluidity leads to a reduced membrane stability and facilitates the electroporation of the cell membrane (Kanduser et al., 2008; Stanley, 1991). The synergetic effect of temperature during pulsed electric field inactivation of microorganisms can be used to improve the inactivation results and/ or to reduce the electrical energy costs (Craven et al., 2008; Riener et al., 2008). Energy savings derive from the lower PEF treatment intensity (treatment time and total specific energy input) required for a certain level of microbial inactivation at increased temperature and from the possibility to recover the electrical energy dissipated during PEF treatment in form of thermal energy for pre-heating the incoming product. PEF treatment in combination with mild heat provides a potential to reduce the total thermal load. A reduction of thermal load could be used to improve the retention of heat sensitive bioactive compounds or to increase the operating time of heat exchangers by reducing the amount of fouling.

A processing concept taking into consideration the impact of temperature on lethality and energy efficiency during apple juice pasteurization by PEF has been proposed by Heinz et al. (2003). An enthalpy diagram for a PEF-process for the pasteurization of apple juice was developed. To preheat the juice to a temperature of 55 °C the enthalpy of the PEF-treated product is utilized in a heat exchanger and cooled to 17 °C. After a startup phase, the pasteurization process can be operated by the input of electrical energy and no additional energy input is needed for heating and cooling.

When applying treatment concepts considering the synergetic effect of PEF and temperature, it is of high relevance to perform an adequate temperature control in order to limit negative thermal effects on heat sensitive compounds. In addition, analytical approaches are required in order to quantify the contribution of the electric field and thermal effects on the overall inactivation results. Such a model for the differentiation of thermal and electric field effects is presented and discussed in research paper III.

1.4. Process-product interactions

Each unit operation and almost each processing step within food processing leads to desired or undesired changes of the food material and product properties (process-product impact). These modifications may be required to establish or improve the processability of a food or a raw material matrix by applying different types of pre-treatments without changing the major characteristics of the food. However, modifications may also be intended to significantly alter the appearance and quality attributes of the raw material matrix in order to produce a certain type of final product. In addition to the impact of food processing on product properties, the product itself and its properties will affect the processing since material properties determine the performance of unit operations in food processing (product-process impact). The following sections will address these bidirectional interactions by presenting general principles as well as examples for such interactions related to the PEF processing of food.

1.4.1. Microbial inactivation

Since the microbial cell is a part of the food product that is subjected to PEF preservation, its permeabilization and inactivation can be analyzed from a process-product interaction point of view. The factors which affect microbial inactivation during PEF treatment are process factors such as electric field intensity, pulse width and shape, treatment time and temperature, microbial factors such as type, shape, size, concentration and growth stage of microorganism and media factors such as pH, antimicrobials and ionic compounds, electrical conductivity and medium ionic strength. The basic mechanism of the electroporation and the inactivation of microorganisms by PEF have been discussed in section 1.1.2. ‘PEF impact on biological cells’.

Effective inactivation for most of the spoilage and pathogenic microorganisms has been shown and colony count reductions depending on treatment intensity, product properties and type of microorganism in the range of 4-6 log-cycles are comparable to traditional thermal pasteurisation. Bacterial spores and viruses are not affected by the PEF treatment (Lelieveld, et al., 2007).

Since the inactivation of microorganisms is based on the electromechanical mechanism of electroporation (Crowley, 1973; Zimmermann et al., 1974) food preservation at moderate temperatures can be realized. However, when increasing the temperature an increased fluidity of the cell membrane leads to a reduced membrane stability and facilitates the electroporation process as discussed in section 1.3. ‘Thermal effects’. This aspect of a

combined PEF and thermal treatment and of the differentiation of the related inactivation effects will be discussed in detail in research paper III.

In order to obtain a maximum of food safety, a direct transfer of cells from the vital to the lethal fraction during the microbial inactivation is favorable. However, since the impact on food quality characteristics limits the applicable treatment intensities, a limited number of dead cells may result. Membrane damage and inactivation of microorganisms due to PEF was firstly considered as an all-or-nothing event (Russel et al., 2000; Simpson et al., 1999), but a differentiated approach is required even if the critical parameters for the electrical breakdown of cell membranes are exceeded. Membrane damage and sublethal injury was found to be repairable under certain conditions and the extent to which cells repair their injuries was reported to depend on the treatment intensity, the microorganism and the treatment medium pH (Garcia et al. 2005; Somolinos et al., 2008; Somolinos et al., 2008a). On the one hand, sublethally injured cell fractions are a risk from a food quality and safety point of view since these cells may recover and regain their initial vitality. On the other hand, sublethally injured fractions have a potential for subsequent complete inactivation by the application of additional hurdles such as suboptimal storage conditions or the application of other inactivation methods such as the application of antimicrobials or other food preservation technologies.

The microbial inactivation rates differ considerably between inactivation in simple media and inactivation in a complex matrix. This was partly attributed to the protective effect of some food compounds such as xanthan (Ho et al., 1995), proteins (Jaeger et al., 2009; Sampedro et al., 2006) or fat (Grahl et al., 1996). Other studies did not reveal differences in the microbial inactivation conducted in buffer or complex media (Dutreux et al., 2000), or did not detect the occurrence of sublethally injured cells after PEF treatment of complex food systems (Walkling-Ribeiro et al., 2008). However, inactivation kinetics obtained from PEF treatment in buffer systems have only limited comparability with real food products. In addition, model microorganisms used in most of the studies and the way of sample preparation differ significantly from the native state of the microbial population present in the real food system. The diverse and heterogeneous microbial flora present in real foods is not comparable to inoculated microorganisms in most cases due to strong variability of microbial species and physiological state of microorganisms. The consideration of microbial growth state, adaptation to the treatment media as well as the existence of inhomogeneous microbial populations with less sensitive subpopulations seem to be the most challenging aspects when transferring inactivation results to real products and industrial implementation.

Research paper I presents investigations on membrane damage and the occurrence of sublethally injured cells using flow cytometry as an analytical tool to evaluate physiological

cell fitness. The role of media complexity and the protective effect of food constituents in microbial inactivation were taken into consideration and will be discussed in the paper in more detail.

1.4.2. Food compounds affected by and affecting PEF performance

PEF as a non-thermal preservation technology for liquid media is mainly applied to complex food matrices containing not only the microbial cells but other food compounds. It is the aim of the process to achieve a maximum level of microbial inactivation while keeping detrimental effects to the surrounding food matrix minimal. Having the phospholipid bilayer as the main target of the electric field application, the treatment will selectively affect membrane structures and microbial cells. However, electric field and related side effects may have an impact on biomolecules that will be discussed below. On the other hand, the presence of food compounds and the fact of having microbial cells suspended in a complex food matrix will have an impact on the inactivation effectiveness of PEF in comparison to inactivation taking place in simple media. In addition, media composition and media properties determine its processability by PEF. Hence, the role of food compounds needs to be examined with regard to this aspect as well.

Effect of PEF on food compounds

When PEF treatment of liquid or semisolid raw materials is used for microbial inactivation, the desired effect is primarily the non-thermal pasteurisation of the food product considering the microbial cell as the target of the treatment. The modification of other food constituents as well as functional food properties may be considered as an unwanted side effect in most cases (Lelieveld et al., 2007; Toepfl et al., 2007). However, potential applications to use the electric field effect in order to modify functional properties of food constituents will gain increasing attention.

The evaluation of the effect of PEF on food compounds is complex. Available reports are limited and different experimental setups and processing parameters make them difficult to compare (Schuten et al., 2004; Van Loey et al., 2002; Yang et al., 2004). The consideration of electric field side effects such as temperature increase and temperature hot spots due to Joule heating effects within a non-uniform electric field (Jaeger et al., 2009, Jaeger et al., 2010) and the occurrence of electrochemical reactions and pH shifts (Saulis et al., 2005; Meneses et al., 2011) is the most challenging aspect within this context. The non-thermal

inactivation of microorganisms by PEF is based on the electroporation of membrane structures. High intensity electric fields are unlikely to affect covalent chemical bonds but the electric field application and related side effects may show an impact on food compounds and process modifications towards the inactivation of microorganisms and enzyme structures are also possible (Aguiló-Aguayo et al., 2010; Martín-Belloso et al., 2005). Due to the application of PEF, changes in the conformational state of proteins might cause changes in protein structure and enzyme activity (Tsong, 1990; Bendicho et al., 2003).

In general, the mechanisms involved in the inactivation of enzymes and the modification of proteins by PEF are not fully understood (Ohshima et al., 2006). Possible mechanisms could entail polarization of the protein molecule; dissociation of non-covalently linked protein subunits involved in quaternary structures; changes in the protein conformation so that hydrophobic amino acid or sulfhydryl groups are exposed; attraction of polarized structures by electrostatic forces, and hydrophobic interactions or covalent bonds forming aggregates (Castro et al., 2001; Perez et al., 2004). Floury et al. (2006) explained the effect of PEF on milk proteins as a result of the modification of the apparent charge after exposure to intensive electric fields and subsequent modification of ionic interactions of the proteins.

Own investigations on the PEF-preservation of milk revealed the potential to achieve a sufficient level of microbial inactivation while maintaining the native antimicrobial systems and avoiding denaturation of whey proteins as occurring during traditional thermal treatment.

Fig. 5 shows the impact of a pulsed electric field treatment of milk on the activity of major bioactive protein compounds. The impact of pulsed electric fields on bioactivity and antimicrobial activity of valuable food constituents like lysozyme, lactoperoxidase or lactoferrin were studied. Results showed residual activities above 90 % after pulsed electric field treatment. Densitometry of IgG and BSA bands after electrophoresis of whey proteins from raw, PEF-treated (38 kV/cm, 22 °C, different energy input in kJ/kg) and pasteurized (75 °C, 30 s) milk showed a significant lower denaturation of immunoglobulin G (IgG) and bovine serum albumin (BSA) for PEF pasteurized samples in comparison to heat treated milk. PEF treatment at high intensity levels decreased the content of native IgG and BSA of about 10 % whereas a reduction of 65 % IgG and 30 % BSA occurred after thermal pasteurization.

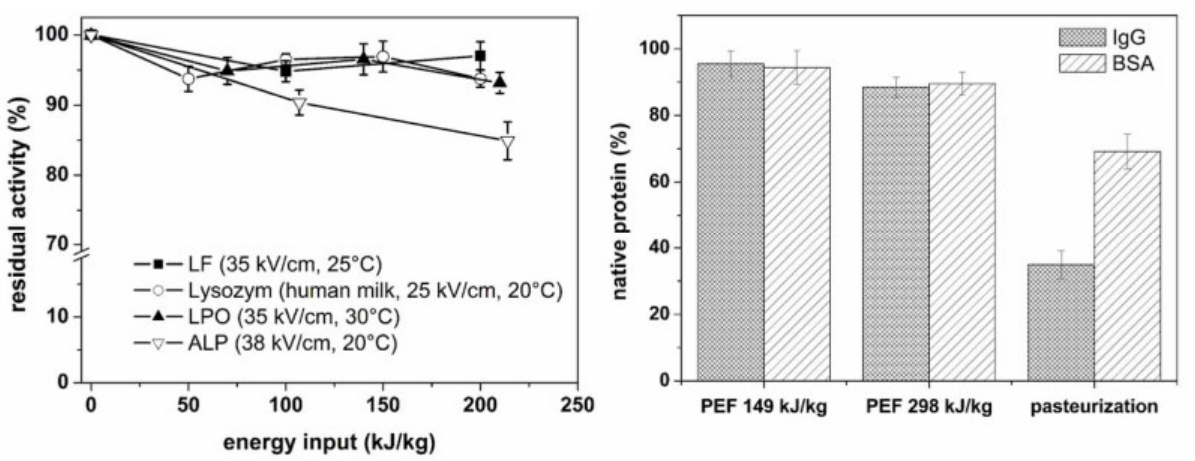


Fig. 5: Impact of PEF-pasteurization on enzymes and bioactive compounds in milk (LF lactoferrin, LPO Lactoperoxidase, ALP alkaline phosphatase) and comparison of the degradation of immunoglobulin G (IgG) and bovine serum albumin (BSA) during conventional thermal and PEF pasteurisation. Based on Jaeger et al. (2009).

As the PEF effect on enzymes and other milk proteins remained small its application could be used to reduce the microbial count in milk for production of raw-milk-type cheese varieties. A shelf life assessment of PEF treated milk was carried out by monitoring microbial growth, showing that the antimicrobial effect of an activated lactoperoxidase-system is retained after a PEF treatment and the synergistic effect of PEF inactivation. The retained antimicrobial activity of native milk constituents effectively prevents microbial growth. Other possible applications include the pasteurization of the above mentioned bioactive components after their isolation from milk for further use as bioactive proteins.

Only few data are available in literature on pulsed electric fields effects on proteins (Barsotti, et al. 2001). Perez et al. (2004) reported a partial modification of the native structure of β-Lactoglobulin when subjecting a concentrate to an electric field of 12.5 kV/cm. Thermal stability of β-Lg was reduced after PEF but gelation rate was shown to be enhanced. Bovine immunoglobulin G subjected to PEF at 41 kV/cm for 54 µs did not show any detectable changes in the secondary structure or thermal stability (Li et al., 2005). No effects of PEF treatment on the physicochemical properties of lactoferrin were found by Sui et al. (2010) for treatment intensities up to 35 kV/cm and a total specific energy input of 41 kJ/kg (treatment time 19 µs) with temperatures below 65 ℃.

Fernandez-Diaz (2000) studied the effects of pulsed electric fields on ovalbumin solutions (2 %; pH 7; 5 mS/cm) and dialyzed egg white (pH 9.2; 4 - 5 mS/cm) applying an electric field strength in the range of 27 – 33 kV/cm. Partial protein unfolding or enhanced SH ionization of ovalbumin was observed after PEF treatment and was found to increase with increasing the total specific energy input.

Recent investigations by Marco-Moles (2009) focused on the PEF effect on protein and lipids in liquid whole egg and the microstructure of these components studied by low temperature scanning electron microscopy (Cryo-SEM). A partial denaturation and insolubilisation of the protein was observed during conventional pasteurization and resulted in a thickening of the lipoprotein matrix as observed by Cryo-SEM. Microstructure of PEF treated samples showed some discontinuities in the lipoprotein matrix.

The modification of milk protein structure may also lead to changes in functional properties, such as coagulation. Yu et al. (2009) studied modifications obtained in a complex milk protein gel resulting after rennet coagulation of PEF treated milk. To follow the coagulation of PEF treated milk, dynamic rheological measurements were performed. Electric field intensity, treatment temperature and pulse number were found to significantly affect rennet coagulation properties of milk. An increase of these parameters resulted in a decrease of curd firmness and an increase of rennet coagulation time. However, the most extreme conditions applied in this study (50 °C inlet temperature and an electric field strength of 30 kV/cm, no information on the total specific energy input and outlet temperature is given) led to similar coagulating effect as thermal pasteurization, indicating that combined effect of long pulse PEF and heat treatment may cause minor impairment of milk coagulation properties. Floury et al. (2006) found a direct impact of the PEF processing at protein components of milk, such as casein, resulting in a decrease of viscosity and an enhancement of coagulation properties working at higher electric field strength levels (up to 55 kV/cm) but lower maximum process temperatures (below 30 °C).

The effect of a pulsed electric field treatment of suspensions of potato and corn starch was under investigation by Han et al. (2009) and Han et al. (2009a). Physicochemical properties of potato and corn starch granules were found to be significantly affected due to the electric field exposure with electric field strength intensities in the range of 30 – 50 kV/cm (no other PEF treatment parameters were reported). Treatment effects included an intragranular molecular rearrangement and a partial loss of the crystalline structure.

During a PEF treatment there are many factors which may have an impact on food compounds besides the electrical field as such. Temperature increase, pH shifts and electrochemical reactions are reported to occur (Morren et al., 2003; Roodenburg et al., 2005; Roodenburg et al., 2005a; Saulis et al., 2005; Jaeger et al., 2009). Especially proteins and enzymes are very sensitive to pH changes and local changes of pH can therefore have a significant impact on enzyme stability during PEF treatment (Meneses et al., 2011). The consideration of such impact parameters is a crucial prerequisite during the study of inactivation kinetics in order to exclude other simultaneously occurring side effects. Research paper II and III will present investigations on the impact of the temperature side effect

occurring during a PEF treatment on the inactivation or retention of heat sensitive food compounds.

Effect of food composition on PEF performance

The effect of food compounds on the microbial inactivation efficiency of PEF and occurring protective effects have already been described in section 1.4.1. 'Microbial inactivation' and will be discussed in detail in research paper I.

The following section will cover the aspect of the optimal PEF processability of foods and the effect of related material properties, such as electrical conductivity, thermal conductivity, viscosity, presence of suspended particles in liquids as well as presence of air inclusions that need to be taken in account. The exact definition of these physical properties and their interaction with fluid flow, heat transfer and electrical treatment of foods is of particular interest for the numerical simulation, which will be discussed in detail in in research paper II.

The electric behavior of foods is not only depending on the chemical composition, such as water content, fat, protein, carbohydrates and minerals, but also on the effects of dissolved and suspended solids or air. Foods containing different phases (i.e., solid particles, air bubbles or large fat globules) with greatly different properties from those of the continuous phase will be considered as heterogeneous. It needs to be taken into account that electric behavior of one phase is essentially different from another phase resulting in different electric field strength distribution, Joule heating rates as well as dielectric strength. Information on the rheological properties of the food products to be PEF treated is required for the proper design of the treatment chamber geometry. Modifications of the treatment chamber geometry can be used to affect the flow behavior and to induce mixing effects that may influence the treatment effects on heat sensitive compounds (Jaeger et al., 2009).

Most food products are complex mixtures of many different chemical compounds. Even the water may be bound in one of several different ways which may alter its effect on the thermal properties (Lewicki, 2004). Based on the composition of a food, it is possible to estimate the thermal properties such as the specific heat capacity (c_p) using prediction equations considering different specific heats and the content of proteins (X_p), fats (X_f), carbohydrates (X_c), water (X_w) and ash (X_a) in the food product as shown by equation (18) according to Heldman et al. (1981):

$$c_p = 1.424 \cdot X_c + 1.549 \cdot X_P + 1.675 \cdot X_f + 0.873 \cdot X_a + 4.187 \cdot X_w \quad (18)$$

Thermal conductivity values and their measurement depend on the structure or physical arrangement of the sample (voids, non-homogeneities, particle-to-particle contact, etc.) as well as on the chemical composition. For most liquid foods, an equation based on water, protein, carbohydrate, fat and ash content appears to be adequate. Choi et al. (1983) developed equation (19) for the calculation of the thermal conductivity (λ) of complex liquid foods depending on their composition:

$$\lambda_p = 0.205 \cdot X_c + 0.20 \cdot X_P + 0.175 \cdot X_f + 0.135 \cdot X_a + 0.61 \cdot X_w \quad (19)$$

For high moisture foods, one could assume that the change in specific heat and thermal conductivity mimics the change in specific heat and thermal conductivity of water with temperature which can be calculated according to Kessler (2002).

Information on the rheological properties of the food products to be PEF treated is required for the proper design of the treatment chamber geometry as well as the numerical simulation of heat transfer phenomena and fluid flow. The latter is of special importance since the flow velocity distribution in the treatment chamber determines the residence time and therefore the treatment time. The viscosity of Newtonian fluids (such as water, milk, and clear fruit juices) is influenced by temperature and composition, but is independent from shear rate and previous shear history (Borwankar, 1992). The rheological behaviour of the fluid food plays an important role in continuous PEF treatment, since it determines the fluid flow and the velocity profile. Equations describing velocity profiles can be used to examine the influence of different rheological models on the velocity distribution and for the determination of the residence time distribution of the fluid particles.

Since the viscosity also depends on the temperature, the control or documentation of the temperature is essential for the determination of the viscosity or for the conduction of experiments and calculations based on viscosity data. As an average value, there is about a 2 % decrease in viscosity for each degree Celsius change in temperature (Lewis, 1987). A product dependent relation between viscosity and temperature has to be determined experimentally, based on empirical equations or on estimations considering hydrodynamic volumes of the component fractions. Detailed information on physical properties can be found in Bertsch (1983) for milk or in Constenla et al. (1989) for fruit juice.

The electric behaviour of liquid foods is not only depending on the chemical composition such as the content of water, fat, protein, carbohydrates and minerals but also on the effects of dissolved and suspended solids which the liquid may contain. Small particle sizes, as the case for orange or tomato juice (homogeneous distribution) or products with randomly dispersed solids of relatively large particle size such as vegetable soup (heterogeneous) may

occur. Although the overall (volumetric) electrical properties of homogeneous and heterogeneous systems of similar moisture, dissolved salts and suspended solids content may be nearly identical the behaviour during PEF treatment is different with respect to effects of particle size, homogeneity and distribution.

The electrical properties such as the electrical conductivity and the electric strength of foods are of key relevance for the PEF treatment, since they determine the maximum applicable electric field intensity as well as the current flow. The coupling and distribution of the electric field strength, the energy and the product's heating is based on the electrical food properties. The mechanism or mode of energy transfer from the electric field to the product is the energy dissipation due to the Joule heating which couples electrical and thermal food properties. Foods conductivity ranges from σ = 0.6 mS/cm (tap water) or even below for pure fats and oils to values in excess of σ = 7 mS/cm for milk ultrafiltrates and other high conductivity products (Barsotti et al., 1999). Treatment chambers with high electrical conductive foods have a poor resistance and it is necessary to produce higher voltage to achieve the same effect of microbiological inactivation that is achieved during processing of low conductive foods. It is also more difficult to build sufficient field strength when the conductivity is too high (Wouters et al., 2001). To obtain the same degree of microbiological inactivation in foods with very different conductivity, the treatment conditions, such as the interelectrode gap in the treatment chamber, the pulse width and the voltage have to be adapted.

On the other hand, the presence of ions appears to be necessary to increase the transmembrane potential (Bruhn et al., 1998). The membrane will be weakened and more susceptible to an electric pulse in media with higher ionic strength, causing higher permeability and structural changes. However, the bactericidal effect of PEF is inversely proportional to the ionic strength of the suspension, namely the inactivation is generally enhanced when the medium has a high electric resistivity (Hülsheger et al., 1981; Mizuno et al., 1988).

Liquid or semisolid foods containing suspended phases (i.e., solid particles, air bubbles or large fat globules) with greatly different properties from those of the continuous phase will be considered as heterogeneous. Electric behaviour of one phase is essentially different from another phase resulting in different electric field strength distribution as well as Joule heating rates. A heterogeneous composition is also relevant for solid foods such as plant raw materials. Especially the inclusion of air in the intercellular space does affect the electric field performance. As it will be shown in the following section, not only compositional but also structural variations in the plant raw material such as differences in the cell size or in the location of target compounds to be recovered are of interest in order to analyse and improve the PEF process performance.

1.4.3. Plant material – structural and cell size effects

Besides the changes occurring on a cellular level, there has been very little research on the effect of PEF treatment on the microstructure of the raw material or the food produced with the PEF treated raw material. During PEF treatment of solid raw materials for cell disintegration and modification of tissue structure, the effect of the treatment on microstructure and texture is the main processing goal in order to improve subsequent processing steps such as drying or extraction (Grimi et al. 2007; Lebovka et al. 2007). It is the aim of the following section to discuss the effect of PEF on food microstructure based on the effects of membrane permeabilization occurring after exposure of biological tissue to an external electric field and the related release of cell content and the loss of turgor. These are the most relevant effects with a direct impact on food microstructure and textural properties. On the other hand, as already discussed for microbial inactivation effects of PEF and the role of food compounds, the food matrix, namely the plant tissue structure may affect the PEF performance in terms of the achievable level of cell disintegration but also in terms of the impact of cell disintegration on subsequent processing steps. Both interactions, PEF-plant tissue and tissue structure-PEF will be discussed in the following sections.

Plant tissue structure affecting PEF performance

PEF treatment of liquid or semisolid products for non-thermal pasteurization application is aimed at the permeabilization of microbial cells. Although variations regarding the sensitivity against PEF may occur within one single microbial population, the microbial cells in the population show an almost homogeneous distribution regarding structural characteristics. This is different in the case of PEF treatment of plant raw materials since cells with different structural properties occur in the same raw material forming different tissue segments. Hence, before applying the PEF technology to plant raw materials it is essential to analyze the specific particularities of the relevant tissue. A modification of structural tissue properties with an impact on subsequent reactions such as the induction of stress responses by reversible permeabilization or the reduction of mass transfer barriers by irreversible permebilization requires accurately defined treatment conditions in order to allow a targeted performance of the PEF process. Effective treatment conditions can only be determined by the consideration of raw material properties.

Fig. 6 shows exemplarily microscopic images from apple, carrot and blueberry tissue as well as from cellular substructures of onion cells. Pictures A, B and C (apple, carrot, blueberry) have the same scale and it is obvious, that major differences exist between the raw materials

regarding the cell size. For apple tissue, an average cell size of around 200 µm was determined and is in accordance with values reported for apple mesocarp cells by Bain (1950) and McAtee (2009). Carrot cell size was found to be around 70 µm and is similar to values reported by Zdunek (2007). When considering the raw material as a whole, the distribution of the cell size is almost homogenous. In addition, differences in the cell structure of apple between the skin and the flesh do not have a large impact since the proportion of the skin is small in comparison to the flesh tissue. This is different for blueberries since the surface-volume-ratio is increased for small fruits and the characteristics of the cells in the skin become more important. For the blueberry, a huge difference in size is found between cells in the skin and cells in the flesh. Whereas the size of skin cells is around 40 µm, cells in the flesh are larger with a cell size of around 140 µm.

Cell size has a significant impact on the cell disintegration either by mechanical means or by PEF treatment. Grinding of tissue with small cells in comparison to large cells will result in a lower degree of cell disintegration since the resulting particles of a given size above cell size, contain a higher number of intact cells. An increase in cell disintegration can be achieved by increasing the grinding intensity leading to a further reduction in particle size. For larger cells, the same particle size distribution after grinding will result in a higher degree of mechanical disintegration already since a higher number of larger cells was destroyed. Hence, the disintegration of carrot cells requires a more severe grinding in comparison to apple cells which is reflected in the processing technology such as the use of colloid mills or homogenizers (Reiter et al., 2003). In the case of processing of blueberries, the inhomogeneous cell size distribution between skin and flesh results in a lower degree of cell disintegration of the skin cells. Hence, in order to disrupt these cells and to facilitate the release of target compounds such as anthocyanins occurring at a high concentration in skin cells, the processing of the mash includes additional thermal and enzymatic treatments (Lee et al., 2002).

Differences in cell size of the raw material have to be taken into account for PEF processing as well. PEF applications such as the disintegration of fruit and vegetable mashes are mainly applied after mechanical disintegration. Depending on the cell size, a different degree of mechanical cell disruption will occur and will affect the PEF performance since a varying fraction of intact cells remains available for cell disintegration by PEF. Hence, the consideration of the cell disintegration index after grinding and mechanical disintegration is essential in order to evaluate the potential of an additional PEF application. This aspect will be discussed in detail in research paper IV.

As discussed in section 1.1.2. ‘PEF impact on biological cells’, the cell size affects the electroporation since the transmembrane potential is proportional to the cell radius. Lower

intensity electric fields will be required to achieve electroporation in larger cells (equation (17) according to Zimmermann et al., 1974).

$\Delta V_m = \frac{3}{2} \cdot E_{ext} \cdot r \cdot \cos(\theta)$	ΔV_m E_{ext} r θ	transmembrane potential [V] external electric field strength [V/mm] radius [mm] angle between radius and electric field direction	(17)

Hence, when dealing with a large variation in cell size, it would be required to optimize the magnitude of the applied electric field for each cell type. This is not possible for structured, complex raw materials. As a consequence, a selective electroporation of large cells will occur in heterogeneous samples. Taking this fact into account for the irreversible electroporation of blueberry cells in order to enhance extraction processes, external electric field intensities need to be sufficiently high in order to allow the permeabilization of the smaller skin cells containing most of the bioactive target compounds such as anthocyanins. The dimensioning of electric field parameters for the reversible permeabilization of inhomogeneous plant raw material is more challenging. Reversible permeabilization is intended to induce a stress reaction in the plant cell aiming at increasing the production of valuable secondary plant metabolites. A targeted treatment can be applied to plant cell cultures (Dörnenburg et al., 1995) or to complex raw material such as potato (Gomez-Galindo et al., 2009) with an almost homogeneous cell size distribution. However, in case of raw material with an inhomogenous cell size such as blueberry it is not possible to achieve a homogeneous reversible permeabilization since larger cells will already be affected by irreversible pore formation to a certain extent. Due to the fact that the target compounds and their synthesis takes place dominantly in the small skin cells, it is impossible to affect this cell type in a reversible manner without causing the formation of permanent pores in the flesh cells leading to the loss of cell vitality. Hence, it is essential to conduct an analysis of effects taking place on the microscale level before developing processing concepts of particular raw materials.

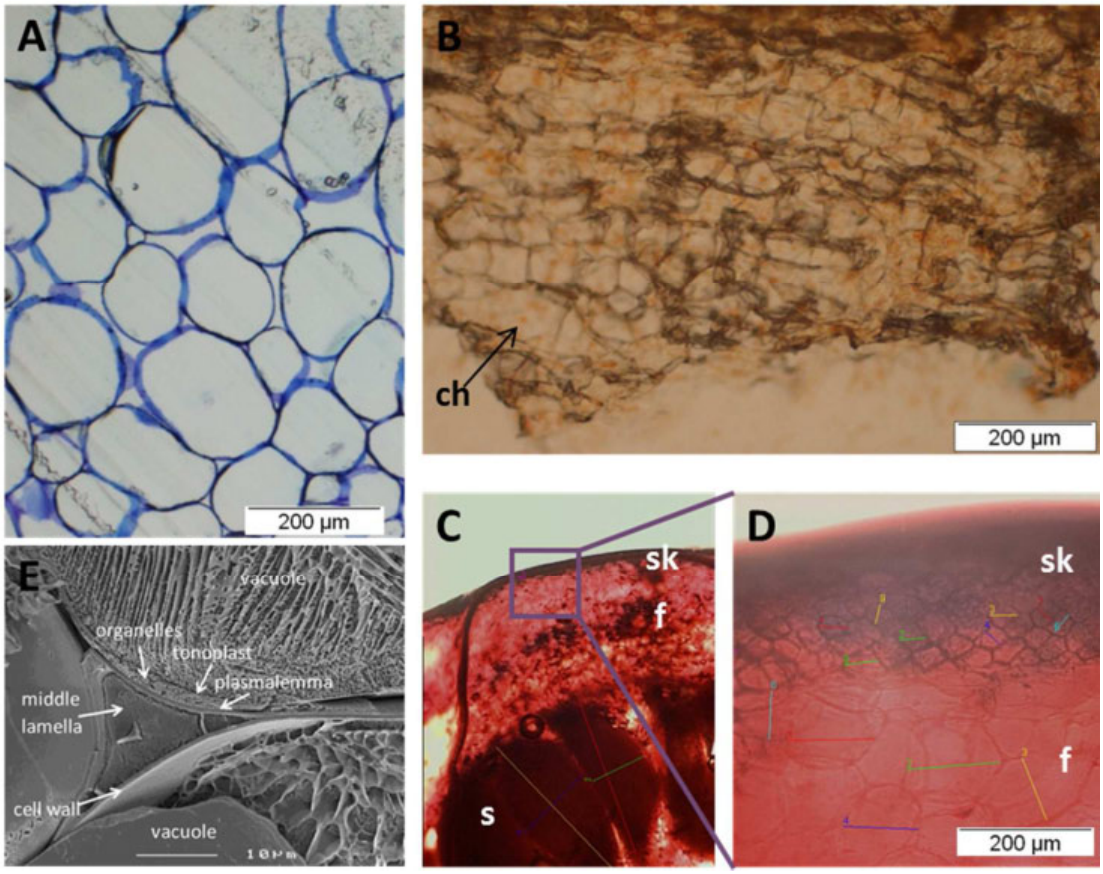


Fig. 6: Microscopic images of A) apple tissue, B) carrot tissue, C and D) blueberry tissue and E) onion cells. Sources: A) Schoessler et al. (2011); B) Sevenich and Salimi (unpublished); C and D) own data; E) Gonzalez et al. (2010). Substructures are shown such as chromoplasts (ch), the skin (sk), the flesh (f) and the seeds (s).

The above discussed aspects were mainly related to cell size and cellular structure. In addition to that, cellular substructures are of interest. The cellular liquid is mainly located in the vacuole and in the cytoplasm and the vacuole is occupying a major part of the cell volume (Taiz et al., 2007). Sugars, organic acids and bioactive compounds such as polyphenols are found at higher concentrations in the vacuolar sap. Other compounds such as carotenoids are located in chromoplasts as indicated in Fig. 6B. The effect of PEF on the cell disintegration of different cell structures is not fully understood yet. Investigations on membrane permeabilization of plant tissue reported by Angersbach et al. (1999), Chalermchat et al. (2010), Fincan et al. (2002) and Lebovka et al. (2001) gave evidence that not only the cell membrane but also the vacuolar membrane is affected. However, smaller cell compartments such as the chromoplasts which have a size of about 10 µm (Straus, 1961; Kim et al., 2010) will not be affected by the applied electrical pulse protocols in the microsecond range (Schoenbach et al., 2004) and a direct effect on the solubilization of crystalline carotenoids from chromoplasts by PEF is unlikely. Hence, thermal treatment or higher intensity mechanical cell disintegration may be required in addition to PEF cell

disintegration in order to affect the release of carotenoids from chromoplasts or the transfer of chromoplasts into extracts or juices. This example points out the importance of the analysis of the location and type of integration of the desired target compound to be released from the cell since it will determine the appropriate processing concept.

Another point of high relevance is the location of different enzymes in the cell and tissue which may contribute to the degradation of secondary plant metabolites. Polyphenoloxidase, an enzyme responsible for the oxidation of polyphenols and the subsequent enzymatic browning of fruits, is located in plastids or chloroplasts in intact cells (Boss et al., 1995; Murata et al., 1997; Mayer, 2006). Polyphenoloxidase occurs in subcellular structures of almost all cells in the fruit. Fig. 6E illustrates the physical separation of onion cells by the cell wall and cell membrane. The vacuole surrounded by the tonoplast occupies almost the total cell volume whereas other cell organelles such as plastids are comparably small in size being around one micrometer (Gonzalez et al., 2010)

A different way of location is found for myrosinase in *Brassicaceae*, an enzyme which catalyzes the hydrolysis of glucosinolates. Myrosinase is accumulated in distinct myrosin cells that are embedded in the tissue (Bones et al., 1996; Husebye et al., 2002). Hence, a cellular separation of myrosinase enzyme and glucosinolate substracte is observed in *Brassicaceae* whereas for the polyphenoloxidase enzyme and the polyphenol substrate a separation takes place on a subcellular level in plants.

Cell disintegration by either mechanical means or PEF is reducing the membrane barrier function of the cell compartments and promotes the contact between enzyme and substrate which may lead to enhanced degradation reactions. However, PEF disintegration is more likely to affect the cell membrane and the tonoplast and to facilitate the release of cytoplasm and vacuolar sap whereas much smaller organelles such as plastids are less affected by the microsecond pulses (Schoenbach et al., 2004).

Own investigations were performed regarding PEF disintegration of *Brassicaceae* tissue composed of myrosin cells containing myrosinase and other cells containing glucosinolates. A decrease of the glucosinolate concentration was found in the tissue but no increase was found in the extract. Hence, the degradation of glucosinolates by myrosinase was facilitated due to PEF cell permeabilization rather than the release. However, for the same tissue containing no myrosinase, an enhanced extractability and higher concentrations of glucosinolates in the extract were found due to the PEF cell disintegration (Fig. 7). Hence, due to the particularities of this type of raw material, PEF is not an appropriate technology for the improvement of the recovery of glucosinolates. Additional measures have to be performed such as the variation of pH or media salt concentration in order to control and

reduce the myrosinase activity before cell permeabilization and enzyme substrate contact takes place. A microscale analysis of structural as well as functional cell characteristics is required in order to complement mesoscale processes such as extraction by PEF cell disintegration.

The effects of PEF regarding a selective release of compounds from different cell compartments and the aspect of the release of enzymes and enzymatic degradation of compounds will be further discussed in research paper IV with focus on polyphenols and polyphenoloxidase.

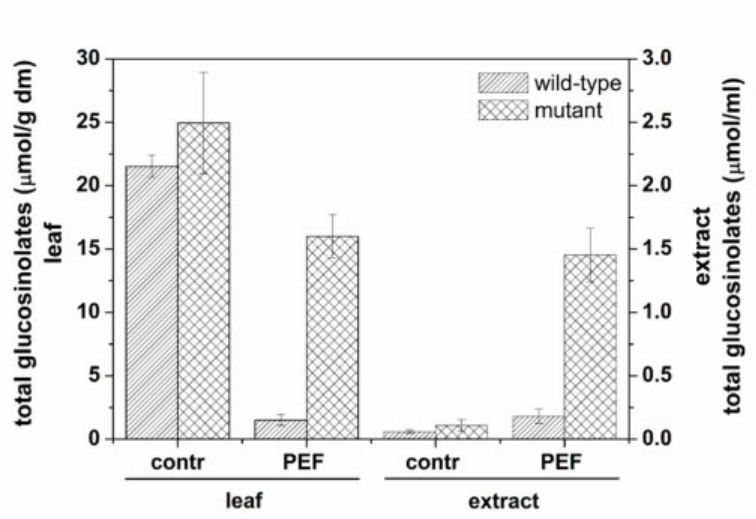


Fig. 7: Glucosinolate content in leaves and leaf extracts of *Arabidopsis columbia* wild-type Col-0 WT and *Arabidopsis columbia* tgg1tgg2 double knock-out mutant (undetectable myrosinase activity) after PEF cell disintegration (3 kV/cm, 5 kJ/kg) and water extraction for 30 min. Based on Lüttich, Jäger, Mewis, Schreiner and Knorr, unpublished.

PEF treatment affecting tissue structure and the performance of connected food processing steps

PEF is affecting the cell membranes and thus can be expected to influence the texture of products in which the structure is largely dependent on the integrity of cells. Release of water in the extracellular space or changes in water retention properties, damage to the cell membranes as well as a possible enhancement of enzyme reactions may occur as immediate or delayed effects. The possible use of pulsed electric fields in food processing has now been investigated for number of years. These studies have mainly been focused on the effect of electric pulses on inactivation of different types of microorganisms in different states and also electric permeabilization of plant cells to increase the yield of different material such as juices. Besides the changes occurring on a cellular level, there has been

little research on the effect of PEF treatment on microstructures of the raw material or the food which is generated during further processing of the PEF treated raw material. Fundamental research on the modification of textural properties of plant and animal raw materials represents the basis for further possible applications.

Lebovka et al. (2004) studied the impact of PEF on apple, carrot and potato tissue. Stress deformation and relaxation tests were performed in order to analyse the changes in tissue texture. PEF treatment in combination with mild heat pre-treatment led to complete elimination of the textural strength of tissue. It was shown that by proper selection of PEF treatment conditions, it was possible to obtain a controlled degree of tissue softening.

A comparison between thermal and PEF treatment as well as combination of both with regard to the textural properties of carrots, potatoes and apples was conducted by Lebovka et al. (2004). Destruction of the cell membranes as well as removal of the cellular turgor were defined as the main impact factors for the modification of texture and viscoelastic properties of plant tissue after PEF treatment. However, application of PEF-treatment will have limited effects on softening of the cell walls and reduction of their integrity when compared to thermal treatment. After the PEF treatment, the tissues lost a part of its initial strength, and both the elasticity modulus and the fracture stress decreased with increasing PEF treatment time. This effect was more pronounced for carrot and apple tissues whereas even highly PEF disintegrated potato tissue showed nearly the same stress–deformation behavior as an intact tissue. Heat treatment not only influenced the turgor component but also the other constituents of the tissue, such as changing of inner chemical structure of the cell wall, their breakdown or swelling, starch gelatinization, protein insolubilization or the expulsion of trapped air.

The modification of tissue physical properties by PEF as described above mainly results from changes in the functional properties of cell membranes. Investigations by Pereira et al. (2009) revealed the impact of size and persistence of PEF created pores on viscoelastic properties of potato tissue. The tan-delta value, defined as the ratio between elastic modulus and viscous modulus, was obtained from dynamic rheological measurements and used to describe the effect of different PEF treatment intensities on tissue structure. More intense PEF treatment results in the formation of larger pores and faster escape of cell liquid and leads to a higher increase of tan-delta values. Reversible permeabilization and pore resealing can be observed by a slow decrease and fast increase of the tan-delta value due to the recovery process and the re-absorbance of leaked cell content which increases the turgor pressure. It was shown that the viscoelastic response may be independent from the degree of permeabilization but affected by the size and distribution of the membrane pores.

The occurring increase in membrane permeability due to PEF treatment positively affects the mass transfer rate and was already shown to enhance drying processes by reducing the drying time significantly (Lebovka et al., 2007, Ade-Omowaye et al., 2003).The effect of the electroporation on the underlying mass transfer phenomena in plant tissue can be well explained on a cellular level. However, information on the permeabilization effect is limited when it comes to the internal transport properties of the complex food raw material structure. Therefore, an investigation of the water transport, distribution of soluble solids and the location of the drying front (marking the interface between saturated and partially dry regions) was conducted on a tissue level (Jaeger et al., 2010).

Apple cubes were subjected to a convective hot air drying (60 °C). The impact of a PEF pre-treatment (3 kV/cm, 11 kJ/kg) on tissue integrity, drying rate, water and fructose distribution was analyzed. The local water distribution in the apple cubes during the drying process was monitored by Magnetic Resonance Imaging. The PEF treatment of the apple cubes at the above given treatment intensity resulted in a cell disintegration index of 0.47 which was determined by electrical impedance measurement (Angersbach et al., 1999) and indicates a membrane permeabilization of 47 % of the cells.

Analysis of the drying curves shows a 21 % reduction of drying time in case of the pre-treated samples in comparison to the control sample in order to reach the common moisture level of industrially dried apple products of 20 % (wet basis). Higher drying rates were observed for the PEF treated samples and the drying front was found to migrate faster towards the core of the sample. A lower standard deviation for the moisture content at different drying times was obtained for the PEF treated samples. This indicates a more homogeneous drying process as a result of the PEF induced cell disintegration which compensates textural differences in the raw material properties.

Since the water in the apple tissue exists as a solution of other substances, such as sugar, the modification of mass transfer phenomena will affect them as well. The average initial moisture content of the apple tissue used for the experiments was 85.0 % (±2.6) on a wet basis and a fructose content of 397 g/kg dry matter was determined.

The migration of fructose depending on drying time and pre-treatment is shown in Fig. 8. Illustrated is the fructose ratio between core and surface of the apple cube whereas values above 1 indicate a higher fructose concentration in the core.

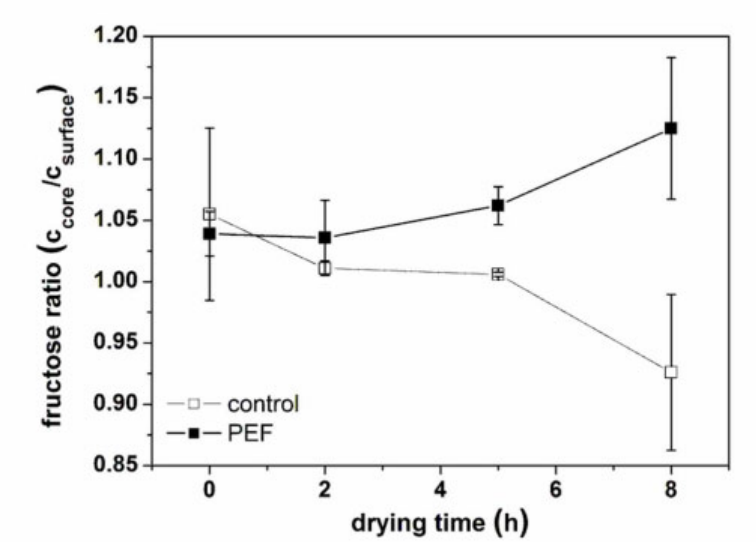


Fig. 8: Effect of PEF pre-treatment on fructose distribution in apple cubes (core and surface layer) during hot air drying. Fructose concentration of the two parts was calculated based on dry matter at the corresponding drying time. Based on Jaeger et al. (2010).

