Impact of Pulsed Electric Fields (PEF) on post-permeabilization processes in plant cells

vorgelegt von

Diplom-Ingenieurin

Anna Winter

von der Fakultät III – Prozesswissenschaften

der Technischen Universität Berlin

zur Erlangung des akademischen Grades

Doktorin der Ingenieurwissenschaften

- Dr.-Ing. -

genehmigte Dissertation

Promotionsausschuss:

Vorsitzender: Prof. Dr. Dipl.-Ing. Frank-Jürgen Methner

1. Berichter: Prof. Dr. Dipl.-Ing. Dietrich Knorr

2. Berichter: Prof. Dr. Sc. agr. Monika Schreiner

Tag der wissenschaftlichen Aussprache: 06.06.2011

Berlin 2011

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Für Oliver, Mae und Milla

Abstract

The exposure of biological cell material to Pulsed Electric Fields (PEF) leads to a spectrum of biophysical and biochemical responses. The most important effect, the electrical breakdown of cellular membranes, realizes the temporary or permanent pore formation in cell membranes, which induces an increase in membrane permeability. The loss of semipermeability enables the transport of non-permeating molecules across the cell membrane. The disintegration of the cell membrane as well as the alteration of structural properties offers numerous options to apply this novel, non-thermal and short-time technique in food- and bioengineering. In this thesis the impact of PEF on plant single cells as well as on vegetable tissues was investigated. In order to understand underlying mechanisms at cellular level and to clarify the influence of cell wall on the degree of cell membrane disintegration, protoplasts from cultured tobacco cells (Nicotiana tabacum b.y.-2) and cells with cell wall were compared during and after reversible as well as irreversible PEF treatment. Results showed higher sensitivity of protoplasts to electric fields related to native cells. Protoplasts sizes were measured before and after different treatment intensities and protoplasts shrinkage was used as an indicator for cell rupture. It could be demonstrated that cell volume decrease is influenced by PEF intensity, initial cell size, cell orientation in the electric field and nucleus position. Focus was also put on the potential of PEF to gentle disintegrate plant tissue and thus to apply this technique in food industry. Hence, the enhancement of mass transfer after irreversible membrane permeabilization from potato and asparagus tissue was examined. Results showed the enhanced release of intracellular molecules from permeabilized tissue as well as improved uptake of low molecular substances into the sample. Sugar, one substrate for the Maillard reaction, was decreased in PEF treated potatoes due to membrane permeabilization and the subsequent release of cell vacuole sugar, while conductivity increased after electroporation and soaking in sodium chloride solution, indicating the improved diffusion of salt caused by PEF. Higher release of cell liquid during drying was noticed additionally. This effect increased with the treatment intensity. Furthermore, it was revealed that PEF application leads to a significant reduction of fat content after deep fat frying of potato stripes and thus provides a potential for the production of low-fat French fries. It was noticed additionally that PEF treatment decreases the content of the biopolymer lignin in white asparagus in order to improve macroscopic characteristics and gain softer texture of the spears. It can be presumed that PEF is a capable assistance to thermal treatments in the processing of potato snack products or in the preserving of asparagus for the achievement of structural modifications and the improvement of process conditions.

Zusammenfassung

Der hochspannungsimpulsinduzierte Aufschluss der Zellmembran und die daraus folgende Änderung der strukturellen Eigenschaften bergen großes Potential für die Anwendung dieses nicht-thermischen und zeiteffektiven Verfahrens in der Bio- und Lebensmitteltechnologie.

Ziel dieser Arbeit war es, den Einfluss von Hochspannungsimpulsen (HSI) auf einzelne Pflanzenzellen als auch auf pflanzliches Zellgewebe zu untersuchen. Zur Erforschung grundlegender Mechanismen auf zellulärer Ebene und zur Klärung des Einflusses der Zellwand auf den Grad der Zellmembranpermeabilisierung wurden Protoplasten kultivierter Tabakzellen (Nicotiana tabacum b.y.-2) und Zellen mit Zellwand bezüglich ihres Verhaltens während und nach reversibler als auch irreversibler HSI-Behandlung untersucht. Es konnte gezeigt werden, dass Protoplasten sich sensibler gegenüber dem elektrischen Feld verhalten als native Zellen. Die Zellgröße der Protoplasten wurde vor und nach verschiedenen HSI-Behandlungsintensitäten gemessen. Die Verringerung der Zellgröße diente als Indikator für den Grad des Zellaufschlusses. Es zeigte sich, dass die Reduktion des Zellvolumens von der HSI-Behandlungsintensität, der Ausgangszellgröße, der Zellorientierung im elektrischen Feld und der Position des Zellkerns abhängt. Zudem sollte das Potential elektrischer Felder zum milden Zellaufschluss von pflanzlichem Gewebe für einen möglichen Einsatz in der Spargel- und Kartoffelindustrie untersucht werden. Verbesserte Stofftransportvorgänge HSI-behandelter Kartoffeln führten sowohl zu einer erleichterten Freigabe von intrazellulären Molekülen als auch zu einer verbesserten Aufnahme von niedermolekularen Substanzen in das Gewebe. HSI-behandeltes Kartoffelgewebe zeigte einen geringeren Gehalt an reduzierendem Zucker, ein Substrat für die Maillard-Reaktion, was sich auf die erleichterte Freigabe des Vakuoleninhalts durch die permeabilisierte Zellmembran zurückführen lässt. Im Hinblick auf die verbesserte Molekülaufnahme in das aufgeschlossene Gewebe wurde eine erleichterte Diffusion von Salzionen in HSI-behandelte Kartoffelscheiben beobachtet. Zusätzlich erhöhte sich der Trocknungsgrad permeabilisierter Kartoffelscheiben mit steigender HSI-Behandlungsintensität. Eine Fettextraktion frittierter Kartoffelstäbchen zeigte, dass eine HSI-Vorbehandlung der Fettaufnahme während des Frittierens entgegenwirkt. Der Einsatz von HSI bei der Herstellung fettreduzierter Pommes frites ist daher denkbar. Bei HSI-behandeltem Spargel war eine Reduzierung des Biopolymers Lignin nachweisbar. Dies könnte die ligninbedingte Verholzung der Spargelstangen bei der Verarbeitung vermindern. HSI-induzierte strukturelle Modifikationen und die dadurch verbesserten Prozessbedingungen lassen den Einsatz von HSI in der Kartoffel- und Spargelverarbeitungsindustrie als viel versprechend erscheinen.

Danksagung

Ich möchte mich an dieser Stelle bei all denen bedanken, ohne deren Hilfe diese Arbeit nicht möglich gewesen wäre.

Ich danke Herrn Prof. Dr. Dietrich Knorr sowohl für Überlassung des Themas und die fachliche Betreuung als auch für seine unkomplizierte und motivierende Art, die mich bei meiner Arbeit sehr unterstützte.

Ich danke Frau Prof. Dr. Monika Schreiner für ihre sofortige Bereitschaft als Gutachterin tätig zu sein und für ihre professionelle Hilfe beim Projektanträge schreiben.

Danke auch an meine Institutskollegen für ihre Hilfsbereitschaft und das freundliche Arbeitsklima. Liebe Ana, danke für die schöne und turbulente Zeit, die wir beim Teilen unserer Büros und beim Bearbeiten unserer Projekte hatten. Bok do Zagreb!

Ganz besonders danken möchte ich Paul und Suse Janositz, nicht nur für uneingeschränkte Unterstützung, sondern auch für ihre grenzenlose und liebevolle Bereitschaft für ihre Enkelinnen zu sorgen, ohne die ich meine Promotion nie hätte fertig stellen können.

Danke, danke, danke an meinen Mann Oliver und die zwei kleinen Mäuse für alles…

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List of Abbreviations

ADF	Acid Detergent Fibre
ADL	Acid Detergent Lignin
AOAC	Association of Official Analytical Chemists
a* value	sample position between red and green
b* value	sample position between yellow and blue
C	capacity (F)
CDI	cell disintegration index
Cl	Cellulases
d	electrode gap
DW	dry weight (g)
E	electric field strength (kV/cm)
f	Frequency (Hz)
Fc	form factor for cells with spherical shape (= 1.5)
IARC	International Agency for Research on Cancer
K_l ; K_l'	electrical conductivity of untreated and treated cell material in a low- frequency field (1-5 kHz)
K_h ; K_h`	electrical conductivity of untreated and treated material in a high- frequency field (3-50 MHz)
L* value	Lightness of a sample
m	sample mass (g)
n	pulse number
PEF	pulsed electric fields
PL	pectin lyases
PPO	polyphenol oxidase
T	pulse width (µs)
TUB	Berlin University of Technology
U	voltage (V)
V_m	transmembrane potential (V)
W	energy input (kJ/kg)
Wpulse	energy per pulse (J)
WC	Water content (g)
κ (T)	electric conductivity (mS/cm)

List of original Articles

This PhD thesis is based on the following publications, which are referred to by their Roman numerals in the text:

Article I Janositz, A. & Knorr, D. (2010). Microscopic visualization of Pulsed Electric Field induced changes on plant cellular level. *Innovative Food Science and Emerging Technologies,* 11, 592–597.

Article II Janositz, A., Noack, A.-K. & Knorr, D. (2011). Pulsed Electric Fields and their impact on the diffusion characteristics of potato slices. *LWT-Food Science and Technology,* 9, 1939-1945.

Article III Janositz, A., Semrau, J. & Knorr, D. (2011). Impact of PEF treatment on quality parameters of white asparagus (Asparagus officinalis L.). *Innovative Food Science and Emerging Technologies,* 12, 269-274.

1. Introduction

The effect of Pulsed Electric Fields (PEF) on cellular material has been one of the most interesting scientific research topics in food- and biotechnology since 1960. First efforts were made concerning the increase of plant tissue permeability (Doevenspeck, 1960) as well as the inactivation of microorganisms due to electroporation (Sale & Hamilton, 1967). The application of PEF involves the subject of biological cell material to a pulsed high voltage field for a very short time, inducing pore formation and subsequent permeabilization of the cell membrane. Electroporation can be reversible or permanent, dependent on the applied treatment intensity. Transient membrane permeabilization maintains cell viability and can be adopted in biotechnology and medicine for the delivery of drugs and genes into living cells (Gehl, 2003; Neumann et al., 1982). Irreversible cell disintegration results in the loss of cell vitality and presents an effective tool for mild pasteurization of liquid foods (Alvarez et al., 2006; Heinz et al., 1999; Jaeger et al., 2009) as well as for the enhancement of mass transfer effectiveness of intracellular substances (Ade-Omowaye et al., 2001a; Bazhal & Vorobiev, 2000; Chalermchat et al., 2004). Based on the various applications of PEF, the emerged non thermal processing method owns a great potential to assist or replace common thermal food manufacturing by producing fresh-like foods with less determinations on nutritional value and thus with a high standard of quality.

Many PEF-assisted operations as extraction, pressing or drying of cellular solid food are based on the irreversible electrical breakdown resulting in pore formation of the semi permeable cell membrane. Thus, mass transfer is positively affected during subsequent processing of food.

The effectiveness of PEF technology depends on several factors which can be classified in technical and chemical process conditions as well as in biological product characteristics. Besides technical factors, including PEF process parameters such as electric field intensity, treatment time, pulse shape and applied energy (Hülsheger et al., 1981; Tatebe et al., 1995; Zhang et al., 1994a) as well as chemical and physical characteristics of treated products, the biological aspects like species, cell size, shape or physiological state influence the degree of membrane permeabilization additionally. Small microorganisms cells were found to be less sensitive against the external electric field, whereas membrane disintegration of larger plant cells occurs in markedly higher percentage by applying same PEF treatment conditions (Sale & Hamilton, 1967).

Although, applications of PEF to improve and modify operations in commercial plant processing have been largely discussed in literature, knowledge about the influence factors of membrane permeabilization and the impact of the cell wall on the degree of cell rupture is still expandable. In order to tap the full potential of PEF technology in food- and bioengineering, it is required to obtain better insight of the PEF-induced changes in the structure of plant tissue at the basic cellular level.

The aim of this thesis is to gain better understanding of the PEF-induced changes in the structure of plant tissue with focus on (i) the permeabilization at cellular level during and after reversible as well as irreversible PEF treatment; (ii) the enhancement of mass transfer as a post-permeabilization process after irreversible cell membrane disintegration.

2. Background

2.1 Biological cell material

2.1.1 Plant tissue

The cell is the structural unit of living organisms (Virchow, 1858). All plant cells are surrounded and structured by a rigid cell wall, providing shape and strength to cell and protecting the plasma from external damage. The firm structure is based on a network of cellulose and hemicelluloses, being associated with pectic material. Each cell has usually one nucleus which is surrounded by cytoplasm. In higher plants the nucleus is enclosed by nuclear membrane. Up to 80 % of the entire plant cell compartment constitutes to parenchyma tissue. In ground tissue systems, large parenchyma plant cells are embedded in a matrix with intercellular spaces between cells and confined by cell walls which are in contact with neighbouring cell walls. Parenchyma cells consist of cytoplasm with plastids and large central fluid-filled vacuoles storing near high amounts of cell sap also ions, sugars, organic and amino acids and other substances. Plant vacuoles are enclosed by a membrane termed tonoplast, which controls the inner vacuole composition due to its highly selectivity in transporting only small molecules through the membrane phospholipid bilayer. Based on this separation the vacuole sap consistence can vary markedly from the cytoplasm content. The solutes in the vacuoles cause an influx of water resulting in the formation of a large internal pressure, the turgor pressure, in plant cells. The maintenance of turgor pressure leads to the rigidity and stability of plant tissue since the pressure is exerts from cell to cell, leading to a large tissue tension.

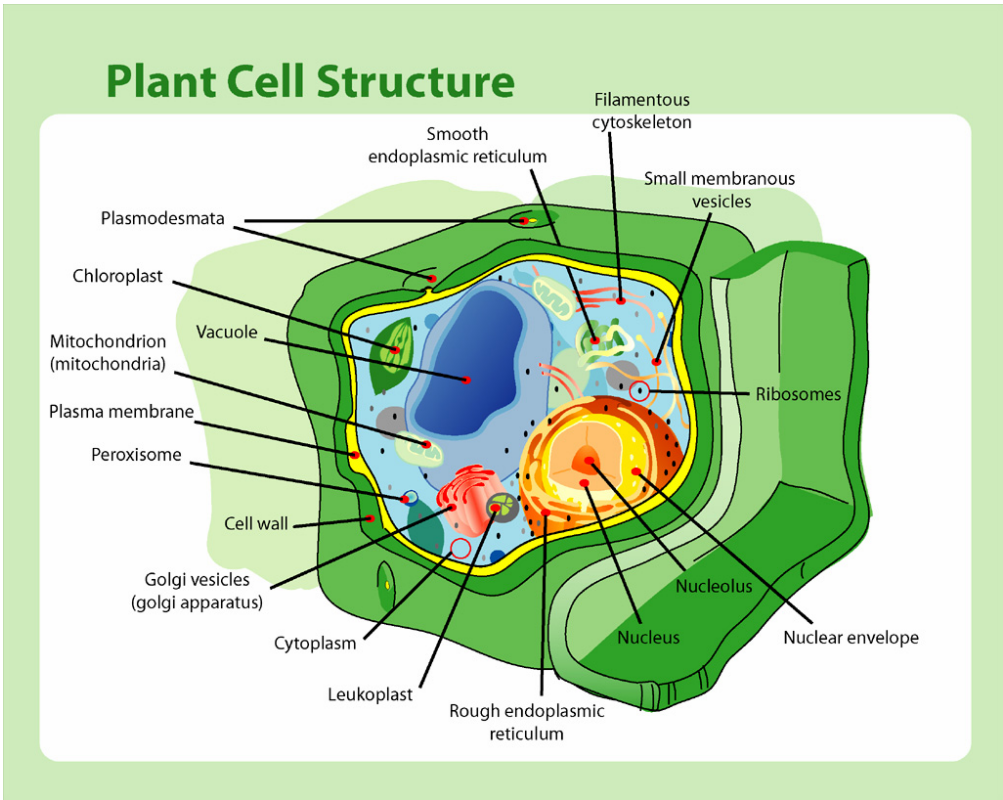

Figure 1: Plant cell structure (http://de.wikipedia.org/).

2.1.2 Plant cell culture

Plant cells, removed from tissues, are able to grow in-vitro if they are supplied with appropriate nutrients and conditions. Plant cell cultures are generally initiated from sterile parts of a whole plant and can be bred as cell suspension cultures in liquid medium or as callus cultures on solid medium. After initial cell division the cells volume increases and, in a batch culture, further expand until limited by some culture variable such as nutrient depletion. Main applications of plant cell cultures are the manufacturing of high-value secondary metabolites (Mewis et al., 2011; Krumbein et al., 2010; Endress, 1994; Knorr, 1994) as well as the production of pharmaceuticals or chemicals from root cultures (Schreiner et al., 2011; Flores et al., 1999; Norton & Towers, 1986) as cost-effective alternatives to classical approaches, using the whole crop as a source. Numerous food additives including flavours, pigments, essential oils, sweeteners and antioxidants have been produced in culture (Dörnenburg & Knorr, 1996; Chung et al., 1994; Swanson et al., 1992; Berlin et al., 1986). Furthermore, the initiation of plant cell culture can be used to realize metabolite extraction from rare and threatened plants contemporary and economically. Plant cells act as independent units and are biosynthetically totipotent, which means that each cell in culture has the ability to retain the full genome and hence can produce the same range of chemicals as

its precursor (Schleiden, 1838; Schwann, 1839). The major benefit in the use of cell culture is the assurance of the uniformity and reproducibility of results. However, the instability of cell lines, insufficient yields and slow growth can be mentioned as long-term problems. In the field of scientific research plant cells can be used as model system in order to understand plant metabolism basics as well as to study the effects of unit operations on plant foods. Relevant non-thermal applications for the food industry as pulsed electric fields, high pressure and ultrasound, which cause the disintegration of biological cell material can be applied to gentle release desired cell metabolites (Cai et al., 2011; Ye et al., 2004). Dörnenburg and Knorr (1993) studied the impact of pulsed electric field and high pressure treatment, respectively on the plant cell cultures *Chenopodium rubrum* and *Morinda citrifolia* and their intracellular pigments, amaranthin and anthraquinones. They found an increased release of 85 % from amaranthin and 5.7 % release of the anthraquinones after PEF application, whereas treatment at pressure level of 350 MPa caused a pigment release of 99 % and 9.4 %. Moreover, novel technologies can be optimized through the use of plant cells as model systems. Studies of process-induced changes of single cells cause better understanding of basic underlying principles and help to tap the full potential of these technologies. The preparation of protoplasts (cells with removed cell wall) from plant tissue or cell suspensions is also of scientific interest. The isolation of cell wall is often performed enzymatically with the cell wall degrading enzymes cellulases and pectinases. The obtained spherical cells must be cultured carefully in an isotonic medium. Near the use of DNA transformation and plant breeding by electrofusion, protoplasts are ideal targets to study membrane biology (Costa et al., 2003; Morse et al., 2004).