The fructose distribution in the sample was studied and found to differ significantly between the PEF pre-treated and the untreated apple cubes during and after drying. For the untreated sample, the fructose ratio decreases showing an increasing concentration of fructose in the surface layer as drying proceeds. This finding was expected since the dissolved fructose migrates and changes its location during drying. Moisture (liquid rather than water vapor) is moving towards the surface of the apple cube and as the solution nears the surface, pure water evaporates from it, leaving behind an increasingly concentrated solution in the outer layer. In the completely dry product, a gradation of percentage of soluble material should be apparent with the highest concentration at the surface of the apple cube.

The fructose migration in the PEF treated sample shows an opposite behavior. The fructose ratio increases during drying indicating higher fructose concentrations in the core of the apple cube. This migration is also more pronounced when drying time proceeds.

Higher drying rates and shorter drying times that were found for the PEF treated sample do not allow a direct conclusion on the movement of water (and soluble solids). In order to investigate the mass transfer and especially the location of the drying front where the water evaporates and leaves behind the dissolved solids, the water distribution in the apple cube was determined by MRI during drying.

The water distribution in the control and PEF treated apple cube after 2 hours of drying is illustrated in Fig 9. PEF pre-treated samples show a higher level of shrinkage at the same drying time since higher water losses occur in comparison to the control sample. A less homogeneous water distribution is also visible for the pre-treated sample indicating a lower

water content in the outer layers of the apple cube. In order to characterize the location of the drying front, the relative water content given as the averaged NMR signal intensity within a rectangular region of interest is shown.

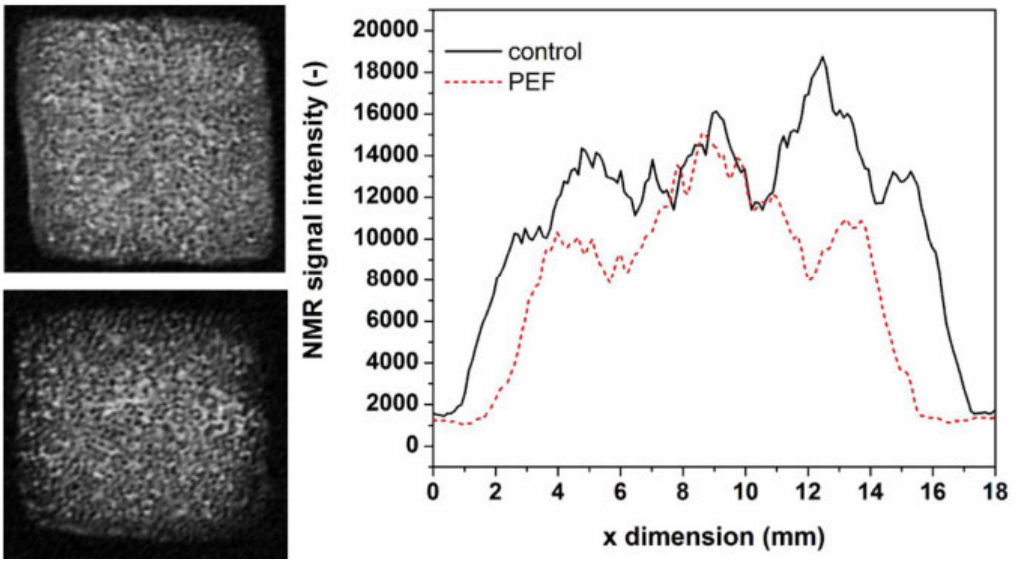


Fig. 9: Left: Water distribution in the middle layer of the apple cube (top: control; bottom: PEF) after 2 hours of drying. White color indicates higher water content. Right: Water distribution along the center line of the apple cube as determined by MRI. Based on Jaeger et al. (2010).

The control sample shows a lower water content within a 3 mm surface layer and a larger high moisture zone within the core of the apple cube. For the PEF treated sample, the surface layer with a lower water content has a dimension in the range of 4 – 6 mm indicating that the drying front is already located closer to the center of the cube. After the wetted area on the surface has totally disappeared (first drying stage) the liquid surface recedes into the capillaries and goes deeper as drying continues. This occurs faster for the PEF treated sample in comparison to the control sample. The evaporation takes place at a certain distance below the food surface (the drying front) and diffusion of vapor occurs from the place of vaporization to the surface.

Since for the PEF pre-treated sample the drying front moves faster and is thus located closer to the center of the apple cube, the evaporation of the water occurs in the deeper layers within the apple cube. The outward mass flow of the liquid component of the tissue through pores, cracks and capillaries takes place as water vapor and the evaporation of water in the deeper layers leaves behind an increasingly concentrated solution in the core of the apple cube which may result in a higher fructose concentration. Hence, the finding of a lower fructose concentration found in the surface layer of the cube could be partly related to differences in the water transport in PEF permeabilized samples.

The example shows that the cell disintegration achieved by PEF is not only affecting the drying process in terms of facilitated water removal. In addition, the modification of water transport also affects the distribution of solutes such as fructose. Despite the reduction of drying times and resulting energy savings and improved product quality the results exemplify the complex interactions between the PEF process and the cell disintegration, the modification of structure and related mass transfer processes and product functional attributes such as sugar content distribution. Furthermore, the obtained data on the fructose distribution reveal the possibility for a tailor-made modification of final product characteristics. Affecting the distribution of soluble compounds may have a potential to increase the storage stability of light or oxygen sensitive constituents by transferring them to the center of the product and to modify sensorial product properties. Investigations performed by Mosca et al. (2010) on the enhancement of sweetness intensity in gels by inhomogeneous distribution of sucrose revealed that samples with an inhomogeneous sucrose concentration were perceived sweeter than samples with the same content of sucrose but with a homogeneous distribution. As a consequence, it was shown that an inhomogeneous distribution of sucrose can be used to reduce sucrose content by 20 % without a decrease in sweetness intensity.

In addition to the example given above, research paper IV will also illustrate the aspect of the creation of tailor-made structural characteristics by applying PEF treatment to plant raw materials. The effect of a PEF treatment of fruit and vegetable mashes on the juice winning properties was investigated. Using mechanical grinding as well as PEF treatment, a process specific induction of a structure modification was shown. Whereas mechanical disintegration was shown to simultaneously modify particle size distributions, the PEF disintegration was independent from particle size modifications. The impact of the different technologies and of the different structural modifications on the juice release properties of the mash will be presented considering mash functionality and particularities of the solid-liquid separation process.

1.5. Process integration

As discussed in section 1.1.3 ‘Applications of PEF in food processing’ and as shown by the various examples given in the previous sections, using the PEF treatment aims at replacing or complementing the existing food processing operations. When PEF is applied for the cell disintegration of plant or animal raw materials, it is not the cell disintegration as such which is in the focus of the process but it is the impact of the cell disintegration on subsequent processing steps such as cutting, drying, extraction or infusion of compounds. On the other hand, unit operations such as grinding of the raw material are applied prior to the PEF

processing and will have an impact on the PEF treatment performance as well as on resulting material properties. Hence, due to the various interactions of the different processing steps, it is essential to analyze not only the PEF process as such but also its integration into complex food processing systems. This aspect is introduced by the three examples given below as well as by research paper IV where the aspect of the adjustment of different processing steps and the integration of the PEF treatment will be discussed in detail.

For the application of PEF for microbial inactivation, the PEF process can be applied as a stand-alone system as an alternative to the traditional thermal pasteurization and interactions with pre- as well as post-PEF processing operations are less pronounced. However, since the resistance of various types of microorganisms and enzymes covers an enormous range, a combination of several preservation methods based on different inactivation mechanisms will provide additional or synergetic inactivation results according to the hurdle concept (Leistner, 1995). Hence, the PEF process integration becomes an important aspect in the field of microbial integration as well since complex systems with different interacting steps will arise.

Process concepts such as the thermal assisted PEF processing have been proposed and were discussed in section 1.3. ‘Thermal effects’ as well as in research paper III. Also, combined treatments such as the application of PEF and antimicrobials have been studied as a potential application of the hurdle concept in food preservation (Nguyen et al., 2007; Mosqueda-Melgar et al., 2008).

The example given below introduces a concept of process combinations including the stepwise application of PEF and ultrasound (US) treatment. The effect of PEF on biological cells and microorganisms was discussed in section 1.1.2. ‘Impact on biological cells’ and 1.4.1. ‘Microbial inactivation’. Ultrasound effects on cells were reported to occur due to cavitation phenomena such as shear disruption, localized heating or free radical formation (Hughes et al., 1962). Cell wall and membrane damage, separation of the cytoplasmic membrane as well as DNA damage may result (Kinsloe et al., 1954; Hughes et al., 1962; Lee et al., 2009). Additive and synergetic inactivation effects were found for combination of ultrasound with pressure or elevated temperatures as well (Knorr et al., 2004).

Hence, the effect of the two processes, PEF and US, on the cell membrane and other targets in the cell, make the combination treatment an interesting approach in order to improve inactivation result of microorganisms in liquid products. Two basic application concepts could be of interest: i) weakening of the cell membrane by ultrasound and facilitating the subsequent pore formation by PEF or ii) cell membrane permeabilization by PEF and

subsequent ultrasound application in order to limit pore resealing and cause further inactivation of sublethally injured cell fractions. In addition, concepts such as the simultaneous application of PEF and ultrasound might be of interest and require further equipment design and investigation.

In order to study the process performance of a combined stepwise PEF and ultrasound application, a pilot scale system was developed for the treatment of liquid food products. The system is shown in Fig. 10 (left) and allowed the flexible positioning of the PEF and ultrasound treatment step in order to study the PEF+US as well as the US+PEF combinations. Additional heat exchangers were implemented in order to control the product temperature and the US treatment chamber also contained a jacket heat exchanger with active cooling in order to remove excessive heat.

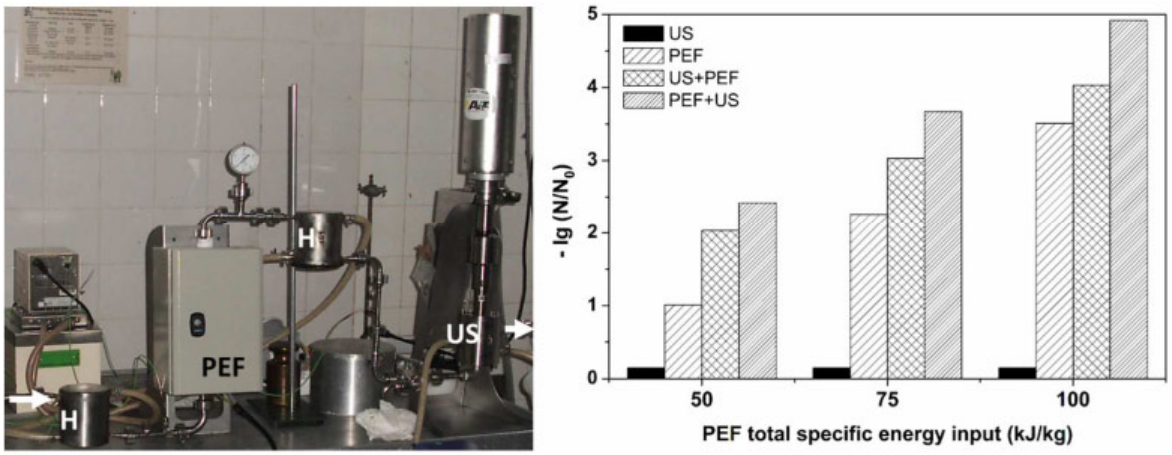


Fig. 10: Left: Pilot scale unit for preservation of liquid foods using a combined stepwise PEF-ultrasound treatment. PEF - pulsed electric field treatment chamber; US - ultrasound treatment chamber; H - heat exchanger. Right: Inactivation of *Streptococcus thermophilus* in Ringer solution using a single PEF and US treatment as well as combination treatments.

A thermotolerant microorganism, *Streptococcus thermophilus*, was chosen in order to limit the impact of additional thermal effects on the inactivation of the microorganism. In addition, the inactivation of thermotolerant microorganisms is of interest since their susceptibility to PEF inactivation may differ from the one related to thermal inactivation due to the different inactivation mechanisms.

Ultrasound treatment of the suspension of the microorganism in Ringer solution was applied using an amplitude of 147 µm and an average exposure time to the sonication field of 2.7 s while the product was pumped continuously through the system and the ultrasound treatment chamber. As shown in Fig. 10 (right) single US processing (inlet temperature 35 ℃) did not result in a relevant level of inactivation of *Streptococcus thermophilus.* The application of a

single PEF treatment (34 kV/cm, 40 °C, different to tal specific energy input as indicated in Fig. 10) lead to inactivation results in the range of 1 – 3.5 log cycles depending on the energy input. Applying the US treatment before the PEF treatment (with intermediate cooling), PEF inactivation results are increased of up to 0.5 – 1 log cycle indicating that pre-stressing of the microbial cell with sonication increases its sensitivity towards PEF inactivation probably due to initial weakening of the membrane. When applying the US treatment after the PEF treatment, a more pronounced increase of PEF inactivation results of 1.4 log cycles is detected. Hence, PEF treated *Streptococcus thermophilus* cells are more sensitive to US treatment when applied after PEF treatment and the inactivation results of the PEF treatment can be improved. Underlying mechanisms could entail the enlargement of PEF induced pores by cavitation and microstreaming effects as well as the deceleration of membrane resealing or the increase of intracellular cavitation effects after electroporation. Huang et al. (2006) reported additive effects only when applying combination treatments of PEF and US to inactivate *Salmonella enteritidis* in liquid whole egg but no synergism was found. However, treatments were performed in batch mode instead of a continuous flow through operation which may have resulted in longer intermediate times between the treatments. Hence, synergetic effects based on temporary weakening of the membrane or reversible pore formation will be less pronounced.

Further studies are required in order to confirm and complement proposed mechanisms of the combination treatment in the microscale on a cellular level. However, the process analysis undertaken in the mesoscale (inactivation level) already revealed differences in the process performance depending on the sequence of the PEF and US treatment showing improved inactivation results for the combination PEF+US in comparison to US+PEF or the single treatments. Differences were related to the process arrangement being an aspect which belongs to the macroscale as described in the introduction section. Hence, this arrangement aspect and the different way of integrating the PEF treatment in the whole processing concept will affect the PEF process performance and can improve process results. The combination of techniques that deliver effective preservation without the excessive use of any single conventional process parameter such as time or temperature allows the selective retention or inactivation of food constituents. The combination of PEF with other stress factors like mild heat, antimicrobial compounds, pH or organic acids as well as the combination with other thermal or non-thermal decontamination techniques will determine further development (Alvarez et al., 2007).

The following two examples of PEF process integration refer to its application for the cell disintegration of plant raw materials covering the PEF assisted juice recovery from fruit and vegetable mashes as well as the PEF assisted recovery of olive oil.

In order to implement the PEF cell disintegration as a processing step into existing processes of juice winning, an integrative approach will be required as discussed in research paper IV. The consideration of pre- and post-PEF processing unit operations such as mechanical disintegration and solid-liquid separation is essential in order to successfully transfer the cell disintegration provided by PEF (microscale) into improved process results such as higher juice yields. Fig. 11 illustrates the different key steps in the production process including the milling, PEF treatment and de-juicing as well as the related process results such as particle size reduction and mechanical disintegration, cell disintegration by electroporation and the solid-liquid separation. Each of the processing steps can be performed using different equipment and as well as variation of the processing parameters. The implementation of the PEF treatment in an existing juice processing line will be simple from a technical point of view concerning the installation of the treatment chamber as well as the availability of industrial scale pulse modulators (Toepfl, 2011). However, the challenging aspect is the adjustment of the material properties of processed mash as well as the adjustment of the process parameters of the connected processing steps. The main impact factors affecting the juice yield are the press design and operation, the raw material properties such as the degree of ripeness, the degree of milling, mash treatment and the number of juice drainage channels opened during pressing (Al-Mashat et al., 1993). Hence, all these parameters need to be taken into consideration when investigating the impact of the pre-treatment of the mash by PEF.

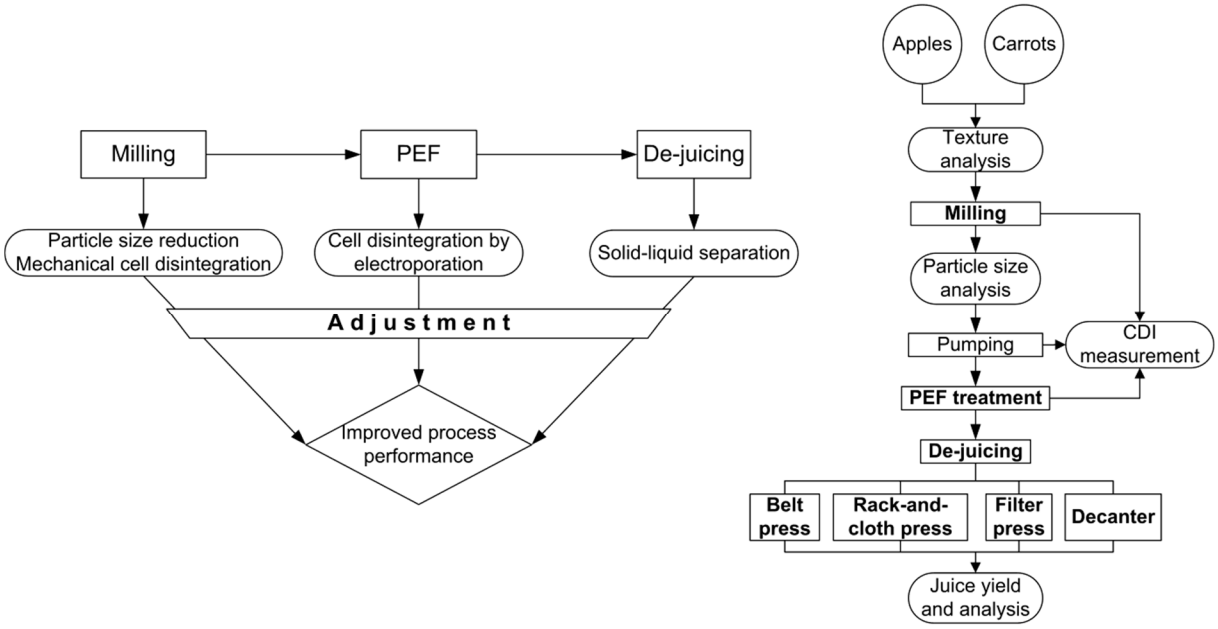


Fig. 11: Left: Concept for the improvement of the process performance of a PEF assisted juice recovery by consideration of connected processing steps. Right: Juice recovery process from apples and carrots: processing steps and related process analysis as performed in research paper IV. CDI refers to ‘cell disintegration index’ as determined by impedance measurement.

Whereas mash characteristics related to particle size or cell disintegration can be mainly controlled by operational parameters such as milling intensity or PEF treatment intensity, the de-juicing process depends very much on the system that is used for the solid-liquid separation. In addition to the operational parameters that can be adjusted for each of the de-juicing systems, the completely different design and working principle require a certain mash structure and pressing behavior on the one hand but offer also the selection of appropriate conditions for a given mash characteristic. Four different de-juicing systems are shown in Fig. 12. The systems are based on different separation principles as well as on design variations regarding the realization of the particular solid-liquid separation. As discussed before, due to the process-product interactions, there will be an impact of the material structure on the separation process, e.g. juice release performance, as well as an impact of the process on the material, e.g. compaction of the mash. Again, phenomena are involved within each scale level as well as related to the process, the structure and the property of the respective raw material. In order to evaluate juice yield results related to different mash structures and PEF treatment intensities, the operating principle of the solid liquid separation system needs to be taken into account. Processing with a belt press is based on a thin layer of mash being squeezed between a perforated moving belt and a series of serpentine winded rollers with decreasing diameter. The applied pressure on the press-cake increases gradually and occurring shearing effects on the cake favor the juice release. For the rack-and-cloth press, the mash is wrapped in coarse-weave press cloths and built up in layers between racks made of wooden slats or metal plates. Pressure is applied to the stack by means of a hydraulic ram. A cylinder-piston system with flexible drainage elements in between is the design principle of the horizontal hydraulic filter press. The press cycle consists of several pressing steps with loosening of the mash between them by backward movement of the piston. The working principle of the decanter is based on the generation of a centrifugal force by rotation of a bowl. The mash is pumped continuously in the bowl and separated into solid particles pressed against the wall and the juice forming a concentric inner layer. The solids are compacted and discharged from the bowl by a screw conveyer.

The consideration and combination of the various parameters related to material properties, disintegration technologies as well as the performance properties of different de-juicing equipment leads to a complex multi-scale and multi-parameter system. The analysis of the occurring interdependencies was shown to be the key factor in order to control the beneficial effect of a PEF treatment of the mash on juice yield. As shown in research paper IV, favorable and unfavorable conditions were identified regarding the mash structure as well as the de-juicing characteristics in order to improve the juice yield increase by PEF. The developed concept can be applied to different multi-step processes including a potential PEF application. A process analysis and optimization provide the basis for the evaluation of the

process performance which in turn is a prerequisite for the decision regarding the use of the PEF treatment for a specific application.

Fig. 12: Pilot scale de-juicing systems. A) belt press; B) rack-and-cloth press; C) horizontal hydraulic filter press; D) decanter.

Increasing the complexity and the scale of the PEF trials requires an appropriate experimental practice in order to allow the evaluation of the impact of the PEF treatment on the overall process performance. An example is given in Fig. 13 showing a pilot system for the PEF assisted recovery of olive oil. The conventional processing consists of the crushing of olives, a malaxation step and the separation of the oil from the remaining pomace using a decanter. The PEF treatment is implemented between the crusher and the malaxer in order to allow a continuous treatment of the olive paste. The malaxation process includes a mechanical agitation at moderate or elevated temperature. It is the aim to induce coalescence and to cause small oil droplets that are released from the olive cell to merge into larger drops in order to facilitate the subsequent separation process (Boskou, 2006). Hence, a high level of cell disintegration achieved during the crushing is a prerequisite for the malaxation since the release of oil droplets is required to a certain extent as a previous step. However, very fine milling provokes the formation of emulsions from which the oil can't be separated by the conventional oil processing techniques (Ranalli et al., 1997). Therefore, the treatment of the paste with PEF has the potential to induce cell disintegration and to facilitate the release of the small oil droplets without having the negative effects of fine grinding. In

addition, the facilitated release of oil from the cell provides the potential to perform the malaxation at lower temperature with beneficial effects on the oil quality (Kalua et al., 2007). In order to study the impact of a PEF treatment of olive paste on oil yield and quality, comparative studies were performed in a pilot plant with and without PEF application at a processing capacity of around 400 kg of paste per hour (Fig. 13.). The aspects discussed below aim at exemplifying the need for an integrative approach considering the performance criteria of the traditional processing steps in order to allow a reliable evaluation of possible PEF effects.

Fig. 13: Setup for a pilot plant to study the PEF assisted recovery of olive oil. A) pulse generator; B) Crusher feed hopper and screw; C) Crusher; D) PEF treatment chamber; E) malaxer; F) decanter.

The most challenging aspects when conducting the pilot plant trials are the limitations resulting from the variations in raw material properties and the time requirements in order to obtain stable processing conditions of all components in the processing line. An appropriate experimental procedure would entail a continuously running pilot plant system with a constant throughput of olive paste and well defined malaxation conditions as well as a steady state decanter performance. In addition to that, the variation of different PEF conditions needs to be realized in order to systematically study the PEF effect at a given configuration of all other processing parameters of the pilot plant. Hence, the knowledge of performance criteria of the traditional processing steps is essential in order to establish an appropriate experimental design. Fig. 14 shows the residence time distribution of the paste in the malaxer. Indicated is the frequency density function E(t) which describes the probability of a particle to spend a given time in the malaxer. The residence time distribution was obtained by measuring the conductivity at the decanter outlet in intervals of 1 min after injection of a highly conductive salt solution (tracer) at the malaxer inlet. F(t) represents the mass fraction of paste which has spent a given time or less in the malaxer. It is obtained by integration of the E(t) function. First tracer particles arrive at the malaxer outlet after 10 min whereas after

90 min, all tracer particles are discharged. According to F(t), 50 % of the tracer particles have left the malaxer after 42 min whereas 40 % of the paste has spent less than 29 min or more than 58 min in the malaxer. The malaxer was filled with 330 kg of paste and a throughput of 330 kg/h was provided which would result in a theoretical residence time of 60 min not considering any mixing effects or variations in the paste flow.

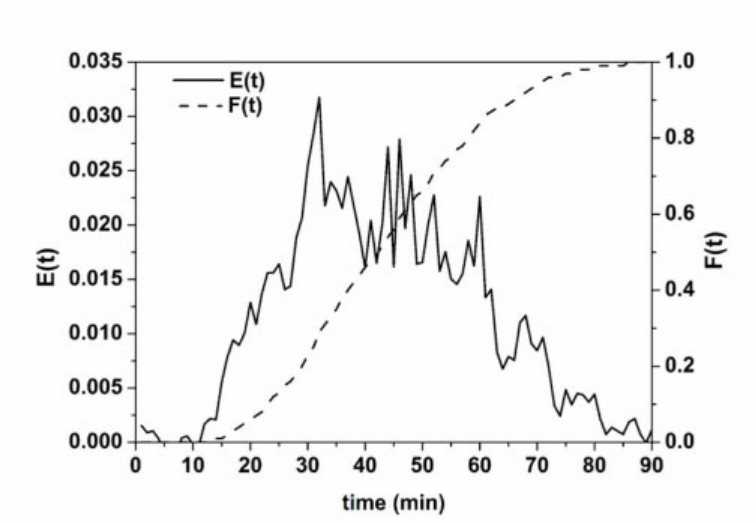


Fig. 14: Residence time distribution of olive paste particles in the malaxer as obtained by the method of pulse injection showing the frequency distribution function E(t) as well as the cumulative distribution function F(t).

Although the residence time of paste particles in the malaxer is inhomogeneous, the composition of the discharged paste will be homogeneous after 90 min of continuous operation. From this time point on, the composition of the paste corresponds to the E(t) function. Hence, the consequence for an appropriate experimental design would be as follows: After complete filling of the malaxer, a time period of 90 min is required in order to guarantee a homogeneous paste composition at the outlet. An experimental run can be started with a given setting of processing parameters (variation of pre-malaxation conditions such as crushing or PEF treatment) and a mass balance can be performed for a defined time interval. If the study of a different parameter setting is required for a second experimental run (e.g. to compare PEF and control treatment), these parameters have to be changed and a time interval of 90 min is required again before a stable mash composition with regard to the new process parameters is obtained. From this time point on, the acquisition of a mass balance can be performed again. Table 1 gives an example of a suitable setup. Depending on the duration of an experimental run to obtain a reliable mass balance for yield calculation, a homogeneous batch of olives of 2 t is required for two comparable trials. Not considered in this calculation are repetitions of the experiments that need to be performed in order to allow a statistical analysis.

Table 1: Example for the requirements regarding time and amount of homogeneous raw material for performing two comparable trials in the pilot plant system. Mass flow rate of olive paste is 330 kg/h, amount of paste in the malaxer is 330 kg, the time for performing a mass balance as a basis for yield calculation is assumed to be 45 min. According to the residence time distribution, malaxation time will be less than 42 min for 50 % of the particles. Step 2b refers to additional time required for the decanter to reach steady state conditions

step number	step description	time requirement (min)	amount of paste (kg)
1	filling of the malaxer	60	330
2a	continuous throughput	90	495
2b	decanter steady state	30	165
3	trial A	45	248
4	parameter change	-	-
5	continuous throughput	90	495
6	trial B	45	248
	total	**360**	**1981**

In addition to the malaxer and its performance characteristics discussed above, the decanter is a key component in the processing line. Especially during the beginning of an experimental run, the decanter requires a certain time to reach steady state conditions after operation parameters are reached and the product is introduced. In the performed trials, an improvement of the separation performance was found to occur for the first two hours of decanter operation. After that time period, a constant oil recovery rate was achieved indicating that steady state conditions were achieved. Hence, for a reliable and repeatable experimental procedure, step 2 needs to be extended in order to achieve the total time required for steady state conditions of the decanter.

The examples discussed above were presented in order to illustrate the complexity of the integration of PEF into multi-stage traditional food processing lines. The focus was less on the PEF treatment as such rather than on the interdependencies of the different components in the system and on the evaluation of performance criteria of the traditional food processing equipment. Both aspects are of high relevance for the setup of an appropriate experimental design to study the PEF performance in the system and for the interpretation of obtained results. The consideration of connected processing steps is required from a technical point of view since each component has a specific range of operating conditions that need to be adjusted. On the other hand, it is crucial to be aware of the properties of the raw material which is passing through the whole processing line and which is modified by each processing step (process-product impact). Depending on this modification, the performance of the subsequent processing step will be affected since a product-process impact occurs (see section 1.4. ‘Process-product interactions’). A successful integration of the PEF technology in

a complex industrial processing environment as well as the evaluation of new application concepts of PEF in food processing will require such a multiscale approach considering the process-structure-property interactions occurring in each single processing step and affecting connected processing steps.

2. Conclusion and outlook

PEF performance characteristics have been investigated focusing on the key priorities of a PEF application in the food industry, namely the non-thermal inactivation of microorganisms and the cell disintegration in order to enhance mass transfer processes. The systematic process analysis approach including aspects related to different levels of scale as well as complexity proved to be a valuable tool in order to reveal potential aspects for a process improvement and a targeted process design. Process-product interactions have been identified and a bidirectional analysis was performed considering PEF effects on food compounds and the raw material matrix as well as considering effects of the product to be PEF treated on the PEF performance.

The transfer of inactivation results from model systems to real foods and the determination of appropriate PEF treatment parameters require the consideration of existing particularities. The occurrence of sublethal damage to microorganisms and the ability of the cells to recover and regain structural integrity, metabolic activity and culturability will limit the PEF inactivation performance. In addition, the crucial impact of food constituents as protective factors against microbial inactivation by PEF is a key aspect relevant for the evaluation of the microbiological safety of a product after PEF treatment. Large sublethally-injured fractions would have an important potential for subsequent complete inactivation by the application of additional hurdles such as suboptimal storage conditions. Hence, the consideration of pre- and post-processing steps is a relevant aspect regarding the process integration in order to increase the susceptibility of the microorganisms to PEF inactivation and to avoid the recovery of injured cells. The pulsed electric field effect on protein structures in milk was found to be small and a high rate of retention of bioactivity was detected. This provides a remarkable potential in order to develop processing concepts for the increase of product shelf life by combinations of PEF inactivation and the antimicrobial effect of native milk constituents such as lactoferrin or the lactoperoxidase system. The study of PEF effects on food compounds was up to now mainly driven by questions concerning detrimental effects occurring during PEF pasteurization of liquids. However, the targeted use of electric fields in order to modify structural and functional properties of proteins or carbohydrates seems to have a large potential to become a promising future field of application for the PEF technology.

Depending on processing parameters and treatment conditions, pulsed electric field side effects such as the temperature increase or the occurrence of electrochemical reactions has to be taken into account to maintain the desired food quality. The numerical simulation of the PEF process with computational tools allows the detection of detrimental side effects and provides the basis for an improvement of the treatment chamber design. An improved efficiency of the microbial inactivation and the avoidance of food over-processing will result. The necessity to determine the temperature in the PEF treatment chamber in addition to the measurement of an average outlet temperature should be considered for a good experimental practice. The determination of a temperature time profile of a complex PEF pasteurization process and information on the corresponding thermal inactivation effects can be used to optimize the PEF treatment. Processing conditions that present a large contribution to the thermal inactivation of heat-sensitive compounds can be avoided and an optimal combination of the application of thermal energy as well as electrical energy can be determined to allow a maximum of microbial inactivation with a minimum of required energy and of degradation of heat-sensitive compounds. The further development of concepts for the evaluation of treatment intensities and the comparison of different treatment chamber designs will be required based on the set of key parameters that were discussed in order to establish standard experimental procedures.

The integration of the PEF treatment into complex processing concepts requires the consideration of connected processing steps. This is of particular relevance for the application of PEF for the disintegration of plant raw materials since the PEF treatment serves as a pre-treatment for subsequent processing steps such as drying, extraction or juice recovery. The consideration of textural properties, particle size and the cell size of the different raw materials is essential in order to evaluate the potential of the PEF application to enhance mass transfer processes such as to increase the juice recovery from fruit and vegetable mashes. PEF treatment allows a tailor made food structure design complementing the mechanical cell disintegration by particle size reduction with cell disintegration by electroporation which is independent from the particle size. The modification of the tissue structure will affect the tissue and product properties as shown for the modification of the sugar distribution in case of drying or the modification of juice release properties during pressing. The adjustment and optimization of related processing steps will be essential in order to control and maximize beneficial PEF effects.

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Sublethal injury

ABSTRACT

The inactivation of *Lb. rhamnosus* by pulsed electric field treatment (PEF) was studied in different fractions of raw milk and Ringer solution in order to evaluate the protective effect of nutrient rich media in comparison to aqueous buffer solutions.

Apart from monitoring of culturability, analysis of the physiological fitness of *Lb. rhamnosus* was conducted aiming to identify sublethally damaged cells. Therefore, flow cytometry and a selective medium plating technique were used and compared to each other. The goal of the study was to apply three different parameters describing the physiological fitness of the model organism *Lb. rhamnosus* after PEF treatment such as culturability, membrane permeability and metabolic activity depending on treatment media and parameters.

A concentration dependent protective effect of the milk protein fraction could be shown and allocated to micellar casein as the major milk protein. Increasing the concentration of whey proteins up to 2% showed a similar impact on limiting the PEF inactivation of *Lb. rhamnosus*.

The evaluation of physiological fitness of cells was based on a determination of structural and functional characteristics by rapid cellular staining using carboxyfluorescein diacetate and propidium iodide. This approach showed good accordance to the conventional selective medium plating technique for the enumeration of sublethally-injured bacteria but flow cytometry provided additional information for the characterisation of this fraction.

The extent of occurrence of dead, sublethal and vital fractions of cells was found dependent on the PEF treatment parameters such as electrical field strength and energy input as well as the different milk fractions used as treatment media.

1. Introduction

Pulsed electric field (PEF) technology is considered as a non-thermal alternative to traditional pasteurisation of liquid foodstuff. The inactivation mechanism is based on electroporation of microbial cell membranes due to repetitive application of short pulses (1–10 μs) of high intensity electric fields (15–40 kV/cm). Effective inactivation for most of the spoilage and pathogenic microorganisms has been shown in fruit and vegetable juices (Heinz et al., 2003; Nguyen and Mittal, 2007), milk (Sampedro et al., 2005; Sepulveda et al., 2005) and model systems (Gómez et al., 2005; Pothakamury et al., 1995; Ulmer et al., 2002) with little or no impact on nutritional and sensorial properties of the food (Elez-Martínez and Martín-Belloso, 2007; Jia et al., 1999).

When exposed to electric field pulses cell membranes develop pores that may be permanent or temporary, depending on the intensity and treatment conditions. Low treatment intensity allows a reversible disturbance of the phospholipid's bilayer, which is routinely used as a tool in molecular biology to introduce polar molecules like DNA into a host cell through the cell membrane (Chang et al., 1992). An irreversible perforation of the cell membrane reduces its barrier effect permanently and causes cell death used for a non-thermal pasteurisation of liquid food (Elez-Martínez et al., 2005; Heinz et al., 2002). Until now there has been no clear evidence on the underlying mechanisms at a cellular level but two main effects have been described to be triggered by the electric field, the ionic punch-through effect (Coster, 1965) and the dielectric breakdown of the membrane (Zimmermann et al., 1973).

Membrane damage and inactivation of microorganisms due to PEF, firstly considered as an all-or-nothing event in some studies (Russel et al., 2000; Simpson et al., 1999; Wuytack et al., 2003; Yaqub et al., 2004), revealed a required differentiated approach even if the critical parameters for the electrical breakdown of cell membranes are exceeded. Membrane damage and sublethal injury are repairable under certain conditions and the extent to which cells repair their injuries was found to depend on treatment intensity, microorganism and treatment medium pH (Garcia et al., 2005). Resealing activities of

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electropermeabilised membranes of human erythrocytes have been reported by Tsong (1990). The impact of cell physiological state and environmental factors on repair capacity of *Escherichia coli* and changes in the survival fraction after PEF treatment and subsequent storage in a citrate–phosphate buffer was investigated by Somolinos et al. (2008a,b) using a selective medium plating technique. They suggested specific pH conditions for sublethally damaged cells to reduce cell recovery and to achieve higher levels of microbial inactivation. Apart from media properties like pH, conductivity and ionic strength that are often reported as parameters influencing PEF inactivation (Jayaram et al., 1993; Vega-Mercado et al., 1996), the impact of food constituents on PEF effectiveness and the occurrence of sublethal injuries are not fully elucidated. Some authors report a protective effect of xanthan (Ho et al., 1995), proteins (Martin et al., 1997; Sampedro et al., 2006) or fat (Grahl and Märkl, 1996). Other studies did not reveal differences in the microbial inactivation conducted in buffer or complex media (Dutreux et al., 2000; Reina et al., 1998), or did not detect the occurrence of sublethally-injured cells after PEF treatment of complex food systems (Walkling-Ribeiro et al., 2008).

Mañas et al. (2001) investigated inactivation of *E. coli* in ovalbumin solution, fish egg suspension, dairy cream and in phosphate buffer and no protective effect of emulsified lipids, soluble proteins or conductive food particulates could be found. Inactivation of *Enterobacter sakazakii* in peptone water and infant formula milk by PEF at 40 kV/cm for 300 µs was found to cause 2.7 log-cycle and 1.2 log-cycle reductions respectively showing an impact of the complex composition of infant formula milk in comparison to peptone water (Pérez et al., 2007). When considering PEF effectiveness as a function of the treatment media, Hülsheger et al. (1981) found the critical field strength and treatment time required to be dependent not only on cell geometry but also on the properties of the media. Selma et al. (2004) studied the impact of recovery conditions on the control of *Lb. plantarum* and *E. coli* after PEF inactivation in broth and orange-carrot juice. Low storage temperature and inoculum size were found to result in a delay in lag-phase after PEF treatment. The delay was more pronounced in the orange-carrot juice than in the MRS broth due to substrate-based stress conditions such as pH and availability of nutrients for the sublethally-injured cell fraction. Inactivation in complex media like an orange juice–milk based beverage was also under investigation by Sampedro et al. (2007) who studied the different PEF process parameters. The authors concluded the need for further investigation on the effectiveness and mechanism of action of the complex food composition during PEF treatment since the inactivation results obtained in the complex orange juice–milk based were lower than in simpler substrates.

Assured food safety and stability, along with a desired level of microbial inactivation require accurately defined treatment intensity followed by a predictable microbial inactivation.

The transfer of inactivation results from model systems to real foods and the determination of appropriate PEF treatment parameters requires the consideration of existing particularities. The occurrence of sublethal damage to microorganisms and the ability to recover and regain structural integrity, metabolic activity and culturability as well as the crucial impact of food constituents as protective factors against microbial inactivation by PEF are relevant key aspects. In addition, it has to be taken into account that the diverse and heterogeneous microbial flora present in real foods may be less sensitive to PEF than inoculated microorganisms due to strong variability of microbial species and physiological state of microorganisms (Michalac et al., 2003).

The study aims to identify the protective effect of different milk constituents against the inactivation of *Lb. rhamnosus* in raw milk and to evaluate the occurrence of sublethally-injured microorganisms. The use of flow cytometry for this purpose is introduced and compared to the selective medium plating technique. *Lb. rhamnosus* served as a model organism, allowing easy cultivation and a well defined methodology for flow cytometry analysis (Ananta et al., 2004) prior to further investigations with spoilage bacteria. Due to the selective inactivation capability of pulsed electric fields depending on cell size of the microorganism, concepts can be developed for the inactivation of spoilage yeast, e.g., in probiotic yoghurt, while selectively preserving probiotic bacteria like *Lb. rhamnosus* (Caroll et al., 2004). Its sensitivity towards pulsed electric fields depending on treatment conditions is therefore of certain interest.

2. Materials and methods

2.1. PEF treatment systems

2.1.1. Micro batch system

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 of 3 Ceramite Y5U 6800Z (Behlke, Kronberg, Germany) capacitors (capacity 19.1 nF in total). A HTS 160–500 SCR, 16 kV, 5 kA, 2 kHz high voltage switch (Behlke, Kronberg, Germany) was used as a switching unit delivering exponential decay pulses. Data acquisition and control were performed on a PC connected by GPIB, using a software developed based on TestPoint (Keithley Instruments, Cleveland, USA), a 100 MHz TDS220 oscilloscope and a P6015A high voltage probe (both Tektronix Inc., Beaverton, USA). For pulsed electric field treatment, micro cuvettes with aluminum parallel plate electrodes, 20 × 10 mm, 2 mm gap, and 400 µl volume (Eppendorf, Hamburg, Germany) were used.

2.1.2. Continuous technical scale pulse modulator

Continuous treatment was performed using a 7 kW modulator (ScandiNova Systems AB, Uppsala, Sweden) providing rectangular pulses in the range of 3–8 µs with a maximum voltage of 50 kV and a repetition rate of 400 Hz. The co-linear type treatment chamber (Berlin University of Technology) was fed with a flow of 5 l/h using a peristaltic pump 323 Du (Watson Marlow, Wilmington USA). The treatment chamber consisted of one central high voltage electrode and two outer grounded electrodes (all stainless steel, inner diameter 6 mm) separated to a distance of 4 mm by two polyoxymethylene insulators with an inner diameter of 4 mm. This geometry provides two treatment zones of a total enclosed volume of 0.22 ml exposed to the electric field, resulting in a total residence time of the medium in the electrical field of 0.15 s at a flow rate of 5 l/h. Adjustment of inlet temperature was conducted by stainless steel cooling coils (Berlin University of Technology) immersed in a VWR 1160S circulating water bath (VWR, Darmstadt, Germany). A Takaoka fiber optic thermometer FT1110 (Chiyoda Corporation, Tokyo, Japan) served as temperature control during PEF treatment. The total specific energy input was chosen as a parameter to describe the treatment intensity and was calculated according to Eq. (1) based on voltage (U) and current (I) signals as well as the mass flow rate ($\dot{m}$) measured during the treatment.

$$W_{\text{specific}} = \frac{1}{\dot{m}} \cdot \int U(t) \cdot I(t) \cdot dt. \quad (1)$$

Milk and milk fractions have been preheated from storage temperature (4 °C) to 30 °C inlet temperature for PEF treatment in order to increase the treatment efficiency by using the synergetic effect between the increased membrane fluidity at the elevated temperature and the electroporation. The applied range of treatment parameters was chosen based on preliminary investigations.

Outlet temperature after treatment did not exceed 60 °C. Cooling to 10 °C was realized within 7 s using a cooling coil with an inner diameter of 2 mm submersed in a water bath (VWR, Darmstadt, Germany) at 5 °C. No thermal inactivation of *Lb. rhamnosus* was found to occur at these conditions.

2.2. Microbial growth conditions and analysis

Lb. rhamnosus E522 was obtained from VTT Biotechnology (Espoo, Finland). For long-term maintenance, the organism was stored as Roti®-Store glass bead cultures (Carl Roth, Karlsruhe, Germany) in a freezer at −80 °C (New Brunswick Scientific, Nürtingen, Germany). One bead of a deep-frozen culture was transferred into de Man, Rogosa, Sharpe–MRS broth (Oxoid, Basingstoke, UK) and incubated for 24 h at 37 °C. An aliquot of this broth was then used to inoculate the final broth, which was again incubated at 37 °C for 24 h to obtain the microorganisms in their stationary growth phase. Cells were harvested and washed once with PBS buffer (phosphate buffer saline) pH 7.0. After washing, the pellet was re-suspended in the final treatment medium to a concentration of 10^7 CFU/ml. After treatment, the collected samples were placed on ice immediately. Colony counts of vegetative cells were determined using a drop plating method on MRS-Agar (Oxoid Ltd., Basingstoke, UK). Selective agar was prepared by the addition of 3% (w/w) NaCl (Merck KGaA, Darmstadt, Germany) to non-selective MRS-Agar. Increasing concentrations in the range of 1–4% of NaCl in MRS-Agar were tested with regard to their impact on growth of *Lb. rhamnosus*. Undiminished growth up to a concentration of 3% was observed, and this concentration level was therefore chosen to assure that injured cells are not able to recover whereas vital cells without membrane damage are growing. On a non-selective medium without the addition of NaCl, cells in both conditions are able to grow. Plates were incubated for 48 h at 37 °C under anaerobic conditions (Anaerocult, Merck, Darsmstadt, Germany).

The inactivation of vegetative organisms was evaluated by calculating the log reduction in a viable colony count compared to the untreated sample. The colony count difference between selective and non-selective media was defined as sublethally-injured cells. The fractions of dead, sublethally-injured and vital cells were expressed as percentages.

2.3. Treatment media

Different fractions of raw whole milk were used to determine their involvement in the total protective effect of milk constituents during PEF inactivation of *Lb. rhamnosus*. Sweet whey was produced by rennet coagulation of raw milk obtained from the Federal Institute for Risk Assessment — Centre for Animal Experiments (Berlin, Germany). Milk composition (fat, protein, and lactose) was determined using a Milcoscan 133B (Foss GmbH, Rellingen, Germany), and measurements of pH (pH-meter CG811, Schott Instruments, Mainz, Germany) and conductivity (conductometer LF95, WTW, Weilheim, Germany) were used to describe media properties. Milk was heated to 32 °C prior to rennet addition (Chr. Hansen, Horsholm, Denmark). After coagulation, whey was separated using cheesecloth and was centrifuged at 2500*g* for 15 min (Megafuge 1.0R, Heraeus, Hanau, Germany) to remove the remaining particles. Concentration of whey proteins and recovery of whey permeate was conducted using a Millipore ProScale Ultrafiltration unit with 10 kDa filter module (Millipore Corporation, Billerica, USA). Micellar casein was obtained from raw milk by ultracentrifugation at 50,000*g* for 1 h (Beckmann Optima LE-80 K, Beckman Coulter Inc., Fullerton, USA). Casein micelles were re-suspended in milk ultrafiltrate to the initial raw milk volume. Adjustments to fat content of milk were done by centrifugal separation at 35 °C for 10 min at 2600*g* (Megafuge 1.0R, Heraeus, Hanau, Germany) and the addition of cream, respectively, to increase fat content. Except lactose (Oxoid Ltd., Basingstoke, UK) all components were recovered from raw milk and used in their native form as treatment media. Ringer solution (Merck KGaA, Darmstadt, Germany) was used as a physiological saline solution, providing similar osmotic conditions to the microbial cell without containing additional protein or fat. Conductivity was adjusted by the addition of deionized water and was determined using a LF95 conductometer (WTW, Weilheim, Germany).

2.4. Flow cytometry

A flow cytometric measurement was performed on a Coulter EPICS XLMCL flow cytometer (BeckmanCoulter Inc., Miami, USA) equipped with a 15 mW, 488 nm aircooled argon laser using cFDA (carboxyfluorescein diacetate) and PI (propidium iodide) for staining of cells. 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. cFDA penetrates through intact membranes and is hydrolysed to carboxyfluorescein (cF) by an active esterase system. cF emits green fluorescence at 530 nm following excitation with laser light at 488 nm, whereas red fluorescence at 635 nm is emitted by PI-stained cells. PI only penetrates into cells through membrane pores and emits fluorescence after binding to DNA. All registered signals were logarithmically amplified. A gate created in the dot-plot of FS vs. SS was applied to discriminate bacteria from artifacts.

Samples were stored at 2 °C after PEF treatment and within 20 min were centrifuged at 2600*g* for 10 min. The obtained pellet was re-suspended in 50 mM PBS buffer. Cells were incubated after the addition of cFDA to a final concentration of 50 mM (Molecular Probes Inc., Leiden, Netherlands) at 37 °C for 10 min to allow intracellular enzymatic conversion of cFDA into cF. Cells were then washed to remove excessive cFDA followed by the addition of PI for a final concentration of 30 mM (Molecular Probes Inc., Leiden, Netherlands) and by incubation in an ice bath for 10 min to allow labelling of membrane-compromised cells. Data were analysed with the software package Expo32 ADC (BeckmanCoulter Inc., USA). Dot-plot analysis of FL1 vs. FL3 was applied to resolve the fluorescence properties of the population. The population was graphically differentiated and gated according to its fluorescence behaviour. A total number of 50,000 cells were counted and results were also numerically expressed by the percentage of cells encountered in the different gates in relation to the total number of detected cells.

An overview of materials and methods including the PEF treatment system, treatment medium and analytical evaluation of process impact on *Lb. rhamnosus* is given in Table 1.

3. Results and discussion

To validate the protective effect of milk in comparison to PEF treatments in an aqueous buffer solution, inactivation of *Lb. rhamnosus* was performed in raw milk (4.3% fat, 3.3% protein, and 4% lactose) and in Ringer solution, both with a pH value of 6.6 and a conductivity of 4.6 mS/cm to guarantee the same electrical properties and therefore the same treatment parameters and process requirements to achieve a comparable PEF intensity. Inactivation of *Lb. rhamnosus* in Ringer solution and in raw milk depending on the total specific energy input is shown in Fig. 1. Treatment at 30 kV/cm and 116 kJ/kg resulted in a 5.5 log-cycle reduction of *Lb. rhamnosus* in Ringer solution, whereas inactivation in milk showed a 2 log-cycle reduction only. The impact of the treatment media at the same treatment parameters and corresponding media properties (pH and conductivity) revealed a significant ($p<0.01$) difference between Ringer solution and raw milk at an energy input of 81 and 116 kJ/kg, indicating a strong protective effect of milk constituents against the microbial inactivation by PEF and, therefore, limiting process effectiveness at the given treatment intensity and energy input.

To determine the contribution of the different fractions of raw milk to this protective effect, PEF treatment of *Lb. rhamnosus* was performed in milk with different fat contents, in whey, in a dispersion of micellar casein, in milk ultrafiltrate and in Ringer solution with and without the addition of lactose. Inactivation results using Ringer solution with and without the addition of lactose (4% final concentration) did not show significant differences ($p>0.05$) as presented in Fig. 1. In both treatment media, inactivation of *Lb. rhamnosus*

Table 1
PEF system, treatment medium and analytical evaluation of the different experiments.

Impact factor on inactivation/analytical evaluation	PEF system	Treatment medium	Detection of process impact
	a) Micro batch system	RM: raw milk	SPT: culturability by plating on MRS with/without NaCl
	b) Continuous technical scale system	WP: whey proteins	FCM: membrane permeabilisation and metabolic activity
		CM: casein	
		RS: Ringer solution	
		L: lactose	
Medium complexity	b)	RM; RS; RS + L	SPT
Raw milk casein	a)	RM; CM, RS	SPT
Whey proteins	b)	WP	SPT
Differentiation of damaged cell fractions by FCM, SPT	a)	RS	FCM; SPT
Sublethal fraction influenced by raw milk	a)	RM; RS	FCM
Physiological state influenced by whey protein/casein media	a)	WP, CM	FCM
Sublethal fraction influenced by treatment intensity	a)	RS	FCM

was comparable and lactose in Ringer solution did not reveal a protective effect against PEF inactivation.

The casein is the major milk protein fraction and is present in the form of casein micelles with a diameter of 20–400 nm. Native micelles obtained from the ultracentrifugation of raw milk were resuspended in milk ultrafiltrate to study the interaction with *Lb. rhamnosus* and the resulting impact on PEF inactivation effectiveness. Due to the limited volume of the ultracentrifuge, a maximum amount of 200 ml of a final dispersion of micellar casein could be obtained, which was not suitable for continuous treatment with the co-linear flow-through system. Therefore, PEF inactivation was performed in a parallel plate batch system, which leads to limited inactivation results in comparison to the continuous system for reasons of missing turbulence during treatment and incomplete treatment due to cuvette design as well as shorter duration of the applied exponential decay pulses. Therefore higher treatment intensities had to be used. Since batch treatment and corresponding cuvette geometry and localisation in the treatment unit allow for a fast heat transfer, temperature increase resulting from the input of electrical energy is far below the usual calculation based on the consideration of the specific heat capacity of the product and the total specific energy input. Maximum temperature in the conducted cuvette treatments did not exceed 35 °C using a maximum frequency of 10 Hz. Inactivation experiments shown in Fig. 2 were done with Ringer solution, raw milk and a dispersion of micellar casein at 30 kV/cm and different numbers of pulses and energy input, respectively. An inactivation of 2 log-cycles is obtained in Ringer solution whereas there were nearly no inactivation results in the casein micelle dispersion. No significant difference in the protective effect of milk and micellar casein is observed. It can therefore be concluded that the protective effect of the casein micelles also occurs without the presence of whey proteins. Casein micelles are also rich in calcium, which mostly occurs in the form of calcium phosphate. However, it is also released from micellar structure in the form of calcium ions, which might have occurred to a certain extent during resuspending of the casein micelles after ultracentrifugation. Hülsheger et al. (1981) reported a protective effect of Ca^{2+} against electric field treatment due to interference with membrane and cellular functions so that their possible additional effect supports the impact of micellar casein. The ion-specific and dose dependent protection of *E. coli* by calcium during high pressure treatment, another non-thermal pasteurisation technology affecting membrane permeability, were reported by Hauben et al. (1998) and were concluded to be due to the stabilization of important cellular targets of high pressure application. Disruption of the casein micelles with a concomitant release of micellar minerals, such as calcium and phosphate, was suggested by Black et al. (2007) to contribute to the protection of *Listeria innocua* during high hydrostatic pressure treatment in milk due to an increased buffering capacity of milk by the solubilization of these minerals, and protection of cell membranes by divalent cations.

PEF treatment of *Lb. rhamnosus* in rennet whey with a protein content of 0.7% showed similar inactivation results to a protein-free ultrafiltrate solution (Fig. 3). This finding confirms the result of the treatment in a whey protein-free, micellar casein dispersion (protein

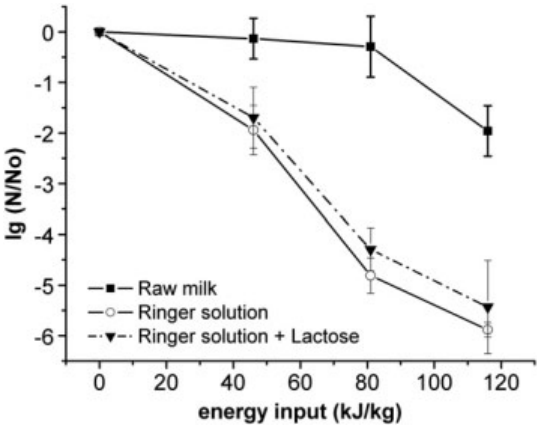

Fig. 1. Comparison of the inactivation results of *Lb. rhamnosus* in raw milk and Ringer solution with and without the addition of lactose (concentration 4%) after continuous PEF treatment with the technical scale pulse modulator at different energy inputs (electric field strength 30 kV/cm, flow 5 l/h, rectangular pulses of 3 µs, energy per pulse 2.5 J, pulse frequency 56 Hz and treatment time 25 µs at an energy input of 100 kJ/kg).

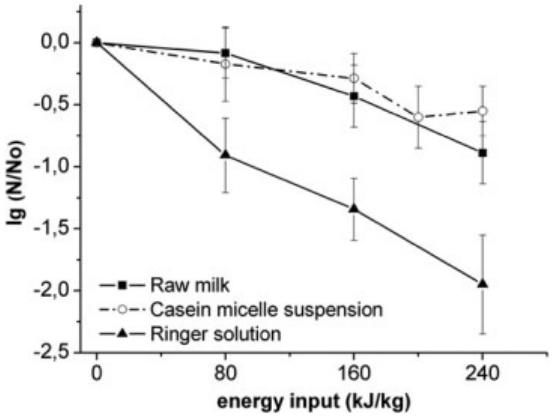

Fig. 2. Inactivation of *Lb. rhamnosus* suspended in a dispersion of casein micelles. PEF treatment at 30 kV/cm and different energy inputs were conducted in the micro batch system using electroporation cuvettes that allowed improved heat transfer, limiting the temperature increase at high energy input (exponential decay pulses, energy per pulse 0.35 J, pulse duration 1 µs, and treatment time 114 µs at an energy input of 100 kJ/kg).

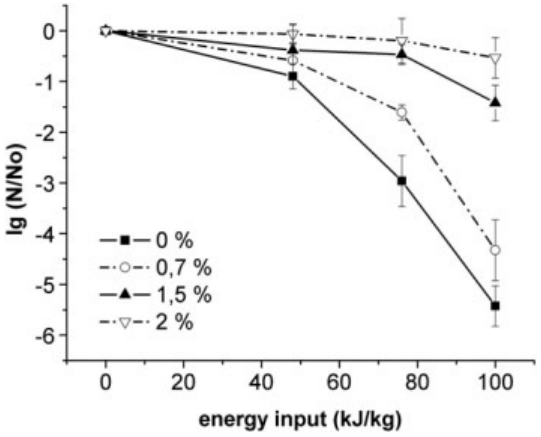

Fig. 3. Inactivation of *Lb. rhamnosus* depending on the concentration of whey proteins and total specific energy input of the continuous PEF treatment performed with technical scale pulse modulator (field strength 30 kV/cm; rectangular pulses; medium conductivity 5.3 mS/cm; pH 6.7; flow rate 5 l/h).

content 3%) where a protective effect similar to raw milk occurred. In order to investigate whether the lack of protection using a whey protein solution was due to the lower protein concentration or due to minor protective abilities, inactivation of *Lb. rhamnosus* was performed in whey containing 0.7 up to 2% protein. Fig. 3 shows inactivation results depending on the protein concentration and clearly indicates a strong correlation between the concentration of whey proteins and the protective effect.

Although fat has an impact on electric field distribution, especially when unhomogenised milk with larger fat globules is considered, the absence of fat in whey and the casein micelle dispersion did not have an influence on the protective effect of milk.

This was in accordance with previous studies where experiments were conducted investigating the inactivation of *Lb. rhamnosus* in milk with a different fat content (0.5–10%) and no difference was found in the log-reduction results obtained (Toepfl, 2006). PEF inactivation of *L. innocua* in skim milk, whole milk and dairy cream was investigated by Picart et al. (2002) and no consistent effect of the fat content on colony count reduction was found. The protective effect of skimmed milk was also under investigation for high hydrostatic pressure inactivation of *E. coli* (Narisawa et al., 2008). There, the protein fraction was also found to be the major component leading to the reduced inactivation results and the higher the skimmed milk concentration the greater the protective effect. In that study it was not possible to identify a particular protein component to contribute to the protective effect but the solid fraction of skimmed milk containing cells were hypothesized to mediate the protective effect. The fact that inactivation of microorganisms is decreased and recovery of injured cells is increased when cells are suspended in nutritionally-rich media containing substances that provide protection against damage or nutrients essential for repair was also under investigation by Gao and Ju (2007). They reported a protective effect of soybean protein during high pressure inactivation of *Bacillus subtilis*. UHT milk was found to decrease pressure sensitivity of *L. monocytogenes* (Patterson et al., 1995) and Martin et al. (1997) reported a less effective inactivation of *E. coli* by pulsed electric fields in skim milk than in buffer solution because of the complex composition and the high protein content. The diminishing of the lethal effect of PEF treatment on microorganisms was concluded to be due to the proteins present in the solution and their ability to absorb free radicals and ions which are active in the cell breakdown.

The results discussed above are based on a determination of viable cell count using non-selective MRS-Agar and can be used to differentiate between the dead and vital fraction of *Lb. rhamnosus* after PEF treatment in the magnitude of up to 7 log-cycles of inactivation. To further extend the information on the mechanism of inactivation, the occurrence of sublethal injuries was taken into account and analysed by using the drop plating method on selective and non-selective agar as well as flow cytometry. Although flow cytometry is based on a single cell detection of up to 50,000 cells per sample, as conducted within the presented study, the number of cells attributed to the different physiological states can only be expressed in the magnitude of 2-3 log-cycles. However, both methods revealed an occurrence of sublethally damaged cells after PEF treatment although they differ regarding the parameters used to describe sublethal injury. Using the plate count technique, those cells are defined to be sublethally damaged that exclusively recover from PEF damage under optimal conditions, i.e., they become culturable again on non-selective agar but not on selective agar containing NaCl as an osmotic stress factor.

In contrast, the flow cytometry method reveals those cells to belong to the sublethal fraction that have electropermeabilised membranes but still possess an active esterase metabolism. Therefore, it is possible to determine fractions of cells in different conditions or states of physiological fitness (Bunthof, 2002) after treatment (Fig. 4). The occurrence of those might be due to inhomogeneous field distribution and different orientations of the cells in the electric field (Grahl and Märkl, 1996) as well as heterogeneous cell population (Kearns and Losick, 2005).

When both methods are applied to the same sample, some differences concerning the detection of a sublethal fraction were expected. However, the data obtained showed good accordance (Fig. 5), thus proving that the esterase metabolism and the culturability of *Lb. rhamnosus* are obviously affected likewise. The standard deviation for the detection of the different cell fractions with flow cytometry and selective plating was determined and is also shown in Fig. 5. The average deviation within the two methodologies was 7.0% for plating and 2.6% for flow cytometry.

Flow cytometric analysis was chosen for further analyses of PEF treated *Lb. rhamnosus* due to the advantages in comparison to the plate count technique like the possibility of rapid and immediate determination of the physiological fitness of cells. The protective effect against PEF inactivation found for casein in milk and the smaller impact of whey proteins due to their lower concentration was assessed, focussing on the comparison of the formation of vital, sublethal and lethal fractions during electropermeabilisation.

When *Lb. rhamnosus* was inoculated in milk and treated with 0–33 kJ/kg at 17 kV/cm, a distinct difference compared to a treatment in Ringer solution became apparent (Fig. 6).

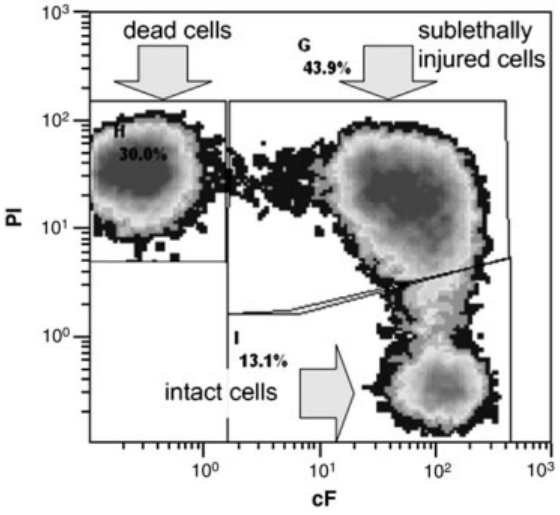

Fig. 4. Flowcytometric analysis of the impact of PEF treatment on *Lb. rhamnosus*. Density plot showing fluorescence intensity of PI and cF. Lethal cells (upper left) showing a loss of membrane integrity and esterase activity, sublethal cells (upper right) showing a loss of membrane integrity but intact esterase system and vital cells (lower right) showing intact membrane and esterase activity. The number of detected cells belonging to the different fractions is given as percentage of the total detected cells.

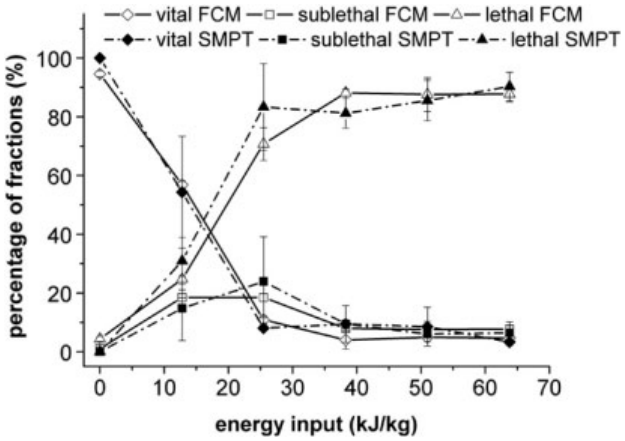

Fig. 5. Comparison of the two different methods used to evaluate PEF inactivation of *Lb. rhamnosus* considering sublethal damage and the protective effect of food constituents. PEF treatment in the micro-batch system at 25 kV/cm and different energy inputs in Ringer solution and subsequent analysis of lethal, sublethal and vital cells by flow cytometry (FCM) and selective medium plating technique (SMPT).

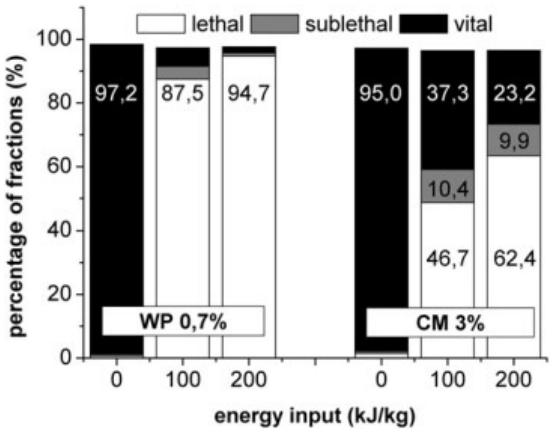

Fig. 7. Impact of PEF treatment on the physiological state of *Lb. rhamnosus* in different treatment media (WP whey protein solution; CM casein micelle dispersion) analysed using flow cytometry (PEF treatment conducted in micro batch system, field strength 30 kV/cm, energy input 100 and 200 kJ/kg).

The vital fraction in milk was reduced by 21% whereas a reduction of 86% was achieved in Ringer solution at similar treatment intensity. This protective effect also seems to help in preventing membrane damage as only a very small sublethal fraction of 2% was found in milk in comparison to 40% in Ringer solution. In addition to providing protection against PEF treatment, milk may represent a suitable recovery medium for sublethally-injured cells so that post-treatment recovery of bacteria happens very fast.

The occurrence of sublethal injury of *L. innocua* in phosphate buffer was also found to depend on energy input and electric field strength by Picart et al. (2002) as well as pH, found by Wouters et al. (1999) and Aronsson et al. (2004), pointing out the importance of injured cells in the post-treatment population regarding aspects of food safety and security, and considering environmental factors to prevent recovery and repair.

Fig. 7 shows the percentage of vital, sublethal and lethal microorganisms after PEF treatment in whey and the dispersion of micellar casein. The white bars indicate irreversibly damaged cells and thus the minimum inactivation success. Grey bars stand for the percentage of sublethally damaged cells. Although permeabilised by the pulsed electric field treatment, these cells are potentially able to survive. In the case of adverse conditions such as disadvantageous medium pH, high osmotic pressure, unfavourable storage temperature or the presence of antimicrobials, these cells finally get inactivated, too. Therefore, the mentioned factors can be used as additional hurdles in combination with the sublethal physiological state of the cell to increase the final inactivation result. Thus, the sum of white (lethal) and grey (sublethal) bars represents the maximum or potentially achievable inactivation.

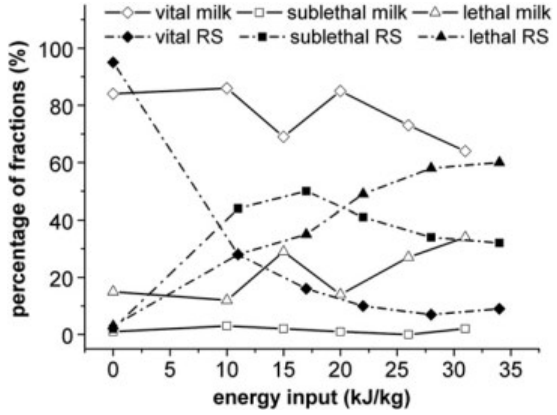

Fig. 6. Influence of complex treatment media (milk in comparison to Ringer solution RS) on the occurrence of sublethally damaged *Lb. rhamnosus* (PEF treatment in the micro batch system at 17 kV/cm, FCM data shown).

As already revealed by determination of the viable colony count using the selective media plating method, inactivation of *Lb. rhamnosus* in casein micelle dispersion is smaller in comparison to inactivation in whey. In both cases, increasing the energy input reduces the percentage of vital cells, this effect being more pronounced for the dispersion of micellar casein since in the case of whey, inactivation already takes place at lower energy input. In this case, inactivation is also more intense as nearly no sublethal fraction occurs and all vital cells are directly transferred to the lethal fraction. For inactivation in the casein micelle dispersion, only a slight increase of the lethal fraction occurs due to the increase of treatment intensity. A noticeable sublethal fraction of 10% could be detected and also higher energy input did not contribute to its reduction. This observation of the differences between the inactivation pathways in media with different compositions leads to the necessity of further investigation of possible optimisation strategies concerning the avoidance of sublethally-injured cells.

As the formulation of food product cannot undergo any substantial changes, the applications of PEF in food processing mainly depend on the technological solutions and correct determination of the process parameters. The impact of the main PEF treatment parameters electric field strength and total specific energy input on the differently damaged fractions of *Lb. rhamnosus* in Ringer solution is shown in Fig. 8, which illustrates an increased percentage of inactivated cells with raising energy input. Comparing the percentages of sublethally damaged cells within the number of total potentially inactivated cells, a higher treatment success can be found for larger energy input rates. The same effect can be found for increased field strength as Fig. 8 shows. Additionally, the phenomena of critical field strength can be observed. The percentage of vital cells is hardly affected after treatment with an electric field strength of 10 kV/cm (95.6% vital cells; non-treated 97.5% vital cells). Increasing the field strength from 15 to 25 kV/cm not only results in the reduction of vital cell population but also in a relative reduction of the sublethally-injured cells as their portion (grey area in the graph) within the total potentially inactivated cells (grey and white area in the graph) is reduced from 69% to 35%. The increase of the field intensity has a more pronounced effect than the increase of the energy input. This finding is in accordance with results obtained by Sampedro et al. (2007) who proposed a high electric field short time (HEST) process.

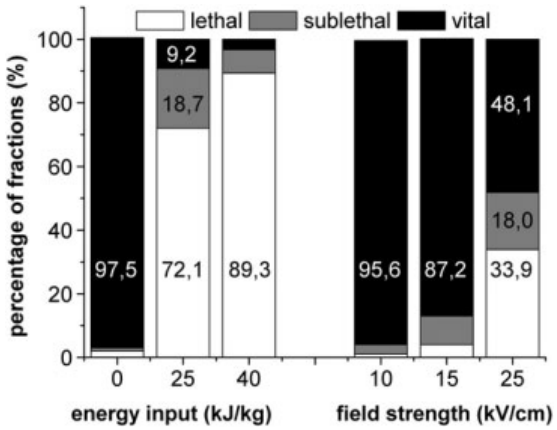

Fig. 8. Influence of energy input and field strength on the occurrence of sublethally damaged *Lb. rhamnosus* in Ringer solution (left: field strength 25 kV/cm at different levels of energy input; right: energy input 12 kJ/kg at different field strengths; micro batch PEF system; FCM data).

The reversible or irreversible rupture of the cell membrane by electroporation was found to be dependent on treatment parameters such as electric field intensity, number and duration of pulses and resulting treatment time and specific energy input in various studies (Abram et al., 2003; Heinz et al., 2002; Wouters et al., 2001). Cell susceptibility and treatment media properties are interacting with the above mentioned processing parameters and lead to the occurrence of intermediate stages during the inactivation of microorganisms by PEF as shown by the obtained results. In addition, it has to be taken into account that pore formation in the cell membrane and resealing of metastable pores is a stochastic process and even the application of homogeneous electric field parameters will therefore lead to inhomogeneous treatment of each cell within the cell population (Saulis & Venslauskas, 1993).

Higher microbial inactivation and the reduction of the sublethally-injured cell fraction were found to occur at higher treatment intensities, either by increased energy input or electric field strength (Fig. 8). However, it has to be considered that severe treatment intensities may also affect sensitive food constituents by electric field and related thermal effects and require mitigation by means of technological optimisation (Jaeger et al., in press).

4. Conclusion

The aim of the study was to evaluate the inactivation of *Lb. rhamnosus* taking into account the mechanistic aspects based on the determination of culturability, membrane permeabilisation and metabolic activity. Differentiation of the cell population after PEF treatment in dead, sublethal and vital cells was possible by either using flow cytometry or a selective media plating technique. The potential of increasing the microbial inactivation could be shown when sublethal fractions of microorganisms are reduced and/or recovery is being avoided by appropriate selection of process parameters, including treatment conditions and food matrix properties. Our studies confirm the thesis that electropermeabilisation is not an all-or-nothing-event. Flow cytometry is a suitable method to determine sublethal damage of microorganisms after a pulsed electric field treatment. The detected parameters differ from those used by the selective medium plating technique and allow a description of the physiological fitness of single cells.

Nevertheless, the effects of food composition on PEF resistance are complex and the cellular basis of this phenomenon still remains unclear. The protective effect of milk could be shown to be due to the milk proteins and to be concentration dependent. As the casein fraction presents approximately 80% of the total milk protein, the protective effect of milk during PEF inactivation of microorganisms is mainly due to micellar casein. Increasing the concentration of whey proteins up to 2% showed nearly the same protective effect as native micellar casein present in milk. A four times smaller reduction of vital cells, and no sublethal damage was found for a treatment in milk when compared to Ringer solution. In order to obtain a maximum of food safety, a direct transfer of cells from the vital to the lethal fraction is favourable. However, since the impact on food quality characteristics limits the applicable treatment intensities, a limited number of dead cells may result. In that case, a large sublethally-injured fraction would have an important potential for subsequent complete inactivation by the application of additional hurdles such as suboptimal storage conditions. However, modified survival properties, a possible increase in the resistance as well as the possibility of the changed virulence of pathogens require consideration and a definite characterisation of the occurring degree of cell injury depending on treatment parameters and media properties will be possible by the presented methods.

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II

Impact of PEF treatment inhomogeneity such as electric field distribution, flow characteristics and temperature effects on the inactivation of *E. coli* and milk alkaline phosphatase

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Impact of PEF treatment inhomogeneity such as electric field distribution, flow characteristics and temperature effects on the inactivation of *E. coli* and milk alkaline phosphatase

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ABSTRACT

High intensity pulsed electric field (PEF) treatment was investigated focusing on the alteration of electric field distribution, flow characteristics and temperature distribution due to the modification of the treatment chamber. The aim was the improvement of the effectiveness of microbial inactivation of *E. coli* and to reduce the PEF impact on alkaline phosphatase (ALP) activity in raw milk. Mathematical simulation of the PEF process conditions considering different treatment chamber setups was performed prior to experimental verification. Finally the impact of the treatment chamber modifications on microbial inactivation and enzyme activity was determined experimentally. Using a continuous flow-through PEF system and a co-linear treatment chamber configuration the insertion of stainless steel and polypropylene grids was performed to alter the field strength distribution, increase the turbulence kinetic energy and improve the temperature homogeneity. The Finite Element Method (FEM) analysis showed an improved electric field strength distribution with increased average electric field strength and a reduced standard deviation along the center line of the treatment zone indicating a more homogenous electric field. The velocity profile was improved resulting in an increase of turbulence kinetic energy due to the insertion of the grids. As revealed by mathematical modeling, the temperature of the liquid was decreased, and formation of temperature peaks was avoided. Measured inactivation of heat sensitive alkaline phosphatase (ALP) was reduced from 78% residual activity to 92% after PEF treatment and it could be shown that thermal effects and temperature peaks have been the main reason for enzyme inactivation due to PEF. At the same time, an increase of microbial inactivation of 0.6 log–cycles could be determined experimentally due to the modification of the treatment chamber design.

Industrial relevance: The application of pulsed electric field as a non-thermal pasteurization technology requires an accurately defined treatment intensity followed by a predictable microbial inactivation. Unavoidable thermal effects occurring during PEF treatment due to ohmic heating have to be minimized to assure the retention of heat-sensitive nutrients and bioactive compounds. The presented investigations contribute to the fulfilment of these requirements for further successful industrial implementation of the PEF technology such as the selective inactivation or retention of enzyme activity in liquid food systems.

1. Introduction

Pulsed electric field (PEF) treatment can be an alternative to traditional thermal pasteurization processes since it is capable of inactivating microorganisms while maintaining fresh-like physical, chemical and nutritional characteristics of food products (Castro, Barbosa-Canovas & Swanson, 1993; Barbosa-Cánovas, Góngora-Nieto, Pothakamury, & Swanson, 1999). One key aspect for maximum PEF effectiveness is the performance of the treatment chamber since this is the part of the PEF unit where direct application of the electric field to the product occurs. Treatment chamber design plays a major role especially for continuous treatment of liquid foods in terms of assured food safety and stability. A maximum of microbial inactivation with a minimum of negative impacts on other valuable food constituents and bioactive components requires an accurately defined treatment intensity including homogenous treatment conditions. Only an appropriate treatment chamber design allows the uniform treatment of food that excludes partial over-processing and is the basis for an implementation at industrial scale. This uniform treatment can be achieved by homogenous electric field distribution, flow velocity distribution and residence time resulting in a homogenous temperature distribution in the treatment chamber.

A linear and uniform electric field distribution can be achieved using a parallel plate electrode configuration although the field intensity has to be considered to depend on product and processing

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parameters such as medium conductivity and constitution and presence of particles or air bubbles (Fiala, Wouters, van den Bosch, & Creyghton, 2001; Gongora-Nieto, Pedrow, Swanson, & Barbosa-Canovas, 2002; Mastwijk, 2004). However, for the reason of the low electrical resistance of the parallel plate treatment chamber configuration resulting in unwanted high current flow and consequently in higher energy requirements to achieve a specific electrical field the applicability of this electrode geometry is limited in some cases. Therefore, alternative configurations like the co-linear treatment chamber are in use having a high load resistance but a less homogenous field distribution depending on constructional properties. The aim of the study was therefore to improve the electric field distribution by modification of a given co-linear treatment chamber geometry. Homogeneity of the pulsed electric field treatment in a continuous treatment chamber is also linked to the flow velocity profile and to a distribution of the temperature increase as a result of the dissipation of electrical energy depending on field intensity, flow behavior and residence time. The coupled phenomena are complex and it is essential to consider flow characteristics as well. To avoid laminar flow and to increase the homogeneity of the flow velocity profile, the formation of turbulence can be achieved by increasing flow velocity for a given geometry resulting in a Reynolds number above the range of 2000–2300 (McComb, 1990; Martin, 2002).

However, higher flow rates require higher power of the pulse modulator as higher pulse frequencies have to be applied to maintain the same treatment time and number of pulses per volume element. It is therefore preferable to create turbulence also at low flow velocities for which manifold methods like insertion of static or dynamic devices, modification of the pipe cross section, curved pipes, etc., are suitable depending on the different requirements.

Important for turbulence generation in a PEF treatment chamber are the following points:

a) Generated turbulence must be homogenous and constant in a certain time interval.
b) Liquid recirculation has to be avoided, since this will produce an over processing of the food.
c) Uncontrolled alteration of the electric field distribution has to be avoided. Improvement of electric field distribution with regard to electric field strength level and homogeneity is desired.
d) Introduction of air has to be avoided.

A possible system to produce turbulence considering the previously named requirements is the insertion of a grid in the treatment chamber. Such a device was used by Cook (1973), Ivanov (1973), Cook (1978), Roach (1987), Zwart, Budwig, and Tavoularis (1997), Janzen, de Souza and Schulz (2003), Lang and Gomez (2004) and Schulz, Janzen and de Souza (2006) to generate turbulence. According to Castro, Marighetti, De Bortoli and Natalini (2003) a grid promotes an improved homogeneity of a velocity profile and produces higher turbulence intensity. The computational models help to obtain good approximations of the relevant physical parameters like flow velocity and distribution prior to experimental setup.

Computational fluid dynamics (CFD) as a computer-based mathematical modeling tool is used for the solution of the fundamental equations of fluid flow and heat transfer. In most instances, the mathematical formulations of the fundamental laws of fluid mechanics and heat transfer are expressed as partial differential equations (PDEs) (Hoffmann & Chiang, 2000). PDEs can be solved by using commercial software packages that are mainly based on two principle numerical methods — Finite Element Method (FEM code) and Finite Volume Method (FVM code). Both methods involve subdividing the flow domain into a large number of finite elements or control volumes and solving the governing equations of fluid flow, i.e. the 3-D Navier–Stokes equations. Within this process a system of algebraic equations is formed and subsequently solved by an iterative method. The numerical methods differ in their derivation and definition of these algebraic equations (O'Callaghan, Walsh & Mc Gloughlin, 2003).

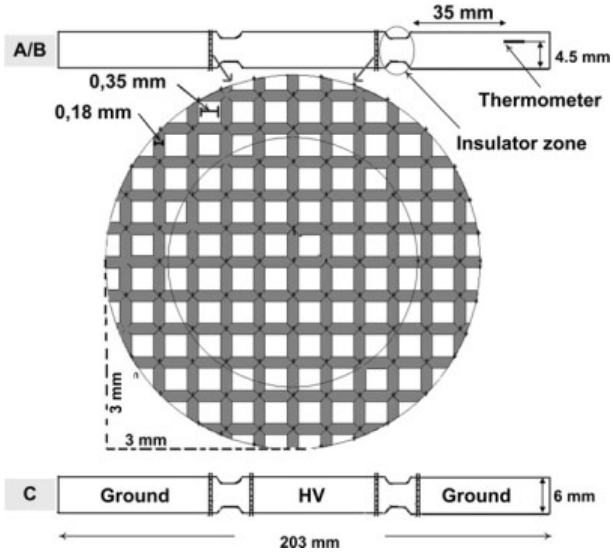

Fig. 1. Grid dimensions and location for models A, B and C as well as position of the fiber optic temperature sensor in the treatment chamber.

Depending on the software package it is possible to describe coupled physical phenomena like fluid flow, heat transfer, electric fields and electrochemical reactions.

Improvement of the electric field distribution, flow behavior and temperature distribution by modification of the treatment chamber geometry as well as the impact of these changes on effectiveness of microbial inactivation and preservation of valuable food constituents were under investigation in the presented study.

2. Materials and methods

2.1. PEF system

Continuous treatment was performed using a 7 kW modulator (ScandiNova Systems AB, Uppsala, Sweden) providing rectangular pulses in the range of 3–8 µs with maximum voltage of 50 kV and repetition rate of 400 Hz. The co-linear treatment chamber (Berlin University of Technology) was fed with a flow of 5 l/h using a peristaltic pump 323Du (Watson Marlow, Wilmington USA). The treatment chamber consisted of one central high voltage electrode and two outer grounded electrodes (all stainless steel, inner diameter 6 mm) separated by a distance of 4 mm using two polyoxymethylene insulators with an inner diameter of 4 mm. This geometry provides two treatment zones of a total enclosed volume of 0.22 ml exposed to the electric field resulting in a total residence time of the medium in the electrical field of 0.16 s at a flow rate of 5 l/h. Adjustment of the inlet temperature in and immediate cooling after treatment was conducted by stainless steel cooling coils (Berlin University of Technology) immersed in VWR 1160 S circulating water baths (VWR, Darmstadt, Germany). The product temperature range for the PEF system used can be 5–90 °C depending on the specific product requirements. A Takaoka fiber optic thermometer FT1110 (Chiyoda Corporation, Tokyo, Japan) served for temperature control during PEF treatment. The measurement point for the small treatment chamber is shown in Fig. 1 and it was varied during experiments in a larger treatment chamber according to Fig. 9 for the validation of the modeling results. Total specific energy input (kJ/kg) was chosen as parameter to describe treatment intensity and calculated by multiplying the energy delivered per pulse with the pulse number divided by the mass of the treated product within the treatment zone. Sterile salt solution adjusted to corresponding treatment media conductivity was used to start up the PEF system prior to product inlet from a second inlet tank and complete

rinse of the salt solution out of the system was assured before product sampling.

2.2. Treatment chamber modification

Insertion of a polypropylene mesh (350 µm aperture width, Stockhausen, Sankt Augustin, Germany) and a stainless steel woven wire cloth (355 µm mesh size and 180 µm wire diameter; Haver & Boecker, Oelde, Germany) at different positions within the treatment chamber was conducted according to Fig. 1. Polypropylene and stainless steel were chosen to determine the primary effect of grid insertion on manipulation of the flow characteristics within the treatment chamber and the additional effect of the conductivity of the mesh material on electric field distribution.

The following models, differing in number and material of grids, have been under investigation (location of the grid is given relative to flow direction):

- Model A: Two grids made of polypropylene, located in front of each treatment zone.
- Model B: Two grids made of stainless steel, located in front of each treatment zone.
- Model C: Four grids made of stainless steel, located in front and behind each treatment zone.

2.3. Microbial growth conditions and analysis

E. coli K12DH5*a* (Hygiene Institut Hamburg, Germany) was stored for long-term maintenance in Roti-StoreÒ cryo-vials (Carl-Roth, Karlsruhe, Germany) in a freezer at −80 °C (New Brunswick Scientific, Nuertingen, Germany). One glass bead with the deep-frozen culture was transferred into ST1-bouillon (Oxoid Ltd., Basingstoke, UK) and incubated for 24 h at 30 °C. An aliquot of this bouillon was then used to inoculate the final broth, which was again incubated at 30 °C for 24 h to obtain the microorganisms in their stationary growth phase. Cells were harvested by centrifugation (2300 *g* for 10 min; Megafuge 1.0R, Heraeus, Hanau, Germany). The pellet was resuspended in the final treatment medium to a concentration of 10^7 CFU/ml. After treatment the collected samples were immediately placed on ice. Viable counts of vegetative cells were determined using drop plating method on Endo-Agar (Oxoid Ltd., Basingstoke, UK). Plates were incubated for 24 h at 30 °C. The inactivation of vegetative organisms was evaluated by comparing viable cell counts in treated and untreated samples and was expressed as log–cycles of microbial reduction.

2.4. Treatment media

Ringer solution (Merck KGaA, Darmstadt, Germany) adjusted to a conductivity of 2.3 mS/cm and raw milk (conductivity of 4.6 mS/cm) were used as treatment media to study geometry effects on microbial inactivation and milk alkaline phosphatase activity. Raw milk was obtained from the Federal Institute for Risk Assessment — Centre for Animal Experiments (Berlin, Germany). Milk composition was determined using Milcoscan 133B (Foss GmbH, Rellingen, Germany) and measurement of pH (pH-meter CG811, Schott Instruments, Mainz, Germany) and conductivity (conductometer LF95, WTW, Weilheim, Germany) were used to describe media properties. In order to study the temperature distribution inside the treatment chamber, saline solution of NaCl adjusted at a conductivity of 4.6 mS/cm was used.

2.5. Alkaline phosphatase activity assay

Determination of alkaline phosphatase (ALP) activity in milk was performed according to Sanders and Sager (1946). This is based on the release of phenol from disodium phenyl phosphate by active phosphatase and the photometrical measurement of phenol using Gibbs' reagent. The experimental procedure was conducted as described by Vester (1962). Activity is expressed as relative value obtained by dividing the measured activity after treatment and the initial activity of the untreated sample.