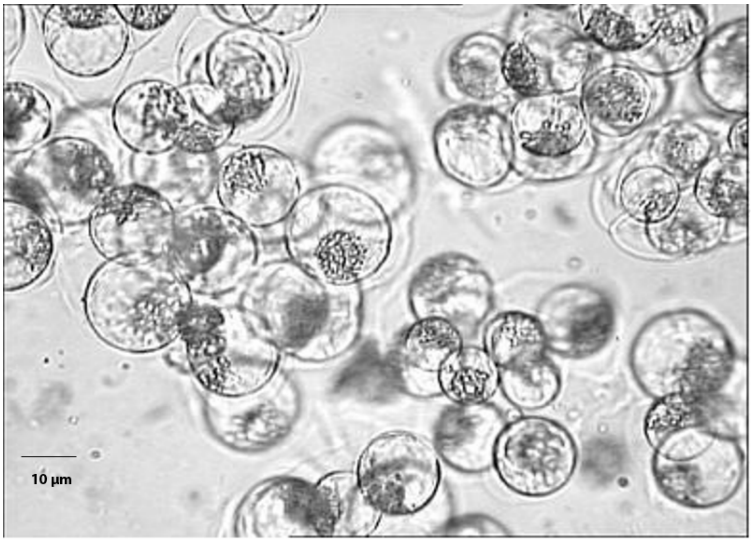

Figure 2: Isolated protoplasts of seven-day-old *Nicotiana Tabacum* cell suspension after enzymatic cell wall degradation (Janositz & Knorr, 2010).

2.2 Pulsed Electric Fields (PEF) Technology

2.2.1 Mechanisms of action

The mechanism of PEF-induced pore formation in cell membrane is not yet fully elucidated. One of the most accepted theories about cell membrane permeabilization caused by an external electric field is related to electrocompression of the cell membrane. The electromechanical model developed by Zimmermann et al. (1974) considers the cell membrane to be a capacitor that separates ionic species and free charges on inner and outer side of the membrane. The different charges on both sides of the membrane cause a natural transmembrane potential in cell. When subjecting biological cell material to an electric field, accumulation and attraction of oppositely charged ions on both sites of the non conductive cell membrane occur. These reactions cause the reduction of membrane thickness. With further increase in the transmembrane potential, as a consequence of the increased electric field, and by reaching a critical value of 1 V, membrane compression intensifies, which lead to the formation of either temporary or permanent pores and the loss of semi-permeability in the cell membrane.

Unlike the theory of membrane compression, other theses are based on molecular realignment within the lipid bilayer and protein channels which cause pore formation in cell membrane when subjecting a cell to an electric field. Based on studies with protoplasts as model systems, it has been suggested that PEF treatment could cause alteration of membrane composition by reorientation of bipolar phospholipids and subsequent membrane permeabilization. These conformational changes could cause destabilization with the loss of membrane semi-permeability and thus the loss of cell vitality (Sale & Hamilton, 1968, Tsong, 1991). Tsong (1991) described the presence of hydrophobic and hydrophilic pores in lipid matrix induced by the electric field and assumed that hydrophilic pores conduct electricity which causes Joule heating. Thus, increase of temperature might cause changes in membrane structure and affect its function as a barrier. Membrane disintegration is believed to be caused by osmotic imbalances and swelling of permeabilized cells. Which means it can be seen as a result of the difference in the permeabilities of ions and macromolecules inside the cell, building up an osmotic pressure that press water into the cells and leads to cell elongation (Fig.3) (Kinosita & Tsong, 1977; Tsong, 1991).

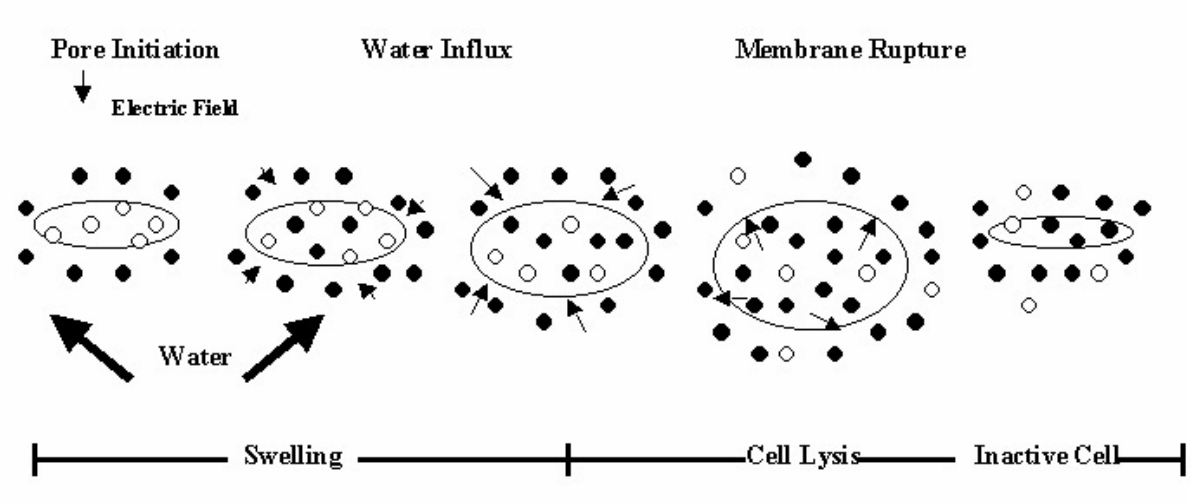

Figure 3: Electroporation of a cell membrane (Tsong, 1991).

Additionally, membrane permeabilization might be a consequence of denaturated protein channels in the lipid layer, since their functionality depends on the natural transmembrane potential. Protein channels getting activated about 50 mV, considerably lower than the critical transmembrane potential. Thus, by exposing cells to PEF, many voltage-sensitive channel proteins might open which induces electrical injury. However, it has to be in mind that protein channel opening may not effectual enough to inhibit an increase in transmembrane potential to equal the breakdown potential of the lipid bilayer. Due to the high current Joule heating or electric modification of the protein channels with subsequent denaturation may occur, identifying that electroporation can take place in protein channel as well as in lipid fraction of the membrane.

2.2.2 Applications

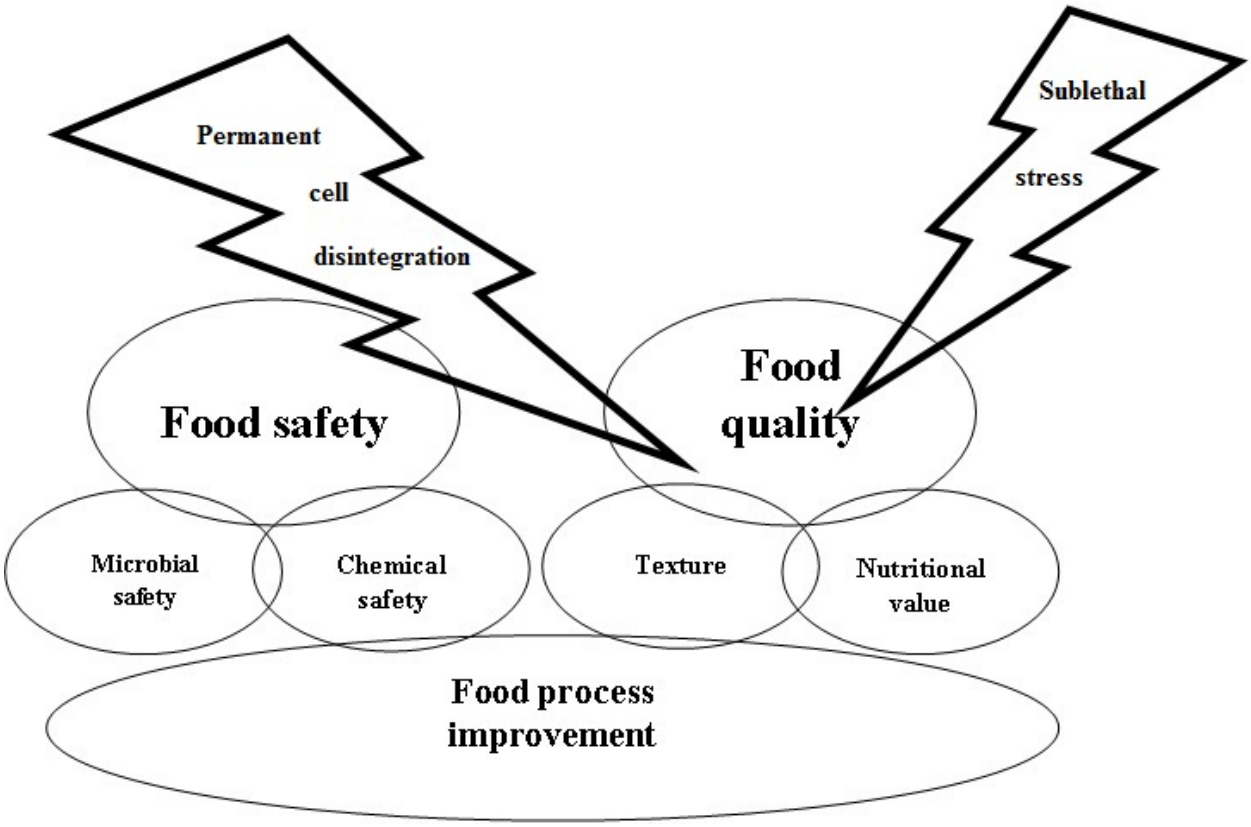

Figure 4: Schematic depiction of food and process improvement due to pulsed electric fields (Janositz, unpublished results).

Most potential applications of PEF in food industry can be referred to the disintegration of the cell membrane. As a mild alternative preservation method to heat pasteurization, PEF can extend shelf-life at sub-lethal temperatures while maintaining physical, chemical and sensory properties of food. Liquid and semi-solid products such as fruit and vegetable juices (Barbosa-Cánovas et al., 1995, Heinz et al., 2003, Molinari et al, 2004), milk (Zhang et al., 1994b, Sampedro et al., 2005), liquid eggs (Amiali et al., 2004, Hermawan et al., 2004) and soups (Vega-Mercado et al., 1996) exposed to PEF in continuous systems showed significant reduction of most pathogenic bacteria. However, studies about PEF-induced pasteurization concerning spores showed only limited inactivation effects (Raso et al., 1998). Especially in recent years, PEF technology research has been investigated not only in microbial safety of food products but also for gentle and controlled modification of plant cell tissue. Very promising results have been achieved concerning the release and production of cell metabolites (Eshtiaghi & Knorr, 2002; Fincan et al., 2004; Guderjan et al., 2005; Puertolas et al., 2010). Due to their function as health related ingredients and/or their use as colouring and flavouring substances in food the recovery of intracellular molecules in its natural state are of

high commercial interest. The improvement of juice yield and rates with simultaneous retaining of fresh-like characteristics in solid-liquid extraction of fruit and vegetables (Knorr et al., 1994; Bouzrara & Vorobiev, 2000; Schilling et al, 2008) as well as the acceleration of mass transport in drying processes (Rastogi et al., 1999, Ade-Omowaye et al., 2001b, Lebovka et al., 2007) are counted among the benefits of PEF employed in food processing. Besides this, PEF treatment offers a potential involving decontamination of waste water (Koners et al., 2004; Kopplow et al., 2004), improvement of textural and sensory properties of cheese made from PEF treated milk (Sepulveda-Ahumada et al., 2000) as well as the prevention of biofouling in cooling water (Abou-Ghazala & Schoenbach, 2000). However, only limited report exists concerning the effect of PEF on enzyme activity. Different conclusions have been drawn varying from significant reduction of some enzymes after PEF application (Schuten et al., 2004) to no effect of PEF on enzyme activity (Van Loey et al., 2001). This contradiction could be referred to difference treatment conditions as well as to the differences in enzyme molecular structure, causing higher sensitivity of some enzymes to PEF than other. Jaeger et al. (2010) found only 5 % reduction of Lactoperoxidase activity due to PEF without thermal effects, but marked that the benefit in maintaining LPO-activity lies in the retention of antimicrobial effect, which can be referred to the presence of LPO.

3. Summary of research methodology

This section summarizes main methodologies that were conducted in the research work.

3.1 Biological raw material

Article I (Janositz & Knorr, 2010) Plant cell studies were carried out with cultured tobacco cells (*Nicotiana tabacum* L. cv Bright Yellow-2), grown in MS medium (Murashige, 1962) for 7 days at 25 °C in the dark with reciprocatory shaking at 120 rpm.

For protoplast preparation, tobacco cells were vacuum filtered and 2 g fresh weight cells were resuspended in 10 ml solution of isotonic buffer W5 (154 mM NaCl, 125 mM $CaCl_2$, 5 mM KCl, 5 mM Glucose, pH 5.7) combined with a mixture of cellulolytic and pectolytic enzymes (0.01 g Rohament Cl, 0.1 g Rohament PL) (AB Enzymes, Darmstadt, Germany) for the residence time of 4 hours. After digestion of cell wall components, the obtained spherical protoplasts were washed twice with 0.6 M mannitol. Isolated protoplasts were finally resuspended in 6 ml unbuffered isotonic mannitol solution to perform pulsed electric field treatment (Fig.1). Buffer was excluded in order to render a low conductivity medium for PEF operation.

Pre-treatment of tobacco cells with cell wall was carried out with vacuum filtration and resuspension of 2 g cells in 6 ml mannitol solution before PEF processing.

Article II (Janositz, Noack & Knorr, 2011), Article III (Janositz, Semrau & Knorr, 2011) Plant tissue experiments were performed with potatoes (*Solanum tuberosum*) and white asparagus (*Asparagus officinalis*). Potatoes were obtained from the potato processing company Lorenz Snack-World GmbH & Co KG (Neu-Isenburg, Germany) and stored in the dark at 8-10 °C. Asparagus spears were bought in a local store in Germany and stored at 4 °C in a refrigerator.

3.2 Experimental set-up and electric field pulses protocol

PEF treatment was performed on plant suspension culture **(Article I)** and plant tissue (**Article II, III)**.

In **Article I**, exponential electric field pulses were applied with the PEF microscope, constructed in the Department of Food Biotechnology and Food Process Engineering (TU Berlin). The microscope (Zeiss Optik, Jena, Germany) enabled the study of direct cell structure changes during the treatment. Main components were a camera (Nikon E 8700, Japan), which was fixed to the microscope, 3 objectives, with a maximum magnification of 400 fold, and a glass slide with two copper foil electrodes (gap 2 mm, length 3 mm, thickness 0.2 mm, area 0.6 mm²). The treatment chamber was connected to the micro pulse modulator, consisting of a power supply FUG HCK, 800 M- 20.000, 20 kV, 80 mA (FUG, Rosenheim, Germany) to a capacitor bank of three capacitors with 6.8 nF each. The pulse parameters were examined by a high voltage and a current probe, coupled to a TDS220 (Sony Tektronix, Beaverton, US) oscilloscope. A PC computer was used to control PEF treatment intensities, namely electric field strength E: 0.25 – 7.5 kV/cm; pulse number n: 10, 20; specific energy input W: 2,206 – 1985 J/g, pulse width τ: 2 -8 µs and frequency f: 2 Hz. The images obtained with the microscope from the samples were recorded with the camera and single pictures of untreated and PEF treated were selected to analyze PEF induced cell disintegration. Camera was activated manually before treatment. For microscopic analysis, each process condition was performed approximately 10 times. Recorded cells per experiment/ picture varied between 1 and 8. Cell area was measured by the program AnalySis 2.11 (Muenster, Germany) from pictures taken from the recorded movie before and after (after the last pulse) PEF treatment. T-tests were used for the analysis of statistical significance. Cell area reduction was calculated by the formula:

(1-(cell size of PEF treated protoplasts/cell size of untreated protoplasts))*100. (1)

In **Article II, III** exponential electric field pulses were applied to a parallel plate treatment chamber for batch-wise operations, which was connected to a capacitor bank of four DP 30560 (GA, San Diego, USA), 15 kV, 2 µF in series. Thus, a total capacity of 0.5 µF was achieved. Capacitors were charged using an ALE802 (Lambda-Emi, Neptune, USA), 40 kV power supply. The applied PEF treatment intensities for potatoes (**Article II**) and asparagus (**Article III**) are listed in table 1:

Table 1: PEF treatment parameter

	Output voltage	Electrode gap	Electric field strength	Pulse number	Pulse duration	Pulse frequency
Article II **Section 4.2**	U= 1000 V	d= 0.2 cm	E= 5 kV/cm	n= 20	τ = 100 µs	*f*= 2 Hz
Section 4.2.1	U= 12000 V U= 20000 V	d= 8 cm	E= 1.5 kV/cm E= 2.5 kV/cm	n= 20	τ = 400 µs	*f*= 2 Hz
Section 4.2.2	U= 9000 V	d= 5 cm	E= 1.8 kV/cm	n= 40	τ = 400 µs	*f*= 2 Hz
Article III **Section 4.3**	U= 15000 V	d= 3 cm	E= 5 kV/cm	n= 20	τ = 400 µs	*f*= 2 Hz

3.3 Examination of cell vitality through impedance measurement

Electrical properties of biological tissue define the impact of PEF on the degree of permeabilization. Thus, the determination of Cell Disintegration Index (CDI) is basically necessary. CDI was analyzed after Angersbach et al. (1999). The method based on the frequency depending conductivity of intact and permeabilized tissue. The cell disintegration index CDI analysis was carried out via impedance measurement equipment (Biotronix GmbH, A. Angersbach, Hennigsdorf, Germany).

CDI was calculated by following equation:

$$CDI = 1 - b\frac{(K'_h - K'_l)}{K_h - K_l} \qquad b=\frac{K_h}{K'_h} \quad 0\leq CDI\leq 1 \qquad (2)$$

where K_l and K_l' indicate the electrical conductivity of untreated and treated cell material in a low- frequency field (1-5 kHz), respectively; and K_h and K_h` indicate the electrical conductivity of untreated and treated material in a high- frequency field (3-50 MHz).

The CDI varies between 0 for intact cells and 1 for total disintegration. Cylindrical pieces were cut out of the tissue and placed into a plastic test tube. The electrode area of the measuring cell was 2 cm². The gap was adjusted to 1.0 cm.

3.4 Determination of sugar content (D- Glucose, D- Fructose)

In **Article II** and **III** sugar content was analysed before and after PEF treatment to examine the influence of PEF on the release of low molecular substances.
For the analysis, samples were washed after treatment in tap water (500 ml), cut, 50 g were mixed with 50 ml distilled water and homogenised with an Ultra- Turax (T 25 digital Ultra-turrax, IKA laboratory technology, Germany) at 15000 rpm for three minutes at room temperature. 5 ml Carrez I solution (3.60 g K4 [Fe(CN)6] x 3H2O (potassium hexacyanoferrate/ 100 ml) and 5 ml Carrez II solution (7.20 g ZnSO4 x 7 H2O (zinc sulfate hepta hydrate/ 100 ml) were added to sample mash (pH= 7.0-7.5). In a volumetric flask 0.3 ml n- Octanol were added to the sample and shook till foam was dissolved. Filtration was performed after addition of distilled water to the mark of 250 ml. Sugar content was analysed spectrophotometrically (Kontron 25/Germany) at 334 nm wavelength.

3.5 Analysis of fat content

In **Article II** the oil uptake of PEF treated potato stripes during frying was analyzed and compared to the fat content of blanched and untreated samples in order to study effect of PEF on the drying behavior during deep fat frying.
Blanching and PEF processing were performed for the comparison of different pre-treatments to reduce fat content during frying. Warm water blanching was accomplished for 2 minutes at 80 °C. Blanched potatoes were cooled in tab water for 10 minutes and dripped of water. After cutting 100 g potato stripes were fried in two liter rapeseed oil for 13 minutes at 190 °C. The frying sieve was shaken to release the surface oil and cooling of the fries was performed for 10 minutes at room temperature. Oil content of potato stripes was determined by 3 h Soxhlet extraction using petroleum ether as a solvent (AOAC, 1995).

3.6 Analysis of lignin content

In **Article III** amount of lignin was measured to analyse impact of PEF on delignification and thus on softer texture.

-Qualitative-

Phloroglucin, a benzentriol (1,3,5- trihydroxybenzene**,** Merck, Darmstadt/Germany), was solubilized in a mixture of ethanol/water (1:1) (w= 5%). For the qualitative detection of lignin, phloroglucin solution was applied to asparagus sample and one drop of hydrochloric acid was added to turn the contained lignin red.

Pictures were recorded by using a light microscope (Nikon Eclipse E400) equipped with a digital camera (JVC, TK -10070E).

-Quantitative-

Lignin content determination is based on Association of Official Analytical Chemists [AOAC] methods (1984) according to the procedures of Goering & Van Soest (1970). The analysis includes the detection of ADF (Acid Detergent Fiber) and ADL (Acid Detergent Lignin).

The freeze dried samples were homogenized in an Ultra- Turrax (T 25 digital ultra-turrax, IKA laboratory technology, Germany) at 24000 rpm for 5 minutes at room temperature. Detection of ADF content: 100 ml of acid detergent dissolution (20 g N-trimethyl-ammonium bromide) were solute in sulphuric acid (c: ½ H_2 SO_4 = 1 mol / l) and added with 0.5 ml Octanol. 1 gram of grounded sample was weighted out and mixed with the solution and boiled for 60 minutes. After boiling, content of the glass beaker was vacuum-filtrated through a filter crucible and washed afterwards with 250 ml hot water and acetone. Filter crucible was dried over night in a drying oven at 100 °C and weighted out after cooling in a dehydrator. The content of ADF was determined by the formula:

$$ADF = \frac{(m2 - m1) * 100}{E} \tag{3}$$

where $m1$ indicates the mass of the filter crucible [g], $m2$ indicates the mass of the filter crucible and ADF [g] and E notifies the initial weight [g].

The filter residue can be used for the detection of raw lignin = ADL (Acid Detergent Lignin).

Determination of ADF content:

Filter crucible with residue of ADF analysis was weight out and placed in a beaker glass. Crucible content was covered with 72 % sulphuric acid, which was cooled to 15 °C. Over a period of three hours, sulphuric acid was refilled and mixture was stirred hourly at a temperature of 20-23 °C. Suction, hot water washing, drying and weighting were performed subsequently. After incineration of organic substances the specimen was weighted again. The annealing loss equates the amount of raw lignin. The experiments were performed in duplicates and replicated five times for statistical purposes.

4. Main findings

This section summarizes the most important results of the three publications.

4.1 Basic principles of PEF on cellular level: Microscopic visualization of cell structure changes

In **Article I (Janositz & Knorr, 2010)** cultured tobacco cells (*Nicotiana tabacum b.y.-2)* were used as model systems and microscopic images were recorded during the PEF treatment to visualize the PEF- induced changes on cell structure. Protoplasts were prepared enzymatically and compared with native cell behaviour in the electric field to identify the influence of cell wall on the degree of cell disintegration. Cell shrinkage was observed for protoplasts after PEF exposure. Thus, cell area was measured before and after PEF treatment and different cell sizes were compared with different treatment intensities. The reduction of cell area served as an indicator for cell membrane permeabilization.

4.1.1 Comparison of protoplasts (digested cell wall) and native cells (intact cell wall)

Visual observation showed higher sensitivity of protoplasts to electric fields than cells with a cell wall. The elimination of cell walls leads to a loss of structural support. Therefore irreversible membrane pore formation after PEF processing of protoplasts was indicated by the reduction of cell size whereas membrane disintegration of cell wall cells could only be noticed with vital dye (phenosafranine) diffusion. Noticeable decrease of protoplast cell area was already shown after the first pulses at quite low treatment conditions ($\geq$ E= 0.5 kV/cm, n= 10, $\geq$ W= 8.824 J/g; Fig.5).

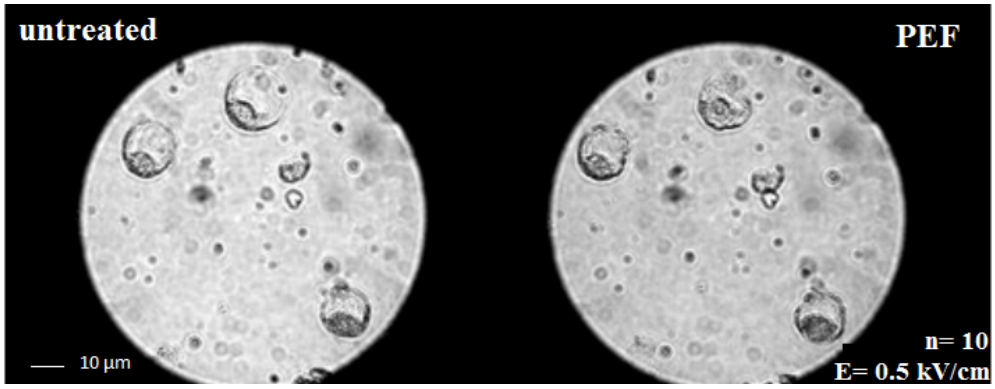

Figure 5: Protoplasts (*Nicotiana Tabacum* L. cv Bright Yellow-2) untreated and after PEF treatment (E= 0. 5 kV/cm, n= 10, f= 2 Hz) (Janositz & Knorr, 2010).

In contrast to cells with cell wall, where the phenosafranine uptake, which indicates irreversible pore formation, was only registered at higher PEF intensities (≥ E= 1.2 kV/cm, n= 20, ≥ W= 2541 J/g; Fig.6).

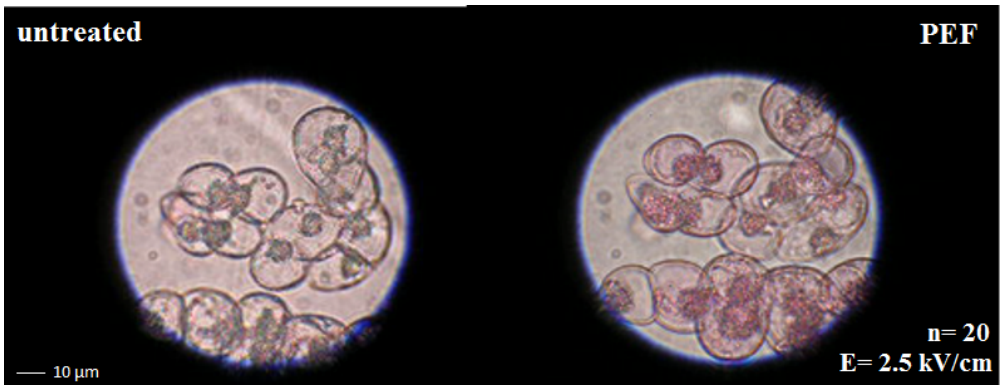

Figure 6: Tobacco cells with cell wall (*Nicotiana Tabacum* L. cv Bright Yellow-2) in vital dye solution (Phenosafranine) untreated and after PEF treatment (E= 2. 5 kV/cm, n= 20, f= 2 Hz) (Janositz & Knorr, 2010).

The cell disintegration index correlated with microscopic observations and demonstrated the intensified effect of PEF on protoplasts (Fig.7). It could be shown that the presence of cell wall highly influence the degree of membrane permeabilization. Both cell types showed higher degree of cell disintegration with the application of higher PEF intensities. The extent of protoplast cell rupture was nearly twice as high compared to the cells with cell wall with same treatment conditions, demonstrating the protective effect of plant cell walls.

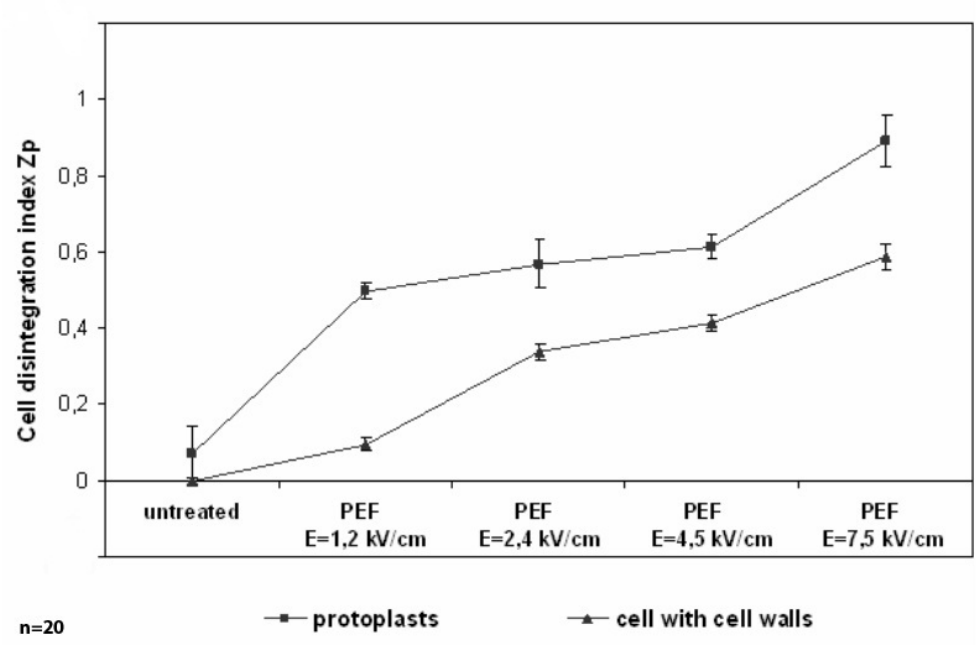

Figure 7: Cell disintegration index of untreated and PEF treated protoplasts and native cells after different PEF treatment conditions (Janositz & Knorr, 2010).

4.1.2 Protoplasts as a model system to visualize influence factors on PEF induced membrane rupture - determinant factors: PEF treatment intensity and cell size

Cell shrinking after irreversible PEF treatment could only be observed in protoplasts. Hence, cell volume of untreated and PEF treated protoplasts can serve to detect the degree of membrane permeabilization. Microscopic analysis during the treatment could visualize the fact that higher PEF intensities cause higher degree of cell rupture, indicated by major cell area reduction at stronger PEF energy inputs (Fig. 8).

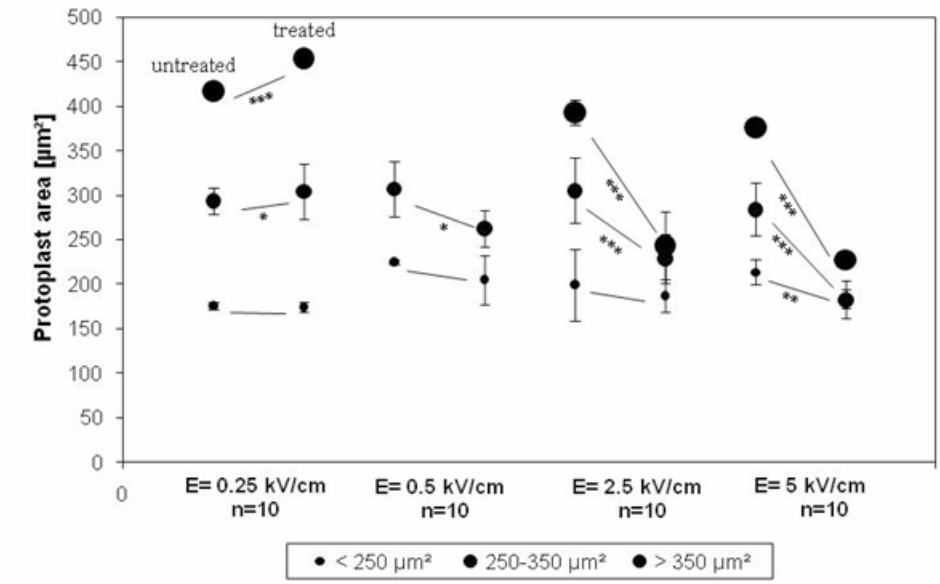

Figure 8: Cell area of untreated and PEF treated protoplasts before and after PEF processing with different treatment conditions. Statistical significance (* P<0.05, ** P<0.01, *** P<0.001) (Janositz & Knorr, 2010).

For cells of 250-350 µm² cell area, application with field strength of 0.5 kV/cm and 10 pulses resulted in cell area reduction of 12.5 % whereas for E= 5 kV/cm and n= 10 the protoplast shrinking reached 34 %. Furthermore, it could be shown that the cell size determines the required external electric field intensity which causes membrane disruption. Larger cells were more affected by the electric field than cells with smaller size. For cells with less than 250 µm² cell area, lower size reduction was noticed after PEF treatment and the different intensities caused minor differences in cell area as it could be monitored for larger cells. The impact of cell size on the effectiveness of PEF treatments is clearly shown in the reduction of the cell size after PEF processing with the highest applied treatment intensity (E= 5 kV/cm, n= 10, W= 883.353 J/g), where cell area differed from 11.8 % for smallest cells (< 250 µm²) to 39.8 % for cell size in the range over 350 µm². The observed effect of cell size on the degree of cell area reduction corresponds with other experimental studies (Sale & Hamilton, 1967; Hülsheger et al., 1983; Zhang et al., 1994b) and is based on the required electric field intensity to induce a given transmembrane potential into a cell.

Another focus of our study was the microscopy of reversible pore formation through following the resealing processes. Furthermore, it was not only possible to visualize irreversible cell disintegration by the reduction of cell size but also to image temporary pore formation in cell membrane after the application of PEF with low energy inputs. The PEF induced stress reaction which causes reversible pores in plasmalemma could be indicated by

cell swelling after the exchange of intra- and extracellular fluids due to slight osmotic imbalance in the medium (Fig.8). Temporary formed pores leads to a break in the osmotic barrier. Subsequently the gradient for osmotic pressure between intra- and extracellular liquids drops to zero. For draining permeabilized cells, a hyperosmotic medium is used. Vice versa, liquid uptake occurs in a hypoosmotic medium.

In figure 8 the differences in protoplast cell area before and after PEF treatment are represented. Whereas treatment conditions higher than E= 0.5 kV/cm and n= 10 led to a reduction of cell area, the utilization of low process parameter (E= 0.25 kV/cm, n= 10, W= 2.206 J/g) resulted in an increase of cell size, which could indicate the resealing of temporary formed pores in membranes after PEF implementation.

4.2 Applications of PEF on plant tissue: Enhanced mass transfer of low molecular substances

In **Article II (Janositz, Noack & Knorr, 2011), Article III (Janositz, Semrau & Knorr, 2011)** PEF were applied on plant tissue with the main aim of irreversible permeabilization of the cell membrane and subsequent improved diffusion of intra- and extracellular molecules.

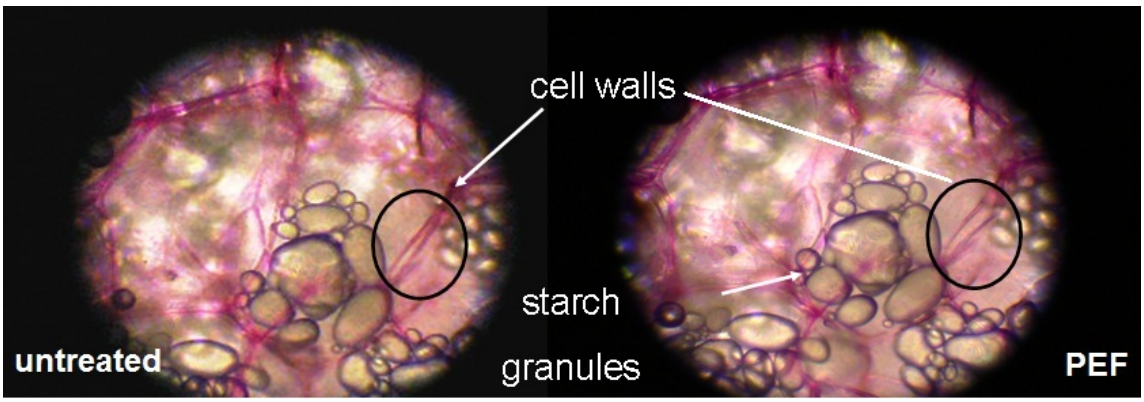

Figure 9: **PEF treated (E= 5 kV/cm, n= 20, 5 min. after treatment) and untreated potato tissue stained with ruthenium red. Light microscope (Nikon Eclipse TS 100, Japan) (Janositz, Noack & Knorr, 2011).**

In Fig. 9 untreated and PEF treated (E= 5 kV/cm, n= 20) potato tissues with stained cell wall pectin are shown. The dye ruthenium red binds to deesterified carboxyl groups and stains pectin in cell wall and middle lamellae. It is seen that the tissue compartment is slightly changed. Still, it is not clear whether cell wall components are changed directly due to the PEF treatment or due to cell membrane disintegration and the release of cytoplasm. However,

it was shown in **Article III** (Janositz, Semrau & Knorr, 2011) that the content of the cell wall biopolymer lignin reduces after PEF application (see 4.3).