Table 1
List of variables and abbreviations.

k	turbulent kinetic energy ($m^2\ s^{-2}$)
U	average velocity ($m\ s^{-1}$)
u	velocity vector ($m\ s^{-1}$)
V	electrical potential (V)
C	electrical charge of the capacitor (C)
C_p	specific heat capacity ($m^2\ s^{-2}\ K^{-1}$)
C_μ:0.09, $C_{\varepsilon 1}$:1.44, $C_{\varepsilon 2}$:1.92	model constants used in the turbulence energy and dissipation equations
D	treatment chamber diameter (m)
E	electric field strength (V/m)
E_{aveg}	average electric field strength (V/m)
f	frequency (Hz)
J	current density ($A\ m^2$)
J^e	externally generated current density ($A\ m^2$)
k	thermal conductivity ($kg\ m\ s^{-3}\ K^{-1}$)
k_t	turbulent heat conductivity ($kg\ m\ s^{-3}\ K^{-1}$)
$\dot{m}$	mass flow rate (kg/s)
n	unit vector normal
N	number of experimental temperatures
P	pressure ($kg\ m^{-1}\ s^{-2}$)
Q	sink or source term ($kg\ m^{-1}\ s^{-3}$)
q	heat flux vector ($kg\ m^{-1}\ s^{-3}$)
r	diameter (m)
R_a	treatment chamber resistance (Ω)
R_i	radius of the inner electrode surface (m)
R_o	radius of the outer electrode surface (m)
Re_{HD}	Reynolds number based on the hydraulic diameter of the treatment chamber
t	time (s)
T	temperature (K)
ΔT	temperature difference (K)
T_0	initial temperature (K)
T_i	experimental temperature (°C)
T_i^*	simulated temperature (°C)
y^+	distance from the wall (m)
Greek letters	
δ_w	layer thickness (m)
∇	Nabla operator
ε	turbulent energy dissipation rate ($m^2\ s^{-3}$)
ε_c	dielectric constant
η	viscosity ($kg\ m^{-1}s^{-1}$)
η_T	turbulent kinematic viscosity ($kg\ m^{-1}s^{-1}$)
ρ	fluid density ($kg\ m^{-3}$)
ρ_M	milk density ($kg\ m^{-3}$)
σ	electrical conductivity ($S\ m^{-1}$)
σ_k:0.9 σ_ε:1.62	k–ε model constants energy and dissipation equations
τ	pulse width (µs)
Abbreviations	
ALP	alkaline phosphatase
CFD	computational fluid dynamics
FEM	finite element method
FVM	finite volume method
HV	high voltage electrode
PDE	partial differential equations
PEF	pulsed electric fields
RMSD	root mean square deviation
RMSE	root mean square error
TKE	turbulence kinetic energy

2.6. Thermal inactivation of alkaline phosphatase in raw milk

Thermal inactivation kinetics of alkaline phosphatase in raw milk were conducted using the capillary tube method as an efficient way to allow fast heat transfer and accurate holding times in the range of seconds as described by Haas, Behsnilian, and Schubert (1996a,b). Glass capillaries (length 100 mm, inner diameter 1 mm, wall thickness 0.15 mm) were filled with 100 µl of raw milk, sealed and immersed in

a water bath VWR 1160 S (VWR, Darmstadt, Germany) at the adjusted temperature for the different holding times (60–70 °C, 0–20 s). After thermal treatment, the capillaries were immediately immersed in ice water to allow rapid cooling.

2.7. Mathematical modeling

The finite element analysis and solver software package Comsol Multiphysics (Comsol Inc., Burlington, USA) was used for calculation of electric field strength, temperature distribution and fluid flow as well as for analysis of coupled phenomena. The variables used and their symbols are summarized in Table 1. A three dimensional simulation was performed for the electric field strength calculations whereas only a two dimensional simulation was applied for joule heating and calculation of turbulence due to available computer capacity. Modeling was performed for the whole treatment chamber consisting of two treatment zones since energy dissipation in the first treatment zone is important to consider. Results are only shown for the second treatment zone since influence of the insertion of the static mixing devices on electric field strength distribution and turbulence will be comparable to the first treatment zone. Extent of temperature increase and importance of temperature distribution is also more distinct in the second treatment zone.

The governing equations for fluid dynamics are adapted from COMSOL (2006).

2.7.1. Fluid flow

The k–ε model describes turbulent flow. The equations for the momentum balances and continuity considering a Newtonian fluid are the following:

$$\rho\frac{\partial U}{\partial t} - \nabla\cdot\left[\left(\eta+\rho\frac{C_\mu}{\sigma_k}\frac{k^2}{\varepsilon}\right)\cdot\left(\nabla U+(\nabla U)^T\right)\right] + \rho U\cdot\nabla U+\nabla P=0 \tag{1}$$

and

$$\nabla\cdot U=0 \tag{2}$$

Where ρ denotes the density of the fluid (kg m^{-3}), U represents the average velocity (m s^{-1}), η the dynamic viscosity (kg m^{-1} s^{-1}), P the pressure (Pa), k the turbulent energy (m^2 s^{-2}) and ε the dissipation rate of the turbulence energy (m^2 s^{-3}). The turbulence energy is given by Eq. (3):

$$\rho\frac{\partial k}{\partial t} - \nabla\cdot\left[\left(\eta+\rho\frac{C_\mu}{\sigma_k}\frac{k^2}{\varepsilon}\right)\nabla k\right] + \rho U\cdot\nabla k=\rho C_\mu\frac{k^2}{\varepsilon}\left(\nabla U+(\nabla U)^T\right)^2-\rho\varepsilon \tag{3}$$

And the dissipation Eq. (4) by:

$$\rho\frac{\partial \varepsilon}{\partial t} - \nabla\cdot\left[\left(\eta+\rho\frac{C_\mu}{\sigma_\varepsilon}\frac{k^2}{\varepsilon}\right)\nabla\varepsilon\right] + \rho U\cdot\nabla\varepsilon=\rho C_{\varepsilon1}C_\mu k\left(\nabla U+(\nabla U)^T\right)^2-\rho C_{\varepsilon2}\frac{\varepsilon^2}{k} \tag{4}$$

The model constants used in the turbulence energy and dissipation equations are set to the following values: C_μ:0.09, $C_{\varepsilon1}$:1.44, $C_{\varepsilon2}$:1.92, σ_k:0.9 and σ_ε:1.62.

The criteria to use the k–ε model instead of a laminar model is not based on the consideration of turbulence according to the Reynolds number, which is between 500 and 1730 at a flow rate of 5 l/h and 20 l/h respectively. The transition value between the laminar and turbulent flow in pipes is between a Reynolds number of 2000–2300 (McComb, 1990; Martin, 2002). However, the treatment chamber geometry, the inserted grid and the insulators generate recirculation zones, allowing a fluid flow that can be considered as turbulent (Fiala et al., 2001). The simulation of temperature was also performed using a laminar model but the k–ε model was in better agreement with the experimental data.

2.7.2. Temperature field

The general Eq. (5) for an energy balance, which considers heat transfer through convection and conduction is:

$$pC_p\frac{\partial T}{\partial t}+\nabla\cdot\left(-(k+k_T)\cdot\nabla T+pC_pT\mathbf{u}\right)=Q \tag{5}$$

$$k_T=C_p\eta_T \tag{6}$$

Where C_p denotes the specific heat capacity (m^2 s^{-2} K^{-1}), T is the temperature (K), k is the thermal conductivity (kg m s^{-3} K^{-1}), k_T is the turbulent heat conductivity, η_T denotes the turbulent kinematic viscosity (kg m^{-1} s^{-1}), ρ is the density (kg m^{-3}), u is the velocity vector (m s^{-1}) and Q is a sink or source term (kg m^{-1} s^{-3}).

2.7.3. Electrical potential

The equations to resolve the electrical potential are based on charge conservation

$$-\nabla\cdot(\sigma\nabla V-J^e)=0 \tag{7}$$

Where σ is the electrical conductivity (S/m), V is the electrical potential (V) and J^e (A/m^2) is an externally generated current density.

The relation between the electrical potential and the electric field is given by Eq. (8):

$$E=-\nabla V \tag{8}$$

Where E is the electric field strength (V/m).

In joule heating, the temperature increase is based on the resistive heating due to the electrical current flow. The generated resistive heat Q is proportional to the square of the magnitude of the electrical current density J. Current density, in turn, is proportional to the electric field, which equals the negative of the gradient of the potential V:

$$Q\propto|J|^2 \tag{9}$$

The coefficient of proportionality is the electrical resistance, which is also the reciprocal of the temperature-dependent electrical conductivity $\sigma=\sigma(T)$. Combining these facts gives the fully coupled relation shown in Eq. (10):

$$Q=\frac{1}{\sigma}|J|^2=\frac{1}{6}|\sigma E|^2=\sigma|\nabla V|^2 \tag{10}$$

2.7.4. Boundary conditions and thermophysical properties

The thermophysical properties are considered to be dependent on temperature.

The variable Q (external source of energy in the heat transfer equation) was multiplied by the factor $f\cdot\tau$, where f is the applied pulse frequency and τ is the pulse width. This calculation is based on the assumption that the ohmic heating only occurs during the duration τ of the ideal square wave pulse (without rise and fall time). All boundary conditions implemented in COMSOL (COMSOL, 2006) are summarized in Table 2. Their location within the treatment chamber is illustrated in Fig. 2. Boundary conditions are the same for both treatment zones in the treatment chamber.

2.7.5. Experimental validation

Experimental validation of the temperature distribution obtained by mathematical modeling was performed by temperature measurement

Table 2
Boundary conditions for the computer models (Boundary numbers according to Fig. 2).

Boundary and number	Value
• Electrostatic model	
HV electrode (5)	$V = V_0$
Ground electrode (4)	$V = 0$
Insulator (6)	$n \cdot \sigma \cdot \nabla V = 0$
Insulator grid (7)	$n \cdot \sigma \cdot \nabla V = 0$
HV grid (8)	$V = V_0$
Ground grid (7)	$V = 0$
• Thermal model	
Inflow (1)	$T = T_0$
Outflow (2)	$n \cdot q = 0,\ q = -k\nabla T$
Wall (2–7)	$n \cdot k\ \nabla T = 0$
• Flow model	
Inflow (1)	$u = u_0,\ k = \frac{3}{2}(u_0 I)^2,\ I \cong 0,16(Re_{DH})^{-1/8},\ \varepsilon \cong 2,35\frac{k^{3/2}}{D}$
Outflow (2)	$u = 0,\ n \cdot \nabla k = 0,\ n \cdot \nabla \varepsilon = 0$
Wall (4,5,6,7)	$n \cdot u = 0,\ n \cdot k = 0$
	$K = \left[\frac{\rho \cdot C_\mu^{0.25} k^{0.5}}{1n(y^+)/0.42 + 5.5}\right] \cdot u$
	$\varepsilon = \frac{C_\mu^{0.75} k^{1.5}}{(0.42\delta_w)}$
• Axial symmetry (3)	$r = 0$

in the treatment chamber. Temperature measurement was conducted with a fiber optic thermometer inserted into the treatment chamber. It was located 1.5 mm from the tube wall and 3.5 cm behind the 2nd insulator. The calculation of the temperature by mathematical modeling was performed for the same location in the treatment chamber. Due to the small dimensions of the treatment chamber (inner diameter of 6 mm) in comparison to the dimension of the fiber optical temperature probe (diameter of 1.5 mm, position of the measuring crystal 5 mm behind the end of the sensor) it was not possible to perform adequate measurements at different locations in the treatment chamber at definite locations and without perturbation of the flow and alteration of the experimental temperature measurement by the measuring device itself. The position of the temperature probe given in Fig. 1 only allowed the measurement in one location for different treatment parameter settings. Therefore only the overall temperature at a definite distance from the treatment zone could be practically measured.

Conformity between measurement and mathematical model is shown in Fig. 8.

To further validate the mathematical model of the temperature distribution, the temperature measurement was performed using a treatment chamber with a larger dimension but with congruent geometry (co-linear type treatment chamber with an inner diameter of 30 mm and an electrode distance of 30 mm). This allowed temperature measurement at three different distances behind the second insulator and at 2 different positions in radial coordinate (wall and center of the treatment chamber) for each distance.

The error between simulated and experimental temperature values was estimated by using the Root Mean Square Error (RMSE), also called Root Mean Square Deviation (RMSD). It is a statistical measure of the differences between values predicted by a model and the values actually observed from experiments and can be expressed by an absolute value (Eq. (11), in this case indicating a difference in °C) and by a relative value according to Eq. (12) given in % (Steel & Torrie, 1960; Bizot, 1983).

$$\mathrm{RMSE} = \sqrt{\frac{\sum_i^N (T_i - T_i^*)^2}{N}} \tag{11}$$

$$\mathrm{RMSE}(\%) = 100 \cdot \sqrt{\frac{\sum_i^N \left[\frac{(T_i - T_i^*)}{T_i}\right]^2}{N}} \tag{12}$$

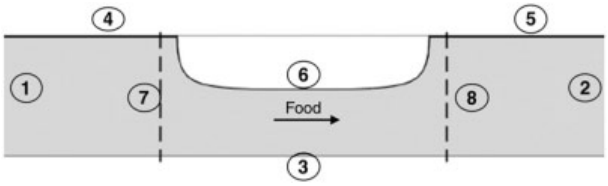

Fig. 2. Treatment chamber and boundary numbers.

Where T_i is the experimental temperature, T_i^* is the simulated temperature, $(T_i - T_i^*)$ is the "prediction error" and N is the total number of experimental temperatures. As classification criteria, a RMSE value below 10% can be considered as adequate to validate the simulation (Cleland & Earle, 1984; Zhang & Cavalieri, 1991; Morales-Blancas, Zuniga & Carrasco, 1999).

3. Results and discussion

The insertion of the grid aimed:

1) to modify the flow characteristics and increase the turbulence intensity to allow a rapid mixing of the fluid.
   Turbulence is seen to be the major factor
   - to reduce inhomogeneous residence times due to lower flow velocity near the chamber wall and consequently temperature increase and over-processing
   - to influence the homogeneity of the treatment intensity
2) to modify the electric field strength distribution to increase the effective field strength due to modification of electrode area by insertion of stainless steel grids
3) to increase electric field strength homogeneity and avoid recirculation to overcome the formation of temperature peaks within the treatment zone.

To evaluate the impact of a polypropylene mesh and a stainless steel woven wire cloth on the distribution of the electric field, numerical simulations were performed, taking into account the dielectric material properties as well as the geometry of the grid as shown in Fig. 3, whereas grids made of polypropylene (model A) or stainless steel (model B) have been inserted in front of each treatment zone and stainless steel grids in front and behind each treatment zone (model C). It could be shown that neither the insertion of an insulator material nor the insertion of the stainless steel grid was altering the electric field in a way, that conduction of experimental investigation would be negatively affected due to the formation of peak electric field strength. Turbulence intensity

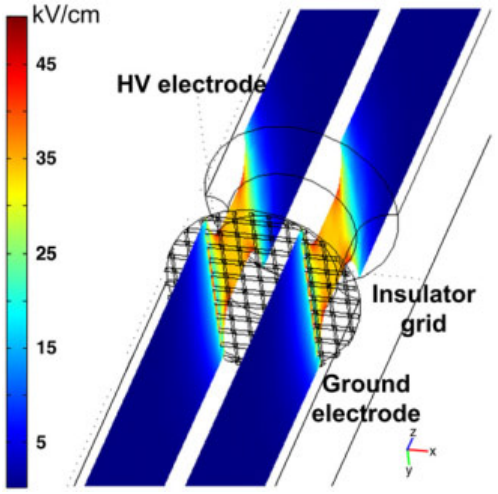

Fig. 3. Numerical simulation of electric field strength in the treatment zone using the treatment chamber modification according to model A (insertion of an insulator grid). Voltage used for calculation was 18 kV/cm.

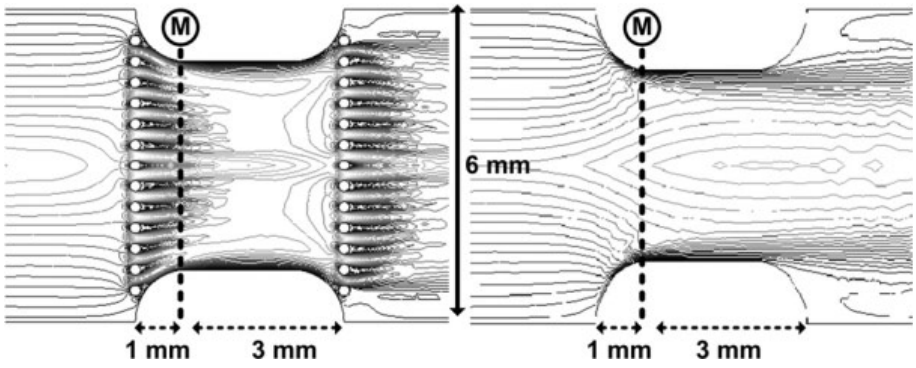

Fig. 4. Streamlines shown with and without inserted static mixing device. Zone (M) of the treatment chamber (2nd treatment zone) where the intensity of turbulence was computationally estimated (see Fig. 5). Illustration shows the location of the grid and zone of calculation of the turbulence exemplarily for model C (grid in front and behind of each treatment zone).

was positively affected in both cases and furthermore the insertion of the stainless steel grids lead to an improvement of electric field distribution homogeneity as will be shown below.

Apart from the alteration of the electric field strength, the impact of the grid on flow behavior and turbulence intensity was studied in the following. Turbulence intensity in a distance of 1 mm from the inserted grid was calculated within the 2nd treatment zone (Fig. 4).

3.1. Grid impact on turbulence kinetic energy (TKE) profile

The turbulence kinetic energy for a treatment chamber without the insertion of mixing devices as well as for the treatment chamber with the inserted grid is shown in Fig. 5 (coordinate where TKE was estimated is according to Fig. 4).

The turbulence kinetic energy is a measure of turbulence intensity and is directly related to the transport of heat, moisture and momentum. It can be generated by fluid shear, friction and eddies in turbulent flows and can be dissipated into heat by the effects of molecular viscosity (Fulachier & Antonia, 1983; Stull, 1989; Neuvazhaev, 1992). As can be seen in Fig. 5, the TKE is improved when grids are used. The increase of TKE improves the mixture of the fluid thus allowing a more homogenous treatment and avoiding temperature peaks (Fiala et al., 2001) as shown in Fig. 11. The average TKE for the model B is $0.0059 \pm 0.0007\ m^2/s^2$ whereas for the model without grids, the average TKE value is $0.002 \pm 0.0009\ m^2/s^2$ indicating a 195% higher average TKE and a 22% lower standard deviation after the insertion of static mixing devices.

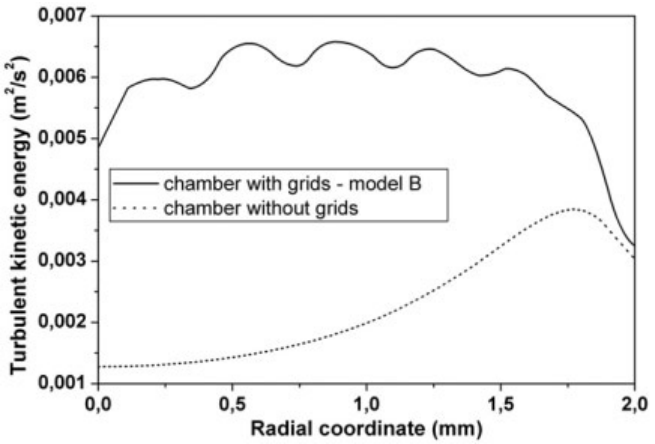

Fig. 5. Turbulent kinetic energy was calculated with COMSOL Multiphysics for a flow rate of 20 l/h using the k–ε model coupled with heat transfer and electrical model. Initial temperature of 30 °C, pulse rate of 200 Hz, 3 µs pulse width.

3.2. Impact on electric field strength

The impact of the insertion of the grids made of an insulating or conductive material on the level and homogeneity of the electric field strength occurring along the center line of the treatment zone was evaluated. The field strength along this line was considered as a suitable parameter to estimate the minimum electric field strength as determined by simulation.

The average electric field strength and its standard deviation were calculated for the center line of the treatment zone (according to Fig. 6) and are shown for the different cases of modification of the treatment zone in Table 3. The standard deviation of the field strength along this line was considered as a parameter to evaluate the homogeneity of the electric field. A geometry dependent factor was calculated for the conversion of the applied voltage into the resulting average field strength in the co-linear treatment chamber (Table 3). This factor is necessary to allow the practical conduction of the PEF treatments performed to determine the temperature distribution as well as microbial and enzyme inactivation.

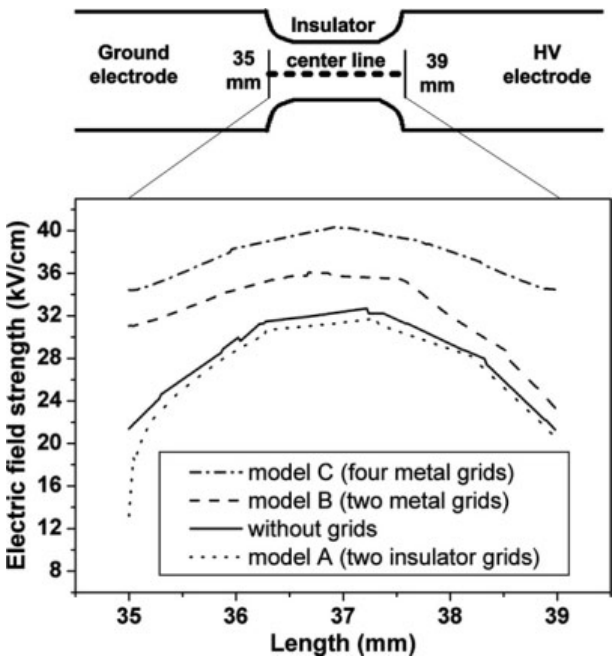

Fig. 6. Comparison of the electric field strength at the center line of one insulator zone for the different treatment chamber setups based on an applied voltage of 18 kV. Steps in the curves are due to incomplete resolution/refinement of the mesh in Comsol and do not present discontinuities.

Table 3
Average electric field strength in the treatment zone after insertion of different grids at a voltage of 18 kV. Calculation performed for the center line of the insulator according to Fig. 6.

Setup	Average electric field strength [kV/cm]	Standard deviation of electric field strength [kV/cm]	Factor for voltage-field strength conversion [-]
Without grid	28.6	3.4	1.6
Case A	27.7	3.7	1.5
Case B	32.6	3.2	1.8
Case C	37.6	1.9	2.1

The insertion of two polypropylene grids each in front of one treatment zone did slightly reduce the average electric field strength and increased the standard deviation of the electric field distribution. Therefore, the insertion of grids with the same geometry but made of a conductive material (stainless steel) was investigated. A positive effect on the electric field distribution and an increase of the electric field strength was expected as the insertion of the metal grids goes along with the extension of the electrode surface. An increase of the average electric field strength of 4 kV/cm was verified by calculation for the same applied external voltage of 18 kV. To further extent the increase of the electric field strength and improve the treatment homogeneity, a modification of the treatment chamber according to case C was performed, where 4 metal grids have been inserted before and after the treatment zone. The electric field strength was increased by 9 kV/cm and the homogeneity of the electric field distribution was enhanced since the standard deviation of the electric field strength along the center line of the treatment zone was reduced from 3.4 kV/cm to 1.9 kV/cm.

A comparison of the electric field strength distribution in case C (insertion of 4 conductive grids) and the unmodified co-linear treatment chamber is shown in Fig. 7 for one treatment zone. As a result of the insertion of the stainless steel grids, a more intense and more homogenous electric field is observed.

3.3. Impact on temperature distribution

Although the PEF treatment is a non-thermal food processing technology, there is a significant temperature increase during PEF treatment due to ohmic heating. Many authors (Fiala et al., 2001; Lindgren, Aronsson, Galt, & Ohlsson, 2002; van den Bosch, Morshuis, & Smit, 2002; Gerlach, Alleborn, Baars, Delgado, Moritz, & Knorr, 2008) have described the temperature distribution in a PEF treatment chamber

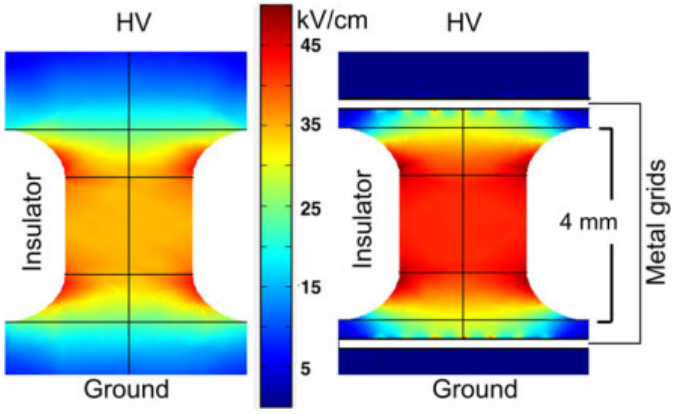

Fig. 7. Comparison between the electric field strength generated in a treatment chamber without grid (left) and with two inserted metal grids (right) at a voltage of 18 kV. The simulation was performed as 3D.

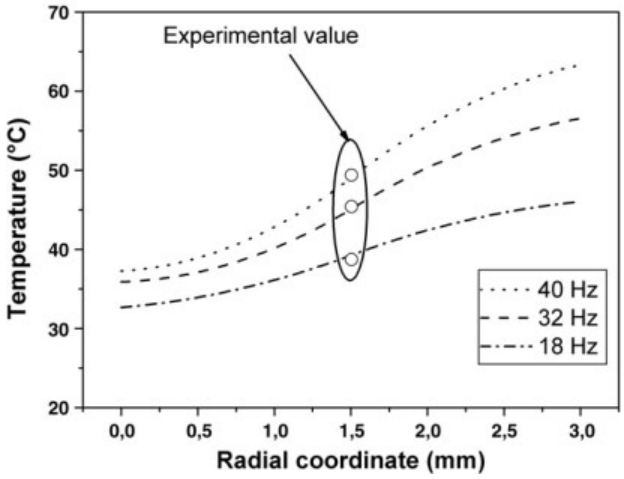

Fig. 8. Temperature as a function of radial coordinate at 3.5 cm behind the 2nd insulator. Calculation and experiment based on a voltage of 18 kV and a conductivity f(T) of 4.6 mS/cm (at 20 °C). Flow rate was adjusted to 4.9 l/h, temperature 30 °C, total specific energy input of 40, 72 and 94 kJ/kg at 18, 32 and 40 Hz respectively. The k–ε model was used for the calculation of the temperature.

and reported the occurrence of high local temperatures due to inhomogeneous field distribution of the electrical field, limited flow velocity and recirculation of the liquid. These temperatures are only detectable using mathematical calculations since measurement in the small volume with peak electric field strength is not possible without interference of the measuring device with the flow and the electric field. Temperature measurement and validation of the values obtained by mathematical modeling was performed in two ways as described in section 2.7.5 Experimental validation:

a) Measuring in the small treatment chamber (inner diameter of 6 mm) at one location for different treatment conditions. The temperature represents one local liquid temperature at the outlet after the PEF treatment chamber (see Fig. 1) and is therefore not suitable to evaluate the temperature effects within the treatment chamber.
b) Measuring in a larger treatment chamber (inner diameter 30 mm) at different locations for one treatment condition.

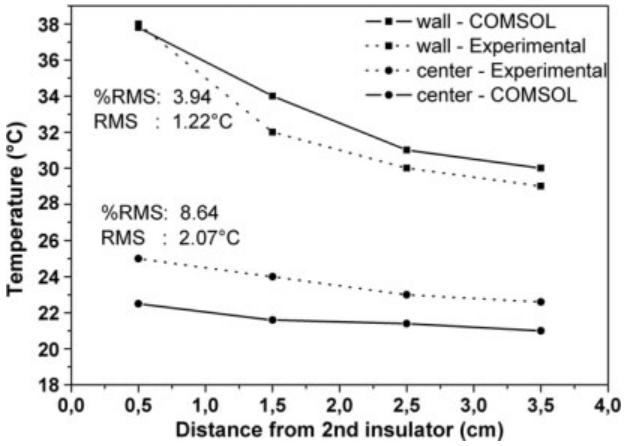

Fig. 9. Temperature obtained by modeling and experimental measurement in a treatment chamber with an inner diameter of 30 mm at the wall and in the centre for three different distances behind the second insulator. Experiment was performed at a flow rate of 60 l/h and an initial temperature of 18.6 °C. Voltage was 18 kV, pulse rate 100 Hz, pulse width 6 µs, conductivity of the salt NaCl solution was adjusted to f(T) 4.6 mS/cm (at 20 °C) and total specific energy input was 87 kJ/kg.

Nevertheless, for experimental performance of PEF treatments the determination of this average outlet temperature is still the most suitable method for an in-line process characterization due to the lack of alternative measuring possibilities. Although, it has to be stated, that even at a distance of 3.5 cm behind the second treatment zone, the fluid still shows an inhomogeneous temperature distribution. However, the measured average temperature at this specific location is in good accordance with the simulation (Fig. 8). As can be seen in Fig. 9, using the large treatment chamber it was possible to detect temperature differences between the wall and the center of the treatment chamber, which could not be measured in the smaller treatment chamber. The experimental and simulated results were evaluated by the RMSE. The RMSE(%) was below the value of 10%, which means that the model can be considered as valid and an average temperature difference between simulated and measured values of 1.2 °C for wall temperatures and 2 °C for the temperatures in the centre of the treatment chamber could be found. A reliable prediction of the treatment temperature based on mathematical simulation also considering high local temperatures is therefore the main task that has to be solved to overcome the limits of temperature measurement and to obtain exact information on thermal exposure of parts of the liquid during PEF treatment.

Fig. 10 illustrates the temperature distribution in the treatment zone as obtained by numerical simulation. Although the average outlet temperature measured at a distance of 3.5 cm from the insulator was 61 °C, there was a high local temperature peak exceeding 80 °C as a result of the high local electric field strength in combination with low flow velocity, lack of turbulence and occurring recirculation and therefore higher residence time at this point (Fig. 4 and 5). Especially when considering heat sensitive components within the treated fluid, a locally extreme thermal inactivation depending on heat inactivation characteristics can occur due to the high temperature. After fluid mixing and sampling at the outlet of the treatment chamber or the cooling system, respectively, this complete inactivation due to thermal effects contributes to a reduced detected overall activity of the alkaline phosphatase, which was the target component under investigation. This leads to the assumption of an inactivation due to the electric field effect since the temperature measured at the outlet of the treatment chamber is below a critical

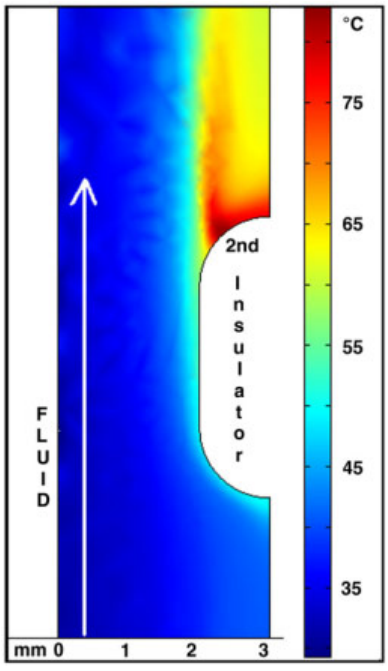

Fig. 10. Temperature contour plot along the 2nd insulator. Calculation based on a voltage of 18 kV, a frequency of 32 Hz and a conductivity $f(T)$ of 4.6 mS/cm (at 20 °C).

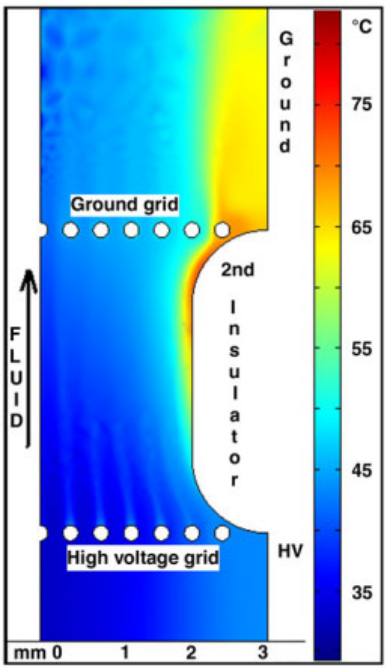

Fig. 11. Temperature contour plot along the 2nd insulator of the treatment chamber with inserted grids. Experiment performed with a voltage of 18 kV, frequency of 32 Hz and a conductivity of 4.6 mS/cm (at 20 °C, dependence on temperature was considered in the calculation).

level and does not suggest any thermal effects. Misinterpretation of the obtained experimental inactivation results may occur.

The maximum temperature increase due to dissipation of electrical energy is reached at the wall since the flow velocity is low and the residence time in the electric field longer. An alteration of the flow behavior and the impact on the temperature distribution by insertion of the grids was investigated aiming to reduce the residence time of the liquid. Since the implementation of two stainless steel grids in each treatment zone showed an improvement of the electric field distribution and had the same impact on the alteration of the flow characteristics as the polypropylene insulator grids, this option was preferred.

Fig. 11 shows the simulation of the resulting temperature distribution after insertion of four grids. The temperature distribution is more homogenous within the treatment chamber which is reached by a reduction of temperature peaks and in the same time by a slight increase of the bulk temperature in the central part of the treatment chamber. The maximum occurring temperature could be reduced from 80 °C to 68 °C due to mixing effects behind the grid. The temperature in the center of the treatment chamber was increased due to the increase of electrical field intensity between the two metal grids and due to mixing effects leading to incorporation of high temperature volume elements. It can therefore be considered that the thermal effects occurring due to extreme temperature peaks are reduced. On the other hand, a more uniform increase of the temperature of large parts of the liquid already takes place in the treatment zone within the electric field. This affects positively microbial inactivation due to synergetic temperature effects on membrane pore formation. However, it has to be stated that the outlet temperature either measured or calculated remained the same and was not affected by the significant local temperature improvements due to the insertion of the grid. This fact shows again the limited capability of the overall temperature measurement as major temperature effects remain unconsidered.

For an experimental evaluation of the improved field strength, flow characteristics and temperature distribution the impact on the inactivation of *E. coli* and raw milk alkaline phosphatase was investigated.

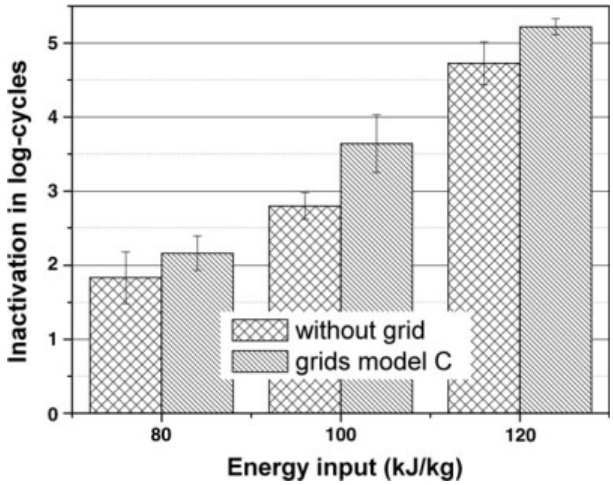

Fig. 12. Inactivation of *E. coli* using a treatment chamber with four inserted stainless steel grids in comparison to a treatment chamber without grid. Voltage was set to 18 kV, average electric field strength according to Table 3. Treatment medium was Ringer solution with a conductivity of mS/cm. Inlet temperature was 30 °C, flow rate 10 l/h, treatment time in the range of 12–19 μs for the different energy input levels.

3.4. Impact on microbial inactivation

The inactivation of *E. coli* in Ringer solution subjected to pulsed electric field treatment in the different treatment chamber configurations at different energy input levels is shown in Fig. 12. The treatment chamber with the inserted grids enhanced the microbial inactivation by an average of 0.6 log–cycles for the regarded treatment intensity range of 80–120 kJ/kg.

The improved inactivation is mainly due to the higher field strength obtained in the treatment chamber with inserted stainless steel grids which was 37.6 kV/cm compared to 28.6 kV/cm both for an initial voltage of 18 kV. The energy input was kept the same for the modified and unmodified treatment chamber configuration. Since a higher current flow was observed in the modified treatment chamber due to the increased electrode surface and reduced treatment chamber resistance, the pulse width was adapted to maintain a constant energy per pulse level and therefore a constant treatment frequency to be able to compare the different treatment chamber configurations under the same treatment conditions. The increase in microbial log-reduction can therefore be considered as a result of the increased electric field strength and the more homogenous field distribution as well as a result of the slightly increased temperature of large parts of the liquid already taking place in the treatment zone.

3.5. Impact on milk alkaline phosphatase (ALP)

Milk alkaline phosphatase is a heat sensitive enzyme used as a heat indicator during conventional pasteurization. To evaluate the improvement of the temperature distribution and the reduction of peak temperatures present in the treatment zone, raw milk was used for PEF treatment and alkaline phosphatase activity was determined for the different treatment chamber modifications including the insertion of four stainless steel grids and the insertion of two polypropylene grids one in front of each treatment zone. Treatment parameters for the different setups are shown in Table 4.

Sampling of raw milk after PEF treatment was performed at the outlet of the cooling system, so that an average phosphatase activity was measured. Although this average value only represents information on the overall phosphatase activity after treatment, it can also be taken as an indicator of the reduction of local high temperature peaks. Considering the low thermal stability of ALP which already shows detectable inactivation at 60 °C for holding times below five seconds

Table 4
PEF parameters used for a treatment chamber with four stainless steel grids, two polypropylene grids or without the insertion of a grid at a voltage of 18 kV for PEF treatment of raw milk at a flow rate of 10 l/h.

Treatment chamber	Without grid	2 Insulator grids	4 Metal grids
Average field strength (kV/cm)	28.6	27.7	37.6
Pulse width (μs)	3.4	3.5	3
T outlet (°C)		61.2	
Frequency (Hz)		96	
Pulse energy (J/pulse)		3.79	
Total specific energy input (kJ/kg)		130	
Total treatment time (μs)	12.5	12.9	11.1

(heat inactivation data not shown), these temperature peaks contribute to a large extent to the overall inactivation due to the extreme temperature level that is reached. Therefore, increased overall phosphatase activity can be interpreted as a result of the improved temperature homogeneity in the treatment chamber. Furthermore, the higher electric field strength that is related to the insertion of the 4 stainless steel grids may also affect the phosphatase inactivation by PEF. According to Castro, Swanson, Barbosa-Cánovas, and Zhang (2001) and Shamsi, Versteeg, Sherkat, and Wan (2008) the electric field strength showed an impact on ALP inactivation by PEF whereas Van Loey, Verachtert, and Hendrickx (2001) did not observe any reduction in alkaline phosphatase activity after PEF treatment.

Relative residual activity of phosphatase in raw milk after PEF treatment is shown in Fig. 13.

For the treatment performed in the chamber without the insertion of grids, an average residual phosphatase activity of 78% was detected. The treatment chamber modification by insertion of two polypropylene grids one before each treatment zone increased the residual enzyme activity after PEF treatment to 87%. Since the treatment parameters in this case are similar to the treatment performed without the addition of the grid (see Table 4) it can be concluded that the pulsed electric field treatment itself did not contribute to the reduced inactivation. Furthermore, the insulator grid located before the treatment chamber and the associated alteration of flow behavior and temperature distribution as shown before can be found to reduce the thermal inactivation effects on heat sensitive compounds during PEF treatment. In contrast, the insertion of metal grids into the treatment chamber leads to a considerable change in electric field properties and to an increase in the average electric field strength of 9 kV/cm. The insertion of two additional grids after the treatment zone also provides an improvement of the fluid mixing and temperature distribution as shown in Fig. 11. Using the modified treatment chamber with four stainless steel grids the enzyme inactivation could be further reduced to a residual enzyme activity after treatment of 92%. Although the field strength was higher in this case, the enzyme inactivation did not increase. This indicates that the

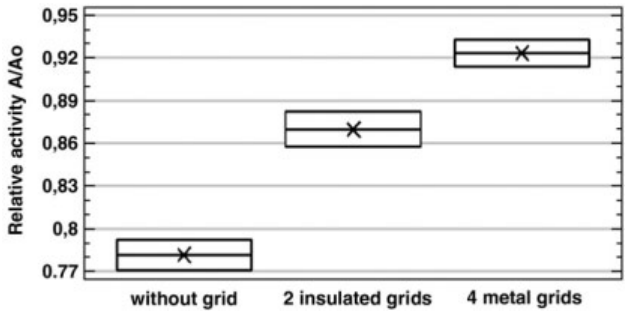

Fig. 13. Impact of the insertion of grids on alkaline phosphatase inactivation in raw milk. Experiment performed at an inlet temperature of 30 °C according to PEF parameters shown in Table 4.

field strength has no impact on ALP inactivation within the investigated range from 29 kV/cm to 38 kV/cm and that the electric field itself (at a level of 38 kV/cm and an energy input of 130 kJ/kg) only leads to a reduction in enzyme activity of 8%. This reduction can be related to thermal treatment resulting from the holding time of the treated raw milk at the PEF outlet temperature of 61 °C for 7 s (heat inactivation data not shown) until appropriate cooling has taken place in the cooling coil used in the experimental setup. The results clearly show that even at increased PEF treatment intensity, nearly no inactivation of alkaline phosphatase in raw milk occurs when temperature effects are considered. The increased retention of alkaline phosphatase activity in the chamber with the insertion of 4 metal grids (92%) in comparison to the chamber without grids (78%) can be related to the improvement of the temperature distribution. Thermal effects were reduced based on the alteration of flow characteristics. Subsequently, the formation of local temperature peaks of up to a 20 °C higher than the measured overall outlet temperature could be avoided. Perturbation of the fluid flow by the grid and resulting turbulence lead to better mixing of the treated liquid and the enzyme, usually exposed to high local temperatures, is less affected as mixing provides also rapid reduction of the locally occurring peak temperature.

4. Conclusion

The numerical simulation of PEF process with computational tools allows the design of an improved treatment chamber and serves to improve the efficiency of microbial inactivation and to avoid food over-processing. A conventional co-linear treatment chamber could be modified by insertion of grids for alteration of electric field strength, flow velocity and temperature distribution. It could be shown that the temperatures occurring within the PEF treatment chamber differ considerably from the measured outlet temperature leading to thermal inactivation of heat sensitive alkaline phosphatase. The insertion of grids in the electric field zone produces a homogeneous and more intense electric field which showed to improve the inactivation of *E. coli*. On the other hand the formation of turbulence at low flow velocities provides a fluid mixing, avoiding the high temperatures generated in the zone near the insulators and leads to a more homogeneous temperature distribution in the treatment chamber. The latter has an important impact on the enzyme activity since alkaline phosphatase inactivation could be reduced significantly. The presented investigations showed the necessity to determine the temperature in the PEF treatment chamber in addition to the measurement of an average outlet temperature. The modification of the treatment chamber by inserting static mixing devices provided a possibility to improve electric field strength and temperature distribution resulting in an increased microbial inactivation and retention of heat sensitive components. The insertion of grids of the dimension used in the study has shown applicability for the treatment of low viscosity products like milk or clarified fruit juices. Other real food systems containing particles or high viscosity products will require different static mixing devices or complete modification of the treatment chamber to allow improvement of treatment homogeneity which is currently under investigation.

Acknowledgements

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III

Model for the differentiation of temperature and electric field effects during thermal assisted PEF processing

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Model for the differentiation of temperature and electric field effects during thermal assisted PEF processing

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ABSTRACT

A model was developed that enables the quantification of thermal and electric field effects during the pulsed electric field (PEF) inactivation of alkaline phosphatase (ALP) and lactoperoxidase (LPO) in milk as well as *Escherichia coli* in apple juice.

The entire PEF process which consisted of pre-heating of the liquid, PEF treatment and rapid cooling to refrigeration temperatures was analyzed with regard to the thermal load to which the product was exposed. Finite volume method (FVM) was used to calculate the heat transfer phenomena within the heating and cooling devices. The temperature increase during PEF treatment due to ohmic heating was calculated using the total specific energy input. A temperature–time profile of the PEF process depending on different treatment conditions was obtained.

Heat inactivation kinetics of native ALP and LPO in raw milk and *E. coli* in apple juice were determined by glass capillary method. A model for the calculation of inactivation levels based on a first order inactivation kinetic was obtained. A MathCad tool was programmed to perform the mathematical comparison of the thermal exposure of ALP, LPO and *E. coli* during the PEF process and their thermal stability. The impact of different temperature–time profiles resulting from the variation of the PEF treatment parameters on inactivation and residual activity were investigated. The quantification of thermal and electric field effects and their contribution to the overall inactivation revealed that thermal effects were the major reason for the enzyme inactivation and had large impact on the inactivation of *E. coli* at elevated temperatures.

The model may be used for the optimization of a PEF process to achieve the minimization of thermal inactivation of heat-sensitive components but also for the beneficial use of thermal effects to improve the microbial inactivation.

1. Introduction

Pulsed electric field treatment is considered as a non-thermal alternative to traditional pasteurization of liquid foods (Lelieveld et al., 2007). The inactivation of microorganisms is based on the electromechanical mechanism of electroporation (Crowley, 1973; Zimmermann et al., 1974) which allows food processing at moderate temperatures. However, the phospholipid bilayer structure of the cell membrane changes from a gel-like to a liquid crystalline state when increasing the temperature and the increased membrane fluidity leads to a reduced membrane stability and facilitates the electroporation of the cell membrane (Kanduser et al., 2008; Stanley, 1991).

When considering the PEF processing as a pure non-thermal treatment, microbial inactivation mainly depends on parameters such as pulse number and treatment time assuming that a critical electric field strength level is exceeded.

Inactivation of *Escherichia coli* in a batch system with exact temperature monitoring was studied by Álvarez et al. (2003) and Amiali et al. (2004) who investigated the PEF inactivation of *E. coli* in liquid egg using a parallel plate treatment chamber with active cooling to keep the treatment temperature below any lethal level. The so obtained inactivation could be completely related to the pulsed electric field effect and a strong influence of the treatment time was found in both studies.

Controversial results exist concerning electric field effects on enzymes. Van Loey et al. (2002) studied the inactivation of LPO and ALP in batch systems. No inactivation of LPO was found (electric field strength of 19 kV/cm, specific energy input of up to 500 kJ/kg, treatment time of 500 μs). Similar results were obtained

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for ALP where no inactivation occurred due to PEF treatment in the batch system (20 kV/cm and 400 μs). However, the increase of the treatment time (and of the specific energy input) up to 8000 μs resulted in a 74% inactivation which was attributed to thermal effects since a maximum temperature of 70 °C was reached during this treatment. PEF inactivation of ALP and LPO (21 kV/cm, 400 kJ/l, 20 pulses, pulse width not reported) studied by Grahl and Märkl (1996) in milk was also minimal as long as the maximum temperature was kept below 50 °C. PEF treatment of purified ALP dissolved in simulated milk ultrafiltrate (cuvette batch system, 22 kV/cm, 70 pulses, 740 μs pulse width) performed by Castro et al. (2001) lead to a 65% reduction in ALP activity. The inactivation was found to be directly related to the ALP concentration and the electric field intensity in terms of electric field strength and number of pulses.

A separation of the contribution of pulsed electric field and thermal energy on the inactivation of *Listeria monocytogenes* was conducted by Fleischman et al. (2004). The experimental approach consisted of the conduction of PEF treatments under isothermal conditions. It allowed the quantification of the microbial inactivation attributed to PEF, thermal energy as well as synergetic effects in terms of increased susceptibility of *L. monocytogenes* to PEF energy at elevated temperature.

Results obtained by El Zakhem et al. (2007) also indicate a synergy between thermal and electric field effects for mild electric field treatments (MEF) of *E. coli* suspended in saline water. A significant reduction of thermal treatment times was achieved when MEF treatment (5 kV/cm) was applied simultaneously.

The synergetic effect of temperature during pulsed electric field inactivation of microorganisms can be used to improve the inactivation results and/or to reduce the electrical energy costs (Craven et al., 2008; Riener et al., 2008). Energy savings derive from:

- The lower PEF treatment intensity (treatment time and total specific energy input) required for a certain level of microbial inactivation at increased temperature.
- The possibility to recover the electrical energy dissipated during PEF treatment in form of thermal energy for pre-heating the incoming product.

A processing concept taking into consideration the impact of temperature on lethality and energy efficiency during apple juice pasteurization by PEF has been proposed by Heinz et al. (2003).

The application of temperature levels above the thermal inactivation limit can occur due to

- The application of a high inlet temperature leading to the development of a PEF assisted pasteurization process with the benefit of the inactivation of thermotolerant microorganisms using the electroporation as an additional inactivation mechanism according to the hurdle concept (Ross et al., 2003).
- The temperature increase during the PEF treatment as a result of the ohmic heating (Lindgren et al., 2002).

In both cases, the high temperature will directly contribute to the inactivation of microorganisms and enzymes but may also lead to the undesired destruction of heat-sensitive components as soon as a certain temperature level is exceeded or the exposure time of the product to the high temperature level is to long.

In order to quantify the contribution of thermal and electric field effects on the inactivation of microorganisms and enzymes during continuous PEF treatment the development of a general model is required considering the temperature levels and the corresponding holding times occurring at each step of the PEF preservation process such as pre-heating, ohmic-heating in the PEF treatment chamber and cooling. Relating this temperature–time profile to the heat inactivation kinetics of the target microorganisms or enzymes, it is possible to calculate an overall thermal inactivation (Lewis and Heppell, 2000). The comparison of this value with the experimentally measured total inactivation after the PEF process (resulting from thermal as well as electric field effects) leads to a PEF-only inactivation as well as a thermal-only inactivation. This differentiation may allow the process optimization to minimize the destruction of heat-sensitive components while achieving effective inactivation of microorganisms using a combination of PEF and elevated treatment temperature.

The present paper aims to introduce such a general mathematical model (emphasized in the Section 2) which is applicable for the differentiation of temperature and electric field effects on the inactivation of microorganisms and enzymes based on heat inactivation kinetics and inactivation results obtained after a PEF preservation process.

2. Materials and methods

Variables and abbreviations used in the text are summarized in Table 1.

Table 1
List of symbols and abbreviations.

Latin letters	
A	enzyme activity (U/l)
A_0	initial enzyme activity (U/l)
A_H	heat transfer area (m^2)
c_p	specific heat capacity ($m^2\ s^{-2}\ K^{-1}$)
d_i	inner pipe diameter of the cooling coil (mm)
d_o	outer pipe diameter of the cooling coil (mm)
D	diameter of the cooling coil (mm)
E	electric field strength (V/m)
E_a	energy of activation (kJ/mol)
h	distance of cooling coil windings (mm)
I	electric current (A)
k	heat inactivation rate constant (s^{-1})
k_0	velocity constant (s^{-1})
k_H	heat transfer coefficient ($Wm^{-2}\ K^{-1}$)
L	pipe length of the cooling coil (mm)
$\dot{m}$	mass flow rate (kg/s)
n	number of windings of the cooling coil (−)
N	viable colony count (CFU/ml)
N_0	initial viable colony count (CFU/ml)
R	universal gas constant ($8314\ Jmol^{-1}\ K^{-1}$)
t	holding time (s)
t_{res}	residence time (s)
t_{treat}	treatment time (s)
T	temperature (K)
T_{ext}	external temperature of the heat exchanger coil (K)
T_{in}	media inlet temperature of the heat exchanger coil (K)
T_{out}	media outlet temperature of the heat exchanger coil (K)
T_{inTC}	product inlet temperature of the treatment chamber (K)
T_{outTC}	product outlet temperature of the treatment chamber (K)
ΔT_{log}	mean logarithmic temperature difference (K)
U	voltage (V)
W_{pulse}	pulse energy (J)
W_{spec}	total specific energy input (kJ/kg)
Greek letters	
ϑ	temperature (°C)
ϑ_0	inlet temperature of the heating and cooling coil (°C)
τ	pulse width (μs)
Abbreviations	
ABTS	2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid)
ALP	alkaline phosphatase
FVM	finite volume method
log Red	reduction of viable colony counts in log-cycles
LPO	lactoperoxidase
PEF	pulsed electric fields
PPO	polyphenoloxidase
rel*A*	residual relative activity
RMSE	root mean square error

2.1. Treatment media

Raw milk for studying the treatment effect on milk alkaline phosphatase and lactoperoxidase was obtained from the Federal Institute for Risk Assessment – Centre for Animal Experiments (Berlin, Germany). Milk composition was determined using Milcoscan 133B (Foss GmbH, Rellingen, Germany) and measurement of pH (pH-meter CG811, Schott Instruments, Mainz, Germany) and conductivity (conductometer LF95, WTW, Weilheim, Germany) were used to describe media properties. Commercial pasteurized clear apple juice (Werder Frucht, Hohenseefeld, Germany) was obtained from a local supermarket and used as the treatment media for *E. coli*.

2.2. Enzyme activity assays and microbial growth conditions

Lactoperoxidase (LPO) activity was assayed according to Sienkiewicz (2006). The method is based on the formation of $ABTS^+$ (2,2′-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) radicals catalysed by LPO in the presence of hydrogen peroxide and ABTS (Fluka, Steinheim, Germany). The reaction mixture consists of 55 mg ABTS diluted in 50 ml phosphate buffer (pH 6) containing 1 ml of 0.03% hydrogen peroxide. 2 ml of the reaction mixture are placed in a semi-micro cuvette with a path length of 10 mm (ratiolab GmbH, Dreieich-Buchschlag, Germany) and the reaction is started by adding 50 μl of an appropriate dilution of the milk sample. The release of $ABTS^+$ radicals per time unit is proportional to the LPO activity and was measured at 420 nm and 25 °C for 2 min using a spectrophotometer (HITACHI, San Jose, USA). A linear regression was applied to determine the slope of the absorbance curve which is directly proportional to the LPO activity considering the dilution of the analyzed milk samples.

Alkaline phosphatase (ALP) activity in milk was determined according to Sanders and Sager (1946). The method is based on the release of phenol from disodium phenyl phosphate by active phosphatase and the photometrical measurement of phenol using Gibbs' reagent. The experimental procedure was conducted as described by Vester (1962).

Enzyme activities are expressed as relative value obtained by dividing the measured activity after treatment and the initial activity of the untreated sample.

E. coli K12DH5α (Hygiene Institut Hamburg, Germany) was stored at −80 °C for long-term maintenance in Roti-Store cryovials (Carl-Roth, Karlsruhe, Germany). After transferring one glass bead with the deep-frozen culture into Standard I Nutrient Broth (Oxoid Ltd., Basingstoke, UK) it was incubated for 24 h at 30 °C. An aliquote of this broth was then used to inoculate the final broth (also Standard I Nutrient Broth) followed by incubation at 30 °C for 24 h to obtain cells in their stationary growth phase. Cells were harvested by centrifugation (2300 g for 10 min; Megafuge 1.0R, Heraeus, Hanau, Germany). The pellet was resuspended in the apple juice to an initial concentration of 10^7 CFU/ml. The samples collected after treatments were immediately placed on ice. The drop plating method was used to determine viable counts of vegetative cells on Endo-Agar (Oxoid Ltd., Basingstoke, UK). Plates were incubated for 24 h at 30 °C. The inactivation of *E. coli* was evaluated by comparing viable cell counts in the treated and untreated sample and was expressed as log-cycles of microbial reduction.

2.3. PEF system

PEF treatment of raw milk and apple juice was performed using a 7 kW pulse modulator (ScandiNova Systems AB, Uppsala, Sweden). Rectangular pulses with a pulse width of 3 μs were used in a continuous co-linear type treatment chamber at a flow rate of 5 l/h provided by a peristaltic pump 323 Du (Watson Marlow, Wilmington USA). The treatment chamber consisted of one central high voltage electrode (length 35 mm) and two outer grounded electrodes (all stainless steal, inner diameter 6 mm) separated by a distance of 4 mm using two polyoxymethylene insulators with an inner diameter of 4 mm. This geometry provides two treatment zones of a total enclosed volume of 0.21 ml exposed to the electric field resulting in a total residence time of the medium in the electrical field of 0.15 s at a flow rate of 5 l/h. Based on numerical simulation of the electric field strength distribution, the zone 1 mm in front and behind the insulator had a relevant electric field intensity and was therefore considered to belong to the treatment zone. The average electric field strength within the treatment zone was calculated and a multiplication factor of 1.71 cm^{-1} was determined for the given geometry to convert the initial voltage into the occurring electric field strength. A detailed description of the treatment chamber and the numerical simulation procedure can be found in Meneses et al. (submitted for publication).

The total specific energy input (W_{spec} in kJ/kg) was chosen as a parameter to describe the treatment intensity. It was calculated according to Eq. (1) multiplying the energy delivered per pulse (W_{pulse}) by the pulse frequency (f) divided by the mass flow rate $\dot{m}$ of the treated product.

The pulse energy was obtained by integration of the voltage $U(t)$ and current $I(t)$ profiles based on the measurement with a TDS 220 oscilloscope (Tektronix Inc., Beaverton, USA):

$$W_{spec} = \frac{f}{\dot{m}} \cdot \int_0^{t_{treat}} U(t) \cdot I(t) \cdot dt. \quad (1)$$

The average pulse number (n) was calculated by multiplying the total residence time (t_{res} = 0.15 s) of the product in the treatment zone with the pulse frequency (f). The total treatment time (t_{treat}) was obtained by multiplying the number of pulses with the pulse width (τ).

Sterile salt solution adjusted to corresponding treatment media conductivity was used to start up the sterile PEF system prior to product inlet from a second tank and complete rinse of the salt solution out of the system was assured before product sampling.

The PEF treatment parameters used for the treatment of milk (ALP and LPO) and apple juice (*E. coli*) are summarized in Table 2.

To study the electric field effect at high specific energy input but without an extensive heating effect, a multiple step processing was applied with repeated treatments using the co-linear continuous treatment chamber system and an intermediate cooling. Treatment conditions marked with an asterisk in Table 2 were applied for a treatment consisting of two cycles for ALP up to a maximum energy input of 214 kJ/kg (maximum treatment temperature 44 °C within one cycle) and a treatment consisting of three cycles for LPO up to a maximum energy input of 210 kJ/kg (maximum treatment temperature 38 °C within one cycle).

2.4. Heating and cooling devices

The adjustment of the inlet temperature and the immediate cooling after the treatment were conducted by stainless steal cooling coils (Berlin University of Technology) immersed in VWR 1160S circulating water baths (VWR, Darmstadt, Germany).

Each cooling coil consists of a stainless steal tube of 2340 mm of total length (L) with an inner diameter (d_i) of 2 mm and a wall thickness of 1 mm. The cooling coil itself has a diameter (D) of 86 mm and a height of 135 mm leading to n = 9 windings with a distance (h) of 15 mm to each other. The geometrical characteristics of the heat exchanger coils can be calculated as follows: d_i/D ratio 0.023 and L/d_i ratio 67.5.

A Takaoka fiber optic thermometer FT1110 (Chiyoda Corporation, Tokyo, Japan) served for temperature measurement during

Table 2
Conditions and parameters used during PEF treatment of ALP and LPO in milk and *E. coli* in apple juice (flow rate 5 l/h, residence time in the treatment zone 0.15 s, pulse width 3 μs). Treatment conditions marked with an asterisk were used for repeated treatment with intermediate cooling.

	ALP					LPO						*E. coli*				
E (kV/cm)	38					34*						35				
W_{spec} (kJ/kg)	97	107*	182	192	202	70*	77	121	169	227	251	24	34	45	55	
W_{pulse} (J)	4.8	4.3*	5.4	5.5	5.5	3.5*	3.8	4.0	4.2	4.5	4.6	2.1	2.1	2.2	2.2	
f (Hz)	28	27*	47	49	51	28*	28	42	56	70	76	16	22	29	35	
Pulse number	4.2	4.1*	7.1	7.4	7.7	4.2*	4.2	6.3	8.4	10.5	11.4	2.4	3.3	4.4	5.3	
t_{treat} (μs)	12.6	12.3*	21.2	22.1	23	12.6*	12.6	18.9	25.2	31.5	34.2	7.2	9.9	13.0	15.8	
T_{in} (°C)	20					20*						50				
T_{out} (°C)	42	44*	60	64	67	38*	40	48	60	75	85	56	58	60	62	

PEF treatment. The measurement point for the final product temperature after treatment was located 3.5 cm behind the second treatment zone.

2.5. Thermal inactivation studies

The capillary tube method as described by Haas et al. (1996a, 1996b) was used to determine thermal inactivation kinetics of ALP and LPO in raw milk as well as *E. coli* in apple juice. Glass capillaries (Kleinfeld Labortechnik GmbH, Stötefeld, Germany; length 100 mm, inner diameter 1 mm, wall thickness 0.15 mm) were filled with 100 μl of sample, sealed and immersed in a water bath VWR 1160S (VWR, Darmstadt, Germany) at the adjusted temperature in the range of 50–85 °C for the different holding times (0–240 s). After thermal treatment, the capillaries were immediately immersed in ice water to allow rapid cooling. It was shown by numerical simulation of the temperature profile in the capillary using Comsol multiphysics (Comsol Inc., Burlington, USA) that the capillary method is an efficient way to allow fast heat transfer and accurate holding times in the range of seconds. The heating up time to a temperature of 0.03 K below the final temperature in the centre of the capillary was 3.75 s showing only a low dependency from the final treatment temperature within the studied range (initial capillary temperature 20 °C, water bath temperatures between 40–90 °C). However, a temperature of 1 K below the final temperature was already reached after a heating up time less than 2.5 s (data not shown).

2.6. Modeling of enzyme and microbial thermal inactivation

The residual enzyme activity (rel$A$) depending on treatment temperature (T) and holding time (t) as well as electric field strength (E) and total specific energy input (W_{spec}) was calculated according to Eq. (2.a) where A_0 denotes the enzyme activity of the untreated sample and A the enzyme activity after thermal treatment and/or after a pulsed electric field treatment. For *E. coli*, N_0 is defined as the initial viable colony count of the untreated sample and N is the colony count after the inactivation treatment. The inactivation of *E. coli* can be also expressed as reduction of viable colony counts in log-cycles (log Red) and is then calculated by Eq. (2.b).

$$\mathrm{rel}A(T,t,E,W_{spec}) = \frac{A}{A_0} = \frac{N}{N_0}, \tag{2.a}$$

$$\log \mathrm{Red} = -lg\lfloor \mathrm{rel}A(T,t,E,W_{spec})\rfloor. \tag{2.b}$$

A first order kinetic based on Eq. (3) was used to describe the thermal inactivation of the enzymes and of *E. coli* for each temperature (T) depending on the holding time (t) based on the values obtained from the thermal inactivation experiments with the capillary tube method.

$$\mathrm{rel}A_{(T)}(t) = \exp\lfloor -k_{(T)}\cdot t\rfloor, \tag{3}$$

k denotes a temperature dependent factor describing the inactivation rate. This factor was determined experimentally based on the known residual relative activity at a specific temperature depending on treatment time. The relation between the inactivation rate (k) and the inactivation temperature was approximated by the Arrhenius Eq. (4), where E_a is the energy of activation, k_0 the velocity constant in s^{-1} for $1/T = 0$ and R the universal gas constant (8.314 $Jmol^{-1}\,K^{-1}$):

$$\ln k(T) = -\frac{E_a}{R}\cdot\frac{1}{T} + \ln k_0 \tag{4}$$

The relation between the inactivation rate and the temperature (Eq. (4)) can be inserted into Eq. (3) and a model for the thermal inactivation of the enzyme or microorganism is obtained (Eq. (5)) including the treatment temperature (T) and the holding time (t):

$$\mathrm{rel}A(T,t) = \exp\left[-k_0\cdot t\cdot\exp\left(\frac{-E_a}{R}\cdot\frac{1}{T}\right)\right]. \tag{5}$$

Table Curve 2D version 4 and Table Curve 3D version 3 (SPSS Inc., Chicago, USA) were used as regression software to fit the experimentally obtained data with the above mentioned mathematical equations.

2.7. Calculation of the product temperature–time-profile in the PEF unit

The total thermal load to which the product is exposed while passing the PEF system can be described by the temperature–time profile. The main sections contributing to a change in temperature or representing a holding section are the tubular heat exchanger used for adjusting the inlet temperature (length 2340 mm), a pipe section (length 400 mm), the treatment chamber itself (length 45 mm) where the electrical energy is dissipated into thermal energy, a second pipe section (length 352 mm) and a second tubular heat exchanger (length 2340 mm) used for product cooling.

FLUENT software (Ansys Inc., Canonsburg, USA) was used for solving fluid flow and heat transfer problems in the cooling coil based on finite volume technique by converting the governing equations to algebraic equations that can be solved numerically. The heat transfer coefficient (k_H) for the cooling coil was determined experimentally and calculated according to Kessler (2002) using Eq. (6) where $\dot{m}$ is the mass flow rate, c_p the specific heat capacity, T_{in} and T_{out} the inlet and outlet temperatures of the cooling coil, A_H the area relevant for the heat transfer (calculated according to Eq. (7) with the outer and inner pipe diameter d_o and d_i and the length L) and $\Delta T\log$ the logarithmic mean temperature difference between the inside and outside (T_{ext}) of the cooling coil (according to Eq. (8)).

$$\dot{m}\cdot c_p\cdot(T_{in}-T_{out}) = k_H\cdot A\cdot\Delta T_{log}, \tag{6}$$

$$A_H = \frac{(d_o-d_i)\cdot\pi\cdot L}{\ln(d_o/d_i)}, \tag{7}$$

$$\Delta T_{log} = \frac{(T_{in}-T_{ext})-(T_{out}-T_{ext})}{\ln\frac{T_{in}-T_{ext}}{T_{out}-T_{ext}}}, \tag{8}$$

The calculated heat transfer coefficient was 613 W m^{-2} K^{-1} for the cooling coil and 1032 W m^{-2} K^{-1} for the heating coil based on the following measured values (values in brackets belong to the heating coil): mass flow rate 5 kg/h, logarithmic mean heat transfer area 150 cm^2 for a model cooling coil with a length of 1660 mm, inlet temperature 63 °C (13 °C), outlet temperature 20 °C (44 °C), external temperature 10 °C (46 °C), specific heat capacity 3.98 kJ kg^{-1} K^{-1}.

A validation of the heat transfer coefficients was performed by comparing the measured and calculated outlet temperatures for the heat exchanger coils. A root mean square error (RMSE) of 3.5% was calculated showing an adequate prediction of the temperature using the determined heat transfer coefficients (Cleland and Earle, 1984).

Implementation of the known characteristics for the heat transfer in FLUENT allowed the simulation of the temperature during the heating and cooling processes.

The governing equations were adapted from FLUENT (2003) and viscosity, density heat capacity and thermal conductivity were considered to be dependent on temperature for the investigated temperature range where relevant changes in the above mentioned physical properties occur. The corresponding relations for milk and apple juice can be found in Bertsch (1983) and Constenla et al. (1989).

The temperature along the centre line of the heat exchanger coils was exported to Table Curve 2D and a regression was calculated for the description of the temperature–time profile of the heating coil and cooling coil. Regressions are shown in Eqs. (9) and (10) for the temperature increase and decrease (with R^2 = 0.979 and 0.998 respectively). Due to the larger temperature difference between cooling medium and product and the improved heat transfer, an exponential equation was found to better describe the temperature–time profile for the cooling coil. Temperature–time profiles are shown exemplarily in Fig. 3:

$$\vartheta(t) = \vartheta_0 + 12.05 \cdot t^{0.5}, \tag{9}$$

$$\vartheta(t) = \vartheta_0 \cdot e^{-0.3422 \cdot t}. \tag{10}$$

The temperature increase within the treatment chamber between the inlet (T_{inTC}) and outlet (T_{outTC}) temperature due to the dissipation of the electrical energy can be calculated based on the energy delivered per pulse (W_{pulse}) and the pulse frequency (f) considering the mass flow rate ($\dot{m}$) and the specific heat capacity (c_p) according to Eq. (11):

$$W_{pulse} \cdot f = \dot{m} \cdot c_p \cdot (T_{outTC} - T_{inTC}). \tag{11}$$

The temperature occurring at a certain time t after the liquid has entered the treatment chamber ($0 < t \leqslant t_{res}$ with t_{res} referring to the total residence time in the treatment chamber) can be calculated according to Eq. (12):

$$T_{outTC} = T_{inTC} + \frac{W_{pulse}}{\dot{m} \cdot c_p} \cdot f \cdot t_{res}. \tag{12}$$

Eqs. (12), (9), and (10) can be combined to calculate the temperature–time profile of the liquid during pre-heating, PEF treatment and cooling using MathCad 2001i Professional (MathSoft Engineering and Education Inc., Cambridge, USA). The temperature did not change significantly in the short pipe-sections that are connecting the different parts of the equipment so that a constant temperature is assumed. Temperature–time profiles for different treatment conditions are shown in Section 3.3.

2.8. Mathematical model for the calculation of the thermal inactivation and differentiation of PEF effects

Finally, the temperature–time profile was related to the thermal inactivation data aiming to correlate the occurring thermal load during the PEF process to a resulting thermal inactivation (Eq. (13)) based on the integration of the temperature dependent inactivation at distinct residence times of the product in different parts of the processing unit.

$$\mathrm{rel}A(t) = \exp\left(\int_0^t -k(T(t))dt\right). \tag{13}$$

The implementation of Eqs. (9), (10), and (12) for the calculation of the temperature–time profile and Eq. (5) for the thermal stability in MathCad allows together with Eq. (13) the calculation of the remaining residual activity after the corresponding heat exposure of the product during the PEF treatment. The total inactivation (including thermal and electric field effects) was determined experimentally by measuring the residual activity in the sample collected at the outlet of the cooling coil behind the PEF unit. The difference between this overall inactivation and the thermal-only inactivation was attributed to a PEF-only inactivation.

Experimental data on thermal inactivation and inactivation after PEF treatment represent the results of two independent trials and are given as mean values with the corresponding standard deviation. Other calculated data are based on these mean values of the experimental data.

3. Results and discussion

3.1. Thermal inactivation of ALP, LPO and E. coli

The thermal inactivation kinetic of milk alkaline phosphatase is shown in Fig. 1. A first order reaction kinetic was applied to model the heat inactivation of the enzymes and *E. coli*. An increase in enzyme inactivation and reduction of *E. coli* colony counts with increasing treatment time was found for each investigated temperature level and was fitted with Eq. (3).

The reaction rate constants $k(T)$ as well as D_ϑ-values obtained from the first order kinetic model using Table Curve 2D are shown in Table 3 for ALP, LPO and *E. coli*. The inactivation rate revealed a strong dependency on the treatment temperature since the reaction rate constants increased for the enzymes as well as for *E. coli* with increasing temperature. The calculated D_ϑ-values showed an increasing thermal sensitivity in the order *E. coli*, ALP and LPO.

D-values obtained for ALP range from 235 s at 60 °C to 5.4 s at 70 °C. Milk alkaline phosphatase is a heat-sensitive enzyme used as a temperature indicator during conventional pasteurization of

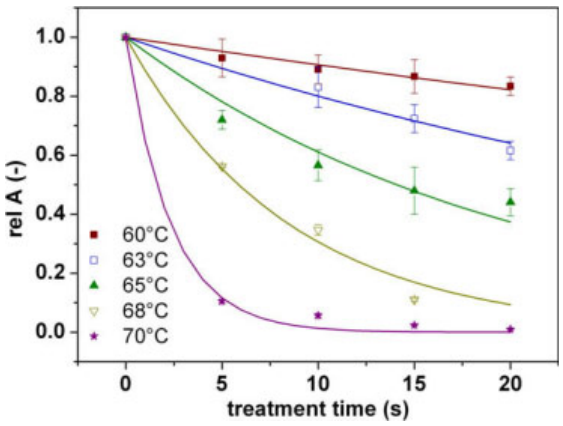

Fig. 1. Heat inactivation kinetics of milk alkaline phosphatase as determined by the capillary tube method for the temperature range of 60–70 °C and holding times of 0–20 s. Data points represent the experimental values, lines are the first order reaction kinetic fits $\mathrm{rel}A_{(T)}(t) = \exp\left[-k_{(T)} \cdot t\right]$.

Table 3
Reaction rate constants k (s^{-1}) and D-values for the thermal inactivation of ALP, LPO in milk and *E. coli* in apple juice. Holding times are 0–20 s for ALP, 0–16 s for LPO and 0–240 s for *E. coli*. First order kinetic models have been applied to fit the experimental data. The coefficient of determination R^2 is given in brackets.

Temperature ϑ (°C)	Reaction rate constant k (s^{-1}) and D_ϑ-value (s); $\mathrm{rel}A_{(T)}(t) = \exp\lfloor -k_{(T)} \cdot t \rfloor$					
	ALP		LPO		*E. coli*	
	k (s^{-1})	D_ϑ (s)	k (s^{-1})	D_ϑ (s)	k (s^{-1})	D_ϑ (s)
50	–		–		0.0310 (0.999)	74.3
55	–		–		0.1030 (0.686)	22.4
60	0.0098 (0.945)	235.0	0.0036 (0.930)	639.7	0.4538 (0.790)	5.1
63	0.0222 (0.980)	103.7	–			
65	0.0492 (0.950)	46.8	–			
68	0.1183 (0.988)	19.5	–			
70	0.4301 (0.996)	5.4	0.0199 (0.998)	115.7		
75	–		0.0511 (0.974)	45.1		
80	–		0.1068 (0.940)	21.6		
85	–		0.2765 (0.963)	8.3		

Table 4
Correlation between the reaction rate constants k (s^{-1}) and the treatment temperature T (in K) for the thermal inactivation of ALP, LPO and *E. coli*. Arrhenius equation was applied to fit inactivation rate constants obtained for different temperatures. The coefficient of determination R^2 is given for each fit. E_a is the energy of activation, k_0 the velocity constant in s^{-1} for $1/T = 0$ and R the universal gas constant (8.314 $Jmol^{-1}K^{-1}$).

	Temperature dependence of the reaction rate constant k (s^{-1}) $\ln k(T) = -\frac{E_a}{R} \cdot \frac{1}{T} + \ln k_0$		
	ALP	LPO	*E. coli*
E_a (kJ/mol)	347.88	171.09	240.06
$\ln k_0$	120.80	56.11	85.83
R^2	0.9748	0.9987	0.9952

raw milk (Wilbey, 1996). LPO is more heat resistant and a lack of LPO in raw milk indicates heat treatments above 80 °C (Griffiths, 1986). A 50% reduction of LPO activity in milk is achieved after a thermal treatment of 14 s at 75 °C in the present case. de Wit and van Hooydonk (1996) reported a treatment time of 30 s necessary to achieve a 50% reduction in LPO activity at 75 °C. Their values were obtained in raw milk with the addition of 10 mM Ca^{2+}. Calcium stabilizes the molecular confirmation of LPO and higher calcium concentrations in milk may result in an increased heat stability of the enzyme (Kussendrager and van Hooijdonk, 2000).

For *E. coli*, a log decimal reduction time of 22 s at 55 °C was found to be lower than the value of 57 s which has been reported by Gabriel and Nakano (2009) for *E. coli K-12* in clear apple juice at the same temperature.

The correlation between the heat inactivation rate and the temperature was described based on the Arrhenius equation. Table 4 gives the kinetic data resulting from the Arrhenius plot (Fig. 2) for *E. coli*, ALP and LPO. The highest energy of activation with 348 kJ/mol was obtained for ALP indicating a fast increase in inactivation with increasing temperature in comparison to *E. coli* and LPO. The highest temperature stability in terms of a low dependency of the level of inactivation resulting from an increase in temperature is indicated by the low energy of activation of 171 kJ/mol found for LPO. Incorporation of the temperature dependent reaction rate constant $k(T)$ in the first order kinetic model for the inactivation of ALP, LPO and *E. coli* leads to the relation between the residual relative activity, the treatment temperature and the treatment time as shown by Eq. (5).

3.2. Temperature–time profile of the product during PEF treatment and related thermal inactivation

Besides the electric field exposure of the food during PEF treatment, the impact of the temperature during the different processing steps has to be taken into account when evaluating the process effect. These steps include (according to Fig. 3) the pre-heating (A),

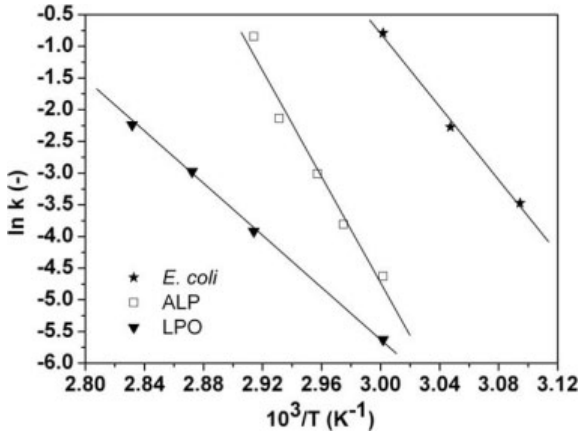

Fig. 2. Arrhenius plot showing the heat inactivation rate constant (k) for ALP and LPO in milk and *E. coli* in apple juice depending on treatment temperature. Lines represent the fit obtained by the Arrhenius model.

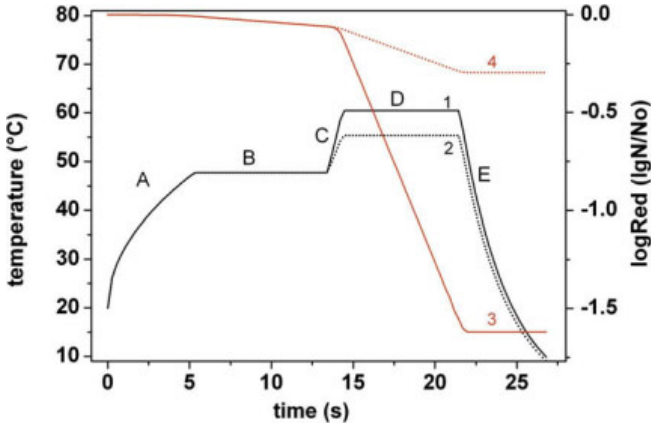

Fig. 3. Calculated temperature–time profile (black lines 1 and 2) of the PEF treatment of apple juice with elevated inlet temperature of 50 °C performed at a total specific energy input of 24 kJ/kg (line 2) and 55 kJ/kg (line 1). Red lines (3 and 4) illustrate the calculated corresponding thermal inactivation of *E. coli* (line 4 for PEF treatment at 24 kJ/kg and line 3 for 55 kJ/kg) in the different sections of the PEF unit. (A) Pre-heating 5.3 s; (B) holding 8.1 s; (C) PEF (heating effect) 0.9 s; (D) holding 7.2 s; (E) cooling 5.3 s. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the ohmic heating during PEF treatment (C), the cooling (E) as well as the holding times occurring in the pipe-sections (B and D) that are connecting the different sections.

The temperature–time profile can be obtained by temperature measurement at different locations in the processing line, or it can be calculated for a more general approach as described in Section 2.7. The combination of the numerical simulation of the temperature increase and decrease in the heat exchangers (Eqs. (9) and (10)) and the calculation of the ohmic-heating effect in the treatment chamber based on Eq. (12) enables the determination of the temperature along the product flow with corresponding holding times.

Since a synergetic effect between temperature and pulsed electric field induced electroporation exists, PEF treatment at elevated temperatures was suggested by various authors in order to reduce the electrical energy consumption and to allow heat recovery to increase the process efficiency and to increase the microbial inactivation results (Heinz et al., 2003; Sampedro et al., 2006; Toepfl et al., 2007).

The application of an elevated inlet temperature of 50 °C and a moderate pulsed electric field treatment in terms of the required total specific energy input of up to 55 kJ/kg lead to a 4.4 log reduction of *E. coli* in apple juice (see Table 2 and Fig. 4 for PEF treatment parameters and inactivation results). A calculated corresponding temperature–time profile of this particular PEF treatment is shown in Fig. 3. Verification was performed by experimental measurement of the temperature at particular locations in the processing line (inlet and outlet of the treatment chamber as well as outlet of the cooling coil). The corresponding temperature data are also shown in Table 2.

The given residence times for the different sections are based on a flow rate of 5 l/h and on the corresponding length of the sections (see Section 2.7).

According to Eq. (12) the temperature increase during the PEF treatment due to the dissipation of electrical energy depends on the total specific energy input (which depends on pulse energy, frequency and mass flow rate in turn). The two different cases shown in Fig. 3 represent two different total specific energy inputs of 24 and 55 kJ/kg resulting in an outlet temperature after the PEF treatment of 56 and 62 °C, respectively (see also Table 2). Based on this temperature–time profile, the thermal inactivation of *E. coli* was calculated using Eq. (13) and the contribution of the different temperature levels and exposure times in the different sections of the PEF processing unit is shown in Fig. 3 (lines 3 and 4). The pre-heating to 50 °C and the subsequent holding time in the pipe section resulted in a minor inactivation of *E. coli* of less than 0.1 log cycles. The ohmic heating during the PEF treatment to 56 and 62 °C depending on the energy input also led to a low thermal inactivation of less than 0.1 log cycles in the PEF treatment chamber itself, since the linear temperature increase took place in a short time interval of only 0.9 s. The highest thermal inactivation occurred in the pipe section (D) which connects the treatment chamber to the cooling coil. The holding time 7.2 s for the distance of 352 mm at 56 or 62 °C resulted in a thermal inactivation of *E. coli* of 0.2 log cycles and 1.5 log cycles, respectively. The rapid cooling in the heat exchanger coil was able to prevent additional thermal inactivation. The overall thermal inactivation of *E. coli* in apple juice resulting from the processing which included the pre-heating to 50 °C and the application of a total specific energy input of 24 or 55 kJ/kg was 0.3 log cycles and 1.6 log cycles, respectively.

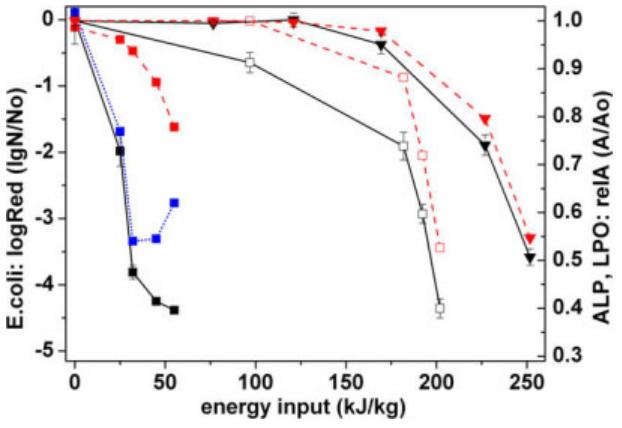

Fig. 4. Inactivation of ALP (□), LPO (▼) and *E. coli* (■) with the differentiation of total inactivation after PEF-thermal-treatment (black solid lines), thermal inactivation based on the temperature–time-profile of the corresponding PEF treatment (red dashed lines) and the calculated remaining electric field effect for *E. coli* (blue dotted line). The treatment conditions as well as the resulting maximum processing temperatures are given in Table 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Overall PEF inactivation, calculation of thermal effects and identification of PEF-only inactivation

3.3.1. Overall PEF inactivation

The overall inactivation of ALP, LPO and *E. coli* after PEF processing of milk and apple juice for PEF treatment times ranging from 7 to 34 μs and field strength levels of 34–38 kV/cm (for exact treatment parameters see Table 2) is shown in Fig. 4 (black solid lines). These are experimental data obtained by the determination of enzyme activity and colony counts in samples taken at the outlet of the PEF processing unit (outlet of the final cooling coil).

Increasing the treatment time (or the total specific energy input) leads to improved inactivation results in each case. Treatment of *E. coli* was performed in apple juice with an inlet temperature of 50 °C. An inactivation of 4.4 log cycles was achieved with a treatment time of 15.8 μs at 35 kV/cm and a total specific energy input of 55 kJ/kg resulting in a maximum process temperature of 62 °C. Similar inactivation results were obtained by Evrendilek et al. (2000) for *E. coli* in apple juice using a field strength of 34 kV/cm, a treatment time of 166 μs and a treatment temperature of 27 °C in an OSU-4A PEF unit with a series of 12 co-field treatment chambers with intermediate active cooling. Heinz et al. (2003) reported a 5.3 log reduction of *E. coli* in apple juice for a treatment at 36 kV/cm and a total specific energy input of 49 kJ/kg. In contrast to the previous results, an energy input of 5341 kJ/kg and a treatment time of 2000 μs were reported by Mosqueda-Melgar et al. (2008) to achieve a 4 log cycle reduction of *E. coli* in apple juice at a frequency of 100 Hz, a field strength of 35 kV/cm and a treatment temperature below 40 °C.

ALP and LPO inactivation by PEF was studied in raw milk. Treatments were performed at an inlet temperature of 20 °C and the treatment time and the corresponding total specific energy input was increased considering maximum processing temperatures of 67 °C for ALP and 85 °C for LPO (see Table 2).

For LPO, no inactivation was found up to an energy input of 121 kJ/kg and a treatment time of 18.9 μs at a field strength of 34 kV/cm and a maximum treatment temperature of 48 °C. Increasing the treatment intensity up to an energy input of 251 kJ/kg and a treatment time of 34.2 μs (resulting in a maximum temperature of 85 °C), inactivation of LPO finally increased leading to a residual activity of only 50% (see Fig. 4).

Alkaline phosphatase showed a reduction in activity of about 26% up to a specific energy input of 182 kJ/kg at 38 kV/cm (treatment time 21.2 μs, maximum temperature 60 °C) whereas a rapid decrease in activity to a residual level of 40% occurred when increasing the total specific energy input up to 202 kJ/kg (23 μs) with a maximum process temperature of 67 °C. Shamsi et al.

(2008) investigated the inactivation of ALP in skim milk using an OSU-4 laboratory scale PEF unit consisting of a series of 4 co-field treatment chambers. A residual activity of 33% was reported for a treatment at 35 kV/cm and 19.6 μs with a maximum temperature of 60 °C.

3.3.2. Thermal effects

The red dashed lines in Fig. 4 represent the thermal inactivation that was calculated based on the temperature–time profile for each PEF processing experiment. The temperature–time profile was dependent on the inlet temperature as well as on the specific energy input that was applied during the PEF treatment. Increasing the number of pulses results in an increase of the specific energy input which leads to a temperature increase in turn. As shown in Fig. 3 for *E. coli* (red lines 3 and 4), a thermal inactivation can be calculated considering the inactivation effect of the elevated inlet temperature as well as of the particular temperature increase due to the ohmic-heating effects.

For ALP an inlet temperature of 20 °C and a specific energy input of 97 kJ/kg leads to a maximum process temperature of 42 °C and does not result in any thermal inactivation. A further increase of the treatment time and the energy input leads to maximum temperatures of up to 67 °C and to a thermal inactivation effect of 47% when considering the relevant holding times in the different sections of the PEF unit.

For LPO at energy inputs up to 169 kJ/kg and a maximum processing temperature of 60 °C no inactivation was found, whereas at 227 kJ/kg and 75 °C a 20% inactivation occurred and increased up to 44% inactivation when the treatment intensity was increased to 251 kJ/kg with a final temperature of 85 °C.

Due to the higher inlet temperature during the processing of apple juice a higher maximum temperature was reached even at low specific energy inputs. The resulting maximum temperatures as well as the higher heat sensitivity of *E. coli* lead to a considerable part of thermal inactivation up to 1.6 log cycles.

3.3.3. Calculated and experimental PEF-only inactivation

The determined thermal inactivation effects revealed a significant contribution to the overall inactivation depending on the processing temperatures reached. Considering the pulsed electric field effect and the temperature effect to be the two main inactivation mechanisms during the treatment, it is possible to determine a PEF-only inactivation by calculating the difference between the overall inactivation (determined experimentally) and the thermal inactivation (calculated using the suggested model based on experimental heat inactivation data). The so obtained PEF-only inactivation is shown in Figs. 4 and 5 (blue dotted lines) for *E. coli* as well as ALP and LPO respectively.