4.2.1 PEF-induced release of intracellular substances ➔ sugar

As demonstrated in **Article II** PEF application on potatoes slices improves the removal of reducing sugars from the tissue (Fig. 10). A significant increase in the release of glucose and fructose was observed after PEF application of potatoes with the field strength E= 1.5 kV/cm and 20 pulses. The enhanced diffusion characteristics after PEF induced electroporation resulted in a one third reduction of fructose content and a nearly bisection of glucose rate.

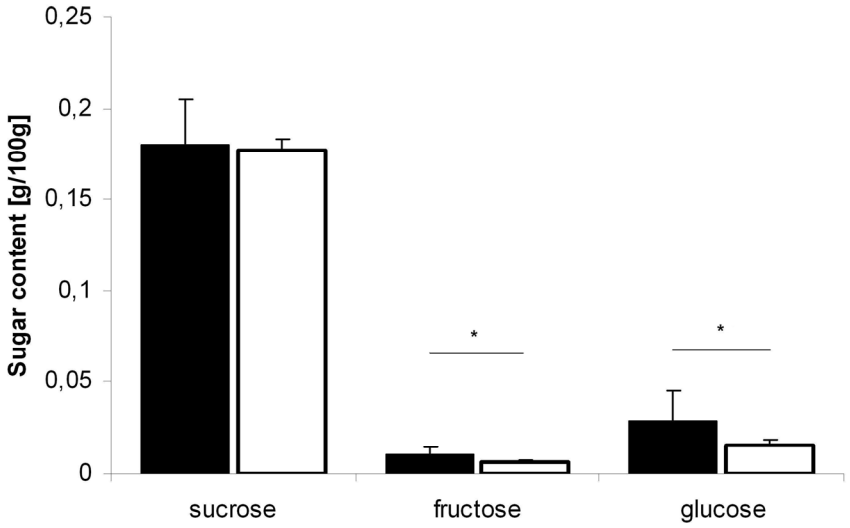

Figure 10: Sugar content in potato slices after PEF treatment (E= 1.5 kV/cm, n= 20) in comparison to untreated samples. □ = PEF treated potato samples, ■ = untreated potato samples. Statistical significance (* P<0.05, ** P<0.01, *** P<0.001) (Janositz, Noack & Knorr, 2011).

In contrast to the PEF-induced sugar release in potatoes, PEF processing on asparagus **(Article III)** did not result in pronounced differences of glucose and fructose content. As represented in figure 11a no alteration of glucose level was found for untreated and PEF treated asparagus directly after treatment. However, on the fourth day of storage, both samples showed significant reduction of glucose content. PEF treated samples amounted 3 g/100g less glucose than the reference.

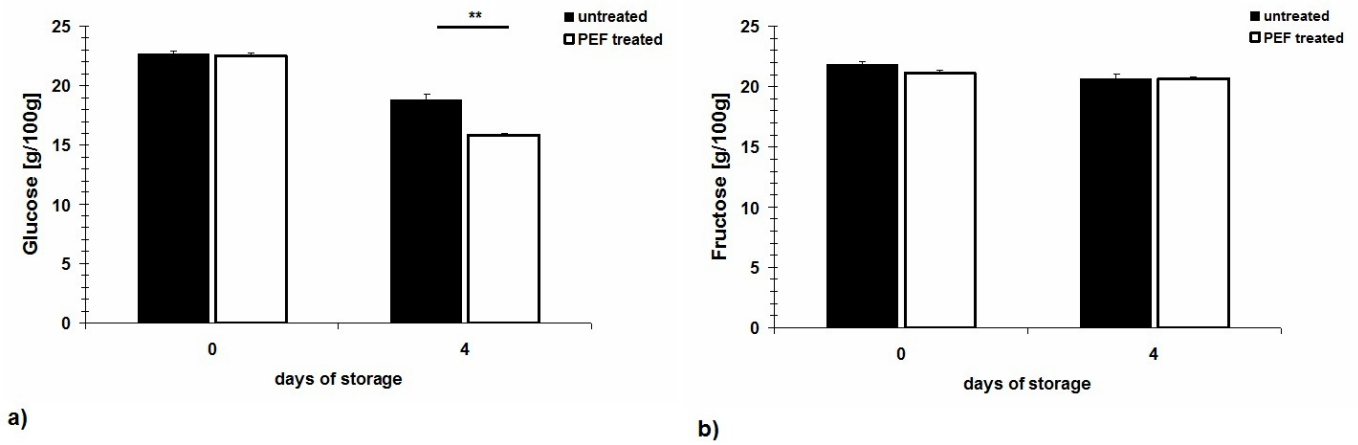

Figure 11: Glucose (a) and fructose (b) content of PEF treated (E= 5 kV/cm, n= 20) and untreated asparagus after 0 and 4 days of storage. Statistical significance (* P<0.05, ** P<0.01, *** P<0.001) (Janositz, Semrau & Knorr, 2011).

The lowering in glucose content during storage could be attributed to respiratory processes, microbial load and the enhanced release of endogenous enzymes due to PEF-induced cell disintegration causing degradation of glucose (Bisson et al., 1926). As demonstrated in figure 11b, only minimal changes in fructose content due to PEF treatment were observed, but no further decrease of fructose in untreated as well as in PEF treated asparagus was noticed. These observations mark the different influence of PEF on different food matrices. The electric field direction can be mentioned as another impact factor, which affects the extent of cell membrane permeabilization. In **Article III**, Cell Disintegration Index (CDI) measurements were performed of PEF processed (E= 5 kV/cm, n= 20) asparagus, treated and measured in longitudinal and diagonal direction.

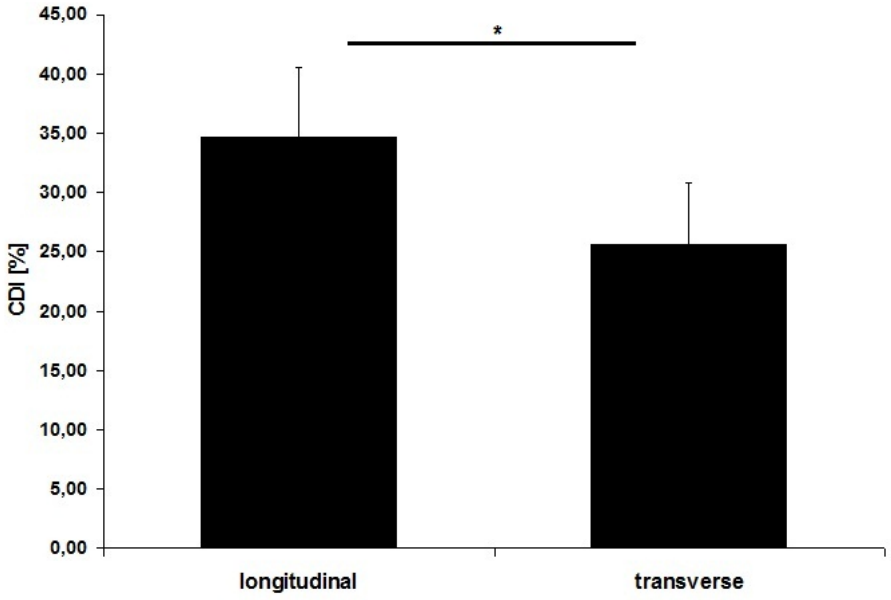

Figure 12: Cell disintegration index of PEF treated asparagus (E= 5 kV/cm, n= 20) with electrode orientation in longitudinal or diagonal path direction. Statistical significance (* P<0.05, ** P<0.01, *** P<0.001) (Janositz, Semrau & Knorr, 2011).

Figure 12 shows that CDI increased by 9.06 % when orientating the electrodes longitudinally relative to the major axis of the tissue. This observation demonstrates that the direction of the electric field has a significant influence on the effectiveness of PEF treatment. Asparagus tissue can be seen as distinctly anisotropic, since the cells have a diameter of approximately 20 µm, but a length of up to 100 µm (Gassner et al., 1989). Electrical conduction along the length of cell, filled with rich ionic intracellular liquid, is thus easier than conduction between the cells in the less conductive extracellular matrix and the non-conductive cell membrane.

4.2.2 PEF-induced release of intracellular substances ➔ cell liquid ➔ lowering of French fries fat content

Several studies reported about the enhancement of drying processes after PEF treatment of plant tissue (Ade-Omowaye et al., 2001b; Taiwo et al., 2002; Lebovka et al., 2007). In **Article II** (Janositz, Noack & Knorr, 2011) higher water loss of PEF treated potato slices after baking in drying oven was found (data not shown). In the present investigation, the effect of PEF pre-treatment on the fat uptake of potato strips during frying was examined. As presented in figure 13 markable fat reduction of 38.66 % was observed for PEF pre-treated samples compared to untreated fried strips. This distinct decrease of fat content could not be found for blanched samples, which showed no significant fat reduction regarding to the reference samples. The blanching-induced layer of gelatinized starch (Moreira et al., 1999) was shown

to be less efficient concerning limitation of oil absorption in comparison to PEF pre-treatment. This finding was attributed to the modified frying characteristics of the PEF treated potato strips. Frying is mainly a drying process that involves heat and mass transfer. After initial heating of the food through the surrounding oil, surface boiling begins including water vaporizing and the formation of bubbles. Moisture is transferred from the surface to the oil and later by diffusion of inner cellular liquid to the surface. The water vapour layer on the potato surface acts as a barrier against the oil and depends on the vapour pressure difference between food moisture and oil, which influence the rate of drying (Jason, 1958). Due to the permeabilized cell membranes of PEF treated tissue cell liquid diffusion from the core to the surface is enhanced, which result in higher vapour pressure difference and thus thicker water vapour layer, reducing dehydration and fat uptake. As revealed visually and haptically the surface of PEF treated potato strips is smooth and flat, which assist additionally the decreased oil uptake during frying and post-frying (Thanatuksorn et al., 2005). Due to the even cut, oil absorption during frying can be reduced in contrast to the more distinct roughness of non PEF treated tissue. During the cooling period PEF treated samples were less susceptible to oil absorption of the adverse crust oil because of the smooth and even outer surface, causing better oil draining (Bouchon & Pyle, 2006).

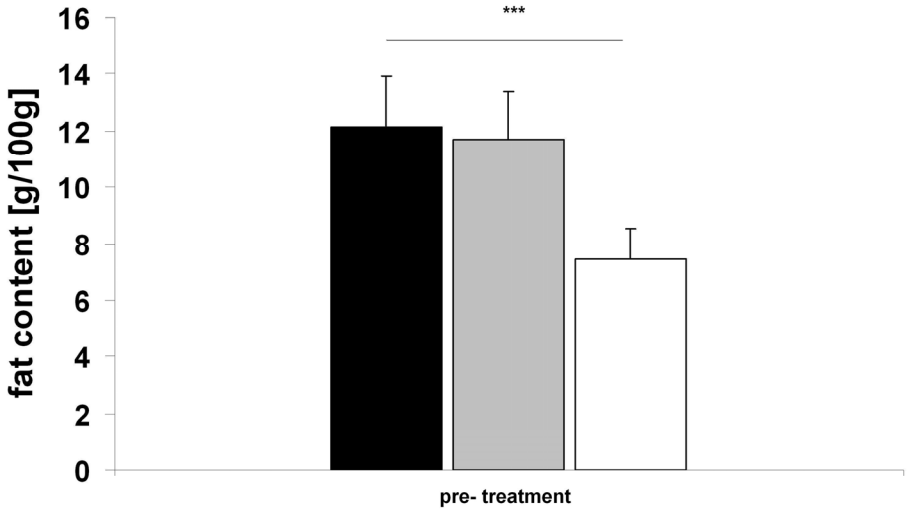

Figure 13: Comparison of blanching (T= 80 °C, t= 2 min.) and PEF (E= 1.8 kV/cm, n= 40) pre-treatment with untreated potato stripes concerning fat uptake during frying. □ = PEF treated potato samples, ▒ = blanched potato samples, ■ = untreated potato samples. Statistical significance (* P<0.05, ** P<0.01, *** P<0.001) (Janositz, Noack & Knorr, 2011).

4.2.3 PEF-induced uptake of extracellular substances ➔ sodium chloride

The uptake of sodium chloride after PEF implementation of potatoes was analyzed in order to examine the potential of PEF to assist the infusion of flavor carrier or pigments in the tissue. Thus, **Article II** focused not only on the enhanced release of molecules out of the cell but also by the increased infusion of substances into the sample. In figure 14 conductivity of untreated and PEF treated potatoes after soaking in sodium chloride solution is presented. It was observed that conductivity of PEF treated samples was higher and increased with residence time, indicating the higher uptake of sodium chloride in the tissue. Two mass processes occurred, water release out of the cells as well as salt diffusion into the tissue dependent on the applied concentration gradient.

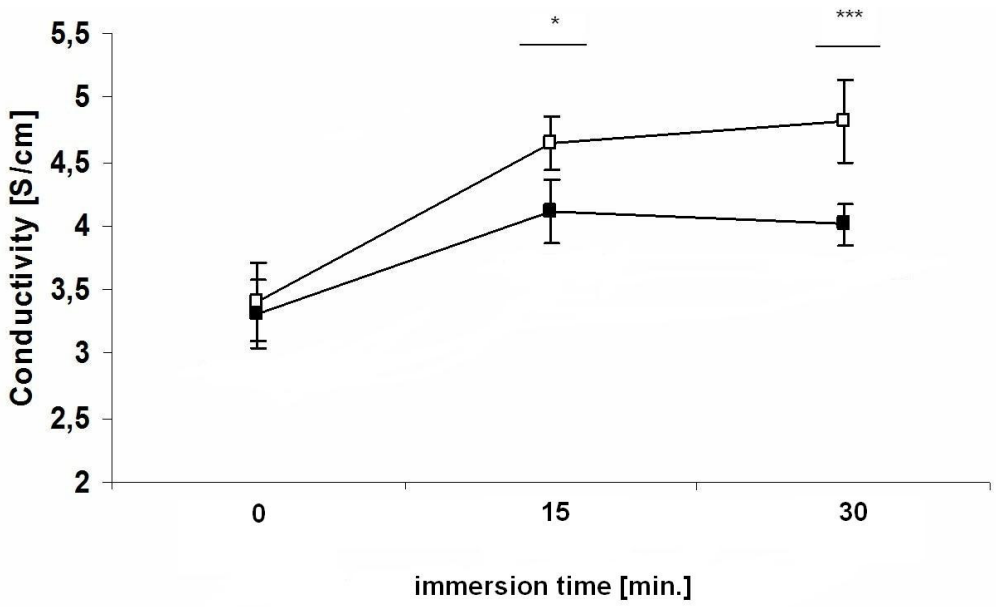

Figure 14: Conductivity of PEF treated (E= 1.5 kV/cm, n= 20) and untreated potato samples without NaCl immersion and after soaking in 1 g/100g NaCl solution for 15 or 30 minutes. □ = PEF treated potato samples, ■ = untreated potato samples. Statistical significance (* P<0.05, ** P<0.01, *** P<0.001) (Janositz, Noack & Knorr, 2011).

4.3 PEF-induced changes on food ingredients ➔ lignin

In **Article III** (Janositz, Semrau & Knorr, 2011) lignin content of PEF treated asparagus was analyzed to clarify impact of PEF on the biopolymer lignin, gaining improved macroscopic characteristics of the spears.

In figure 15 asparagus tissue with red stained lignin is shown. The chemical reaction with phloroglucin and sulphuric acid was performed to visualize the distribution of lignin in the

spear. It is seen that lignin is particularly abundant in the pod (a) and located in longitudinal direction of the spear. This was clarified by viewing cross-sectional imaging (b). Lignin deposition was noticed as compact and bundled grown in asparagus tissue.

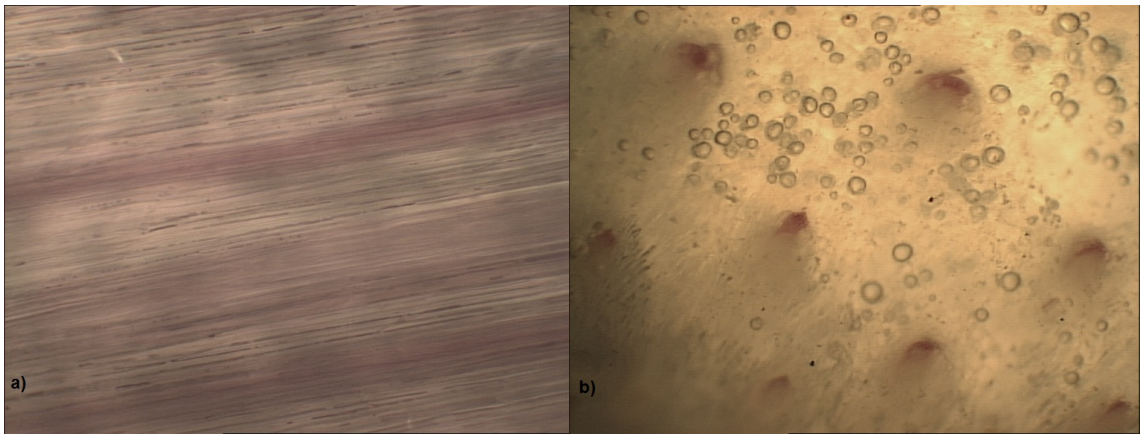

Figure 15: Cross section of asparagus spear (a) and longitudinal cut of asparagus pod (b) performed after reaction with phloroglucin to visualize lignin (Janositz, Semrau & Knorr, 2011).

PEF application was found to have an influence on lignin content in asparagus. As represented in figure 16, the amount of raw lignin decreased from 12.6 % (± 0.08) in untreated asparagus sample to 10.2 % (± 0.34) in the PEF treated asparagus base section. The behaviour of macromolecules exposed to an intense electric field is not well understood (Neumann, 1986). Lignin, a complex phenolic polymer, is seen as highly resistant to biodegradation (Crawford, 1981). Its chemical structure is branched and the macromolecule is bonded with various lignin cross-links and also linkaged between lignin and polysaccharides as cellulose and hemicellulose (Eriksson et al., 1980). Application of PEF may be able to enhance separation of cellulosic material from lignin. High voltage pulses may be effective to break intermolecular and intramolecular bonds within or between the cellulose, hemicellulose, and lignin (Navapanich & Giorgi, 2008). Explanation for the breakage could be that cellulose microfibrills contain large number of hydroxyl groups on the surface causing interactive force attraction with the hydroxyl and methoxyl groups of coumaryl, coniferyl, and sinapyl alcohols from lignin (Houtman & Atalla, 1995). These findings indicate that the dominant force connecting lignin and cellulose is caused by electrostatic dipole-dipole interactions. Subsequent delignification can occur when the bonds are cleaved resulting in solubilization of polymer fragments (Goring, 1971).