For *E. coli* the inactivation was found to depend mainly on the pulsed electric field treatment time (up to 9.9 μs with a specific energy input of 34 kJ/kg) up to a maximum processing temperature of 58 °C where a 3.8 log reduction of *E. coli* was achieved in apple juice at 35 kV/cm.

These expected results are in accordance with inactivation kinetics studied by other authors who also found a correlation between microbial inactivation and treatment time when investigating pulsed electric field effects in a controlled temperature range avoiding thermal inactivation effects (Álvarez et al., 2003; Amiali et al., 2004). The effect of the initial treatment temperature on the PEF induced inactivation of *E. coli* in apple juice is also shown by Heinz et al. (2003) and Toepfl et al. (2007) who reported a decreasing PEF treatment intensity necessary to achieve similar levels of microbial inactivation at higher treatment temperature.

As soon as the PEF treatment intensity and the temperature increase due to ohmic-heating reaches a level at which thermal inactivation occurs, one can observe the superposition of two different inactivation mechanisms.

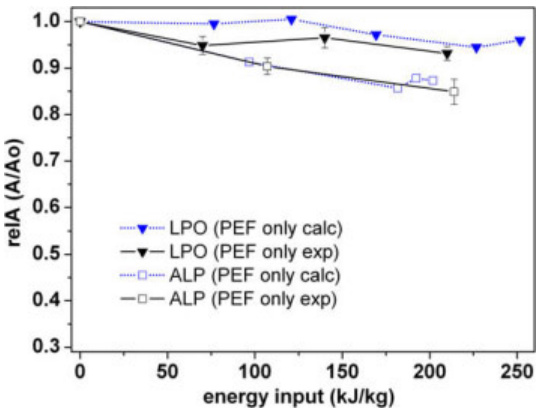

Fig. 5. PEF-only inactivation of ALP (□) and LPO (▼) determined by calculation (blue dotted lines; based on total and thermal inactivation illustrated in Fig. 4) and determined experimentally (black solid lines) based on multiple step treatments with intermediate cooling. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

When increasing the treatment time and the specific energy input, the pulsed electric field contribution to the overall inactivation is reduced (kink in the PEF-only inactivation curve in Fig. 4) and is further decreased as long as the maximum temperature due to the higher treatment intensity increases. Whereas at a treatment time of 9.9 μs (maximum temperature 58 °C, specific energy input 24 kJ/kg) almost 88% of the total inactivation are due to the pulsed electric field effect, the value decreases to 63% at a treatment time of 15.8 μs and a maximum temperature of 62 °C (specific energy input of 55 kJ/kg).

Based on the obtained data, it is possible to differentiate between the inactivation resulting from pulsed electric field and thermal effects during a PEF treatment at elevated temperatures. On the other hand, the model can be used to identify different inactivation mechanisms during a combined temperature–PEF treatment. Up to a certain specific energy input, the occurring microbial inactivation can be attributed to pulsed electric field effects namely electroporation. Exceeding a certain treatment intensity (in terms of treatment time and specific energy input) the contribution of thermal effects is constantly increasing. A synergetic effect of the temperature during the PEF inactivation would be detectable by an increase of the PEF-only inactivation at increased inlet temperature. This effect was not shown in the presented study since the experiments were performed at one elevated temperature level only and a further temperature increase occurred as a result of the electric field application.

The reduction in raw milk LPO activity caused by the pulsed electric field was found to be between 0.5% and 5.5% (electric field strength 34 kV/cm, specific energy input 0–250 kJ/kg and treatment time 0–34.2 μs) showing no correlation to the treatment time and specific energy input (blue dotted line for LPO in Fig. 5). A PEF treatment (34 kV/cm, 70 kJ/kg, 12.6 μs, see also Table 2 for treatment conditions) was repeated three times with intermediate cooling to 20 °C so that a final total specific energy input of 210 kJ/kg was achieved without exceeding a temperature of 38 °C (below thermal inactivation temperature of LPO). The experimental PEF-only inactivation results (Fig. 5, black solid line with triangle) do not show an activity loss higher than 7% and do not reveal any dependency from the treatment time. This is in good accordance with the calculated PEF-only inactivation results.

Inactivation of ALP due to pulsed electric field application (38 kV/cm, treatment time 12.6–18.9 μs, 97–202 kJ/kg) was in the range of 9–14% whereas a slight increase of the PEF inactiva-

tion was found when increasing the treatment time. A PEF treatment (38 kV/cm, 107 kJ/kg, 12.3 μs, see also Table 2 for treatment conditions) was repeated two times with intermediate cooling to 20 °C so that a final total specific energy input of 214 kJ/kg was achieved without exceeding a temperature of 44 °C (below thermal inactivation temperature of ALP). The experimental PEF-only inactivation results for ALP (Fig. 5, black solid line with square) show an activity loss of up to 15% which is in good accordance with the calculated PEF-only inactivation results.

The results discussed above are in accordance with results obtained by Van Loey et al. (2002) as well as Grahl and Märkl (1996) who studied the inactivation of LPO and ALP in batch systems and who found only minimal PEF effects as long as the maximum treatment temperature was below a thermal inactivation level. In contrast, the results of Castro et al. (2001) indicate a PEF based ALP inactivation that is directly related to the ALP concentration and the electric field intensity in terms of electric field strength and number of pulses. Riener et al. (2009) reported a 29% inactivation of ALP in raw milk after PEF treatment at 35 kV/cm and 75 μs in a continuous system but the authors did not find any inactivation of LPO.

This finding is similar to the results obtained in the presented study where ALP shows a higher PEF-only inactivation in comparison to LPO evaluated based on the measured maximum treatment temperature. Apart from electric field effects on ALP activity, the occurrence of temperature hot spots in the treatment chamber that are not considered by the calculated or experimental PEF-only inactivation results have an impact on overall enzyme inactivation especially when considering heat-sensitive enzymes such as ALP (Jaeger et al., 2009).

These controversial results show that numerous effects need to be considered when studying the PEF effect on enzymes. Especially during continuous treatment at high specific energy inputs and in case of inhomogeneous temperature distribution, thermal effects will have a relevant contribution to the overall inactivation which can not be related to pulsed electric field effects.

4. Conclusion and outlook

Depending on the pre-heating temperature and the PEF intensity in terms of the applied total specific energy input, a temperature–time profile can be created for each specific PEF processing experiment. This temperature–time profile and the information on the corresponding thermal inactivation effects can be used to optimize the PEF treatment since processing conditions that present a large contribution to the thermal inactivation of heat-sensitive compounds can be avoided. An optimal combination of the application of thermal energy as well as electrical energy can be determined to allow a maximum of microbial inactivation with a minimum of required energy and of degradation of heat-sensitive compounds. The differentiation of electric field and thermal effects is the basis for the evaluation of inactivation mechanisms occurring during a thermal assisted PEF treatment. Inactivation results obtained for *E. coli* showed that thermal effects will have a dominant inactivation effect as soon as the temperature increase due to ohmic-heating exceeds a lethal level at high total specific energy input.

Although a 50% reduction of LPO activity was found after PEF treatment, the differentiation of occurring effects by the presented model revealed a calculated PEF-only inactivation of 5–7%. This was confirmed experimentally by a multiple step treatment with intermediate cooling which proved to be a suitable way to achieve high specific energy inputs at low maximum temperature when performing the treatment in continuous operation.

The overall inactivation results of ALP illustrated in Fig. 4 show a reduction in enzyme activity of up to 60% after the pulsed electric field treatment. A large part of this inactivation could be related to the overall thermal load to which the product was exposed during the PEF processing considering the temperature increase due to ohmic heating.

However, the model used to calculate the temperature–time profile is based on the estimation of a homogeneous temperature increase in the treatment chamber. This assumption has certain limitations as shown by Jaeger et al. (2009) who investigated the temperature distribution in a co-linear treatment chamber. A correlation between the occurrence of high local temperatures due to inhomogeneous field distribution, limited flow velocity and recirculation of the liquid and the resulting thermal inactivation of ALP due to these hot spots could be shown. These hot spots are not considered in the thermal inactivation model and the obtained results for the thermal-only inactivation are likely to be underestimated especially for *E. coli* and ALP since they are more heat-sensitive than LPO. The inactivation defined as PEF-only in this study still contains the thermal effect of these hot spots in the treatment chamber. Further experimental investigations as well as the implementation of available heat inactivation kinetic data into the numerical simulations of the temperature distribution within the treatment chamber itself are required for the exact determination of the contribution of these hot spots to the total inactivation and for the improvement of the presented model for the differentiation of thermal and electric field effects during PEF processing at elevated temperatures.

The presented model enables to quantify the thermal and pulsed electric field effects during the PEF processing. It allows the determination of synergetic temperature effects as well as lethal effects occurring above a certain temperature level and provides a valuable tool for the evaluation of involved inactivation mechanisms. Based on this information, it may be possible to develop a concept for the design of a thermal assisted PEF process with an optimal combination of thermal and electrical energy to allow an energy efficient and gentle inactivation process.

5. Industrial relevance

Pulsed electric field treatment is a promising non-thermal pasteurization technology for liquid food products aiming to inactivate microorganisms while maintaining heat-sensitive bioactive components. A combination of the PEF process with traditional lethal heat treatments or with the synergistic effect of temperature below the lethal heat inactivation level is a suitable way to improve the process effectiveness by reducing electrical energy consumption, using heat recovery and increasing microbial inactivation results. The differentiation of thermal and PEF effects has to be undertaken to limit negative effects on heat-sensitive food constituents and to clearly define the microbial inactivation pathways from a food safety point of view.

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IV

Adjustment of milling, mash electroporation and pressing for the development of a PEF assisted juice production in industrial scale

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Adjustment of milling, mash electroporation and pressing for the development of a PEF assisted juice production in industrial scale

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ABSTRACT

Pulsed electric field (PEF) assisted juice recovery from apple and carrot was performed. Different mash structures were subjected to PEF treatment at two different treatment intensity levels. Solid–liquid separation was performed using four different systems: belt press, rack-and-cloth press, hydraulic filter press and decanter. The combination of milling and PEF treatment provided a method for the independent control of particle size and cell disintegration. An increase of juice yield after PEF treatment was found for apple mash in the range of 0–11% and for carrot mash in the range of 8–31% depending on mash structure and de-juicing system. It was the aim of the study to investigate the interdependency of the aforementioned processing steps in order to develop adjustment concepts for the maximization of the juice yield increase by PEF and to evaluate the impact on related juice quality parameters.

Industrial relevance: Pulsed electric field (PEF) treatment of fruit and vegetable mashes and the resulting cell disintegration is a promising alternative to conventional enzymatic maceration or thermal disintegration to improve juice yields. PEF equipment is commercially available for industrial scale processing of fruit and vegetable mashes. Despite the uncomplicated technical implementation of the PEF system in the existing processing line, related processing steps such as milling and pressing need to be adjusted in order to maximize the beneficial PEF effect on the juice yield. The present study provides the basis for the required process analysis and optimization.

1. Introduction

Juice recovery processes from fruits and vegetables strongly depend on mass transfer phenomena. They can be enhanced by the disintegration of the raw material using traditional mechanical methods as well as a subsequent enzymatic and/or thermal treatment of the mash (Ashurst, 1995).

Pulsed electric fields (PEF) can be applied as an alternative method for cell disintegration. Biological tissues exposed to high electric field pulses develop pores in the cell membrane resulting in increased membrane permeability and a facilitated loss of the cell content (Knorr et al., 2001; Vorobiev & Lebovka, 2008). Concepts for electric field applications as an alternative for cell disintegration in fruit juice production have been reported more than 40 years ago (Flaumenbaum, 1968).

An increase in juice yield (Grimi et al., 2009; Schilling, Alber, Toepfl, et al., 2007), the reduction of processing times and energy requirements in comparison to enzymatic and thermal treatments of mashes (Toepfl et al., 2006), an enhanced release of secondary plant metabolites (Fincan, DeVito, & Dejmek, 2004; Lebovka, Praporscic, & Vorobiev, 2003) as well as the possibility to recover native pectins from apple pomace (Schilling, Toepfl, Ludwig, et al., 2008) can be considered as the beneficial attributes of cell disintegration by PEF.

Although the PEF treatment of fruit and vegetable mashes was shown to increase the juice yield and to improve the juice composition in laboratory scale, the application in industrial scale is still limited. Published results of one long term industrial scale application (10 t/h) of PEF in apple juice production showed a consistent increase of juice yield (Mueller et al., 2007). However, no systematic study is available to date taking into account the entire juice production process including complex interactions between mash structure, cell disintegration by PEF and the final solid–liquid separation performed with various de-juicing systems.

The press design and operation, the raw material properties such as degree of ripeness, the degree of milling, mash treatment and the number of juice drainage channels opened during pressing can be considered as the main impact factors affecting the juice yield (Al-Mashat & Zuritz, 1993).

Despite the experimental and theoretical studies on PEF cell disintegration more information is required regarding these aspects in order to apply bench-scale data on PEF assisted juice recovery to commercial size equipment. Therefore, the objectives of the present study are the following:

i) characterization of the mash structure and the mash particle size distribution,

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ii) investigation of the impact of mechanical grinding and PEF treatment on the degree of cell disintegration, and
iii) evaluation of the impact of the aforementioned aspects on juice yield and quality considering different solid–liquid separation systems.

This information will be essential for the integration of the PEF technology in industrial scale juice production since the adaptation of the conventional process parameters may be required in order to optimize the PEF assisted juice production.

2. Materials and methods

2.1. Raw material

Apples of the variety Jonagold were obtained after a 7 month post-harvest storage in ultra-low-oxygen-atmosphere from Obstland Dürrweitzschen AG (Grimma, Germany). Fresh commercial carrots (variety Bolero) were obtained from Keuthmann GmbH & Co. KG (Berlin, Germany).

Since raw material firmness is affecting its milling behavior and the resulting particle size characteristics, a texture analysis was performed for the measurement and documentation of characteristic values. Raw material firmness in turn is mainly affected by variety and degree of ripeness and storage time and conditions.

In order to provide relevant data for the raw material used in the study, a puncture test was applied for the characterization of apple tissue firmness using a TA-XT2 texture analyzer (Stable Micro Systems Ltd., Surrey, UK). A cylindrical probe (SMS P5, 5 mm diameter, 19.63 mm²) was inserted around 5 mm with a penetration speed of 1 mm/s (threshold value 0.1 N) into the peeled and smooth flesh vertically to the equatorial zone on two opposite sides of the apple. Yield point and slope to yield point were determined based on the force-deformation curve. The obtained force-deformation curves were in accordance with type B curves reported by Bourne (2002) for apples held in cold storage for several months and showed similar shape as examples given by Chen et al. (1996) for skinless apples. The apples used in the present study can be characterized by the following textural parameters: force at yield point 3.4 N (±0.4), deformation at yield point 1.2 mm (±0.2), and slope to yield point 2.8 N/mm. The average water content of the apples was 85.7 ± 1.0% (w/w). It was determined by drying a thin layer of 2 g of grinded apple at 120 °C using a Sartorius MA 35 Moisture Analyzer (Sartorius, Göttingen, Germany).

Carrot textural properties were determined by a Warner-Bratzler shear test (Llorca et al., 2001; Rastogi, Nguyen, & V.M.B., 2008). Force–time curves were recorded by cutting the carrot in radial direction at a point in the middle of its axial length (penetration speed 1 mm/s, threshold value 0.5 N) using a Warner-Bratzler blade. Relative carrot firmness was expressed by calculating the ratio between maximum shear force and diameter of the carrot at the point of cutting. 12 carrots have been measured. The average relative carrot firmness was found to be 2.2 N/mm (±0.2) with maximum shear force levels in the range of 44–79 N and carrot diameters between 22 mm and 37 mm. The average water content of the carrots was determined as described for apples and resulted in a value of 88.4 ± 3.2% (w/w).

2.2. Grinding

A centrifugal mill (Type Moloch, Hollmann, Wetzlar, Germany) with replaceable stainless steel screens with a hole size of 9 mm, 5 mm and 2 mm was used for grinding apples and carrots. In order to produce very fine carrot mash, a centrifugal mill (Type MCH20 K, Stephan Microcut, Stephan und Söhne GmbH & Co., Hameln, Germany) with a screen of 0.35 mm gap size was used. The degree of mechanical grinding and the resulting mash structure was chosen according to the requirements of the solid liquid separation system and controlled by using aforementioned screens with different hole size. Mash types used depending on the different solid–liquid separation systems are indicated in Table 1.

2.3. Mash particle size distribution

The particle size distribution of the mash after grinding was determined by sieve analysis using a vibratory sieve shaker (Fritsch GmbH, Idar-Oberstein, Germany) at amplitude level 6. A column of 17 test sieves with apertures in the range of 6.3–0.15 mm was used and a sample of 200 g of mash was poured into the top sieve. Wet sieving was performed for 3 min using a water feed of 1.7 L/min distributed by a sprinkler on the top sieve. In order to remove retained water, sieving was performed for an additional 3 min without water addition. Sieves have been weighed afterwards and the net weight of each sieve in wet condition was subtracted in order to determine the amount of mash captured on each sieve. The percentage of retained mash particles was calculated for each aperture by relating the weight of mash on a particular sieve to the cumulative total amount of mash. The cumulative particle weight fraction was then plotted against the aperture of the sieves.

2.4. Cell disintegration index

An impedance measurement and the calculation of a cell disintegration index was applied for the characterization of the degree of cell disruption by mechanical grinding as well as by pulsed electric field treatment according to Angersbach, Heinz, and Knorr (1999). The impedance analyzer (Biotronix, Hennigsdorf, Germany) was working in the frequency range of 10^3–10^7 Hz. The measuring cell consisted of two cylindrical stainless steel electrodes (diameter 10 mm) separated to a distance of 10 mm by a polyethylene tube containing a cylinder of intact apple or carrot tissue or the apple and carrot mash respectively. A cell disintegration index between 0 (intact tissue) and 1 (complete cell rupture) was defined.

Table 1
Conditions and parameters used during PEF treatment of apple and carrot mash. Initial voltage 14.4 kV, electric field strength 3 kV/cm, pulse width 3 µs. Values in brackets refer to the high intensity PEF level.

	Belt press	Rack-and-cloth press	Filter press	Decanter
Apple mash				
Mass flow ($\dot{m}$ in kg/h)	250	250	250	50
Pulse energy [J]	2.7	3	3	3.1
Total specific energy input ($W_{specific}$ in kJ/kg)	2 (12)	2 (12)	2 (12)	4 (12)
Frequency (f in Hz)	51 (305)	47 (278)	47 (278)	16 (54)
Residence time (t_{res} in s)	0.61	0.61	0.61	3.05
Treatment time (t_{treat} in µs)	93 (559)	86 (509)	86 (509)	146 (494)
Type of mash	9 and 5	9 and 5	9 and 5	5
Carrot mash				
Mass flow ($\dot{m}$ in kg/h)	100	70	70	50
Pulse energy [J]	3.9	3.9	3.9	3.9
Total specific energy input ($W_{specific}$ in kJ/kg)	2 (12)	3 (12)	3 (12)	4 (12)
Frequency (f in Hz)	16 (85)	16 (60)	16 (60)	16(43)
Residence time (t_{res} in s)	1.53	2.18	2.18	3.05
Treatment time (t_{treat} in µs)	73 (389)	105 (392)	105 (392)	146 (394)
Type of mash	5 and 2	2 and 0.35	2 and 0.35	2 and 0.35

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2.5. PEF treatment

PEF treatment of apple and carrot mash was performed using a 7 kW pulse modulator (ScandiNova Systems AB, Uppsala, Sweden). Rectangular pulses with a pulse width of 3 μs were used in a continuous co-linear type treatment chamber at a flow rate of 50–250 kg/h provided by a screw pump. The electrical conductivity of the apple mash was 1.3 mS/cm, the carrot mash had an electrical conductivity of 2.3 mS/cm.

The treatment chamber consisted of one central high voltage electrode and two outer grounded electrodes (all stainless steel, inner diameter 34 mm) separated by a distance of 30 mm using two polyoxymethylene insulators with an inner diameter of 30 mm. This geometry provides two treatment zones with a total enclosed volume of 42.4 cm^3 exposed to the electric field. A numerical simulation of the electric field strength distribution in the treatment zone was performed. The average electric field strength within the treatment zone was calculated and a multiplication factor of 0.21 cm^{-1} was determined for the given geometry to convert the initial voltage (14.4 kV) into the occurring average electric field strength (3 kV/cm). As a result of the treatment chamber geometry and the inhomogeneous electric field distribution, a local minimum electric field strength of 2.1 kV/cm and a local maximum value of 7 kV/cm occurred. Based on the calculated electric field strength distribution within the entire treatment zone, a standard deviation of 0.5 kV/cm from the calculated average value of 3 kV/cm was obtained. Hence, electric field strength levels above the threshold for the permeabilization of apple and carrot tissue in the range of 0.4–0.8 kV/cm (Angersbach, Heinz, & Knorr, 2000) were reached in the entire treatment zone. A detailed description of the underlying numerical simulation procedure can be found in Meneses et al. (2011).

The total specific energy input $W_{specific}$ [kJ/kg] was chosen as a parameter to describe the treatment intensity whereas other parameters such as the pulse frequency or the treatment time were adjusted accordingly considering different electrical properties of the mash as well as different flow rates based on the requirements of the de-juicing system. The total specific energy input was calculated according to Eq. (1) where f [s^{-1}] is the pulse frequency, $\dot{m}$ [kg/s] the mass flow rate of the mash, U [V] the electrical voltage, I [A] the electrical current in the treatment chamber and τ [s] the pulse width. Values for voltage and current were obtained and integrated by a TDS 430 oscilloscope (Tektronix Inc. Beaverton, USA).

$$W_{specific} = \frac{f}{\dot{m}} \cdot \int_0^{\tau} U(t) \cdot I(t) \cdot dt \quad (1)$$

A low treatment intensity level with a total specific energy input of 2–4 kJ/kg and a high treatment intensity level with 12 kJ/kg have been defined for the trials.

The treatment time t_{treat} [μs] was calculated by multiplying the total residence time t_{res} [s] of the product in the treatment zone with the pulse frequency and the pulse width τ [μs].

The parameters for PEF treatment for the different trials are summarized in Table 1. The parameters were chosen in order to achieve the different levels of cell disintegration in the tissue. However, no optimization of the PEF parameters was performed in the present study. Further work is required in order to define the optimum treatment parameter combination suitable to reach a desired cell disintegration level or juice yield increase at lowest treatment intensity possible.

2.6. Solid–liquid separation

Four different systems were used for the solid–liquid separation. Continuous de-juicing was possible by a belt press and a decanter. In this case, the continuous PEF treatment was coupled directly with the solid liquid separation process. A hydraulic filter press and a rack-and-cloth press were used in batch mode. In this case, continuous PEF treatment of the mash was performed before loading of the press. Apart from the mode of operation (continuous or batch) the different systems have been chosen due to their different working principle.

2.6.1. Belt press

A single belt press type EBP 500 (voran Maschinen GmbH, Pichl, Austria) was used for de-juicing of apple and carrot mash. The tension of the belt (width 500 mm, length 6530 mm) was controlled pneumatically using a pressure of 2 bar. The belt velocity was set at 1.2 m/min (level 1) resulting in a mash contact time of 3 min. The belt press was fed with 250 kg mash per hour resulting in a central belt area of 25% that was covered with mash.

2.6.2. Rack-and-cloth press

The rack-and-cloth press type Triumph 1 (Hollmann, Wetzlar, Germany) was used for pressing batches of 20 kg of apple and carrot mash. The mash was stacked in 5 layers (600×600×20 mm) using the packer frame and press cloths. Metal plates were placed between the layers. Pressure was applied to the stack by means of a hydraulic ram. One pressing cycle consisted of 4 pressing periods of 2 min each at pressure levels of 1.5, 3.0, 4.5 and 7.5 bar (hydraulic pressure of 20, 40, 60 and 100 bar). Total pressing time was 8 min.

2.6.3. Hydraulic filter press

A HP14 hydraulic filter press (Bucher Unipektin, Niederweningen, Switzerland) with press chamber volume of 14 L and two drainage elements was used for de-juicing apple and carrot mash. After filling the press with 10 kg of mash, one pressing cycle was performed. Subsequently, the pressing piston was moved backwards and intensive loosening of the mash was done manually before running a second pressing cycle. Afterwards, another loosening step was performed and a third pressing cycle was applied. The three pressing cycles were performed at a pressure of 5.5 bar and a holding time of 2 min each resulting in a total pressing time of 6 min.

2.6.4. Decanter

A decanter type CA 150-01-33 (Westfalia Separator AG, Oelde, Germany) was used for continuous solid–liquid separation of the mash. The drum diameter was 150 mm, the rotation speed was set to 5500 rpm working at a differential speed of 5 rpm. The regulating disk diameter was set to 120 mm (level 9, low position). Decanter feed was performed with 50 kg/h for best de-juicing conditions. Due to the limitations of the pulse modulator and a possible minimum pulse frequency of 16 Hz, a total specific energy input of 4 kJ/kg resulted for the low treatment intensity level as indicated in Table 1. Residence time of the mash in the decanter was less than 2 min.

2.7. Collection of juice and yield calculation

The amount of mash, pomace and juice was weighed using a platform scale (DE 150 K 50 NL, Kern und Sohn GmbH, Balingen-Frommern, Germany). The gross juice yield Y [% (w/w)] was defined as amount of juice [kg] obtained per 100 kg of mash. In order to consider a varying content of total suspended solids TSS [% (w/w)] a corrected juice yield $Y_{TSScorr}$ [% (w/w)] was calculated according to Eq. (2). It allows comparing the juice yield of different pretreatments and de-juicing systems independent from the content of suspended particles.

$$Y_{TSS_{corr}} = Y \times \left(1 - \frac{TSS}{100}\right) \quad (2)$$

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Since the content of total dissolved solids *TDS* [°Brix] of the resulting juice was depending on the mash structure, the pre-treatment as well as the de-juicing system, a net juice yield was calculated in relation to the lowest content of total dissolved solids TDS_{ref} [°Brix] of 12.8°Brix for apple juice and 7.8°Brix for carrot juice according to Eq. (3).

$$Y_{TSS_{corr}}^{TDS_{ref}} = Y_{TSS_{corr}} \times \frac{TDS}{TDS_{ref}} \quad (3)$$

For apple juice, an amount of 200 mg of ascorbic acid (Merck KgaA, Darmstadt, Germany) was added per liter of juice immediately after juice recovery. Juice samples were collected and stored at −20 °C for further analysis.

Each juice winning experiment was performed in duplicate. Hence, mean values and given standard deviation of the juice yield are calculated based on values from 2 independent de-juicing trials (same de-juicing system, mash type, PEF intensity).

2.8. Juice quality analysis

Samples were collected from each de-juicing trial and each sample was analyzed in duplicate. The standard deviation of an analytical value from a sample analyzed by repeated measurements is given in Section 2.8. Mean values and standard deviation for the analytical parameters (TSS, TDS, TP, carotenoids) given in Section 3.4 are calculated based on the final values obtained for each of the repeated juice winning experiments.

2.8.1. Total suspended solids (TSS)

The content of total suspended solids results from the transfer of cellular matter from the mash into the juice and was determined by centrifugation of approximately 6 g of juice at 15,000×*g* (Sorvall RC -5B, DuPont Instruments, Wilmington, USA) for 60 min. The liquid phase was decanted and the centrifuge tube with the solid pellet was turned upside down to allow remaining liquid to drain for 5 min. The weight of the pellet (suspended solids) was related to the weight of the juice sample and expressed as TSS content [% (w/w)]. By using this procedure, the TSS content of a sample could be determined by repeated measurements with a standard deviation of ±0.11% (apple juice) and ±0.09% (carrot juice).

2.8.2. Total dissolved solids (TDS)

The content of total dissolved solids mainly reflects the content of sugars and organic acids and was determined at 20 °C using a digital refractometer (RFM 80, Bellingham & Stanley Ltd., Kent, UK) and expressed in °Brix. Juice samples were centrifuged at 2665×*g* for 20 min (Megafuge 1.0R, Heraeus, Hanau, Germany) in order to remove particles before the measurement. By using this procedure, the TDS content of a sample could be determined by repeated measurements with a standard deviation of ±0.03°Brix (apple juice) and ±0.02°Brix (carrot juice).

2.8.3. Total polyphenols

In apple juice, the content of total polyphenols (TP) was determined according to the Folin–Ciocalteu method (Singleton & Rossi, 1965). Juice samples were centrifuged at 2665×*g* for 20 min (Megafuge 1.0R, Heraeus, Hanau, Germany). In order to remove remaining ascorbic acid, an ascorbate oxidase spatula (Roche Diagnostics GmbH, Penzberg, Germany) was dipped into 4.5 mL of juice and left for 6 min with intermediate stirring. The juice sample was diluted (1:10 v/v with water) and 0.2 mL of the dilution were added to 1 mL of freshly prepared Folin–Ciocalteu Reagent (Sigma-Aldrich, St. Louis, USA) (1:10 v/v with water). The mixture was allowed to equilibrate for 5 min and then mixed with 0.8 mL of 75 g/L sodium carbonate solution. After incubation at 40 °C for 30 min, the absorbance of the mixture was read at 760 nm (Uvikon 922, Kontron Instruments AG, Schlieren, Germany) against a blank using water instead of the juice sample in the assay. The TP content (c) was expressed as mg of gallic acid equivalents (GAE) per L of sample based on a calibration curve obtained with gallic acid. In order to compare the release of TP from the mash independent from any dilution effect occurring due to increased juice yields, the TP content was referred to a reference apple juice of 12.8°Brix (TDS_{ref}) and a juice yield of 75% (w/w) giving a corrected TP content (c_{corr}) according to Eq. (4).

$$C_{corr} = c \times \frac{Y_{TSS_{corr}}^{TDS_{ref}}}{75} \quad (4)$$

By using this procedure, the total polyphenol content of a sample could be determined by repeated measurements with a standard deviation of ±4.65 mg/L.

2.8.4. Carotenoids

In carrot juice, carotenoids were determined according to Fish, Perkins-Veazie, and Collins (2002) and Davis, Fish, and Perkins-Veazie (2003). 2 mL of carrot juice was mixed with 5 mL of BHT (2,6-Di-tert-butyl-4-methylphenol) solution (Sigma-Aldrich, Steinheim, Germany) (0.05% w/v in Acetone), 5 mL ethanol and 10 mL n-hexane. Samples were shaken on ice in the dark for 15 min. 3 mL of distilled water was added and shaking was performed for another 5 min. Sample tubes were left for 5 min in order to allow phase separation. An aliquot of the upper hexane phase was removed and absorbance was measured at 453 nm (Uvikon 922, Kontron Instruments AG, Schlieren, Germany). Hexane was used as blank. The carotenoid content was expressed as mg ß-carotene per L of juice and calculated based on Beer–Lambert law with a molar extinction coefficient of 139,000 L mol^{-1} cm^{-1} and a molecular weight of 536.88 g/mol for ß-carotene. The cuvette thickness was 1 cm. In order to compare the release of carotenoids from the mash independent from any dilution effect occurring due to increased juice yields, the total carotenoid content was referred to a reference carrot juice of 7.8°Brix (TDS_{ref}) and a juice yield of 75% (w/w) according to Eq. (4). By using this procedure, the carotenoid content of a sample could be determined by repeated measurements with a standard deviation of ±0.49 mg/L.

3. Results and discussion

3.1. Particle size distribution in apple and carrot mash

The size distribution of the particles in apple and carrot mash was determined by sieve analysis and is shown in Fig. 1. By increasing the level of mechanical size reduction using different configurations of the centrifugal mills, the particle size spectra are shifted to the left towards smaller particles. However, no major difference was found between the size distribution of apple and carrot mash for particles obtained from milling with the same adjustment of the centrifugal mill (2 mm and 5 mm screen) indicating that the grinding system is unsusceptible against the different raw materials. Defined mash particle size characteristics were obtained and can be used as basis for the subsequent processing steps.

Data in literature on the particle size distribution of fruit and vegetable mashes are limited. Schobinger (2001) presents a particle size spectrum of *apple mash* obtained after grinding with a centrifugal mill (Bucher Unipektin, Niederweningen, Switzerland). The centrifugal mill is widely used in industry especially for grinding of apples resulting in an ideal mash structure for de-juicing with different press types. When comparing the particle weight fraction distribution obtained from this system with the mash particle size spectra obtained from the centrifugal mills used in the present study, the mash type 2 and type 5 are found to be the most similar. For the

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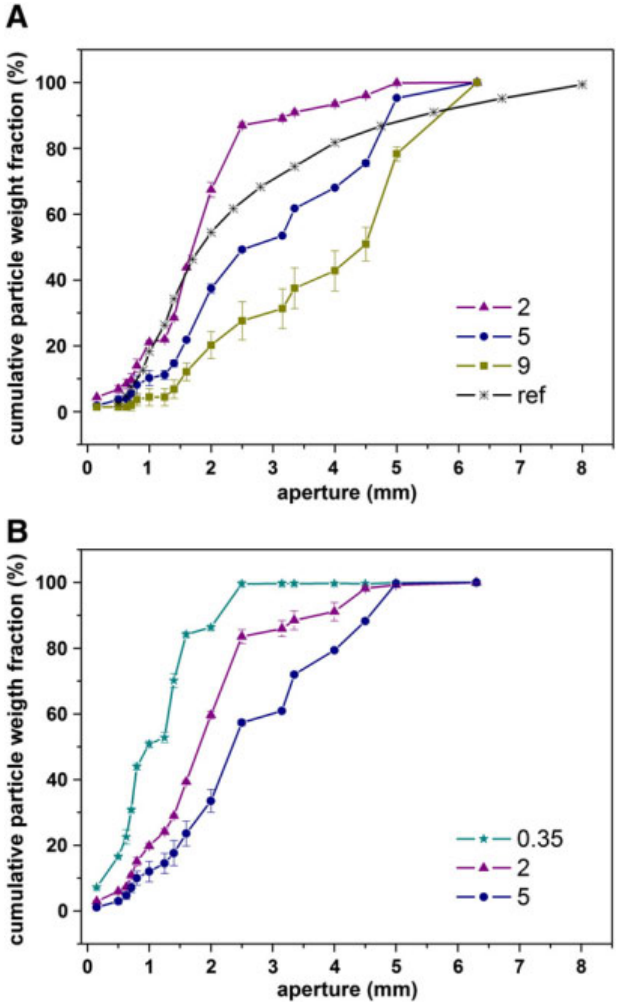

Fig. 1. Particle size distribution of apple mash (A) and carrot mash (B) resulting from grinding using different screens (hole size 0.35, 2, 5, 9 mm) as determined by sieve analysis. Values for apple mash redrawn from Schobinger (2001) are given as a reference (ref) for comparison.

system characterized by Schobinger (2001), fine particles <0.8 mm only occur to a small extent. The presence of two main particle size fractions between 1.25 mm and 2 mm and around 4 mm is found (values were obtained from the relative particle weight fraction plot, graph not shown) and characterized as favorable for optimal de-juicing conditions of the apple mash. However, the particle size needs to be adapted to the textural properties of the raw material. The softer the raw material is, the larger the particle size should be in order to allow optimal drainage in the mash during pressing. Due to the fact that apples were used after a 7 month post-harvest storage and for reasons discussed in the section Cell disintegration index, it was decided to use apple mash types with a particle size shifted towards larger particles (mash type 5 and type 9) in comparison to the distribution given by Schobinger (2001). For the mash type 5, two main particle size fractions around 2 mm and 5 mm occurred to almost the same extent whereas for mash type 9, the peak at a particle size of 2 mm was decreased and particles in the size range around 5 mm and above became more dominant (values were obtained from the relative particle weight fraction plot, graph not shown).

For *carrot mash*, grinding in industrial scale is mainly performed using a centrifugal mill or hammer mill followed by a fine grinding with a colloid mill or homogenizer system in case a decanter is in use for solid liquid separation (Reiter, Stuparic, Neidhart, et al., 2003). As already mentioned, the particle size distribution of the carrot mash type 2 and type 5 is very similar to the one obtained for apple mash produced with the same centrifugal mill configuration. The mash type 2 has a maximum relative particle weight fraction at 2.5 mm. For mash type 5, additional peaks also occur in the range of 3.35 mm to 5 mm. By using the grinding element type 0.35 for the centrifugal mill, it was possible to shift the particle size spectrum towards particles around and below 1.6 mm with dominant fractions occurring in the size range of 0.5 mm and 0.8 mm.

No data on the particle size distribution of carrot mash was available from literature for comparison. Ludwig et al. (2003) presents a visual characterization of carrot mash obtained from a hammer mill and a colloid mill. The particle size of carrot pomace suspensions after fine grinding is reported by Stoll et al. (2003). The largest particle fraction is found at a size of around 150 μm. It can be concluded, that the average size of the mash particles was higher since the measured pomace suspension was subjected to an additional grinding step. Hence, a relevant number of intact cells (carrot cell size of around 70 μm) will be present in the mash even after severe grinding of the carrots.

3.2. Cell disintegration index

The mechanical grinding is not only reducing the particle size in order to increase the surface–volume-ratio and to facilitate the juice release, it is also causing cell disintegration by mechanical destruction of cells at the cutting area. The cell disintegration in terms of cell membrane disruption was quantified by using an impedance measurement method.

A cell disintegration index of 0.86 was found for *apple mash* type 2 (fine grinding) indicating that the majority of cells (86%) was destroyed by grinding already (Fig. 2). Therefore, this mash type was not used for further processing since no additional cell disintegration could be achieved by applying the PEF treatment. Reducing the grinding intensity results in larger particles and therefore in a lower degree of mechanical cell disruption. For apple mash type 5 and mash type 9 a large proportion of intact cells remain in the particles and are susceptible to further cell disintegration by PEF treatment. The application of an electric field of 3 kV/cm and 2 kJ/kg results in an increase of the cell disintegration to final values of 74% for mash type 5 and 86% for mash type 9. The higher increase for mash type 9 might be due to the higher number of intact cells in the larger particles available for electroporation in the coarse mash. Increasing the total specific energy input to 12 kJ/kg leads to a maximum cell disintegration value of 86% and 89% for mash type 5 and type 9 respectively. Hence, these mash types have a similar level of cell disintegration after PEF treatment as mash type 2 after mechanical grinding. However, independent from the similar cell disintegration level, all mash types have kept the r particle size distribution as shown in Fig. 1. This is of high relevance since the mash structure resulting from the particle size distribution is directly linked to the performance characteristics of the solid–liquid separation systems and to the mash drainage properties, namely the extra-particular mass transfer. The cell disintegration itself in turn is linked to the juice release characteristics of each single mash particle, namely the intra-particle mass transfer. An independent control of both parameters, particle size by mechanical means and cell disintegration by PEF allows the optimization of the de-juicing step considering particular requirements of the de-juicing system as presented in the following section 'Juice yield'.

Considering the *carrot mash*, the mash type 2 shows a cell disintegration level of 37%. Although this mash has a similar particle size distribution like the apple mash type 2, the resulting cell disintegration is considerably different (86% for apple mash). This is due to the fact that the carrot cells are much smaller in size than apple cells. As analyzed by light microscopy, an average cell size of 70 μm was found for carrot whereas apple cells in the mesocarp had an average size of 200 μm. Similar values were reported by Zdunek et al. (2007)

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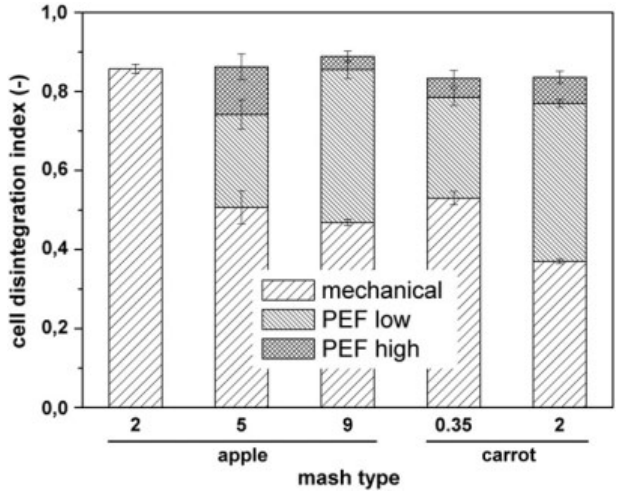

Fig. 2. Cell disintegration index of apple and carrot mash for the different mash types depending on the mechanical grinding intensity (indicated is the hole size of the screens used in the centrifugal mill) and depending on the PEF treatment intensity (low 2–4 kJ/kg, high 12 kJ/kg).

for carrot cells and by Bain and Robertsen (1951) and McAtee et al. (2009) for apple cells.

As a result of the difference in size of apple and carrot cells, the cell destruction in carrot mash particles of the same size as apple mash particles is lower since a larger number of smaller cells are enclosed and not affected by the grinding process at the cutting area. However, further reduction of the carrot mash particle size (mash type 0.35) increases the degree of mechanical cell disintegration to 53%. By applying PEF treatment additional cell disintegration can be achieved reaching values of up to 84%.

Impedance measurement was successfully used by various authors for the quantification of cell disintegration in fruit and vegetable tissue after electroporation. Bazhal, Lebovka, and Vorobiev (2003) reported a maximum disintegration index of around 0.9 for both, apple and carrot tissue, after PEF treatment at 1.5 kV/cm and treatment times in the range of milliseconds. A dependency on the electric field strength as well as on the treatment time (number of pulses) was identified.

However, data are limited regarding the level of cell disintegration in fruit or vegetable mashes depending on grinding intensity and particle size. Cell disintegration index values given for potato tissue by Angersbach et al. (1999) are 0.55 (coarsely ground potato, strips with cross section area of 2.5 × 0.6 mm) and 0.84 (fine-grained potato, particle size 0.3–0.7 mm). An estimation of potato cell size of around 150 μm can be obtained from Konstankiewicz and Zdunek (2001).

Based on the characterization of the mashes by determining the particle size distribution and the resulting degree of cell disintegration and in accordance with the mash structure requirements of the solid–liquid separation systems, the following apple mash types were selected for de-juicing: mash type 5 for the decanter since large particles are unfavorable for efficient juice recovery by the decanter. In addition to mash type 5, mash type 9 was used for all other de-juicing systems since an improved performance for larger particles can be expected when reducing possible compaction of the press-cake with negative effects on the juice transport properties of the mash and on the juice yield. Mash type 2 was discarded since maximum cell disintegration was reached by mechanical means already. For the carrot mash, mash type 0.35 and type 2 were found to be suitable for all solid–liquid separation systems except for the belt press, where an increased transfer of solid particles through the belt was observed. In this case, mash type 5 was used additionally.

The impact of mechanical grinding on particle size and cell disintegration as well as the impact of PEF treatment on cell disintegration was discussed above. In addition to that, it has to be mentioned that the PEF treatment of the mash is affecting its rheological properties. The loss of turgor pressure of the intact cells of the mash particles after electroporation causes a softening of these particles (Lebovka, Praporscic, & Vorobiev, 2004a,b). In addition, the facilitated juice release will result in a slightly higher percentage of liquid fraction in the mash before pressing. These effects have to be taken into account for the evaluation of the performance of the solid–liquid separation systems and the interpretation of the juice yield results.

The PEF application and the resulting cell disintegration by electroporation was investigated with regard to its potential to increase the juice yield and to act as an alternative to conventional mechanical cell disintegration by particle size reduction. It was the aim of the experimental setup to compare the de-juicing properties of untreated and PEF treated mash considering the mechanical disintegration and the resulting decrease in particle size as well as the PEF disintegration with maintained particle size. This may provide a valuable tool for a tailor-made mash structure design by combining particle size reduction and cell disintegration independent from each other. It could be used to improve the performance of the different de-juicing systems by de-linking the intra- and extra-particular mass transfer properties of the mash.