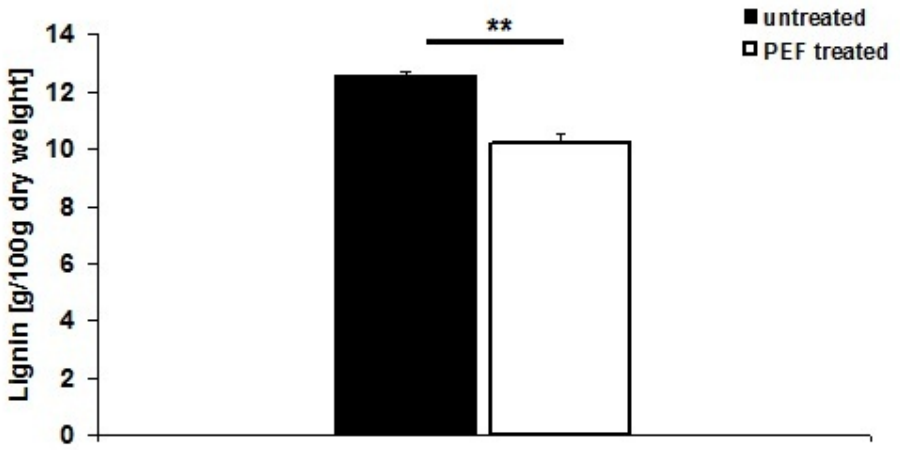

Figure 16: Amount of Acid Detergent Lignin (= raw lignin) of PEF treated (E= 5 kV/cm, n= 20) and untreated asparagus. Statistical significance (* P<0.05, ** P<0.01, *** P<0.001) (Janositz, Semrau & Knorr, 2011).

5. Conclusions

This PhD thesis is focused on the basic principles underlying PEF technology and the applications of PEF particularly in the enhancement of mass transfer processes.
The following conclusions can be drawn:

Basic research

- *A novel microscopic technique allows the in situ analysis of plant cell material under PEF treatment.*
  Microscopic analysis of cell structure changes during PEF treatment is a useful tool to gain a better insight in the permeabilization mechanism of plant cell material. The microscope connected with a pulse modulator aims to achieve further information concerning the influence factors of PEF-induced cell membrane rupture.

- *Protoplasts as model systems are adequate facilities for PEF basic research.*
  Plant cells with removed cell wall can help to understand the basic effects of PEF on plant cell components and to study the impact of cell wall on cell protection in the electric field.

- *Plant cell walls have a protective effect against the electric field.*
  Protoplasts show higher sensibility to the electric field than suspension cells with intact cell wall. Thus, the presence of cell wall highly influences the degree of cell membrane permeabilization.

- *Changes of cell size can serve as an indicator for cell vitality.*
  Protoplast cell size is reduced after irreversible cell disruption and slightly increased after reversible membrane permeabilization. Determination of cell viability can help to evaluate the effectiveness of applied technology on biological material and to assay different process conditions during process development.

- *The direction of the electric field influences degree of cell disintegration.*

Because of the anisotropy of asparagus tissue the electric field orientation has a significant influence on the degree of electroporation of asparagus. These findings confirm the relevance of electrode orientation in order to ensure the efficiency of tissue permeabilization.

Applications

- *PEF improve the removal of reducing sugars*

  PEF treatment on potato slices causes an increase in reduction of glucose and fructose. It can be considered that PEF pre-treatment is a capable assistance or alternative to conventional thermal processing for the removal of reducing sugars, which represents relevant substrates for the Maillard reaction and acryl amide formation. However, the effectiveness to release low molecular substances depends on food matrix. No pronounced sugar reduction was noticed after PEF processing of asparagus.

- *PEF improve drying rates and thus reduce fat uptake of potato strips during deep fat frying*

  PEF application enhances diffusion coefficients within potato tissue and causes higher release of cell liquid during oven drying of potato slices. Improved drying characteristics of the disintegrated food matrix may the reason for the reduction of fat content in PEF pre-treated French fries, as well. Thus, PEF treatment provides a potential to be implemented in potato processing in order to apply a non-thermal method for the production of low-fat French fries, energy and water saving and with only minimal losses on the basic product.

- *PEF enhance infusion of sodium chloride*

  PEF assist the infusion of common salt into potato tissue. In agreement with the observations of Toepfl and Heinz (2007), who reported about improved diffusion of salt and nitrite into pork haunches after PEF treatment, PEF application is considered to be a method able to target insert pigments or flavour carrier not only in animal but also in plant tissue.

- *PEF reduce lignin content in white asparagus*
  PEF application decreases amount of lignin in white asparagus spears. Thus, PEF may be applied as a pre-treatment before preserving to minimize lignification in order to improve macroscopic characteristics and gain softer texture of the spears.

5.1 Outlook and Future work

Basic research

- *PEF-microscopy enables in situ analysis of how the electric field influences cell wall substances*
  The application of pulsed electric fields in combination with simultaneous microscopic visualization provides a promising tool to observe cell structure changes instantaneously during treatment. This technique offers new ways to study the immediate effects of PEF on cellular level and to identify influencing factors on the degree of cell membrane disintegration. The development of innovative methods for the examination of cell vitality shall help to convert the basic knowledge into effective processes. Based on the different characteristics of protoplasts and native cells in the electric field it is of great interest if PEF application influences biopolymers in cell wall. With regard to the reduction of lignin content in PEF treated asparagus spears and the possible separation of cellulosic material from lignin due to PEF, it should be tested if the external electric field has an influence on other macromolecules like celluloses, hemicelluloses and pectin. It can be supposed that PEF affect glycosidic bonds, polar hydroxyl groups and/or the charged carboxyl groups on the molecule pectin.

Applications

- *The enhanced mass transfer in potato and asparagus tissue due to PEF treatment needs to be examined at industrial scale processes*

  PEF treatment was shown to be effective for the enhancement of mass transfer in potato slices and asparagus spears. To apply PEF in food industry, more studies in technical scale need to be performed. Thus, PEF equipment design and treatment conditions should be optimized. This includes PEF treatments with continuous PEF treatment chambers that are high in diameters in order to achieve flow-rates up to 5 t/h.

- *Consumer acceptance of PEF treated potato products with lower fat content and hardened crust texture needs to be evaluated*

  Another aspect of great importance is the consumer acceptance of PEF pre-treated French fries. Altered drying characteristics of the PEF- treated potato stripes leads to lower fat content but also to a harder crust structure. Due to extensive sensory evaluations, it can be clarified to which extent the cross texture is noticed and accepted by the human taste.

- *PEF application improves the removal of reducing sugars. Future studies shall clarify whether PEF treatment improves the diffusion of amino acid as well and to which extent this reflects reduced acrylamide formation.*

  As far as PEF enhance the release of low molecular substances it should be tested whether PEF treatment increases the removal of amino acids (asparagine, glutamine) from potato tissue likewise. This is of great interest, because reducing sugars and amino acids represent relevant substrates for the Maillard reaction. Finally, the acrylamide formation after processing of PEF pre-treated French fries and potato crisps should be determined.

- *The effect of electrode positions on the level of mass transfer in PEF- treated asparagus should be examined*

  It was shown that PEF treatment with longitudinal electrode orientation causes higher cell disintegration degrees in asparagus than placing the electrodes in longitudinal direction. Thus, it is of relevance to analyze effects of PEF on asparagus characteristics additionally by orientating the electrodes in longitudinal direction relative to the major axis of the spear. This would help to optimize PEF processing and achieve more effective process conditions.

Acknowledgements

This work has been supported by an EU-integrated Project NovelQ “Novel Processing Methods for the Production and Distribution of High-Quality and Safe Food”, FP6-CT-2006-015710, Priority 5 `Food Quality and Safety`.

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Curriculum Vitae

Anna Winter née **Janositz**

PhD Student, Dipl.-Ing. (M.Eng) Food Engineering

Biography:

Since 06/2005 Ph.D. student at Berlin University of Technology, Prof. D. Knorr,
Thesis: Impact of Pulsed Electric Fields on post-permeabilization processes of plant cells.

04/2005 Dipl.-Ing. (M.Eng.) Food Technology, Berlin University of Technology
Thesis: Auswirkungen von Hochspannungsimpulsen auf das Schnittverhalten von Kartoffeln (Solanum tuberosum).

1998-2005 Studies of Food Technology at Berlin University of Technology

Date of birth May, 20th, 1977

Children two daughters (*29/01/07, *18/06/09)

List of Publications:

Janositz, A., Semrau, J. & Knorr, D. (2011). Impact of PEF treatment on quality parameters of white asparagus (Asparagus officinalis L.). *Innovative Food Science and Emerging Technologies*, 12, 269-274.

Janositz, A., Noack, A.-K. & Knorr, D. (2011). Pulsed Electric Fields and their impact on the diffusion characteristics of potato slices. *LWT-Food Science and Technology,* 9, 1939-1945.

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Jaeger, H., **Janositz, A**. & Knorr, D. (2010) The Maillard reaction and its control during food processing. The potential of emerging technologies. *Pathologie Biologie (Paris)*, 58, 3, 207-213.

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Oral and poster presentations:

Balasa, A., **Janositz, A.** & Knorr, D. (2009) Pulsed electric field treatment of plant tissue: an overview , EuroFoodChemXV 2009, Copenhagen/Denmark.

Janositz, A., Toepfl, S. & Knorr, D. (2006) The impact of Pulsed Electric Field treatment on the reduction of potato cutting energy. Food is life, IUFoST, Nantes/Frances.

Janositz, A. & Knorr, D. (2006) Factors of influence in plant cell membrane permeabilization. COST meeting 928-300606, Cost, Reykjavik/Iceland.

Janositz, A., Toepfl, S. & Knorr, D. (2006) Impact of pulsed electric fields on membranes on a cellular level. Food factory of the future, SIK - The Swedish Institute of Food and Biotechnology, Gothenburg/Sweden.

Janositz, A. & Knorr, D. (2006) Die Reduzierung der Zellfläche als Indikator der HSI-induzierten Permeabilisierung. BioPerspectives im Fokus: Nahrung für die Zukunft, Industrie und Handelskammer (IHK) Potsdam, Potsdam/Germany.

Janositz, A., Smetanska, I. & Knorr, D. (2006) Factors of influence in plant cell membrane permeabilization. Workshop Molecular Interactions, Max-Planck-Insitut für Molekulare Genetik, Berlin/Germany.

Janositz, A., Toepfl, S. & Knorr, D. (2005) Microscopic visualization of plant tissue during pulsed electric field treatment. Nonthermal Processing Division, IFT –institute of food technology, Philadelphia/USA.

Janositz, A., Toepfl, S. & Knorr, D. (2005) Mikroskopische Visualisierung von pflanzlichem Gewebe während der Behandlung mit Hochspannungsimpulsen. FEI Diskussionstagung, FEI, Berlin/Germany.

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Ich erkläre an Eides statt, dass die vorliegende Dissertation in allen Teilen von mir selbständig angefertigt wurde und die benutzten Hilfsmittel vollständig angegeben worden sind.

Anna Winter

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Innovative Food Science and Emerging Technologies 11 (2010) 592–597

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Microscopic visualization of Pulsed Electric Field induced changes on plant cellular level

A. Janositz *, D. Knorr

Technical University of Berlin, Institute of Food Technology and Food Chemistry, Department of Food Biotechnology and Food Process Engineering, Germany

ARTICLE INFO

Article history:
Received 22 April 2009
Accepted 23 July 2010

Keywords:
Pulsed Electric Fields (PEF)
Plant cell culture
Protoplast
Microscope
Cell size
Stress induction

ABSTRACT

The effects of Pulsed Electric Fields (PEF) on protoplasts from cultured tobacco cells (*Nicotiana tabacum* b.y.-2) in comparison to the changes on cultured plant cells with cell walls were visualised in order to study the direct impact of PEF on cell components and to clarify the influence of the cell wall on electroporation. Optical microscopic analyses were carried out and images were recorded during PEF treatment. Results showed higher sensitivity of protoplasts to electric fields related to cells with a cell wall. Protoplasts sizes were measured before and after different treatment intensities and protoplasts shrinkage was used as an indicator for cell rupture. It could be demonstrated that cell volume decrease is influenced by PEF intensity, initial cell size, cell orientation in the electric field and nucleus position.

Industrial relevance: Since the beginning of the 20th century the relevance of Pulsed Electric Fields (PEF) technology in food- and biotechnology has increased substantially. However, the mechanism of membrane permeabilization and the PEF induced changes in cell structure remain poorly understood, diminishing the optimal use in food industry. In this study the direct effects of PEF on cultured plant cell material and influencing factors of the degree of membrane disintegration were visualized and identified. The development of new methods to examine cell vitality shall help to convert the basic knowledge into effective processes.

1. Introduction

During the 1990's, industrial interest in developing gentle food technologies, to replace the currently common thermal processing, has increased substantially. The non-thermal application of Pulsed Electric Fields (PEF) is counted among these emerging processes and received considerable relevance in bio- and food technology. It involves the exposure of biological cell material to short repeated pulses of a high voltage with the result of pore formation in cell membrane leading to membrane permeabilization and cell rupture. The benefit of this approach is an important aspect of process and product development because it is aimed to protect quality food attributes, such as sensory quality and nutrition value, as well as to control the microbial safety with minimal or no changes during processing. Besides the use of non thermal technologies to inactivate microorganisms through mechanical destruction of cellular structure (Ho & Mittal, 2000; Wouters, Alvarez, Angersbach & Knorr, 2001; Heinz, Alvarez, Angersbach & Knorr, 2001) the main field of interest is the treatment of plant cell material, for cell membrane disruption leading to increased membrane permeability and to improved mass transfer of inner liquid and cell components (e.g. health related plant metabolites, pigments) from the intracellular vacuoles. Processes such as drying, osmotic dehydration and extraction are facilitated by PEF treatment (Angersbach, Heinz & Knorr, 1998; Ade-Omowaye, Angersbach, Taiwo & Knorr, 2001). The application of osmotic dehydration can be used as a pre-treatment to conventional drying, or freezing for the enhancement of diffusion characteristics with simultaneous maintenance of fruit product attributes and the reduction of energy consumption. Prior PEF treatment intensifies the desired effect due to the improvement of water and solution mass transfer into and out of the tissue. Rastogi, Eshtiaghi and Knorr (1999) investigated the impact of PEF on the dehydration characteristics of carrots and found out that PEF processing of carrot cubes caused a lowering of moisture content during osmotic dehydration. Further successful results have been gained concerning apple slices (Taiwo, Angersbach & Knorr, 2002), mango (Tedjo, Taiwo, Eshtiaghi and Knorr, 2002), and bell peppers (Ade-Omowaye, Rastogi, Angersbach & Knorr, 2002).

Several studies reported on the gentle recovery of sensitive vacuole components such as flavours and dyestuffs in different plant food and on the increase of extraction yield after PEF processing (Eshtiaghi & Knorr, 1999; Bouzrara & Vorobiev, 2000; Bazhal, Lebovka & Vorobiev, 2001; Guderjan, Toepfl, Angersbach & Knorr, 2005). These low energetic cost and short treatment time applications offer alternative possibilities to thermal processes and therefore to optimize process control on food sector. Additionally, the employment of mild heat can be used to intensify the desired target and

* Corresponding author. TU Berlin, Department of Food Biotechnology and Food Process Engineering, Koenigin-Luise-Str. 22, D-14195 Berlin, Germany. Tel.: +49 30 314 71411.
E-mail address: anna.janositz@tu-berlin.de (A. Janositz).

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doi:10.1016/j.ifset.2010.07.004

therefore to obtain synergistic effects with the combination of both treatments (Schilling et al., 2008). Another benefit of PEF processing lies in the treatment with mild conditions to induce the production of secondary metabolites with the maintenance of cell viability. The stimulation caused by PEF can be used to target influence the plant for the generation of health related compounds as a stress response of the electric field (Guderjan et al., 2005).

The most accepted theory about the permeabilization mechanism is conceived to be related to electrocompression of the cell membrane (Zimmermann, 1986). Due to the electric field, accumulation and attraction of oppositely charged ions on both sites of the non conductive cell membrane occur, causing membrane thickness reduction. With further increase in the transmembrane potential, as a consequence of the increased electric field, a critical value is reached and membrane compression is intensified leading to the formation of pores and the loss of semi-permeability in the cell membrane. This pore formation can be temporary (reversible) or irreversible (permanent), depending on treatment intensity and sample composition (Zimmermann, 1986). Reversible pore formation takes place when the external electric field is removed while the critical membrane potential is reached and the generated pores are small related to the membrane surface. Characteristic for the processing with mild PEF conditions is that the cell retains its viability in contrast to high energy input treatment (for plant cells, $E \geq 1$ kV/cm), which results in the loss of cell vitality. The effectiveness of PEF technology to permeable cell membranes depends on several factors which can be classified in technical and chemical process conditions as well as in biological product characteristics. The technical factors include PEF process parameters such as electric field intensity, treatment time, pulse shape and applied energy, whereas the electric field intensity has been described as the most relevant factor defining membrane rupture by pulsed electric fields (Hamilton & Sale, 1967; Hülsheger, Potel & Niemann, 1981; Schoenbach, Joshi, Stark, Dobbs & Beebe, 2000; Zhang, Monsalve-González, Barbosa-Cánovas & Swanson, 1994; Tatebe, Muraji, Fujii & Berg, 1995). The chemical and physical characteristics of treated products have also an important impact on the efficiency of PEF. Further product parameters are the composition of treated media, including pH, temperature and especially ionic strength, which is responsible for the conductivity of treated media (Jayaram, Castle & Margaritis, 1993; Vega-Mercado, Pothakamury, Chang, Barbosa-Cánovas & Swanson, 1996). Biological characteristics such as species, size, shape or physiological state influence the degree of membrane permeabilization additionally. Therefore, small microorganism cells were found to be less sensitive against the external electric field, whereas membrane disintegration of larger plant cells occurs in markedly higher percentage by applying same PEF treatment conditions (Sale & Hamilton, 1967; Zhang, Monsalve-González et al., 1994).