3.3. Juice yield

3.3.1. Apple juice

The net juice yield for apple juice is shown in Fig. 3 independent from the amount of total suspended solids (TSS) and in relation to a concentration of total dissolved solids (TDS) of 12.8°Brix. The impact of the mash pre-treatment on TSS and TDS will be discussed separately in section 'Juice quality parameters'. An increase of net juice yield after mash electroporation can be observed for all de-juicing systems and mash types except for fine mash (mash type 5) processed with the filter press under the applied conditions. The increase of juice yield after PEF treatment was more pronounced for coarse mash (mash type 9).

Solid–liquid separation by belt press resulted in an increase of juice yield depending on the PEF treatment intensity and the corresponding degree of cell disintegration.

The same applies for the rack-and-cloth press where a higher initial juice yield was achieved already due to a longer effective pressing time and a higher and more homogeneous pressure in comparison to the belt press. The juice yield was again found to be lower for the coarse mash indicating that the juice release from larger particles was hindered. In contrast to juice yield results obtained with the belt press, a further increase of the PEF treatment intensity did not result in an increase of juice yield for fine mash. The level of cell disintegration achieved with the low PEF treatment intensity seems to result in a maximum improvement of juice release already. In addition, the high treatment intensity may have probably caused softening of the mash particles to an extent that unfavorable de-juicing conditions such as compaction and closing of the capillaries in the mash were resulting. For the filter press, no increase of juice yield after PEF treatment was found for the fine mash under the given processing conditions. However, for the coarse mash, PEF treatment at both intensity levels was able to increase the juice yield. Using the decanter for the solid–liquid separation, an increase of juice yield was obtained for the PEF pre-treatment at the low intensity level. However, the higher PEF treatment intensity of 12 kJ/kg did not cause a further yield increase. This indicates that the cell disintegration level achieved with the low PEF treatment intensity resulted in the most favorable de-juicing properties of the mash for the subsequent processing by the decanter.

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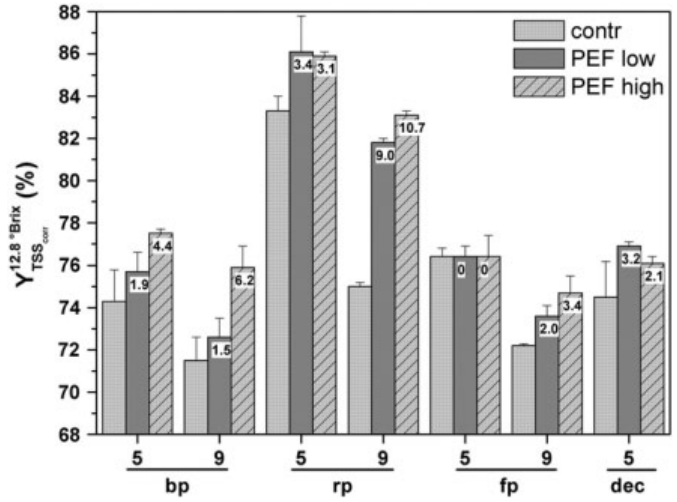

Fig. 3. Net juice yield for apple juice. A fine (mash type 5) and a coarse (mash type 9) mash were processed with the belt press (bp), rack-and-cloth press (rp), filter press (fp) and decanter after pre-treatment with PEF at 2 kJ/kg (PEF low) and 12 kJ/kg (PEF high) or without pre-treatment (contr). The numbers indicated in the graph represent the increase of juice yield achieved by PEF pre-treatment given in per cent (%) increase in relation to the untreated control sample.

Processing of PEF treated mash with a belt press of the same type used in the present study was performed by Turk, Vorobiev, and Baron (2009). Treatment intensity was set at an electric field strength level of 1 kV/cm and a total specific energy input of 57 kJ/kg (3 kV/cm and 2 kJ/kg or 12 kJ/kg in the present study) and two mash types (fine and coarse) have been processed. Two different fractions of juice were collected from the belt press, one at an early stage of pressing below the first roller and a second fraction was obtained from the three subsequent rollers.

For the first roller section, a juice gross yield of around 62% was obtained for the coarse mash and around 65.5% for the fine mash. PEF treatment of the coarse mash increased the juice yield to 64.5% whereas a not significant decrease to 63% was observed for the fine mash. However, during the second pressing stage (three roller section) gross yields of around 7.4% for the control samples were increased to 9% and 10.2% for coarse and fine mash respectively. In this case, for the fine mash, the increase of juice yield was more pronounced for the second pressing stage which is in contrast to the findings of the present study that will be discussed in the section Kinetics of juice recovery. However, no detailed information on mash structure and raw material properties is available in order to allow a direct comparison. With regard to the overall juice yield, as also observed in the present study, coarse mash showed a more pronounced increase after PEF treatment. A mash structure dependent difference in the effect of PEF on juice yield and quality indicating an increase of PEF efficiency for larger mash particles with a higher number of intact cells in the mash was also confirmed by Turk, Baron, and Vorobiev (2010) in laboratory scale.

Schilling et al. (2008) obtained gross yields of up to 84.9% in a hydraulic filter press after pressing apple mash for 90 min. No significant changes of juice yield were observed for enzymatic or PEF treatment. The filter press was considered as less appropriate under the given experimental processing conditions in order to transfer the cell disintegration achieved by PEF into a juice yield increase. It was concluded that there is a need for testing other solid–liquid separation systems.

Regarding the mash structure, it was stated by Janda (1985) that coarse mash has the advantageous properties of a low resistance of the press-cake to the juice drainage since a large cross-section of drainage channels will result due to a larger particle size. In addition, a lower degree of mechanical stress acting on the mash as well as the lower sum of cutting surfaces was suggested to result in lower pectin content in the juice with positive effect on juice viscosity and drainage properties. However, the presence of larger particles has two major disadvantages:

i) a lower degree of cell disintegration due to a higher number of intact cells in the particle and thus a low quantity of free running juice, and
ii) a longer intra-particle mass transfer distance for the juice in order to reach the surface of the particle (small surface–volume-ratio).

Therefore, any mash modification should be aimed at combining the advantages of coarse and fine mash while reducing the respective disadvantages.

The PEF treatment is reducing the mass transfer barrier of the cell membrane and facilitates the juice release from the particle. However, a second prerequisite for improving the overall juice yield is a favorable juice transfer through the mash which can be mainly achieved by a porous mash structure and thin layers of mash during the pressing.

De-juicing of the fine mash with the filter press did not show an increase in juice yield under the applied processing conditions. The filter press is designed as a cylinder–piston system with flexible drainage elements in between. The press cycle consists of several pressing steps with loosening of the mash between them by backward movement of the piston. However, an unfavorable mash structure and softening of the mash particles due to PEF cell disintegration may have had an adverse effect on the performance of the solid–liquid separation in the filter press. A higher degree of mash compaction and blocking of the drainage elements can be considered as possible result.

Based on the results of the present study, it can be concluded that in addition to the disintegration of the cell structure by PEF, optimal drainage conditions in the press-cake have to be maintained. Thin layers of mash being squeezed between a perforated moving belt and a series of serpentine winded rollers, alternating and gradually increasing pressure and occurring shear stress are the main characteristics of the belt press. They result in effective de-juicing performance by creating new channels for the juice to flow out from the mash (Downes, 1999). Hence, the cell disintegration achieved by PEF and the facilitated release of juice from the particle can be converted in a higher juice yield.

3.3.2. Carrot juice

The net juice yield for carrot juice is shown in Fig. 4 independent from the amount of total suspended solids (TSS) and in relation to a concentration of total dissolved solids (TDS) of 7.8°Brix. The impact of the mash pre-treatment on TSS and TDS will be discussed separately in section 'Juice quality parameters'.

An increase of net juice yield after mash electroporation can be observed for all de-juicing systems and the increase of juice yield by PEF treatment is much higher in comparison to apple juice. As in the case of apple juice, the increase of juice yield is also more pronounced for coarse mash in comparison to finer mash structures.

The belt press in combination with PEF pre-treated (12 kJ/kg) carrot mash (mash type 2) resulted in the highest juice yield of all carrot mash trials amounting to 75.9%. However, also the low intensity PEF treatment (2 kJ/kg) increases the juice yield already from 61.2% for the untreated to 72.8% for the PEF treated mash.

For mash type 5 the improvement of the juice yield by PEF is more pronounced but a further increase of the treatment intensity from 2 kJ/kg to 12 kJ/kg does not lead to a further increase of juice yield. This is also true for the rack-and-cloth press (mash type 0.35 and type 2) as well as for the filter press and the decanter (both mash type 0.35). In all these cases, the increase of juice yield is achieved with the low intensity PEF treatment already whereas a further increase of the total specific energy input is not contributing to a further increase in juice yield although an increase of cell disintegration in the range of 6% is still observed.

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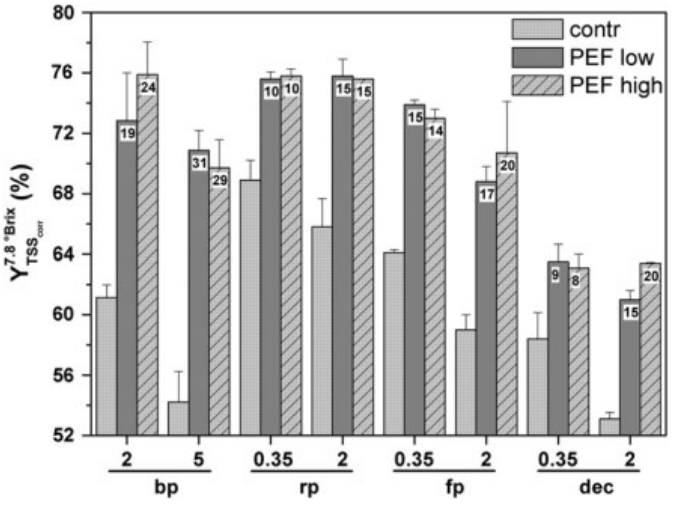

Fig. 4. Net juice yield for carrot juice. Three different mash types (mash type 0.35, 2 and 5) were processed with the belt press (bp), rack-and-cloth press (rp), filter press (fp) and decanter after pre-treatment with PEF at 2 kJ/kg (PEF low) and 12 kJ/kg (PEF high) or without pre-treatment (contr). The numbers indicated in the graph represent the increase of juice yield achieved by PEF pre-treatment given in per cent (%) increase in relation to the untreated control sample.

However, when processing coarse mash (mash type 2) with the filter press, the increase of the total specific energy input from 2 kJ/kg to 12 kJ/kg results in an increase of juice yield. A dependency of the juice yield from the PEF treatment intensity was also found for the coarse mash processed with the decanter.

The PEF treatment proved to be more effective in terms of juice yield increase for carrot mash in comparison to apple mash. The carrot mash particles contain a higher number of intact cells due to the smaller cell size and the texture of the particles is much harder compared to apple mash. Therefore, the cell disintegration achieved by PEF as well as the mash particle softening due to the electroporation show to be more beneficial for facilitating the juice release from the carrot mash.

Values for the juice yield from carrot mash available in literature differ considerably based on the processing technology. Results reported by Knorr et al. (1994) showed an increase in carrot juice yield from 30% to 70.3% for coarse mash (particle size 3 mm) and from 51.3% to 76.1% for fine mash (particle size 1.5 mm) due to PEF treatment at 2.6 kV/cm with 50 pulses and subsequent pressing at 10 MPa for 5 min.

Electric field application by an electroplasmolysis treatment investigated by Rayman, Baysal, and Demirdöven (2011) resulted in an increase in juice yield of 10.6% but juice yields were in a low range (44–57%) probably due to the laboratory de-juicing system. The temperature increase was reported to be in the range of 5 °C.

Yield values for industrial scale processing of carrot juice including thermal and enzymatic mash pre-treatment and a solid–liquid separation process either by pressing or decanter are reported to reach maximum values up to 75–78% (Kardos, 1975; Schobinger, 2001). Juice yields around 76% were obtained after PEF treatment and de-juicing with the belt press or rack-and-cloth press and are therefore in the range of industrial values obtained with traditional processing. However, despite of comparable juice yields, traditional processing steps such as thermal treatment and acidification have a major impact on juice stability (Reiter, Neidhart, & Carle, 2003) and further investigations are required in order to evaluate the stability of juices from PEF pre-treated mash and to develop modified processing concepts if required.

3.3.3. Kinetics of juice recovery

Material properties of the mash and processing parameters affect the kinetic of juice expression from biological tissue and attempts for the description and modeling of related phenomena have been made (Lanoisellé et al., 1996; Schwartzberg, 1997). Since the PEF treatment is affecting the mash particles in terms of the disintegration of cells and related tissue softening a change of material properties will result and could have an impact on the kinetic of juice release which may require the adjustment of processing parameters.

Hence, the pressure and pressing time dependent release of the juice during the pressing sequence was recorded for de-juicing trials with the rack-and-cloth press. It was aimed to obtain information on how the degree of mash compaction, changes in drainage properties as well as the reduced presence of a liquid phase in the press-cake will affect the solid–liquid separation process for untreated and PEF treated mash during the course of pressing.

Fig. 5 shows exemplarily the pressing curves for the coarse apple and carrot mash (mash type 9 for apple and mash type 2 for carrot) for which a considerable increase in the total amount of recovered juice after pressing was observed for the PEF treated samples.

Now, the gross juice yield (Y) is shown since no data on TSS and TDS were recorded for the different juice fractions during a pressing sequence. The slope of the pressing curve is a measure for the magnitude of juice release since it correlates to the amount of juice released during a corresponding pressure holding interval.

For *apple mash*, final gross yields of 76.0% were obtained for the control sample whereas 82.1% and 82.6% were recovered from PEF treated mash (total specific energy input of 2 kJ/kg and 12 kJ/kg respectively). As expected and shown by the pressing curve, the amount of juice released during the course of pressing is decreasing for all samples. This is due to the reduced presence of a liquid fraction in the press-cake and due to increasing compaction with declined drainage properties. However, differences exist between the control and PEF treated samples regarding the kinetic of the juice release. According to the pressing curve the higher amount of juice recovered from the PEF treated samples is released during the first stages of pressing. Whereas for the control sample a yield of only 63.1% is achieved after the first pressing step (1.5 bar for 2 min), the PEF treated samples have already released more juice resulting in a yield of 67.2% and 67.3% for 2 kJ/kg and 12 kJ/kg respectively. The speed of juice release is much higher for the PEF treated samples as indicated by the slopes of the pressing curve. Whereas an average yield of 31.5% is obtained per minute during the first pressing step for the control sample, values for the PEF treated samples are higher. The same is true for the second and third pressing step, where the PEF treated samples still show a higher magnitude of juice release from the mash in comparison to the control sample. However, in the last pressing step, no distinct difference is found anymore and almost the same amount of juice is released per minute independent from the pre-treatment of the mash as indicated by similar slopes of the pressing curve. The increase of the slope of the pressing curve for all samples during the last pressing step is due to a higher pressure increase (3 bar instead of 1.5 bar as for the other pressing steps).

Regarding the pressing curves for the *carrot mash*, the same trend as for the pressing behavior of apple mash is visible. The difference in final gross juice yields is a result of the higher release of juice from the PEF treated mashes taking place during the first two pressing steps. In the first pressing step, the highest amount of juice is released and PEF treated mashes show a considerable higher magnitude of juice release. In the second pressing step (3.0 bar for 2 min) slopes of the pressing curve for the PEF treated samples are still higher in comparison to the control sample but differences between control and PEF treated mash regarding the juice release become less pronounced for the subsequent third and fourth pressing step where similar values for the slope of the pressing curve are obtained.

Different observations are reported by Bazhal, Lebovka, and Vorobiev (2001), Bouzrara and Vorobiev (2003) and Praporscic et al. (2007) who investigated the application of a PEF treatment at different stages during the compression. Bazhal et al. (2001) studied the juice release behavior of fine-cut apple raw material during simultaneous PEF

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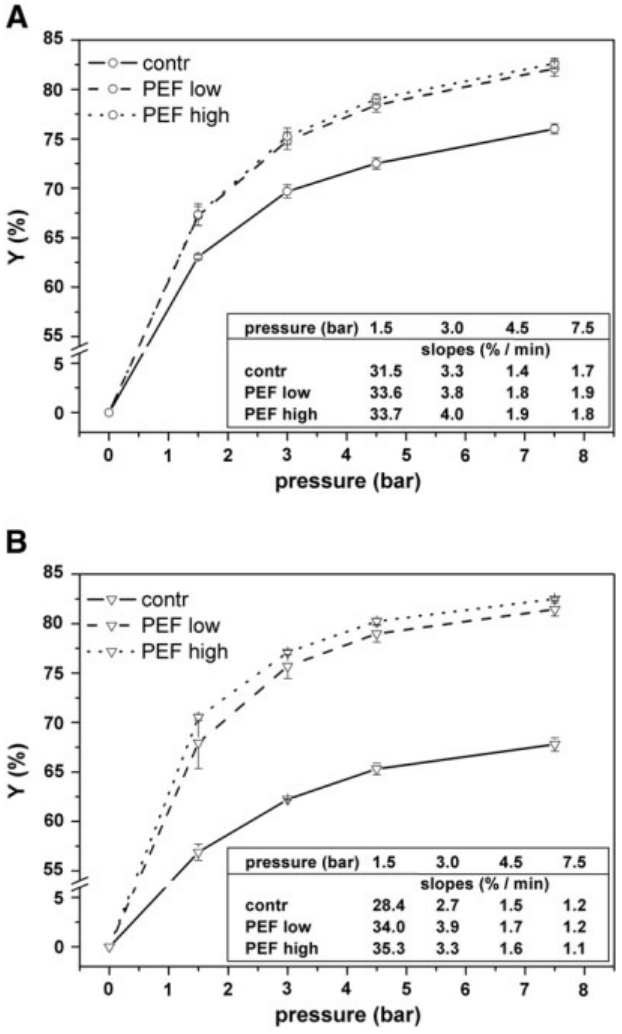

Fig. 5. Pressing curve for apple (A) and carrot (B) showing the juice yield (gross yield) obtained from apple mash type 9 and carrot mash type 2 in the rack-and-cloth press depending on applied pressing pressure (course of pressing, total pressing time 8 min, holding time of 2 min for each intermediate pressure level of 1.5, 3.0, 4.5 and 7.5 bar). The slopes for the yield increase per minute of holding time during each pressing step are also indicated for the control as well as for the PEF treated samples.

(0.1–0.52 kV/cm, treatment time 5 ms) and pressure treatment (3 bar) and identified optimal stages of pressing for the PEF application. A 3-step change in the food material structure was proposed during pressing:

i) pre-compaction and expulsion of extra-particle air–liquid mixture,
ii) mechanical rupture of residual cells and expulsion of liquid from ruptured cells
iii) final compression of the press-cake.

An enhancement of juice yield by PEF was achieved independent of the time of application but optimal juice quality resulted when applying PEF treatment after a pre-compression. This finding was confirmed by Praporscic et al. (2007) for apple where again a dependency in the juice yield increase on the time of PEF application during pressing was found. However, the same experiments conducted for carrots did not show differences in the final yield in case of PEF application after 30 s or 1000 s during the whole pressing time of 10,000 s at a pressure of 5 bar.

Based on the findings of the present study concerning the juice release behavior of apple and carrot mash during the course of pressing it can be concluded, that the effect of the PEF pre-treatment on juice yield increase is mainly due to beneficial juice release during the early pressing phase. However, the PEF treatment was applied before the pressing and may have already affected material properties of the mash. This may lead to differences in its pressing behavior which makes comparison with the results from studies on combined pressing and PEF treatment difficult. As mentioned before, the PEF treatment is causing cell disintegration in the mash particles and facilitates intra-particle juice transport. However, as a second prerequisite for the transfer of this effect into an increase in juice yield, the mash structure and drainage properties need to be suitable in order to allow the transport of the released juice through the mash in the extra-particle space. This extra-particle flow, namely the drainage properties of the mash, is reduced as pressing is progressing since a higher level of mash compaction and the reduction of mash porosity occurs. This fact needs to be taken into account and requires further investigation on how existing pressing concepts can be adapted in order to allow an optimal juice release from the mash particles and an optimal juice transfer through the mash when working with PEF treated mash. The application of pressing aids may be considered and the revision of industrial pressure cycles applied in the operation of hydraulic filter presses or rack-and-cloth presses is essential in order to adapt the rate of compression to the particular requirements of a PEF treated mash.

Research work undertaken by Al-Mashat and Zuritz (1993) supports the presented findings of the interaction of mash and pomace structure and the juice yield. The investigations on the stress relaxation behavior of apple pomace and the effect of temperature, pressing aids and compaction rate on juice yield as undertaken by Al-Mashat and Zuritz (1993) provide a basis for the optimization of the design and operation of juice presses.

Further research work in this field is required considering the particularities of PEF treated mash in order to adapt the operating conditions of the de-juicing systems or to modify mash and press-cake properties.

3.4. Juice quality parameters

3.4.1. Apple juice

The content of total suspended solids (TSS), total dissolved solids (TDS) and total polyphenols (TP) was determined in apple juice and results are shown in Table 2.

The content of total suspended solids (TSS) refers to cell matter particles that have been transferred from the mash into the juice. Values measured for the juice are in the range of 1.2–1.8% for the pressing systems and between 0.7% and 0.8% for the decanter. Values were lower for juice resulting from fine mash and no distinct impact of PEF treatment was found.

The highest TSS values were obtained from the belt press. PEF treatment did slightly increase the TSS content for the fine mash whereas for the coarse mash, differences were in the range of the experimental deviation. The effect of lower TSS values for fine mash in comparison to coarse mash was also found for the rack-and-cloth press. No distinct effect of PEF treatment on TSS content was found. The filter press showed similar TSS contents for the juice resulting from fine and coarse mash being in the range of 1.2–1.5%. Only for the coarse mash, a slight increase of TSS values in the juice was observed after PEF treatment. The lowest values were found in the juice from the decanter separation. No distinct effect of the PEF treatment of the mash (mash type 5) on TSS content of juice was found.

The impact of the decanter process parameters on TSS content was investigated by Beveridge, Harrison, and Gayton (1992). TSS values in apple juice between 2 and 3% were defined as a common range for solid–liquid separation systems and as the upper limit for apple juice obtained with the decanter. Increasing the mash flow rate (feed rate of the decanter) was found to increase the TSS content in the juice. Based on the low TSS values obtained in the present

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Table 2
Total suspended solids (TSS), total dissolved solids (TDS) and total polyphenols (TP) content of apple juice depending on de-juicing system, mash type and PEF treatment intensity. Given are mean values of 4 replicates (samples from 2 independent de-juicing trials analyzed in duplicate) and the corresponding standard deviation in brackets. TP content is given as corrected value by consideration of a standard net juice yield of 75% (w/w).

Press	Mash type	PEF	TSS in % (w/w)	TDS in °Brix	TP in GAE mg/L
Belt press	5	0	1.39 (±0.00)	12.7 (±0.2)	484.55 (±7.77)
		Low	1.58 (±0.04)	12.7 (±0.2)	499.55 (±4.88)
		High	1.57 (±0.13)	12.9 (±0.1)	392.38 (±61.24)
	9	0	1.71 (±0.20)	12.8 (±0.2)	267.38 (±16.96)
		Low	1.81 (±0.06)	12.7 (±0.2)	287.14 (±16.53)
		High	1.81 (±0.02)	13.1 (±0.1)	482.11 (±9.12)
Rack-and-cloth press	5	0	1.30 (±0.03)	13.3 (±0.2)	305.37 (±16.39)
		Low	1.18 (±0.04)	13.3 (±0.4)	340.60 (±23.52)
		High	1.23 (±0.05)	13.1 (±0.0)	351.58 (±19.16)
	9	0	1.63 (±0.21)	12.8 (±0.0)	261.55 (±11.85)
		Low	1.59 (±0.05)	12.9 (±0.0)	334.17 (±15.57)
		High	1.48 (±0.01)	13.0 (±0.1)	388.19 (±6.49)
Filter press	5	0	1.25 (±0.14)	13.2 (±0.0)	216.23 (±33.13)
		Low	1.23 (±0.03)	13.2 (±0.0)	254.43 (±10.10)
		High	1.49 (±0.50)	13.1 (±0.0)	271.88 (±30.24)
	9	0	1.27 (±0.06)	12.8 (±0.0)	197.97 (±35.87)
		Low	1.33 (±0.01)	12.9 (±0.0)	323.47 (±12.15)
		High	1.43 (±0.04)	13.0 (±0.2)	339.46 (±12.73)
Decanter	5	0	0.77 (±0.08)	13.2 (±0.5)	401.84 (±8.11)
		Low	0.72 (±0.01)	13.1 (±0.1)	429.85 (±25.56)
		High	0.75 (±0.04)	12.8 (±0.1)	429.94 (±28.12)

study, it can be concluded that there is further potential to increase the decanter feed rate in order to improve the machine capacity.

Lower TSS values for juices obtained from fine mash for different solid–liquid separation systems are probably due to better filtration properties of the press cake in comparison to coarse mash. Although fine mash is composed of smaller particles, solids transfer in the juice is reduced as a more dense structure is formed during compaction and the capillary structure is reduced leading to a higher retention of fine particles.

In addition, a higher release of insoluble pectin may result from the fine grinding and will increase the juice viscosity which will have an impact on juice drainage properties (Janda, 1985). The PEF effect mainly concerns the release of low viscous cytoplasm and vacuole sap and will affect the content of insoluble pectin to a lower extent. Lower juice viscosity in turn will facilitate the transfer of suspended particles from the mash in the juice and may explain the slightly higher TSS values obtained for juices from PEF treated mash from the belt press or filter press.

Differences in the filtration properties of the press cake could also affect the transfer of dissolved solids in the juice since adsorption and adhesion properties depend on the structure of the mash as concluded by Praporscic et al. (2007) but no effect was found in the present study as it will be discussed below.

The content of total dissolved solids (TDS) shows only minor differences between the various de-juicing systems, mash types and PEF treatment intensities. Values are in the range of 12.7–13.3°Brix.

Lowest values are obtained for the belt press. The lower TDS content of juices obtained from this pressing system is probably due to the belt cleaner which is flushing the screen belt and thus wetting the belt which results in a continuous transfer of small amounts of water into the mash and juice. PEF treatment is slightly increasing the TDS content in the juice for the higher PEF treatment intensity level.

Juice resulting from fine mash processed with the rack-and-cloth press shows the highest TDS value of all de-juicing trials. Juice obtained from coarse mash had lower TDS values but showed a slight increase for PEF treated mash. The same is true for the filter press whereas for the decanter, the PEF effect was within the experimental deviation of the TDS values obtained.

The content of total dissolved solids depends very much on the variety of the processed apples as well as on their degree of ripening. Therefore, a comparison of absolute values with literature data is limited.

A total dissolved solids content of 12.2°Brix is given by Schilling et al. (2008) for cloudy apple juice obtained with a hydraulic filter press. No difference was found between control and PEF treated samples. Turk et al. (2010) also reported no significant differences in the TSS content between control and PEF samples and between juices obtained from fine or coarse mash. The values ranged between 12.8 and 14.0°Brix. However, Praporscic et al. (2007) found an increase of the TDS value when PEF cell disintegration was applied during pressing. For coarse particles, the TDS value increased from 11°Brix to around 11.8°Brix immediately after PEF treatment and decreased back to slightly higher values as before PEF treatment. For the fine mash, initial TDS values were around 11.4°Brix and increased to about 12°Brix due to PEF treatment. The effect was independent from the time of PEF application during pressing.

Dissolved solids are mainly present in the vacuole of the apple cell as it is also the case for the polyphenols (Yamaki, 1984). Therefore, similar principles for their release will apply and will be discussed together with the TP content of the juice later in the text.

Values for the content of total polyphenols (TP) range from 198 mg/L to 500 mg/L. A trend towards an increase due to PEF treatment can be shown for all de-juicing systems and mash types except for fine mash processed with the belt press. A lower release of polyphenols was found for the coarse mash in comparison to the fine mash (for untreated samples). For PEF treated samples the increase of TP values of the juice was higher for coarse mash in comparison to fine mash.

Highest TP values of 484–500 mg/L were obtained for mash type 5 processed with the belt press. A decrease in the content of TP in the juice was observed for the mash treated with 12 kJ/kg but the decrease was most likely due to deviations in the experimental procedure as indicated by the high standard deviation. Values for juice from coarse mash are lower (267–287 mg/L) whereas a considerable increase to 482 mg/L is observed after PEF treatment at 12 kJ/kg. Juice from the rack-and-cloth press shows lower content of TP. Juice from fine mash gives a total polyphenol content of 305 mg/L. This value is increased by 11–15% due to PEF treatment. A higher increase of 27–48% can be observed for the coarse mash. The filter press gave the lowest TP values. However, PEF treatment resulted in an increase in TP values for both mash types but more pronounced for the juice from coarse mash. In this case an increase of about 63–71% was found for PEF treatment whereas for fine mash, TP values were increased in the range of 17%–26%. Processing of mash type 5 with the decanter resulted in juice with considerably high content of TP. The control sample showed 402 mg/L whereas the PEF treated samples were around 430 mg/L showing an increase which was however still within the range of the experimental deviation.

Turk et al. (2009) also investigated the polyphenol content of apple juice obtained from a belt press and the impact of mash structure, PEF treatment and stage of pressing (first and subsequent section of the belt press). Polyphenols were determined by HPLC and a total amount was calculated based on the content of the analyzed single compounds. Values were in the range of 909–1595 mg/L.

Juices from the first section had a higher TP content (1304–1595 mg/L) than juices from the second section (909–1108 mg/L). For the juice from the first section a reduction of TP for PEF samples was found for both mash types mainly due to lower contents of hydroxycinnamic acids and compounds from the favan-3-ol family. Juice obtained from the subsequent pressing stages showed a slight increase in polyphenols for PEF treated fine mash but no difference between PEF and control for coarse mash. However, a significant increase was found for phloridizin in juice from the second section due to PEF treatment for both mash types. It was concluded that

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differences in the content of phenolic compounds in the juice between PEF and control were mainly related to polyphenoloxidase (PPO) activity increased by PEF. The compounds found in a lower concentration in PEF samples (hydroxicinnamic acids, catechins) are part of a PPO induced degradation, whereas phloridzin, which was found in higher concentrations in juices from the second pressing stage, is a bad substrate for PPO.

Results reported by Turk et al. (2010) indicated a negative effect of increasing the mash size on TP content in juice probably due to a lower release of highly polymerized procyanidins. Whereas a total polyphenol content of 215.2 mg/L in juice from fine mash was found, the value decreased to 122.7 mg/L for juice from coarse mash. When PEF treatment was applied, a TP content in the juice of around 100 mg/L resulted for both mash types.

Hence, the findings regarding the negative PEF effect on TP content of the juice are in contrast to the ones obtained in the present study which is probably mainly due to differences in the experimental procedure, i.e. longer pressing times and no addition of ascorbic acid to the juice. The decrease of polyphenol content in the juice after mash electroporation reported by Turk et al. (2010) was mainly related to oxidation phenomena during the pressing period (60 min) and more pronounced for particles with a higher level of disintegration either due to smaller particle size or due to PEF treatment.

However, Schilling et al. (2008) detected a content of 487 mg GAE/L in the control juice obtained from a hydraulic filter press (pressing time 90 min). By applying PEF to the mash, the TP content in the juice could be increased considerably to 594 mg GAE/L. A similar increase of 22% is found for the juice from mash type 5 processed with the filter press in the present study.

Theoretical approaches on the cell membrane disintegration and related reduction of mass transfer barriers have been developed and linked to experimental data on a facilitated release of the liquid cell content by various authors. However, although reported in several studies, concepts concerning the understanding of the effect of a PEF treatment on the release of polyphenols are limited. A first hypothesis was suggested by Turk et al. (2010) which will be further developed in the following section.

The activity of polyphenoloxidase seems to play a major role and changes in the polyphenol concentration in the juice might not be due to changes in the release but due to a different degree of degradation by polyphenoloxidase.

Polyphenols occur as dissolved substances in vacuoles of plant cells (De, 2000) and vacuoles occupy up to 90% of the total cell volume (Taiz & Zeiger, 2007).

Yamaki (1984) studied the distribution of sugars, organic acids and phenolic compounds in the vacuole, the cytoplasm and the extracellular free space of immature apple tissue. Fructose and glucose were the most abundant sugars in the cell and almost all of the fructose and glucose was located in the vacuole. More than 90% of the organic acids and almost all of the phenolic compounds were deposited in the vacuole. Based on the number and volumes of the different cell compartments, the solute concentration was estimated to be 888 mM in the vacuole (mainly fructose, glucose, phenolic compounds and malic acid), 57 mM in the cytoplasm (mainly sucrose and malic acid) and 73 mM in the free space (mainly sorbitol, sucrose and glucose).

Hence, from a mass transfer point of view, any change in TDS content of the apple juice is related to a changed ratio between the release of highly concentrated vacuole sap and the release of less concentrated cytoplasm and liquid from the free space. Most of the liquid and most of the compounds contributing to the TDS content of the juice are located in the same cell compartment, namely in the vacuole, in a constant concentration. Changes of the physical release properties, e.g. by disintegration of the vacuole, will facilitate the release of the vacuole sap but will not change the concentration of the contained compounds.

Any changes in the ratio of TDS compounds must be related to additional effects rather than the mass transfer only. The following additional effects responsible for the selective release of specific compounds are suggested:

i) Changes in temperature and facilitated release of poorly water soluble compounds as well as increased reaction rates,
ii) Facilitated release of bound compounds due to native enzyme activity,
iii) Decrease of the content of single compounds by enzymatic or non-enzymatic degradation or
iv) Location of a compound in a separate cell compartment that is disintegrated.

ad i) Changes in temperature may improve the release of poorly water soluble compounds but may also increase reaction rates. Depending on the total specific energy input, PEF treatment of the mash will result in a temperature increase. The maximum energy input of 12 kJ/kg applied in the present study leads to a temperature increase of about 3 °C. Although this is almost negligible in the present study, it may become relevant in case of higher treatment intensities or in the case of combined thermal and PEF treatments. According to Rechner (2000) only 43% of the total polyphenols are transferred from the apple to the juice. Especially for phloridzin, procyanidin and catechin the transfer rate is limited due to their poor water solubility. van der Sluis et al. (2002) reported a high amount of flavonoids (including catechin and phloridzin) to be retained in the pomace without being released or degraded. An improved release of phloridzin among other polyphenols from apple mash was shown by Spanos, Wrolstad, and Heatherbell (1990) due to an increase in extraction temperature. However, a temperature increase will also have an effect on the hereafter mentioned reactions and their reaction rates.

ad ii) The enhancement of the release of flavonols as a consequence of enzymatic mash treatment and the depolymerization of the cell wall was discussed by Schilling et al. (2007). Mihalev et al. (2004) reported an increase of the polyphenol content of apple juice after non-oxidative mash maceration with pectolytic enzymes.

Depending on the degree of cell disintegration and the time between the mash preparation and the solid–liquid separation, the activity of native pectolytic enzymes in the apple mash may result in depolymerization of structural cell components and may affect the release of polyphenols.

ad iii) Polyphenoloxidase (PPO) is the main responsible enzyme for the oxidation of polyphenols to their corresponding quinones which are further polymerized to form brown pigments. PPO is located in plastids or chloroplasts in intact cells (Mayer, 2006; Murata et al., 1997) but may also solubilize to a certain extent (Barrett, Lee, & Liu, 1991). Cell disintegration by either mechanical means or PEF is reducing the membrane barrier function of the cell compartments and promotes the contact of PPO and polyphenols as well as the access to oxygen. PEF disintegration is more likely to affect the vacuole and to facilitate the release of vacuolar sap whereas much smaller organelles such as plastids are less affected by the applied electrical pulse protocols (Schoenbach et al., 2004).

The content of native polyphenols in the juice depends on the extent and speed of the two competing reactions during the de-juicing process, namely the facilitated release of vacuolar sap due to PEF cell disintegration on one hand and the oxidation on the other hand.

Oxidation may take place in the cell after destruction of the

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compartmentalization, it may take place in the liquid fraction of the mash due to released PPO and polyphenols and finally it may take place in the juice due to transferred PPO and polyphenols.

Oxidation in the cell may result in the formation of polymer products which have different properties regarding their water solubility and release (Rechner, 2000) and may lead to a decreased transfer of polyphenols in the juice.

The extent of oxidation in the liquid fraction of the mash or in the recovered juice will be determined by the concentration of released PPO and polyphenols as well as by the susceptibility of different polyphenols to act as a substrate for PPO.

Whereas in untreated mash, a given ratio of PPO and polyphenols will be released due to mechanical destruction of polyphenol containing vacuoles and PPO containing plastids, this ratio will be shifted towards the release of vacuolar sap in PEF treated samples due to the disintegration of the vacuoles. Hence, a similar amount of PPO will be released into an increased amount of vacuolar sap which will result in a lower PPO concentration in the juice. This will mainly reduce the degradation of polyphenols in the juice whereas the aforementioned aspects of oxidation in the cell and in the liquid phase of the mash will be less affected.

Occurring reactions mainly depend on the mechanical cell disintegration based on the grinding system and resulting mash particle size and on the PEF induced cell disintegration as well as on the characteristics of the de-juicing system such as the duration of solid liquid separation, the contact time of juice and mash and the access of oxygen. The given concept is still speculative and requires further systematic investigations on the impact of cell disintegration on the release of polyphenols and PPO and their interactions.

ad iv) The aspect of location of compounds in a higher concentration in separate cell compartments is more relevant for carrot cells where the carotenoids are stored in a higher concentration in chromoplasts. This aspect will be discussed for carrots in a later section. The aspect is also relevant for fruits with a higher surface–volume ratio than apples in which polyphenols are mainly located in the skin cells that are less susceptible to disintegration (such as grapes or berries). The selective disintegration of such cells or compartments within the cell will then result in an increase of polyphenol concentration in the juice released from the entire fruit.

Since the vacuole of apple cells contains most of the liquid fraction of the cell as well as most of the polyphenols at a given concentration, the release of juice is directly linked to the release of polyphenols at this given concentration. However, without the permeabilization of the vacuolar membrane, an increase of juice yield may be obtained due to the transfer of cytoplasm. The polyphenol content of the juice will not be affected as long as the vacuole is intact. An additional permeabilization of the vacuolar membrane will result in the facilitated release of vacuole sap adding a higher concentrated liquid to the juice.

Pressure application during the solid–liquid separation process will express juice from the internal pores of the mash particles first. Thus, a decrease of TDS during the course of pressing will occur. This was shown by Praporscic et al. (2007). As the compaction of the press-cake proceeds, the destruction of intact cells will occur and vacuole sap will be released which leads to an increase of TSS (Praporscic et al., 2007). The same effect will result for the PEF treatment but in a more pronounced manner leading to the destruction of intact vacuoles and resulting in the release of vacuole sap with a higher concentration of TDS. The increase of TDS (and polyphenols) is therefore clearly not related to the disintegration of the cell membrane. Instead, this phenomenon is clearly coupled to the disintegration of the vacuolar membrane and the facilitated release of vacuolar sap with a high TDS concentration. However, the increase of juice yield itself may be partly explained by the disintegration of the cell membrane since this will promote the release of the cytoplasm on the one hand and will be the prerequisite for the transfer of the vacuole sap into the extra-cellular space on the other hand.

3.4.2. Carrot juice

The content of total suspended solids (TSS), total dissolved solids (TDS) and carotenoids was determined in carrot juice and results are shown in Table 3. For de-juicing trials with the belt press, mash types 2 and type 5 were used whereas mash type 0.35 and type 2 were used for all other separation systems.

Regarding the content of TSS, values range from 1.3–3% where the lowest values are obtained from juices produced with the filter press (1.3–1.7%).

Juices obtained from the belt press show a TSS content which is independent from the mash type (1.7–2.2% for mash type 2 and 1.9–2.1% for mash type 5) and affected by PEF treatment in the way that high intensity treatment leads to juices with the lowest TSS values. For juices resulting from the rack-and-cloth press, TSS values are higher for fine mash but PEF treatment of the mash shows no clear effect on TSS content of the juice obtained. This is different for the juices from the filter press where PEF treatment of the mash led to a decrease of the TSS content of the resulting juices. Decanter separation resulted in similar TSS values for fine and coarse mash. PEF effects were within the range of the experimental deviation.

Average TSS values for carrot juice as obtained after solid–liquid separation are reported by Handschuh (1994) to be in the range of 1–2% as determined by centrifugation at $4000 \times g$ for 10 min. Values of commercial carrot juice are increased to 8–10% and achieved by blending with carrot puree (Reiter et al., 2003). In the present study, no distinct effect of PEF treatment or mash type could be found and regarding the solid–liquid separation system, only the filter press affected the TSS content by giving juices with a lower value in comparison to the other de-juicing systems. TSS values for carrot juice were higher than for apple juice for all de-juicing systems.

Table 3
Total suspended solids (TSS), total dissolved solids (TDS) and carotenoid content of carrot juice depending on de-juicing system, mash type and PEF treatment intensity. Given are mean values of 4 replicates (samples from 2 independent de-juicing trials analyzed in duplicate) and the corresponding standard deviation in brackets.

Press	Mash type	PEF	TSS in % (w/w)	TDS in °Brix	ß-carotene in mg/L
Belt press	2	0	2.21 (±0.15)	7.7 (±0.2)	20.60 (±0.42)
		Low	2.45 (±0.51)	7.8 (±0.3)	25.51 (±0.14)
		High	1.69 (±0.21)	7.9 (±0.4)	27.47 (±0.52)
	5	0	2.09 (±0.05)	7.8 (±0.4)	12.97 (±0.11)
		Low	2.08 (±0.07)	7.9 (±0.2)	16.17 (±2.53)
		High	1.89 (±0.13)	7.8 (±0.3)	16.96 (±1.56)
Rack-and-cloth press	0.35	0	2.97 (±0.08)	7.3 (±0.0)	35.88 (±0.81)
		Low	2.44 (±0.03)	7.3 (±0.0)	39.47 (±6.77)
		High	2.96 (±0.15)	7.4 (±0.0)	43.07 (±0.69)
	2	0	2.25 (±0.29)	7.8 (±0.2)	37.90 (±3.29)
		Low	2.04 (±0.06)	7.4 (±0.1)	48.46 (±0.13)
		High	2.04 (±0.15)	7.3 (±0.0)	48.52 (±1.96)
Filter press	0.35	0	1.60 (±0.13)	8.1 (±0.0)	40.24 (±1.13)
		Low	1.41 (±0.06)	8.1 (±0.0)	32.72 (±4.24)
		High	1.39 (±0.10)	7.9 (±0.1)	38.68 (±1.54)
	2	0	1.66 (±0.05)	8.0 (±0.0)	30.73 (±2.99)
		Low	1.36 (±0.04)	8.1 (±0.0)	39.05 (±1.09)
		High	1.31 (±0.32)	8.1 (±0.0)	37.08 (±4.02)
Decanter	0.35	0	2.26 (±0.60)	7.4 (±0.1)	44.79 (±6.21)
		Low	1.97 (±0.41)	7.4 (±0.1)	36.83 (±3.34)
		High	1.65 (±0.14)	7.3 (±0.0)	33.60 (±3.93)
	2	0	2.18 (±0.28)	7.4 (±0.0)	39.78 (±4.24)
		Low	2.57 (±1.17)	7.3 (±0.0)	38.43 (±0.07)
		High	1.95 (±0.12)	7.3 (±0.0)	31.03 (±0.88)

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Whereas for apple juice, a slight increase of TSS was found for juice from PEF treated mash, this is not the case for carrot juice.

Regarding the TDS content, all obtained juices were in the range of 7.3–8.1°Brix. The lower values were obtained for rack-and-cloth press and decanter whereas highest values were achieved by de-juicing with the filter press. PEF treatment of the mash did not show a distinct effect on TDS content of the juice and no major difference was found between fine and coarse mash. Juices obtained from the belt press showed comparable high standard deviations in TDS values. This may be partly related to manipulation of the juice composition by water transfer from the belt due to the belt cleaning system.