Although background knowledge and theories about the PEF induced plasmolysis increased (Cruzeiro-Hanson, 1988; Dimitrov, 1984; Sugar & Neumann, 1984), the mechanism of membrane permeabilization and the changes on cell level after PEF processing remain poorly understood, degrading the full potential use of this technology. One problem in the study of biological cells is that the dynamic process of electroporation is extremely rapid. Pore building occurs within 10 ns at sites where the membrane potential reaches/obtain 1 V (Dimitrov & Jain, 1984). This celerity causes difficulties in the visualization of pore formation and the subsequent exchange of intra- and extracellular compounds. Membrane recovery can be happen in a broader range, from 0.1 ms to 2.8 h (Ho & Mittal, 1996). Furthermore, research not only about effects of PEF on cell membranes but also on other cell materials as cell walls is still scarce. Bazhal, Lebovka and Vorobiev (2003) analysed textural parameters of PEF treated apples and reported that the electric field affects not only plasmalemma membranes but also the cell wall integrity. Moreover, the function of the cell wall as a possible protection for the cell against the electric field as well as the interaction between cytoplasm and cell wall during post permeabilization are largely unknown.

In the study undertaken, enzymatic protoplasts (cells without cell wall) have been prepared from a tobacco plant cell culture to create a model system for the analysis of PEF induced membrane changes on cell level. The cells were exposed to a microscope with an integrated PEF unit in order to visualize membrane disintegration not only afterwards but also during the period of PEF processing. To gain better inside of the permeabilization mechanism, specific methods for the indication of cell vitality of plant cell cultures after PEF treatment were developed. Additionally, protoplasts were compared with cell wall cells to analyse the impact of PEF on different cell types and to explain the role of cell wall in electric cell rupture.

2. Materials and methods

2.1. Plant cells and protoplasts

Cultured tobacco cells (*Nicotiana tabacum* L. cv Bright Yellow-2) (Takebe, Otsuki & Aoki, 1968; Mathur & Koncz, 1998; Nagata & Kumagai, 1999) were grown in MS medium (Murashige, 1962) for 7 days at 25 °C in the dark with reciprocatory shaking at 120 rpm.

For protoplast preparation, tobacco cells were vacuum filtered and 2 g fresh weight cells were resuspended in 10 ml solution of isotonic buffer W5 (154 mM NaCl, 125 mM $CaCl_2$, 5 mM KCl, 5 mM Glucose, pH 5.7) combined with a mixture of cellulolytic and pectolytic enzymes (0.01 g Rohament Cl, 0.1 g Rohament PL) (AB Enzymes, Darmstadt, Germany) for the residence time of 4 h. After digestion of cell wall components, the obtained spherical protoplasts were washed twice with 0.6 M mannitol. Isolated protoplasts were finally resuspended in 6 ml unbuffered isotonic mannitol solution to perform pulsed electric field treatment (Fig. 1). Buffer was excluded in order to render a low conductivity medium for PEF operation.

Pre-treatment of tobacco cells with cell wall was carried out with vacuum filtration and resuspension of 2 g cells in 6 ml mannitol solution before PEF processing. The measured conductivity of both cell types (protoplasts and cells with cell wall) mixed with mannitol was 3.3 mS/cm.

2.2. Tobacco cells

2.2.1. Staining

For the visualization of cell rupture from cells with cell wall after PEF processing, vital dyes were needed, to penetrate into permeabilized cells and indicate irreversible membrane disintegration. Therefore, freshly prepared solution (0.1%) of the vital dye Phenosafranine (dry content 80%, Sigma-Aldrich, USA) was added to cell suspension in a ratio 1:2 directly before treatment.

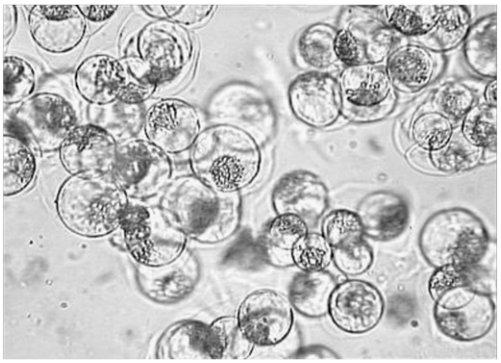

Fig. 1. Isolated protoplasts of seven-day-old *Nicotiana tabacum* cell suspension after enzymatic cell wall degradation.

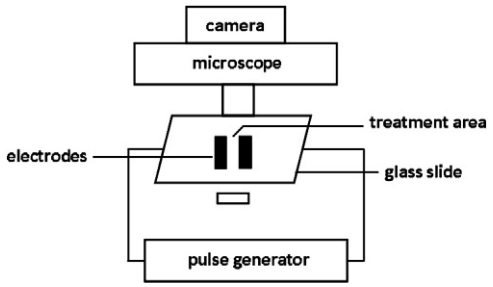

Fig. 2. Schematic set-up of pulsed electric field treatment chamber combined with a microscope.

2.2.2. Experimental set-up and electric field pulses protocol

The exponential electric field pulses were applied with the PEF microscope, schematic drawing shown in Fig. 2, constructed in the Department of Food Biotechnology and Food Process Engineering (TU Berlin). The microscope (Zeiss Optik, Jena, Germany) enabled the study of direct cell structure changes during the treatment. Main components were a camera (Nikon E 8700, Japan), which was fixed to the microscope, 3 objectives, with a maximum magnification of 400 fold, and a glass slide with two copper foil electrodes (gap 2 mm, length 3 mm, thickness 0.2 mm, area 0.6 mm²). The treatment chamber was connected to the micro pulse modulator, consisting of a power supply FUG HCK, 800 M–20.000, 20 kV, 80 mA (FUG, Rosenheim, Germany) to a capacitor bank of three capacitors with 6.8 nF each. The pulse parameters were examined by a high voltage and a current probe, coupled to a TDS220 (Sony Tektronix, Beaverton, US) oscilloscope. A PC computer was used to control PEF treatment intensities, namely electric field strength E: 0.25–7.5 kV/cm; pulse number n: 10, 20; specific energy input W: 2206–1985 J/g, pulse width τ: 2–8 µs and frequency f: 2 Hz. The images obtained with the microscope from the samples were recorded with the camera and single pictures of untreated and PEF treated were selected to analyze PEF induced cell disintegration. Camera was activated manually before treatment. For microscopic analysis, each process condition was performed approximately 10 times. Recorded cells per experiment/picture varied between 1 and 8.

Cell area was measured by the program AnalySis 2.11 (Muenster, Germany) from pictures taken from the recorded movie before and after (after the last pulse) PEF treatment.

T-tests were used for the analysis of statistical significance.

Cell area reduction was calculated by the formula:

(1−(cell size of PEF treated protoplasts / cell size of untreated protoplasts))*100.

2.2.3. Examination of cell vitality through impedance measurement

The extent of cell membrane permeabilization was determined using the cell disintegration index (CDI) (Angersbach, Heinz & Knorr, 1999). The method is based on the frequency dependence of conductivity of intact and permeabilized tissue.

The cell disintegration index Z_p was calculated by:

$$Z_p = 1-b\frac{K`h-K`l}{K_h-K_l} \quad b = \frac{K_h}{K`_h} 0 \le Z_p \le 1$$

where K_l and K_l' indicate the electrical conductivity of untreated and treated cell material in a low-frequency field (1–5 kHz), respectively; and K_h and $K_h`$ indicate the electrical conductivity of untreated and treated material in a high-frequency field (3–50 MHz).

The CDI values between 0 for intact cells and 1 for total disintegration.

Impedance measurement of protoplasts and cell with cell wall at low and high frequencies within the frequency range of 3 kHz and 100 MHz was carried out via impedance measurement equipment (Biotronix GmbH, A. Angersbach, Hennigsdorf, Germany). The electrode area of the measuring cell was 2 cm². The gap was adjusted to 1.3 cm.

3. Results and discussion

3.1. Effect of PEF on protoplasts (digested cell wall) and native cells (intact cell wall) — a comparison

Visual observation showed higher sensibility of protoplasts to the electric field compared to cells with cell walls (Figs. 3 and 4). The elimination of cell walls leads to a loss of structural support. Therefore irreversible membrane pore formation after PEF processing of protoplasts was indicated by the reduction of cell size whereas membrane disintegration of cell wall cells could only be noticed with vital dye (phenosafranine) diffusion. Noticeable decrease of protoplast cell area was already shown after the first pulses at quite low treatment conditions ($\geq E = 0.5$ kV/cm, $n = 10$, $\geq W = 8824$ J/g; Fig. 3). In contrast to cells with cell wall, where the phenosafranine uptake, which indicates irreversible pore formation, was only registered at higher PEF intensities ($\geq E = 1.2$ kV/cm, $n = 20$, $\geq W = 2541$ J/g; Fig. 4). The cell disintegration index correlated with microscopic observations and demonstrated the intensified effect of PEF on protoplasts (Fig. 5). It could be shown that the presence of cell walls highly influence the degree of membrane permeabilization. Both cell types showed higher degree of cell disintegration with the application of higher PEF intensities. The extent of protoplast cell rupture was nearly twice as high compared to the cells with cell wall with same treatment conditions, demonstrating the protective effect of plant cell walls.

3.2. Protoplasts as a model system to visualize influence factors on PEF induced membrane rupture

Cell shrinking after irreversible PEF treatment could only be observed in protoplasts. Hence, cell volume of untreated and PEF

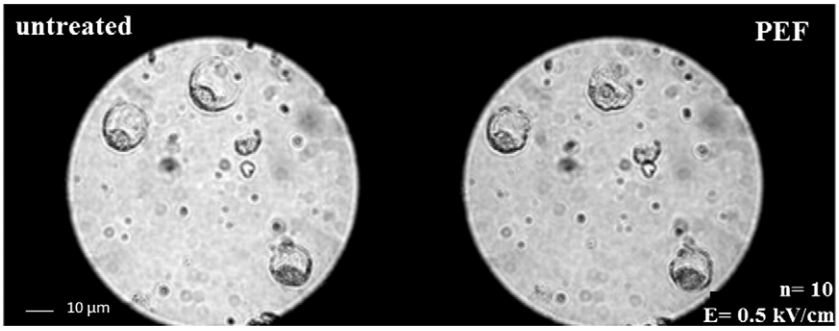

Fig. 3. Protoplasts (*Nicotiana tabacum* L. cv Bright Yellow-2) untreated and after PEF treatment ($E = 0.5$ kV/cm, $n = 10$, $f = 2$ Hz).

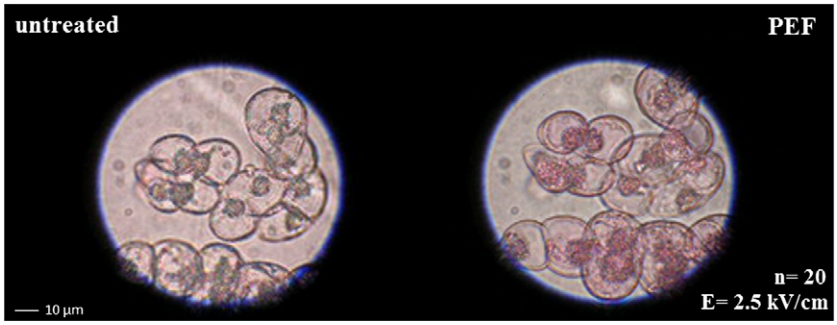

Fig. 4. Tobacco cells with cell wall (*Nicotiana tabacum* L. cv Bright Yellow-2) in vital dye solution (Phenosafranine) untreated and after PEF treatment ($E = 2.5$ kV/cm, $n = 20$, $f = 2$ Hz).

treated protoplasts can serve to detect the degree of membrane permeabilization.

3.2.1. Determinant factors: PEF treatment intensity and cell size

Microscopic analysis during the treatment could visualize the fact that higher PEF intensities cause higher degree of cell rupture, indicated by major cell area reduction at stronger PEF energy inputs (Fig. 6). For cells of 250–350 µm² cell area, application with field strength of 0. 5 kV/cm and 10 pulses resulted in cell area reduction of 12.5% whereas for $E = 5$ kV/cm and $n = 10$ the protoplast shrinking reached 34%. Furthermore, it could be shown that the cell size determines the required external electric field intensity which causes membrane disruption. Larger cells were more affected by the electric field than cells with smaller size. For cells with less than 250 µm² cell area, lower size reduction was noticed after PEF treatment and the different intensities caused minor differences in cell area as it could be monitored for larger cells. The impact of cell size on the effectiveness of PEF treatments is clearly shown in the reduction of the cell size after PEF processing with the highest applied treatment intensity ($E = 5$ kV/cm, $n = 10$, $W = 883{,}353$ J/g), where cell area differed from 11.8% for smallest cells (<250 µm²) to 39.8% for cell size in the range over 350 µm². The observed effect of cell size on the degree of cell area reduction corresponds with other experimental studies (Sale & Hamilton, 1967; Hülsheger et al., 1983; Zhang, Chang & Barbosa-Cánovas, 1994), and is based on the required electric field intensity to induce a given transmembrane potential into a cell, which can be calculated by the equation

$$V_m = f^* a^* E_c$$

(Schwan, 1957) (1).

Where V_m is the transmembrane potential induced by an external field of the strengthE_c [kV/cm], a [µm] is the cell radius and f is the form factor for spherical shape (= 1.5). This formula enables the calculation of the induced potential for tobacco protoplasts of different cell sizes. The calculated relation between transmembrane potential to cause disruption of cell membrane and cell diameter is represented in Fig. 7. The graph shows cells of different diameters sizes evaluated from the field strength $E = 2.5$ kV/cm. It is shown that external electrical fields induce higher transmembrane potentials in larger cells and that the potential rises proportional to the increase in cell size. Thus, the average cell diameter of the smallest protoplast group was 14.5 µm and the associated transmembrane potential 2.72 V, whereas for larger cells (>18 µm) the mean cell diameter was 20.73 µm with a transmembrane potential of 3.89 V.

Another focus of our study was the microscopy of reversible pore formation through following the resealing processes. Furthermore, it was not only possible to visualize irreversible cell disintegration by the reduction of cell size but also to image temporary pore formation in cell membrane after the application of PEF with low energy inputs. The PEF induced stress reaction which causes reversible pores in plasmalemma could be indicated by cell swelling after the exchange of intra- and extracellular fluids due to slight osmotic imbalance in the medium (Fig. 8). Temporary formed pores leads to a break in the osmotic barrier. Subsequently the gradient for osmotic pressure between intra- and extracellular liquids drops to zero. For draining permeabilized cells, a hyperosmotic medium is used. Vice versa, liquid uptake occurs in a hypoosmotic medium.

In Fig. 8 the differences in protoplast cell area before and after PEF treatment are represented. Whereas treatment conditions higher than $E = 0.5$ kV/cm and $n = 10$ led to a reduction of cell area, the utilization of low process parameter ($E = 0.25$ kV/cm, $n = 10$, $W = 2206$ J/g) resulted in an increase of cell size, which could indicate the resealing of temporary formed pores in membranes after PEF implementation.

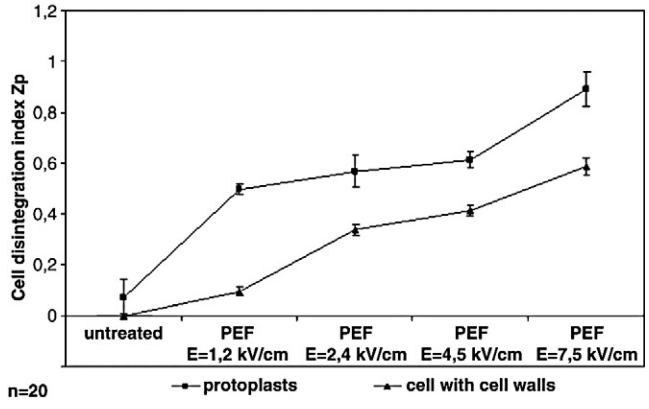

Fig. 5. Cell disintegration index of untreated and PEF treated protoplasts and cells with cell wall after different PEF treatment conditions.

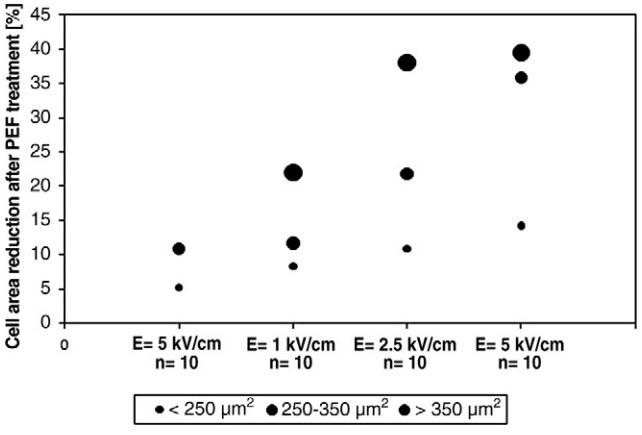

Fig. 6. The effect of different PEF treatment conditions on cell size reduction of protoplasts.

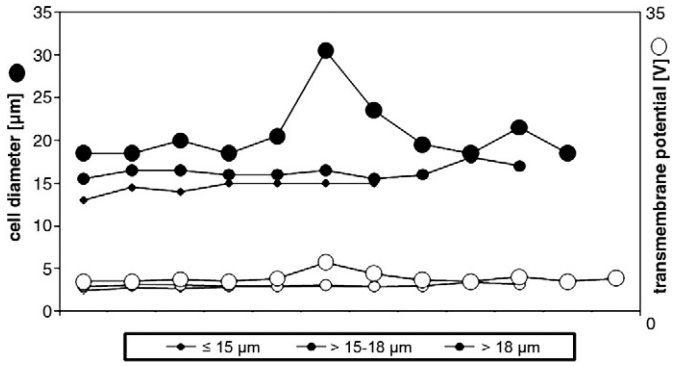

Fig. 7. Exemplary example for the relationship between cell size and transmembrane potential, induced at the field strength of $E = 2.5$ kV/cm and 10 pulses.

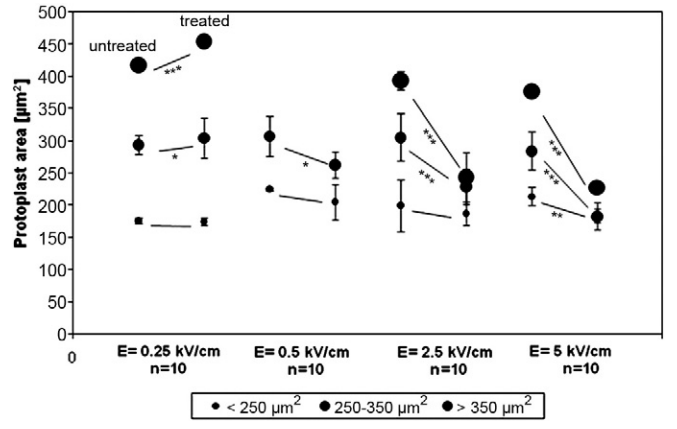

Fig. 8. Cell area of untreated and PEF treated protoplasts before and after PEF processing with different treatment conditions. Statistical significance (* $P<0.05$, ** $P<0.01$, *** $P<0.001$).

3.2.2. Determinant factors: electric field direction and cell nucleus

The *in situ* study of plant cell permeabilization aimed to achieve some further information concerning the influence factors of PEF induced membrane rupture. On the basis of microscopic analyses it could be frequently noticed that cell shrinking started at the cell poles, which were nearest to the electrodes (Fig. 9). An observation, which was described by some research groups (e.g. Kinosita et al., 1988; Hibino, Itoh & Kinosita, Jr, 1993). Electroporation does not occur uniformly across the cell membrane. The areas nearest the electrodes are easily electroporated, whereas others still remain intact, based on different build transmembrane potentials.

The beginning of cell size decrease at these areas can be seen as an indicator for the initiation of pore formation on the membrane sites facing to the electrodes.

Furthermore, it was assumed that membrane disruption depends on cell nucleus position (Fig. 10). The nucleus stabilizes the cell which results in higher membrane damage probability at the opposite side. In contrast to the described increase of transmembrane potential due to the position of the cell in the electric field, it cannot be concluded that pore formation starts at the obverse membrane side were the cell core is located, but only to nucleus induced cell firmness. Further investigations have to be performed to intensify the possibility of nucleus induced cell stability.

4. Conclusions

Microscopic analysis before, during and after PEF treatment is a useful tool to gain a better insight in the permeabilization mechanism of plant cell material. The comparison between protoplasts and native plant cells concerning their PEF induced cell structure changes helps to determine the effect of PEF on the cell wall. Visual observation showed higher sensibility of protoplasts to the electric field in contrast to cells with cell wall. The cell disintegration index correlated with microscopic analysis and indicated higher degree of disintegrated cells for protoplasts.

It was detected that protoplast cell size is reduced after irreversible cell disruption and slightly increased after reversible permeabilization. Hence, it was concluded that measurement of cell size before and after treatment can serve as an indicator for cell vitality. It could be demonstrated through the measurement of cell size and the visual analysis that cell area decrease is influenced by PEF intensity, cell size, electrode situation and nucleus position.

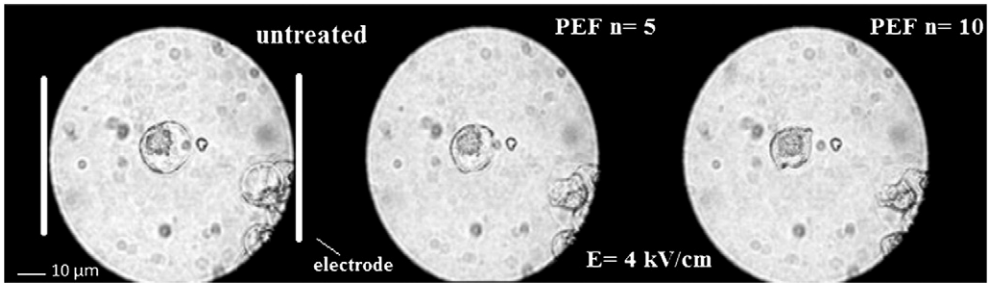

Fig. 9. Recorded images of untreated and PEF treated ($E = 4$ kV/cm, $n = 10$, $f = 2$ Hz) Tobacco protoplasts at different treatment times.

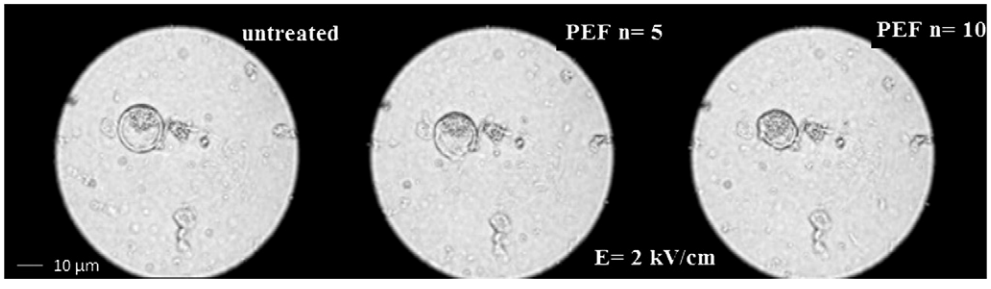

Fig. 10. Recorded images of untreated and PEF treated ($E = 2$ kV/cm, $n = 10$, $f = 2$ Hz) Tobacco protoplasts at different treatment times.

Acknowledgments

This work has been supported by an EU-founded Integrated Project NovelQ "Novel Processing Methods for the Production and Distribution of High-Quality and Safe Food", FP6-CT-2006-015710, Priority 5 `Food Quality and Safety`.

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Pulsed electric fields and their impact on the diffusion characteristics of potato slices

A. Janositz*, A.-K. Noack, D. Knorr

Department of Food Biotechnology and Food Process Engineering, Institute of Food Technology and Food Chemistry, Technical University of Berlin, Germany

ARTICLE INFO

Article history:
Received 6 October 2010
Received in revised form
13 April 2011
Accepted 18 April 2011

Keywords:
Pulsed electric fields (PEF)
Potato
Diffusion
Reducing sugars
Drying

ABSTRACT

Mass transfer in potato slices and strips after Pulsed Electric Fields (PEF) treatment was examined to evaluate potential application of PEF in potato processing. PEF treatment on cell material leads to pore formation in cell membrane and thus modifies diffusion of intra- and extracellular media. Results showed enhanced release of intracellular molecules from permeabilized tissue as well as improved uptake of low molecular substances into the sample. Sugar, one substrate for the Maillard reaction, was decreased in PEF treated potatoes, while conductivity increased after electroporation and soaking in sodium chloride solution, indicating the improved diffusion of salt caused by PEF. Higher release of cell liquid during drying of PEF treated potatoes was noticed in comparison to untreated potato slices. This effect increased with the treatment intensity. Furthermore, it was revealed that PEF application leads to a distinct reduction of fat content after deep fat frying and thus provides a potential for the production of low-fat French fries. It can be presumed that PEF is a capable assistance to thermal treatments in the processing of potato chips or French fries for the achievement of structural modifications and improved process conditions.

1. Introduction

Mass transfer processes are important unit operations in food industry requiring the disintegration of biological material. Especially the processing of plant cells is of great commercial interest because of the high amount of health related ingredients, pigments and cell liquid in the vacuoles but also due to the diversified potential to be further manufactured. Cell membranes can be seen as a barrier in diffusion processes, which are therefore strongly influenced by the degree cell membrane permeabilization. To soften the tissue, disrupt cell membranes and to release intracellular content out of the vacuoles, diffusion processes are often applied with thermal, enzymatic or mechanical exposures. However, these pre-treatments are connected with some drawbacks because processes such as extraction, osmotic dehydration or drying are leading to high energy consumption, long holding times and determination on nutritional value (Carlsson-Kanyama & Faist, 2000; Santos, Veggi, & Meireles, 2010; Tangka, 2003).

1.1. PEF processing

The newly emerged application of Pulsed Electric Fields (PEF) constitutes an alternative to conventional processing of plant cell material, with the main aim of mass transport enhancement through permeabilization of the cell membrane. PEF processing is a non-thermal technology to treat plant cell material with less degradation of nutritional compounds. The application of PEF includes the implementation of short repeated high voltage pulses to biological cell material resulting in pore formation of cell membranes. Two different kinds of pore structure are leading to different application fields of PEF. Mild reversible PEF treatment ($E = 0.5–1$ kV/cm) maintains the viability of cells and can be used to target stimulate plant cells to produce secondary metabolites (Guderjan, Toepfl, Angersbach, & Knorr, 2005). Higher energy pulsing ($E > 1.0$ kV/cm) causes permanent membrane disintegration and therefore the mechanical destruction of the cell. Irreversible pore formation offers several fields of application in the non-thermal treatment of plant cell material. Mass transfer processes are enhanced by PEF treatment due to the facilitated release of intracellular liquid out of the cell after membrane disruption. Improvement of juice rates and intracellular metabolite extraction (Bazhal & Vorobiev, 2000; Bazhal, Lebovka, & Vorobiev, 2001; Knorr, Geulen, Grahl, & Sitzmann, 1994; Sensoy & Sastry, 2004) or the enhancement of drying efficiency (Ade-Omowaye,

* Corresponding author. TU Berlin, Department of Food Biotechnology, and Food Process Engineering, Koenigin-Luise-Str. 22, D-14195 Berlin, Germany. Tel.: +49 30 314 71411.
E-mail address: annajanositz@yahoo.de (A. Janositz).

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doi:10.1016/j.lwt.2011.04.006

Angersbach, Esthiahgi, & Knorr, 2001) can be mentioned besides the use of PEF as a pasteurization method to decontaminate solid and liquid food (Alvarez, Condon, & Raso, 2006; Heinz, Alvarez, Angersbach, & Knorr, 2002; Wouters & Smelt, 1997).The critical external field strength is highly dependent on cell size as well as on cell orientation in the field (Heinz et al., 2002). Compared to small bacterial cells (1–10 μm), which require high treatment conditions to disrupt (critical electric field strength of 10–14 kV/cm), lower energy input is needed for the PEF induced membrane permeabilization (critical electric field strength of 1–2 kV/cm) of plant cells, due to their larger cell size, which is in the range of 10–100 μm (Janositz & Knorr, 2010; Knorr, Angersbach, Eshtiaghi, Heinz, & Lee, 2001). The advantage of using mild process conditions for plant cells is based on the easier generation of transmembrane potential in large cells, which is required to form pores in the phospholipid layer.

1.2. PEF assisted mass transfer

The employment of PEF in the treatment of plant tissue can be used to increase extraction yield in the production of fruit and vegetable juice. PEF applied as a pre-treatment before pressing retains product quality and results in the production of fresh taste juices with a high concentration of heat-sensitive ingredients. It was demonstrated by Knorr et al. (1994) that PEF assisted extraction of carrot juice increased not only extractions rate but also preserved natural composition of functional compounds as β-carotene. Higher availability of β-carotene was attained as well as an improvement in extraction efficiency from 51% to 67% by using PEF. PEF processing can be employed to enhance wine extraction and the amount of phenolic compounds (Puértolas, López, Condón,Álvarez & Raso, 2010). An increased wine yield after pressing of PEF treated white grapes was found by Praporscic, Lebovka, Vorobiev, and Mietton-Peuchot (2007). They showed that high-quality wine can be produced after PEF application due to rapid expression of low oxidized must with smallest possible rate of mire formation. Besides the use of PEF to enhance extraction yield and composition in the production of fruit and vegetable liquids, the implementation of PEF in sugar processing has also attracted attention. The disaccharide sucrose, located in the intracellular structure of the sugar beet, is traditionally extracted by thermal treatment for 10–20 min at high temperatures, about 75–80 °C to achieve denaturation of cellular membranes (Van der Poel, Schiweck, & Schwarz, 1998). This thermal treatment leads near high water consumption and energy costs to some undesirable effects on the extracted product, as it reduces the juice purity after thermal destruction of cell wall components and transformation of high-molecular-weight substances. Several studies demonstrated the improved sugar extraction of PEF treated sugar beets (Lebovka, Shynkaryk, El-Belghiti, Benjelloun, & Vorobiev, 2007). Eshtiaghi and Knorr (2002) reported after PEF treatment of sugar beets about a 97% sugar yield and a 2–3 times faster extraction rate as that achieved by conventional thermal processing. Also López, Puértolas, Condón, Raso, and Álvarez (2009) investigated in PEF assisted extraction of sucrose from sugar beets and found out that temperature of thermal treatment could be reduced from 70 °C to 40 °C after PEF pre-treatment. Loginova, Vorobiev, Bals, and Lebovka (2011) demonstrated that previous PEF treatment enhances the exhaust of sugar beet pulp and increases pulp dryness. Another point of view that contributes to the great importance of the facilitated sugar extraction after PEF application is the potential cancer-causing agent acrylamide. Acrylamide can be formed in thermally processed food at temperatures above 120 °C as a result of the Maillard reaction, which occurs between amino acids as asparagine or methionine and reducing sugars as fructose or glucose (Ledl & Schleicher, 1990; Rosén & Hellenäs, 2002; Stadler et al., 2002). Maillard reactions are important in food processes such as baking or frying. The formed Maillard products are partly responsible for color and aroma of many food products. In processes such as drying, pasteurization and sterilization Maillard reactions are undesirable and cause major food spoilage due to the darkening of color, the reduced nutritional availability of certain amino acids and the formation of unfavored reaction products (Zhang & Zhang, 2007). The formation and reduction of acrylamide in the Maillard reaction mainly depends on the variables: temperature, processing time, pH, water activity, product composition and the availability of reactants, which were regulated by the processing of food material (Rufia'n-Henares, Delgado-Andrade, & Morales, 2009). Since temperature and time of food process count as the most important factors affecting Maillard reaction, the application of PEF provides a new concept to reduce thermal energy and treatment time with the combination of target control of food composition. Jaeger, Janositz, and Knorr (2010) demonstrated the advantageous use of PEF in the processing of potato chips. A higher release of sucrose, glucose and fructose was observed after PEF lab scale application based on the enhanced diffusion of cell water and low molecular substances out of the cells. A 50% increase in reduction of glucose content could be shown after PEF treatment in comparison to untreated potato slices. Better diffusion after PEF implementation was not only indicated by the enhanced sugar release out of the cell but also by the improved enzyme infusion of glucoseoxidase in the potato. It was possible to increase the enzymatic decrease of glucose content from 52% to 65% and therefore to present an effective method to improve enzyme diffusion as well as enzyme substrate accessibility (Jaeger et al., 2010).

The enhanced diffusion properties after PEF induced electroporation can also be used to accelerate the drying process efficiency of plant cell material. A shorter drying time of PEF treated potato cubes during fluidized bed drying was reported by Angersbach and Knorr (1997). Ade-Omowaye,Rastogi, Angersbach & Knorr (2003) studied the effect of PEF on red bell pepper slices and compared the dehydration characteristics with other pre-treatments. They reported about significant effects on drying time after PEF treatment. Drying process time decreased from 360 min without pre-treatment to 220 min with PEF pre-treatment and thus could be reduced to one third. Lebovka, Praporscic, and Vorobiev (2003) and Lebovka, Shynkaryk, and Vorobiev (2007) demonstrated an essential influence of PEF treatment at moderate electric field strengths on potato drying rates and showed that PEF application allows performing thermal pre-treatment at milder temperatures. For the process of osmotic dehydration several studies reported about PEF improved water and solution diffusion. Rastogi, Eshtiaghi, and Knorr (1999) investigated the impact of PEF on dehydration of carrot cubes and described a decrease in moisture content during osmotic treatment. Similar observations were made for mango (Tedjo, Taiwo, Eshtiaghi, & Knorr, 2002) and apple slices (Taiwo, Angersbach, & Knorr, 2002).

In the study undertaken, PEF were applied to show the potential of this technology as a disintegration method to improve mass transfer processes of potato slices. Potato food matrix was used to study the different effects of facilitated diffusion after PEF pre-treatment with regard to potential application of PEF in potato chips processing. Drying of potato slices was investigated as well as the enhanced release of monosaccharides, which represent relevant substrates for the Maillard reaction. Furthermore, oil content of fried potato strips was examined to analyze the influence of PEF pre-treatment on fat uptake during frying. The study focused not only on mass transfer from but also to the tissue. The uptake of sodium chloride after PEF implementation of potatoes was analyzed in order to examine the potential of PEF to assist the infusion of flavor carriers or pigments into the tissue.

2. Material and methods

2.1. Sampling

Potato tubers, *Solanum tuberosum*, varieties `Karlena' (mid-early maturing Dutch-German variety, BSA 1993) and `Saturna' (mid-late maturing Dutch-German variety, BSA 1993) were obtained from the potato processing company Lorenz Snack-World GmbH & Co KG (Neu-Isenburg, Germany) and stored in the dark at 8–10 °C. The analyses of sugar content and the uptake of sodium chloride were performed with the variety `Karlena', `Saturna' potatoes were investigated additionally in drying experiments. These cultivars have similar starch content (ca. 18 g/100 g, Kita, 2002) and are capable products for the processing of potato chips. Fat analyses were realized with potato tubers, *S. tuberosum*, of the variety `Russet Burbank' (late maturing US variety) which is often used in French fries production. Samples were obtained from a potato cropping farm in Sachsen-Anhalt/Germany. Before treatment, unpeeled potato tissue slices, 1.5–2 mm in thickness × approx. 5 cm in diameter, were sampled with a stainless steel vegetable slicer and washed in a defined volume (500 ml) of tap water for 5 s. Experiments in lab scale were performed with ca. 200 g specimen (15 potato slices = five potatoes with three slices of each potato) larger scale studies were carried out with 1 kg potatoes per test cycle (75 potato slices = 25 potatoes with three slices of each potato). For the analysis of fat content (see 2.8), PEF treatment was performed on whole tubers (ca. 200 g each) and blanching was carried out on potato halves. After treatments samples were subsequently cut into stripes (10 × 10 × 40 mm) by a French fry cutter with a stainless steel cutting grid.

2.2. Experimental set-up and electric field pulses protocol

PEF treatment was applied with exponential pulses. The treatment chamber was connected to a capacitor bank of four DP 30560 (GA, San Diego, USA), 15 kV, 2 μF in series was used to achieve a total capacity of 0.5 μF and was charged using an ALE802 (Lambda-Emi, Neptune, USA), 40 kV power supply. The parallel plate treatment chamber for laboratory batch-wise operation was built with an electrode size of 20 × 7 cm. Larger scale batch-wise PEF applications were performed in a parallel plate treatment chamber which was built with an electrode size of 49.5 × 32 cm and 45 × 29.5 cm for the upper and lower electrode, respectively. Samples were treated at room temperature in tab water. The applied PEF treatment parameters are listed in Table 1. The field strengths of $E = 1.5–2.5$ kV/cm were selected in order to achieve irreversible cell disintegration at a low energy consumption. Zimmermann (1986) reported that field strengths $E > 1$ kV/cm are sufficient to result in permanent pore formation of plant cell membranes. The microscopic study (see 2.3) was performed with the higher field strength of $E = 5$ kV/cm Janositz, Semrau, and Knorr (2011) demonstrated that the cell wall component lignin was reduced in white asparagus after PEF treatment with the same treatment intensities. Thus, parameter were chosen to have conditions that enable the breaking of covalent bonds within the cell wall and allow the investigation of PEF induced effects in this cell compartment. Temperature increase during PEF treatments was considered to be negligible (Knorr & Angersbach, 1998). Each process condition was performed at least 5 times. Mean values were calculated and presented together with standard deviation in the figures. Student's *t*-tests were used for the analysis of statistical significance.

Table 1
Parameter of different potato PEF treatments.