TDS values depend very much of the carrot variety and processing technology but values obtained in the present study were in accordance with values reported by Stephens et al. (1971) for juice from raw (7.0–7.5°Brix) and heated (7.6–8.2°Brix) carrot mash.

Praporscic et al. (2007) studied the changes of TDS content for carrot juice when applying PEF treatment during pressing. A drastic increase in TDS in the juice was found immediately after PEF application for coarse mash when PEF was applied in later pressing stages (TDS around 7.2°Brix for the control juice and 9°Brix for the PEF treated juice). When PEF was applied during earlier pressing stages, the increase in TDS occurred delayed. For fine mash, the same trend was found but less pronounced than for coarse mash. Knorr et al. (1994) reported almost identical values for pH and total acidity in juices from PEF treated mash compared to untreated samples.

As discussed in detail in Section 3.4.1. TDS content mainly depends on sugars and organic acids that are located in the vacuole. The PEF treatment during pressing will destroy intact cells and vacuoles leading to an immediate facilitated release of highly concentrated vacuole sap and an increase of TDS as reported by Praporscic et al. (2007). Hence, the increase in TDS is due to a selective release of vacuole sap in comparison to the release of cytoplasm and the liquid from the free space which is expressed already during earlier pressing stages. Different release characteristics may apply when the PEF application is performed as a pre-treatment. Since most of the cellular liquid is contained in vacuoles, the increase of juice yield as observed for carrot mash is due to the release of vacuole sap with a given TDS concentration and an increase of the TDS content of the juice is not likely to occur.

Carotenoid values in the carrot juice were in the range of 13.0–48.5 mg/L for all de-juicing systems and experimental setups under investigation. An increase of carotenoids of the juice due to PEF treatment of the mash was observed for the belt press and rack-and-cloth press and for the filter press (mash type 2).

For the belt press, PEF treatment of the mash type 2 at 2 kJ/kg and 12 kJ/kg increased the carotenoid content of the juice by 24% and 33%. The initial carotenoid content of juice from mash type 5 was lower but a similar percentage of increase (25% and 31%) was achieved by PEF treatment. Mash type 5 was only used for the belt press. All other separation systems were supplied with mash type 0.35 and type 2.

Juice from the rack-and-cloth press obtained from mash type 0.35 had a considerably higher content of carotenoids of 35.9 mg/L. An increase of 10% and 20% was achieved by PEF treatment of the mash at 2 kJ/kg and 12 kJ/kg. Coarse mash (type 2) processed with the rack-and-cloth press showed a final carotenoid value of 48.5 mg/L for both PEF treatment intensities which represents an increase of 28% based on an initial content of 37.9 mg/L in the control sample.

PEF treatment of the mash processed with the rack-and-cloth press resulted in juice with a lower carotenoid content but the effect was in the range of the experimental deviation. An increase of the carotenoid content was obtained in juice from mash type 2 after PEF treatment. The content of the control juice (30.7 mg/L) was increased by 27% and 21% to values of 39 mg/L and 37 mg/L (low and high PEF specific energy input level respectively).

High initial carotenoid values were obtained in juices from the decanter. PEF treatment of the mash reduced the carotenoid content in the juice by up to 25%.

Conventional mechanical pressing of carrot mash results in carotene rich pomace since the transfer of the water insoluble carotene from the cells into the juice is limited. Thermal and enzymatic mash treatment is commonly used in carrot juice production in order to improve the release and to increase the carotene content of the juice.

Liao et al. (2007) reported an increase in carotenoid content in carrot juice from 42 mg/kg to 68 mg/kg due to enzymatic mash treatment. Values for carotene content of carrot juice produced in industrial scale are given by Kardos (1975) in the range of 12–81 mg/kg or Handschuh (1994) with a range of 70–100 mg/kg.

Although the PEF cell disintegration resulted in an increase of the carotenoid content in the juice reaching final values of up to 48.5 mg/L, improved solubilization and release of carotene was lower than the one reported for juices obtained after thermal and enzymatic treatment. However, no direct comparison can be made since not only processing conditions but also raw material properties have a major impact on carotene content of the final juice.

Carotenoids in carrots are located in crystalline form in chromoplasts which have a size of about 10 μm (Kim et al., 2010; Straus, 1961). Although PEF cell disintegration is affecting larger membrane structures such as the membrane of the cell or vacuole, smaller cell compartments such as the chromoplasts will not be affected by the applied electrical pulse protocols (Schoenbach et al., 2004) and a direct effect on the solubilization of contained carotenoids is unlikely. The effect of PEF on the cell disintegration of different cell structures is not fully understood yet. Investigations on membrane permeabilization of plant tissue reported by Angersbach et al. (1999), Fincan and Dejmek (2002) and Lebovka, Bazhal, and Vorobiev (2001) gave evidence that not only the cell membrane but also the vacuolar membrane is affected. However, no information is available on the disintegration of subcellular organelles such as chromoplasts by PEF in vegetable tissue.

In addition to the permeabilization of the cellular membrane by PEF, particle size reduction as well as thermal means may be required in order to improve the release of carotenoids from chromoplasts due the size of these subcellular organelles and the crystalline structure of the carotenoids.

4. Conclusion

The consideration of textural properties, mash particle size and the cell size of the different raw materials is essential in order to evaluate the potential of the PEF application to increase the juice recovery from fruit and vegetable mashes. PEF treatment allows a tailor made mash structure design complementing the particle size reduction and cell disintegration achieved by mechanical means with additional cell disintegration. Hence, particle size and the level of cell disintegration become independent variables.

For apple juice, high improvements of the juice yield were obtained for PEF treated mash processed with the belt press and the rack-and-cloth press. In the case of carrot mash, belt press and filter press were most appropriate to transfer the PEF cell disintegration into higher juice yields. For carrot mash, a PEF treatment at a low specific energy input level (2–4 kJ/kg) proved to be sufficient to result in maximum juice release whereas for apple mash, further increase of the energy input to 12 kJ/kg did further increase the juice yield. Maximum juice yields obtained from apple mash after PEF treatment were in the range of 77.5% (belt press) and 86.1% (rack-and-cloth press) for fine mash types. Treatment of carrot mash with PEF resulted in final juice yields of up to 76% which is in the same range as reported for conventional carrot processing using thermal and enzymatic mash treatment. However, further investigations are required on carrot juice quality and storage stability. Blanching of

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carrots, thermal mash treatment as well as acidification improves color and cloud stability of the juice during storage and a revision of the conventional process regarding these aspects in combination with the implementation of the PEF treatment requires further investigation.

Minor impact of PEF treatment on TSS and TDS content of the final juices was found whereas total polyphenol content in apple juice was increased in most cases and carotene content in some cases due to PEF pre-treatment. Further investigations are required with regard to the manifold aspects of the PEF impact on the release of polyphenols as well as polyphenoloxidase in order to understand and control occurring interactions.

The adjustment and optimisation of pressing programs including the consideration of compression rates or the application of pressing aids are necessary in order to further improve beneficial PEF effects. For this, future research work on the impact of mash structure and PEF pre-treatment on press-cake properties and related drainage characteristics is required. In addition, the optimization of the PEF treatment parameters in order to minimize the PEF power consumption while maintaining a desired degree of cell disintegration will further improve process efficiency. Regarding the industrial implementation, the consideration of the total energy input of the entire juice winning process needs to be performed based on industrial data. The potential of increasing juice yield, shortening pressing times and increasing the throughput need to be evaluated from an economical point of view in order to compare the different juice winning alternatives as well as the benefits of assisting the juice winning with PEF.

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Meneses, N., Jaeger, H., Moritz, J., & Knorr, D. (2011). Impact of insulator shape, flow rate and electrical parameters on inactivation of *E. coli* using a continuous co-linear PEF system. *Innovative Food Science & Emerging Technologies*, *12*, 6–12.

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Toepfl, S., Mathys, A., Heinz, V., & Knorr, D. (2006). Review: Potential of emerging technologies for energy efficient and environmentally friendly food processing. *Food Reviews International*, *22*, 405–423.

Turk, M., Vorobiev, E., & Baron, A. (2009). Pulsed electric field assisted pressing of apple mash on a continuous pilot scale plant: Extraction yield and qualitative characteristics of cider juice. *Presented at International Conference on Bio and Food Electrotechnologies, Compiegne, France*.

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Curriculum vitae and list of publications

HENRY JÄGER – CURRICULUM VITAE

MAIN RESEARCH FIELD

- Non-thermal microbial inactivation in liquid foodstuffs using pulsed electric fields (PEF)
- Impact of PEF-treatment on bioactive compounds and identification of related mechanisms
- PEF treatment chamber design, process analysis and optimisation
- PEF application for cell disintegration of plant material and recovery of bioactive compounds
- Process integration in industrial scale for energy efficient and sustainable food processing

EDUCATION

Since 06/2006	Technische Universität Berlin, Department of Food Biotechnology and Food Process Enigneering Research associate, PhD candidate Thesis under the supervision of Prof. Dr. D. Knorr: Process performance analysis of pulsed electric field (PEF) food applications
02/2006	M. Eng. (Dipl.-Ing.) Food Technology Thesis: Impact of Pulsed Electric Fields on the Activity of Enzymes and the Inactivation of Selected Microorganisms in Milk Diploma degree *1.2 (passed with distinction)*
10/2003 – 07/2005	Technische Universität Berlin Student assistant in the Team of Mathematical Statistics working in the EU-Project WINE DB "Establishing of a Data Bank for Analytical Parameters for Wines from Third Countries"
10/2000 – 02/2006	Technische Universität Berlin Studies of Food Technology, emphasis on Dairy Technology

RESEARCH PROJECTS

- Enhancement of drying processes for plant raw materials via process induced reduction of mass transfer barriers. *AiF17161, German Federation of Industrial Research Associations.*
- PROMETHEUS: Process contaminants: Mitigation and elimination techniques for high food quality and their evaluation using sensors and simulation. *FP 7 EU project.*
- OILPULSE: Increased virgin olive oil yield and quality by complementing existing and new olive oil mills with pulsed electric field (PEF) technology. *Project coordinator. FP 7 EU project.*

RESEARCH PROJECTS (continued)

- BioMed: Improving dietary glucosinolate production, processing and characterization of potential health effects for the prevention of colon cancer. *Subproject leader. BMBF Federal Ministry of Education and Research.*
- FriPlas: Application of plasma for the gentle preservation of perishable foods. Subproject C: Product quality and systems integration. *BLE Federal Institute for Agriculture and Nutrition.*
- Transparent_Food: Quality and integrity in food: a challenge for chain communication and transparency research. WP3 Food Quality and Safety. *FP 7 EU project.*
- Track_Fast: Training requirements and careers for knowledge-based food science and technology in Europe. *FP 7 EU project.*
- Process-induced increase in the yield of secondary metabolites from bilberry and comparison of their stability in multi-capsules versus conventional products. *Project leader. AiF15610, German Federation of Industrial Research Associations. Part of the DFG/AiF-Cluster on Bioactive ingredients from microstructured multi-capsule systems.*
- Application of high voltage pulsed electric fields for extraction of apple juice without enzymatic treatment, subsequent gentle preservation in pilot scale and evaluation of the substantial equivalence to conventionally processed products. *Project leader. AiF179, German Federation of Industrial Research Associations.*
- Impact of PEF treatment on whey proteins. *Project leader. BfR Federal Insitute for Risk Assessment.*

AWARDS

05/2011 ICEF11 Scientific Presentation Award. 11th International Congress on Engineering and Food – Food Process Engineering in a Changing World. Athens, Greece.

10/2010 Supervisor of the team *Mr. Chocolate*, winner of the European Student Award for Food Innovation (TROPHELIA Europe 2010). SIAL 2010, Paris, France.

05/2010 2nd prize of the Julius Maggi Research Award 2010 sponsored by Nestlé Product Technology Centre Singen. European Federation of Chemical Engineering (EFCE), 4th European Workshop on Food Engineering and Technology. Belgrade, Serbia.

RESEARCH GRANTS

08/2008 Travel grant of the International Union of Food Science & Technology for participation at 14th World Congress of Food Science & Technology 2008. Shanghai, China.

10/2008 Travel grant of the Australia's Commonwealth Scientific and Industrial Research Organisation (CSIRO) for participation at FIESTA 2008 - Food Innovation: Emerging Science, Technologies and Applications, 4th Innovative Foods Conference. Brisbane, Australia.

ORGANIZATION OF INTERNATIONAL SCIENTIFIC CONFERENCES

11/2011 EFFoST Conference 2011. European Federation of Food Science and Technology Annual Meeting. Berlin, Germany.
Member of the local organizing committee.

09/2010 BerlinFood 2010 – European PhD Conference on Food Science and Technology. Berlin, Germany.
Member of the local organizing committee.

02/2010 ISP-PhD Congress 2010. International Society on Pulsed Power Applications 1st International PhD Congress. Karlsruhe, Germany.
In charge of the congress organization.

REVIEWER ACTIVITIES

Innovative Food Science and Emerging Technologies; Journal of Food Engineering; LWT-Food Science and Technology; International Journal of Food Microbiology; Italian Journal of Food Science; The Encyclopedia of Biotechnology in Agriculture and Food; Journal of the Science of Food and Agriculture; Journal of Food Science and Technology; Food Microbiology; Journal of Agricultural and Food Chemistry; International Dairy Journal; International Journal of Food Science and Technology

CO-SUPERVISION OF DIPLOMA THESIS

- Protective effect of food constituents during PEF pasteurisation
- Preservation of human milk with PEF - impact on bioactive compounds
- Modelling of electric field and thermal effects during PEF-treatment
- PEF assisted soy protein extraction
- PEF assisted recovery of fruit and vegetable juices
- Controlling ethanol yield and methanol content in distillates of fermented apple pomace
- Impact of electroporation pre-treatment on sugar distribution in apple tissue after drying
- Process- and storage stability of anthocyanins in blueberry products
- Establishing a suspension culture of *Vaccinium myrtillus*
- Impact of chemical and physical stress factors on glucosinolate production in *Brassica* sprouts
- Process stability of glucosinolates in *Brassicaceae* and impact of myrosinase activity
- Effect of isostatic high pressure treatment and pH on myrosinase activity in *Brassicaceae*
- Coating of fruits for the reduction of water loss during thawing
- Spray drying of probiotics – stabilisation effect of milk constituents
- Impact of physical pre-treatments on enzymatic cross-linking of milk and soy proteins
- Size reduction of fibre particles in soy bean slurry
- Application of non-thermal atmospheric pressure plasma for surface decontamination of fruits
- Application of electronically activated water for sanitation in the food industry

TEACHING ACTIVITY

- Emerging technologies in food processing (integrated seminar)
- Fruit juice technology (practical course)
- Recovery of food constituents (practical course)
- Food additives (integrated seminar)
- Convenience products (integrated seminar)

MEMBERSHIP IN SCIENTIFIC ORGANIZATIONS

- German Association of Food Technologists (GDL e.V.)
- International Society on Pulsed Power Applications (ISP e.V.)
- European Federation of Food Science and Technology (EFFoST), Special Interest Group Young Scientists

INTERNSHIP EXPERIENCE

12/2005 –	Hielscher Ultrasonics GmbH, Teltow, Germany
03/2006	Manufacturer of ultrasound equipment, student assistant in the R&D lab
08/2004 –	Fonterra Co-operative Group, Hawera and Whareroa site, New Zealand
12/2004	Dairy company, intern student, section milk powder, milk fat and cheese
08/2003 –	Milchwirtschaftliche Lehr- und Untersuchungsanstalt (MLUA)
09/2003	Oranienburg, Germany. Dairy technology centre, intern student
02/2003 –	Bäckerei Raddatz GmbH, Gröditz, Germany
03/2003	Bakery, intern student, section bread and cake production
09/2001	Heinrichsthaler Milchwerke GmbH, Radeberg, Germany Dairy factory, intern student, section cheese and butter production
07/2000 –	Campina GmbH, Elsterwerda, Germany
09/2000	Dairy factory, intern student, section yoghurt and dessert production

LANGUAGES

German	Native
English	Fluent
French	Fair

SCHOOL EDUCATION

1992-1999	Manfred-von-Ardenne-Gymnasium Riesa/Saxony, Germany High school, final grade *1.2 (very good)*
1987-1992	Elementary school

DATE AND PLACE OF BIRTH

11/09/1980 Riesa/Saxony, Germany
Nationality German

CONTACT DETAILS

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ANNEX

List of Publications

Berlin, 08.08.2011

PUBLICATIONS

BOOK CONTRIBUTIONS (8)

Jaeger, H., Meneses, N. and Knorr, D. (2012), Pulsed electric field technology, In: Encyclopedia of Food Safety, Motarjemi, Y., Gorris, L. (Eds.), Elsevier: Oxford, submitted.

Schlueter, O., Meda, V. and Jaeger, H. (2012). Emerging technologies: Non-thermal processing. In: ISEKI Food Series: Food Processing. Kristbergsson, K., Otles, S. (Eds.). Springer: Heidelberg, submitted.

Jaeger, H., Reineke, K., Schoessler, K. and Knorr, D. (2012), Effects of emerging processing technologies on food material properties, In: Food Materials Science and Engineering, Bhandari, Bhesh; Roos, Yrjo (Eds.), Wiley-Blackwell: Indianapolis, in press.

Meneses, N., Jaeger, H. and Knorr, D. (2011), Computational Fluid Dynamics Applied in Pulsed Electric Field Preservation of Liquid Foods, In: Innnovative Food Processing Technologies - Advances in Multiphysics Simulation. Knoerzer, Kai; Juliano, Pablo; Roupas, Peter; Versteeg, Cornelis (Eds), ISBN 9780813817545, Wiley-VCH: Weinheim.

Meneses, N., Jaeger, H. and Knorr, D. (2011), Basics for Modeling of Pulsed Electric Field Processing of Foods, In: Innnovative Food Processing Technologies - Advances in Multiphysics Simulation. Knoerzer, Kai; Juliano, Pablo; Roupas, Peter; Versteeg, Cornelis (Eds), ISBN 9780813817545, Wiley-VCH: Weinheim.

Jaeger, H. and Knorr, D. (2010), Pulsed Electric Fields (PEF): Mass Transfer Enhancement, In: Encyclopedia of Agricultural, Food, and Biological Engineering, Heldman, D.R. (Ed.), Taylor and Francis: London.

Knorr, D., Balasa, A., Boll, D., Jaeger, H., Mathys, A., Oba, E., Richter, J. and Volkert, M. (2009), Alternative Processing Methods for Functional Foods, In: An integrated Approach to New Food Product Development. Moskowitz, Howard; Saguy, Sam; Straus, Tim (Eds.), Taylor and Francis: Boca Raton.

Jaeger, H., Balasa, A. and Knorr, D. (2008), Food Industry Applications for Pulsed Electric Fields, In: Electrotechnologies for Extraction from Food Plants and Biomaterials, Series: Food Engineering Series, Vorobiev, Eugene; Lebovka, Nikolai (Eds.), Springer: New York.

JOURNAL ARTICLES - Peer reviewed (20)

2011

Jaeger, H., Schulz, M., Lu, P., Knorr, D. (2011). Adjustment of milling, mash electroporation and pressing for the development of a PEF assisted juice production in industrial scale. Innovative Food Science and Emerging Technologies, submitted. Impact factor 2.174

Schoessler, K., Jaeger, H., Knorr, D. (2011). Effect of continuous and intermittent ultrasound on drying time and effective diffusivity during convective drying of apple and red bell pepper. Journal of Food Engineering, accepted manuscript in press. Impact factor 2.081

Barba, F., Jaeger, H., Meneses, N., Knorr, D. (2011). Impact of High Pressure and Pulsed Electric Fields Processing on Physicochemical and Nutritional Characteristics of Blueberry Juice during Refrigerated Storage. LWT-Food Science and Technology, submitted. Impact factor 2.292

Meneses, N., Saldana, G., Jaeger, H., Raso, J., Lanzarote, I., Sebrian, G., Knorr, D. (2011). Modelling of polyphenoloxidase inactivation by pulsed electric fields considering coupled effects of temperature and electric field. Journal of Agricultural and Food Chemistry, submitted. Impact factor 2.470

Meneses, N., Jaeger, H. and Knorr, D. (2011). pH-Changes during Pulsed Electric Field Treatments – Numerical Simulation and in situ Impact on Polyphenoloxidase Inactivation. Innovative Food Science and Emerging Technologies, accepted. Impact factor 2.174

Meneses, N., Krauss, J., Jaeger, H., Ertunc, Ö., Rauh, C., Knorr, D. and Delgado, A. (2011). Modeling, simulation, optimization and impact measurement of pulsed electric field processing: A review. Trends in Food Science and Technology, submitted. Impact factor 4.051

Meneses, N., Jaeger, H. and Knorr, D. (2011). Minimization of thermal impact by application of electrode cooling in a co-linear PEF treatment chamber. Journal of Food Science, accepted. Impact factor 1.601

Knorr, D., Froehling, A., Jaeger, H., Reineke, K., Schlueter, O., Schoessler, K. (2011) Emerging technologies in food processing. Annu. Rev. Food Sci. Technol. 2: 203-235.

Meneses, N., Jaeger, H., Moritz, J. and Knorr, D. (2011). Impact of insulator shape, flow rate and electrical parameters on inactivation of E. coli using a continuous co-linear PEF system. Innovative Food Science & Emerging Technologies 12: 6-12. Impact Factor 2.174

Moritz, J., Balasa, A., Jaeger, H., Meneses, N. and Knorr, D. (2011). Investigating the potential of polyphenol oxidase as a temperature-time-indicator for pulsed electric field treatment. Food Control, submitted. Impact factor 2.463

JOURNAL ARTICLES - Peer reviewed (continued)

2010

Cai, Z., Riedel, H., Thaw Saw, N.M., Kütük, O., Mewis, I., Jäger, H., Knorr, D. and Smetanska, I. (2010). Effects of Pulsed Electric Field on Secondary Metabolism of Vitis vinifera L. cv. Gamay Fréaux Suspension Culture and Exudates. Applied Biochemistry and Biotechnology 4: 443-453. Impact factor 1.420

Jaeger, H., Meneses, N., Moritz, J. and Knorr, D. (2010). Model for the differentiation of temperature and electric field effects during thermal assisted PEF processing. Journal of Food Engineering 100:109-118. Impact factor 2.081

Jaeger, H., Janositz, A. and Knorr, D. (2010). The Maillard reaction and its control during food processing. The potential of emerging technologies. Pathologie Biologie 58:207-213. Impact factor 0.960

2009

Jaeger, H., Schulz, A., Karapetkov, N. and Knorr, D. (2009). Protective effect of milk constituents and sublethal injuries limiting process effectiveness during PEF inactivation of Lb. rhamnosus. International Journal of Food Microbiology 134:154-161. Impact Factor 2.581

Jaeger, H., Meneses, N. and Knorr, D. (2009). Impact of PEF treatment inhomogeneity such as electric field distribution, flow characteristics and temperature effects on the inactivation of E. coli and milk alkaline phosphatase. Innovative Food Science & Emerging Technologies 10:470-480. Impact Factor 2.174

Forina, M., Oliveri, P., Jäger, H., Römisch, U., and Smeyers-Verbeke, J. (2009). Class modeling techniques in the control of the geographical origin of wines. Chemometrics and Intelligent Laboratory Systems, 99(2), 127-137. Impact factor 1.940

Römisch, U., Jäger, H., Capron, X., Lanteri, S., Forina, M., and Smeyers-Verbeke, J. (2009). Characterization and determination of the geographical origin of wines. Part III: multivariate discrimination and classification methods. European Food Research and Technology, 230(1), 31-45. Impact factor 1.622

Smeyers-Verbeke, J., Jäger, H., Lanteri, S., Brereton, P., Jamin, E., Fauhl-Hassek, C., Forina, M., and Römisch, U. (2009). Characterization and determination of the geographical origin of wines. Part II: descriptive and inductive univariate statistics. European Food Research and Technology, 230(1), 15-29. Impact factor 1.622

2008

Schilling, S., Schmid, S., Jäger, H., Ludwig, M., Dietrich, H., Toepfl, S., Knorr, D., Neidhart, S., Schieber, A. & Carle R. (2008). Comparative study of pulsed electric field and thermal processing of apple juice with particular consideration of juice quality and enzyme deactivation, J. Agric. Food Chem. 56(12), p. 4545-4554. Impact Factor 2.532

JOURNAL ARTICLES - Peer reviewed (continued)

2007

Roemisch, U.; Jaeger, H. and Vandev, D. (2007). Application of Regularized Discriminant Analysis. Pliska Stud. Math. Bulgar., 18, 327-339

JOURNAL ARTICLES - Others (3)

Schulz, M., Jäger, H., (Lu, P.), Knorr, D. (2011). Abstimmung von Zerkleinerungsgrad, Elektroporation und Entsaftungssystem auf die Saftausbeute von Apfel- und Karottensaft – Part 1 and 2. In: Flüssiges Obst, confructa medien GmbH, Flüssiges Obst 06-2011 and 07-2011, 222-227 and 268-272

Jäger, H., Knorr, D. (2007). Einsatzmöglichkeiten gepulster elektrischer Felder zur Haltbarmachung hitzeempfindlicher Produkte in der Milchindustrie – Part 1 and 2. In: Deutsche Molkerei Zeitung – DMZ, AVA Agrar-Verlag Allgäu GmbH, dmz 24/25, 22-25

Töpfl S.; Jäger H.;Heinz V.;Knorr D. (2006). Neues Verfahren zur Haltbarmachung von Milch. In: Deutsche Molkerei Zeitung - DMZ, AVA Agrar-Verlag Allgäu GmbH, dmz 2, 24-28

PROCEEDINGS (12)

Jaeger, H., Schulz, M., Lu, P., Knorr, D. (2011). Adjustment of milling, mash electroporation and pressing for the development of a pulsed electric field (PEF) assisted juice processing in industrial scale. In: Proceedings of the International Congress on Engineering and Food 2011 (ICEF11). Taoukis, P.S., Stoforos, N.G., Karathanos, V.T., Saravacos, G.D. (Eds). ISBN 978-960-89789-3-5. Athens, Greece.

Moussa-Ayoub, T., Jaeger, H., Knorr, D., El-Samahy, S., Rohn, S., Kroh, L.W. (2011). Impact of traditional and innovative technologies on some characteristics and bioactive compounds of Opuntia macrorhiza juice. In: Proceedings of the International Congress on Engineering and Food 2011 (ICEF11). Taoukis, P.S., Stoforos, N.G., Karathanos, V.T., Saravacos, G.D. (Eds). ISBN 978-960-89789-3-5. Athens, Greece.

Meneses, N., Jaeger, H., Knorr, D. (2011). Understanding enzyme inactivation mechanisms during pulsed electric field treatments. In: Proceedings of the International Congress on Engineering and Food 2011 (ICEF11). Taoukis, P.S., Stoforos, N.G., Karathanos, V.T., Saravacos, G.D. (Eds). ISBN 978-960-89789-3-5. Athens, Greece.

Knorr, D., Jaeger, H., Reineke, K., Schoessler, K., Schlueter, O. (2011). Emerging technologies for targeted food processing. In: Proceedings of the International Congress on Engineering and Food 2011 (ICEF11). Taoukis, P.S., Stoforos, N.G., Karathanos, V.T., Saravacos, G.D. (Eds). ISBN 978-960-89789-3-5. Athens, Greece.

PROCEEDINGS (continued)

Barba, F., Esteve, M.J., Jaeger, H., Meneses, N., Knorr, D., Frigola, A. (2011). Color Modifications in Fruit Juice Mixed with Milk Beverages Processed by Different Non-Thermal Technologies. In: Proceedings of the 6th International CIGR Technical Symposium – Section 6, Towards a Sustainable Food Chain, Food Process, Bioprocessing and Food Quality Management. Nantes, France.

Meneses, N., Jaeger, H., Reineke, K., Schoessler, K. and Knorr, D. (2010). Simulation of non-thermal technologies. In: Proceedings of the International Conference on Food Innovation - foodInnova2010. Fito, P., Toldrá, F. (Eds.). ISBN 978-84-693-5010-2.Valencia, Spain.

Barba, F.J., Meneses, N., Jaeger, H., Esteve, M.J., Frígola, A. and Knorr, D. (2010). Impact of pulsed electric fields on colour modifications in blueberry juice during refrigerated storage. In: Proceedings of the International Conference on Food Innovation - foodInnova2010. Fito, P., Toldrá, F. (Eds.). ISBN 978-84-693-5010-2. Valencia, Spain.

Barba, F.J., Meneses, N., Jaeger, H., Esteve, M.J., Frígola, A. and Knorr, D. (2010). Impact of high pressure processing on total anthocyanins, monomerics and polymerics in blueberry juice. In: Proceedings of the International Conference on Food Innovation - foodInnova2010. Fito, P., Toldrá, F. (Eds.). ISBN 978-84-693-5010-2. Valencia, Spain.

Barba, F.J., Meneses, N., Jaeger, H., Esteve, M.J., Frígola, A. and Knorr, D. (2010). Colour modifications in orange juice milk processed by ultrasound. In: Proceedings of the International Conference on Food Innovation - foodInnova2010. Fito, P., Toldrá, F. (Eds.). ISBN 978-84-693-5010-2. Valencia, Spain.

Jaeger, H., Zwiens, C., Regier, M., Knorr, D. (2010). Electroporation of apple cubes affecting drying rate, water and fructose distribution during hot air drying. In: Proceedings of the 17th International Drying Symposium (IDS 2010). Tsotsas, E., Metzger, T., Peglow, M. (Eds.), Mujumdar, A.S. (Series Ed.). ISBN: 978-3-86912-037-9. Magdeburg, Germany.

Jaeger, H., Meneses, N. and Knorr, D. (2009). Pulsed electric field preservation of heat sensitive products - food safety and qualtiy aspects. In: Proceedings of the International Conference on Bio and Food Electrotechnologies (BFE). Vorobiev, E., Lebovka, N., Van Hecke, E., Lanoiselle, J.-L. (Eds.). ISBN 978-2-913923-31-7. Compiègne, France.

Meneses, N., Jaeger, H., Moritz, J. and Knorr, D. (2009). Simulation and optimization of PEF treatment chamber geometry considering different processing conditions. In: Proceedings of the International Conference on Bio and Food Electrotechnologies (BFE). Vorobiev, E., Lebovka, N., Van Hecke, E., Lanoiselle, J.-L. (Eds.). ISBN 978-2-913923-31-7. Compiègne, France.

PARTICIPATION AT SCIENTIFIC CONFERENCES

INVITED TALKS (8)

Nicht-thermische Konservierungsverfahren zum Erhalt sensorischer und funktioneller Lebensmitteleigenschaften. DLG-Forum Taste meets Technology. Anuga FoodTec. March 2009, Cologne, Germany.

La reaction Maillard et l'industrie alimentaire – Can the food processing industry optimize its practices? Société Francaise des Antioxydants, Réaction de Maillard, Glycation & Industrie Agro-Alimentaire. December 2008, Paris, France.

Nonthermal food preservation throughout a hurdle approach. NPD-IFT/Effost Joint Workshop: Innovative Applications of Nonthermal Technologies in Foods, November 2008. Madrid, Spain.

Hochspannungsimpulse - Zellaufschluss und Stressinduktion zur Gewinnung von Sekundärmetaboliten aus pflanzlichen Materialien. Forschungskolloquium „Aktuelle Aspekte in der Ernährungs- und Lebensmittelforschung". Institut für Humanernährung und Lebensmittelkunde, Christian-Albrechts-Universität zu Kiel, November 2008, Kiel, Germany.

PEF treatment – evaluation and improvement of process effectiveness. Seminar given at Food Science Australia, Innovative Foods Centre. 2008, Werribee/Melbourne, Australia.

New technologies for a gentle food processing.
Journées Aliment & Santé, 7ième edition, June 2008, La Rochelle, France.

PEF-Behandlung von Lebensmitteln – Anwendungen und rechtliche Zulassung; International Society on Pulsed Power Applications e.V.; 11th General Meeting, 15 February 2008, Gelsenkirchen, Germany.

Die Entwicklung der Hochspannungsimpulstechnik – Ein Überblick
DIL e.V. Seminar: Pulsed Electric Field Processing, October 2007, Quakenbrück, Germany.

PRESENTATIONS (12)

2011

Adjustment of milling, mash electroporation and pressing for the development of a pulsed electric field (PEF) assisted juice processing in industrial scale. International Congress on Engineering and Food 2011 (ICEF11). May 2011, Athens, Greece.

2010

Electroporation of apple cubes affecting drying rate, water and fructose distribution during hot air drying. 17th International Drying Symposium (IDS 2010). October 2010, Magdeburg, Germany.

PRESENTATIONS (continued)

Juice factory of the future – Adjustment of milling, mash electroporation and pressing for the development of a pulsed electric field (PEF) assisted juice production in industrial scale. Food Factory 2010 5th International Conference on the Food Factory for the Future. June 2010, Gothenburg, Sweden.

Pulsed electric fields for the gentle preservation of liquid foods – process analysis and optimization for the minimization of thermal effects. The 4th European Workshop on Food Engineering and Technology. European Federation of Chemical Engineering (EFCE), Section Food. May 2010, Belgrade, Serbia.

2009

Pulsed electric field preservation of heat sensitive products – Food safety and quality aspects. International Conference Bio & Food Electrotechnologies (BFE 2009). October 2009, Compiegne, France.

Pulsed electric field assisted extraction of plant raw materials. 5th CIGR International Technical Symposium on Food Processing, Monitoring Technology in Bioprocesses and Food Quality Management. September 2009, Potsdam, Germany.

Non-thermal technologies for energy efficient cell disintegration of plant raw materials and by-products. Total Food 2009, Sustainability of the Agri-Food Chain. April 2009, Norwich, UK.

Gepulste elektrische Felder zur Keiminaktivierung in Lebensmitteln – Prozessanalyse und Optimierung von Behandlungszellen. DECHEMA VDI-GVC ProcessNet Jahrestreffen des Fachausschusses Lebensmittel-Verfahrenstechnik. March 2009, Lausanne, Switzerland.

2008

PEF for the recovery and gentle preservation of phyto- and lacto-bioactives. EFFoST First European Food Congress 2008. November 2008, Ljubljana, Slovenia.

Pulsed electric fields – non-thermal technology for cell disintegration and food preservation. 14th World Congress of Food Science and Technology 2008. October 2008, Shanghai, China.

Pulsed electric field technology for non-thermal inactivation of pathogens and spoilage bacteria in heat sensitive products. FOOD MICRO 2008 Evolving microbial food quality and safety. September 2008, Aberdeen, UK.

2007

Betrachtung neuer lebensmitteltechnologischer Verfahren nach Novel-Food-Verordnung am Beispiel der Milchkonservierung mittels Hochspannungsimpulsen.
GDL-Kongress Lebensmitteltechnologie. October 2007, Hamburg, Germany.

POSTER PRESENTATIONS (30)

2011

Jaeger, H., Froehling, A., Gruber, A., Schlueter, O., Schulz, A., Voigt, E., Knorr, D. (2011). Bactericidal effects of electrochemically activated water – inactivation kinetics and mechanisms. ISEKI_Food 2011 2nd International ISEKI_Food Conference. Milan, Italy. *Accepted.*

Surowsky, B., Zuelicke, F., Jaeger, H., Schlueter, O., Knorr, D. (2011). Impact of cold plasma on quality-determining ingredients in apple juice. ISEKI_Food 2011 2nd International ISEKI_Food Conference. Milan, Italy. *Accepted.*

Martinez Osorio, A., Meneses, N., Barrena, J., Jäger, H., Knorr, D. (2011). Application of nonthermal technologies for the processing of Camu Camu (Myrciaria dubia). Tropentag 2011 International research on food security, natural resource management and rural development – Development on the margin. Bonn, Germany. *Accepted*

Luettich, K., Jaeger, H., Knorr, D. (2011). Verbesserte Gewinnung und Verarbeitung diätetischer Glucosinolate sowie Charakterisierung ihrer potenziellen Funktion in der Prävention von Darmkrebs – Glucosinolatgewinnung aus Brokkolisprossen nach Vorbehandlung mit Ohmschen Erhitzen *(Improving dietary glucosinolate production, processing and characterization of potential health effects for the prevention of colon cancer – Recovery of glucosinolates from broccoli sprouts after Ohmic heating).* 2nd BMBF Statusseminar, Federal Ministry of Education and Research. Potsdam, Germany.

2010

Jaeger, H., Zwiens, C., Regier, M., Knorr, D. (2010). Drying rate and soluble solute distribution in apple cubes during hot air drying affected by an electroporation pre-treatment. 2010 EFFoST Annual Meeting. Dublin, Ireland.

Meneses, N., Jaeger, H., Knorr, D. (2010). Improvement of Pulsed Electric Field Treatment Considering Retention of Heat Sensitive Compounds. 2010 EFFoST Annual Meeting. Dublin, Ireland.

Jaeger, H., Froehling, A., Voigt, E., Schulz, A., Gruber, A., Schlueter, O., Knorr, D. (2010). Microbial inactivation by electrochemically activated water – mechnisms of action on a cellular level. BerlinFood 2010 – European PhD Conference on Food Science and Technology. Berlin, Germany.

Luettich, K., Jaeger, H., Sebastian, K., Tuenge, R., Knorr, D. (2010). Glucosinolate production from broccoli (*Brassica oleracea var. italic*) sprouts after the application of high pressure. BerlinFood 2010 – European PhD Conference on Food Science and Technology. Berlin, Germany.

Surowsky, B., Jaeger, H., Schlueter, O., Knorr, D. (2010). Impact of non-thermal atmospheric pressure plasma on Vitamin C. BerlinFood 2010 – European PhD Conference on Food Science and Technology. Berlin, Germany.

POSTER PRESENTATIONS (continued)

Meneses, N., Jaeger, H., Knorr, D. (2010). Temperature control in pulsed electric field treatments. BerlinFood 2010 – European PhD Conference on Food Science and Technology. Berlin, Germany.

Hartyani, P., Dalmadi, I., Cserhalmi, Zs., Jaeger, H., Knorr, D. (2010). Physico-chemical and sensory properties of pulsed electric field and high pressure treated apple and orange juice. BerlinFood 2010 – European PhD Conference on Food Science and Technology. Berlin, Germany.

Meneses, N., Jaeger, H., Knorr, D. (2010). pH-changes during pulsed electric field treatments - Numerical simulation and in situ impact on polyphenoloxidase inactivation. Food Innovation: Emerging Science, Technologies and Applications - FIESTA 2010, 5th Innovative Foods Centre Conference. 2010. Melbourne, Australia

Jaeger, H., Meneses, N., Knorr, D. (2010). PEF preservation of raw milk – impact on milk proteins. IFT Annual Meeting 2010. Chicago, USA.

Meneses, N., Jaeger, H., Knorr, D. (2010). Design of pulsed electric treatment chambers. IFT Annual Meeting 2010. Chicago, USA.

Meneses, N., Jaeger, H., Knorr, D. (2010). Pulsed electric field processing – Modelling and design. Food Factory 2010 5th International Conference on the Food Factory for the Future. Gothenburg, Sweden.

Meneses, N., Jaeger, H., Knorr, D. (2010). Enzymes as temperature-time indicators and integrators for pulsed electric field processing. Final workshop COST 298. Naples, Italy.

2009

Meneses, N., Jaeger, H., Barba, F., Saldaña, G., Raso, J., Knorr, D. (2009). Modelling and Simulation of Pulsed Electric Field Considering Enzyme Inactivation. 2009 EFFoST Annual Meeting - New Challenges in Food Preservation. Budapest, Hungary.

Meneses, N., Jaeger, H., Moritz, J., Knorr, D. (2009). Comparison between Pulsed Electric Field and Thermal Effects during Lactoperoxidase and Alkaline Phosphatase Inactivation. International Conference Bio & Food Electrotechnologies (BFE 2009). Compiegne, France.

Jaeger, H. and Knorr, D. (2009). Pulsed electric fields to control microbial growth in raw milk. 5th CIGR International Technical Symposium on Food Processing, Monitoring Technology in Bioprocesses and Food Quality Management. Potsdam, Germany

Meneses, N., Jaeger, H., Knorr, D. (2009). Pulsed electric field assisted extraction of secondary plant metabolites. Total Food 2009, Sustainability of the Agri-Food Chain. Norwich, UK.

POSTER PRESENTATIONS (continued)

2008

Jaeger, H., Meneses, N., Moritz, J., Knorr, D. (2008). Differentiation of thermal and electric field effects occurring during PEF pasteurisation. IFT-NPD/EFFoST Nonthermal Processing Workshop: Innovative Applications of nonthermal technologies in foods: Technology, safety, health and consumer acceptability. Madrid, Spain.

Meneses, N., Jaeger, H., Knorr, D. (2008). Improvement of PEF treatment by insertion of a static mixing device in the treatment chamber. IFT-NPD/EFFoST Nonthermal Processing Workshop: Innovative Applications of nonthermal technologies in foods: Technology, safety, health and consumer acceptability. Madrid, Spain.

Meneses, N., Jaeger, H., Knorr, D. (2008). CFD and heat transfer in pulsed electric field treatments. EFFoST First European Food Congress. Ljubljana, Slovenia.

Jaeger, H., Meneses, N., Moritz, J., Knorr, D. (2008). Impact of PEF on enzymes – Role of electric field and coupled thermal effects considering treatment chamber geometry and processing conditions. Food Innovation: Emerging Science, Technologies and Applications - FIESTA 2008, 4th Innovative Foods Centre Conference. Brisbane, Australia.

Jaeger, H., Meneses, N., Moritz J., Knorr, D. (2008). Pulsed electric fields technology - a forward looking method for gentle, non-thermal preservation of food. Food Factory 2008 4th International Conference on the Food Factory for the Future. Laval, France.

2007

Jaeger, H., Pfister, M. K.-H. & Knorr, D. (2007). Einfluss der Hochspannungsimpuls-Konservierung von Milch auf Milchproteine *(Impact of PEF preservation of raw milk on milk proteins)*. DECHEMA Tagung Lebensmittelwissenschaft im Fokus, Frankfurt, Germany.

Sienkiewicz, T., Jaeger, H., Driemel, G., Kroh, L.W., Knorr, D. (2007). Einfluss physikalischer und chemischer Vorbehandlungen auf die Vernetzbarkeit von Milchproteinen mit Transglutaminase *(Impact of physical and chemical pre-treatments on cross-linking behavior of milk proteins using transglutaminase)*. Milchkonferenz 2007. Vienna, Austria.

Sienkiewicz, T., Jaeger, H., Driemel, G., Kroh, L.W., Knorr, D. (2007). Vernetzung von Proteinen zur Verbesserung der Adsorption an anorganischen Oberflächen *(Cross-linking of proteins to improve adsorption properties on anorganic surfaces)*. GDL Kongress Lebensmitteltechnologie. Hamburg, Germany.

Jaeger, H., Schulz, A., Raschke, D., Knorr, D. (2007). Pulsed electric fields-utilization for the induction of stress response, cell permeabilization and microbial inactivation. VAAM annual meeting. Osnabrück, Germany.

2006

Toepfl, S., Jaeger, H., Heinz, V., Knorr, D. (2006). Milk preservation by pulsed electric fields-utilization of native antimicrobial activity. IUFOST, Nantes, France.

Eidesstattliche Erklärung

Hiermit versichere ich an Eides statt, dass ich die Dissertation selbständig verfasst habe. Alle benutzten Hilfsmittel und Quellen sind aufgeführt.

Weiter erkläre ich, dass ich nicht schon anderweitig einmal die Promotionsabsicht angemeldet oder ein Promotionseröffnungsverfahren beantragt habe.

Veröffentlichungen von irgendwelchen Teilen der vorliegenden Dissertation sind von mir, wie in der vorstehenden Publikationsliste aufgeführt, vorgenommen worden.

Berlin, 8.8.2011

Henry Jäger