PEF parameter	Section 2.3	Section 2.4–2.7	Section 2.8
Field strength E [kV/cm]	5.0	1.5; 2.5	1.8
Pulse number n	20	20	40
electrode distance d [cm]	0.2	8	5
Pulse form	exponential	exponential	exponential
Pulse duration τ [μs]	100	400	400
Pulse frequency [Hz]	2	2	2

2.3. Microscopic visualization of PEF treated potato tissue

Potato tissue (Ø 20 mm, thickness 30 μm) was stained with 20 μl of ruthenium red (Sigma–Aldrich, Germany, dry content 50%) and washed with distilled water after 15 min. Subsequent PEF treatment was applied with a PEF microscope, constructed in the Department of Food Biotechnology and Food Process Engineering (TU Berlin). The microscope (Zeiss Optik, Jena, Germany) enabled the study of direct cell structure changes during PEF treatment. Main components were a camera (Nikon E 8700, Japan), which was fixed to the microscope, 3 objectives, with a maximum magnification of 400 fold, and a glass slide with two copper foil electrodes (gap 2 mm, length 3 mm, thickness 0.2 mm, area 0.6 mm^2). The treatment chamber was connected to the micro pulse modulator, consisting of a power supply FUG HCK, 800 M- 20.000, 20 kV, 80 mA (FUG, Rosenheim, Germany) to a capacitor bank of three capacitors with 6.8 nF each. The pulse parameters were examined by a high voltage and a current probe, coupled to a TDS220 (Sony Tektronix, Beaverton, US) oscilloscope. A PC was used to control PEF treatment intensities. The images obtained with the microscope from the samples were recorded with the camera and single pictures of untreated and PEF treated potato tissue were selected. Camera was activated manually before treatment.

2.4. Determination of cell vitality through impedance measurement

Cell disintegration index (CDI) was analyzed after Angersbach, Heinz, and Knorr (1999). The method based on the frequency depending conductivity of intact and permeabilized tissue. The cell disintegration index CDI analysis was carried out via impedance measurement equipment (Biotronix GmbH, A. Angersbach, Hennigsdorf, Germany).

CDI was calculated by following equation:

$$CDI = 1 - b\frac{(K_h' - K_l')}{K_h - K_l} \quad b = \frac{K_h}{K_h'} \quad 0 \le \mathrm{CDI} \le 1 \tag{1}$$

where K_l and K_l' indicate the electrical conductivity of untreated and treated cell material in a low- frequency field (1–5 kHz), respectively; and K_h and K_h' indicate the electrical conductivity of untreated and treated material in a high- frequency field (3–50 MHz).

The CDI varies between 0 for intact cells and 1 for total disintegration. Cylindrical pieces were cut out of the potato sample and placed into a plastic test tube. The electrode area of the measuring cell was 2 cm^2. The gap was adjusted to 1.0 cm.

2.5. Determination of sugar content (sucrose, D- glucose, D- fructose)

Sugar analyses were performed with an enzymatic uv test (Boehringer Mannheim/R-Biopharm, Germany). PEF processed potato slices were washed after treatment in tap water (500 ml), cut, 50 g were mixed with 50 ml distilled water and homogenized with an Ultra- Turax for 3 min 5 ml Carrez I (3.60 g K4 [Fe(CN) 6] × 3H2O (potassium hexacyanoferrate/100 ml) and 5 ml Carrez II solution (7.20 g ZnSO4 × 7 H2O (zinc sulfate hepta hydrate/100 ml) were added to potato mash (pH = 7.0–7.5). In a volumetric flask 0.3 ml n- Octanol were added to the sample and shook till foam was dissolved. Filtration was performed after addition of distilled water

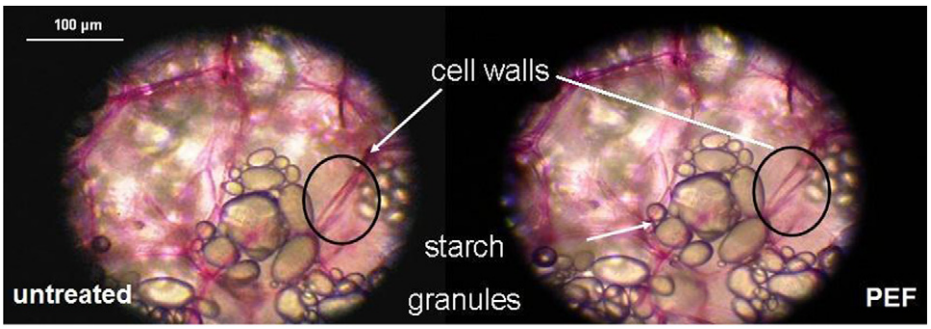

Fig. 1. Untreated and PEF treated ($E = 5$ kV/cm, $n = 20$, 5 min after treatment) potato tissue stained with ruthenium red.

to the mark of 250 ml. Sugar content was analyzed spectrophotometrically (Kontron 25/Germany) at 334 nm wavelength.

2.6. Determination of salt uptake

Surface water of sliced PEF and untreated potatoes was dabbed with a cloth. Each kind of sample was immersed in 1 g/100 mL solution of Sodium Chloride (NaCl) for 0 (no immersion), 15 and 30 min, respectively. Subsequent, potatoes were mashed in a blender and conductivity was measured.

2.7. Determination of drying efficiency

Potato slices were weighted before PEF treatment. Pre drying of untreated and PEF treated samples was performed in a drying oven (Heraeus, Hanau, Germany) for 10 min at 100 °C. After subsequent weighting, slices were sprayed with 200 µl (0.177 g) rape oil. Last drying cycle was carried out for another 20 min at 200 °C and output weight was determined. The degree of dryness was calculated by

$$DM[\%] = \frac{DM}{(WC + DM)} \times 100 \quad (2)$$

Water Content WC = initial weight of untreated sample− output weight after drying [g].

Dry Matter DM = output weight after drying− tara [g].

2.8. Analysis of fat content

Blanching and PEF processing were performed for the comparison of different pre-treatments to reduce fat content during frying. Warm water blanching (potato/water ratio 1:3) was accomplished for 2 min at 80 °C. Blanched potatoes were cooled in tab water for 10 min and dripped of water. After cutting, 100 g potato stripes were fried in 2 L rapeseed oil for 13 min at 190 °C. The frying sieve was shaken to release the surface oil and cooling of the fries was performed for 10 min at room temperature. Oil content of potato stripes was determined by 3 h Soxhlet extraction using petroleum ether as a solvent (AOAC, 1995).

3. Results & discussion

PEF application on plant cell material results in an improved mass transfer of intracellular substances. In Fig. 1 untreated and PEF treated ($E = 5$ kV/cm, $n = 20$) potato tissues with stained cell wall pectin are shown. The dye ruthenium red binds to deesterified carboxyl groups and stains pectin in cell wall and middle lamellae. It is seen that the tissue compartment is slightly changed. The observation that the cell wall is effected by PEF treatment brings novel aspects in the research of non-thermal technology. Still, it is not clear whether cell wall components are changed directly due to the PEF treatment or due to cell membrane disintigration and the release of cytoplasm. However, it was shown in a recent study (Janositz et al., 2011) that the content of the cell wall biopolymer lignin reduces after PEF application. Lignin degradation may be occurred due to the effective break of intermolecular and intramolecular bonds within or between the cellulose, hemicellulose, and lignin.

3.1. Effect of PEF on diffusion of low molecular substances

3.1.1. Reducing sugars (glucose, fructose), sucrose

It could be demonstrated that pre-treatment of PEF on potato slices in technical scale led to a reduction of sugar content (Fig. 2). A significant increase in the release of glucose and fructose was observed after PEF application of 1 kg potatoes with the field strength $E = 1.5$ kV/cm and 20 pulses. The enhanced diffusion characteristics after PEF induced electroporation resulted in one third reduction of fructose content and a nearly bisection of glucose rate. The observations correspond with the study of Jaeger et al. (2010). The authors demonstrated an enhanced release of reducing sugars in potatoes after PEF processing in lab scale. It can be considered that PEF pretreatment is a capable assistance or alternative to conventional thermal processing for the removing of reducing sugars, which represents relevant substrates for the Maillard reaction and acrylamide formation. The performed investigations in technical scale shall help to conduct the novel processing technology in industrial scale for the PEF assisted production of potato chips.

3.1.2. Sodium chloride

Better diffusion characteristics after PEF processing were not only noticed by the enhanced sugar release out of the cell but also

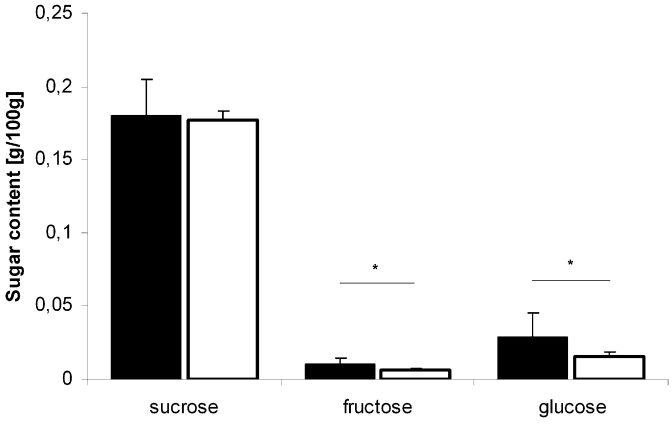

Fig. 2. Sugar content in potato slices after PEF treatment ($E = 1.5$ kV/cm, $n = 20$) in technical scale in comparison to untreated samples. □ = PEF treated potato samples, ■ = untreated potato samples. Statistical significance (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

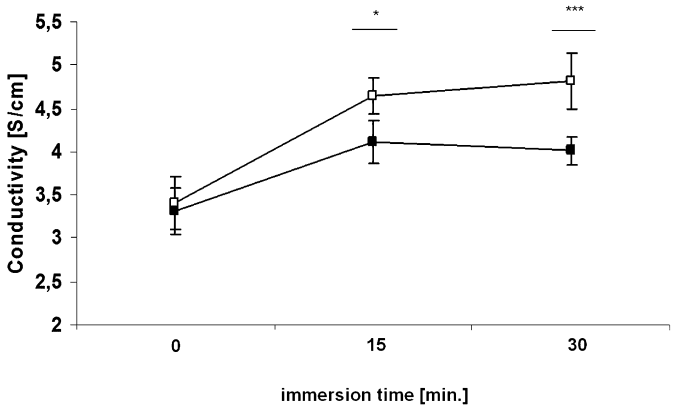

Fig. 3. Conductivity of PEF treated (E = 1.5 kV/cm, n = 20) and untreated potato samples without NaCl immersion and after soaking in 1 g/100 mL NaCl solution for 15 or 30 min □ = PEF treated potato samples, ■ = untreated potato samples. Statistical significance (*P < 0.05, **P < 0.01, ***P < 0.001).

by the increased infusion of sodium chloride in the sample. In Fig. 3 conductivity of untreated and PEF treated potatoes after soaking in sodium chloride solution is presented. It was observed that conductivity of PEF treated samples was higher and increased with residence time, indicating the higher uptake of sodium chloride in the tissue. Two mass processes occurred, water release out of the cells as well as salt diffusion into the tissue dependent on the applied concentration gradient. The observations of enhanced salt uptake correlated with investigations published by Toepfl and Heinz (2007). The authors reported improved diffusion of salt and nitrite into pork haunches after PEF treatment of E = 3 kV/cm and 5 kJ/kg. The research work shows that PEF application provides a potential for the target uptake of flavor and color components not only in animal but also in plant tissue. In case of potato tissue, PEF treatment might be applied as a pre-treatment before frying in potato chips processing.

3.2. Effect of PEF on drying efficiency

PEF treatment decreased water content of potato slices after baking in drying oven. Fig. 4 presents different moisture degrees of untreated and PEF treated potato slices after hot air drying. In comparison to untreated samples, PEF processed potatoes (Field strength E = 1.5 kV/cm, n = 20, variety `Saturna') show 3.89 g/100 g higher loss of water content after 10 min baking at 100 °C and 8.15 g/100 g higher water reduction after further 20 min baking at 200 °C.

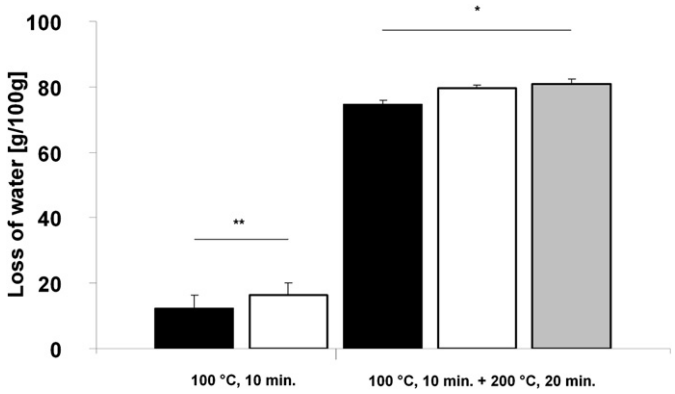

Fig. 4. Water loss of PEF treated (E = 1.5 kV/cm, E = 2.5 kV/cm, n = 20) and untreated potato slices after baking in drying oven (100 °C for 10 min + 200 °C for 20 min). □ = PEF treated potato samples (E = 1.5 kV/cm, n = 20), ■ = PEF treated potato samples (E = 2.5 kV/cm, n = 20), ■ = untreated potato samples. Statistical significance (*P < 0.05, **P < 0.01, ***P < 0.001).

Table 2
Cell disintegration index (CDI) of PEF treated potatoes (E = 1.5; 2.5 kV/cm, n = 20).

	PEF	PEF
	E = 1.5 kV/cm	E = 2.5 kV/cm
	n = 20	n = 20
CDI	0.37	0.57
	(±0.064)	(±0.041)

Faster drying of PEF treated potato samples is based on cell membrane permeabilization due to the electric field and subsequent improved diffusion of intracellular liquid out of the tissue. It was noticed that stronger treatment conditions (E = 2.5 kV/cm, n = 20) result in greater water loss during potato baking. The degree of cell disintegration was calculated by impedance measurement and clarified the relation between field strength and water decrease (Table 2). Higher treatment intensities cause higher degree of cell disintegration. Cell disintegration index amounted to 0.37 (±0.064) for the field strength of E = 1.5 kV/cm and increased to 0.57 (±0.041) after PEF treatment with E = 2.5 kV/cm. However, it became evident that the effect of PEF pre-treatment on potato slices is less pronounced than it was demonstrated in previous studies dealing with PEF induced improvement of potato samples during drying (Angersbach & Knorr, 1997; Lebovka, Shynkaryk, El-Belghiti, et al., 2007; Lebovka, Shynkaryk, & Vorobiev, 2007). This might be referred to the minor thickness and the large surface of the crisps affecting the process of drying. Hot air drying is a rapid dehydration method in which the drying rate seems to be influenced primarily by baking temperature and thickness of the potato slices, and to a lesser extent by PEF pre-treatment. Nevertheless, PEF assisted drying of potato slices seems to be promising because it allows the use of milder thermal conditions and thus, avoids undesirable changes in pigments, vitamins and flavoring agents (Aguilera, Chiralt, & Fito, 2003).

3.3. Effect of PEF on fat uptake

In the present investigation, the effect of PEF pre-treatment on the fat uptake of potato strips during frying was examined. As presented in Fig. 5 PEF pre-treated samples contained 4.69 g/100 g less fat than untreated fried strips, which equates a fat reduction of 38.66% due to PEF treatment compared to untreated samples. This distinct decrease of fat content could not be found for blanched samples, which showed with 0.46 g/100 g lower oil content (3.79% reduction) only minimal fat reduction regarding to the reference samples. The blanching-induced layer of gelatinized starch (Moreira, Castell-Perez, & Barrufet, 1999, p. 202) was shown to be less efficient regarding limitation of oil absorption in comparison to PEF pre-treatment. This

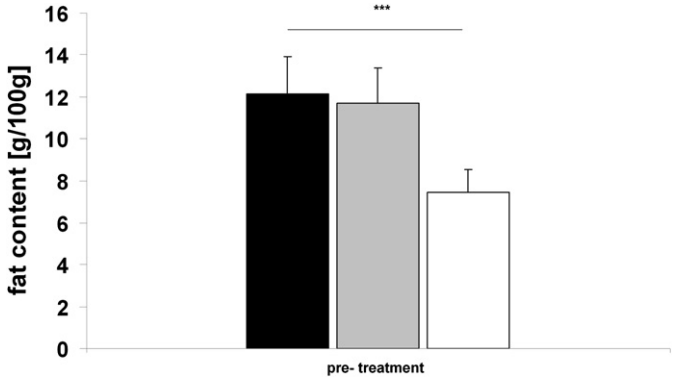

Fig. 5. Comparison of blanching (T = 80 °C, t = 2 min) and PEF (E = 1.8 kV/cm, n = 40) pre-treatment with untreated potato stripes concerning fat uptake during frying. □ = PEF treated potato samples, ■ = blanched potato samples, ■ = untreated potato samples. Statistical significance (*P < 0.05, **P < 0.01, ***P < 0.001).

finding was attributed to the modified frying characteristics of the PEF treated potato strips. Frying is mainly a drying process that involves heat and mass transfer. After initial heating of the food through the surrounding oil, surface boiling begins including water vaporizing and the formation of bubbles. Moisture is transferred from the surface to the oil and later by diffusion of inner cellular liquid to the surface. The water vapour layer on the potato surface acts as a barrier against the oil and depends on the vapour pressure difference between food moisture and oil, which influence the rate of drying (Jason, 1958). Due to the permeabilized cell membranes of PEF treated tissue cell liquid diffusion from the core to the surface is enhanced, which result in higher vapour pressure difference and thus thicker water vapour layer, reducing dehydration and fat uptake. As revealed visually and haptically the surface of PEF treated potato strips is smooth and flat, which assist additionally the decreased oil uptake during frying and post-frying (Thanatuksorn, Pradistsuwana, Jantawat, & Suzuki, 2005). Due to the even cut, oil absorption during frying can be reduced in contrast to the more distinct roughness of non PEF treated tissue. During the cooling period PEF treated samples were less susceptible to oil absorption of the adverse crust oil because of the smooth and even outer surface, causing better oil draining (Bouchon & Pyle, 2006).

4. Conclusions

PEF processing can be applied in potato processing to improve diffusion characteristics of intracellular liquid and low molecular substances into and out of the tissue. Technical scale PEF treatment of potatoes decreases the content of reducing sugars and disaccharides and thus removes substrates for the Maillard reaction. PEF assisted infusion of molecules into potato tissue was demonstrated by enhanced salt uptake after electroporation. Therefore, PEF treatment can be considered as a novel method to insert color and flavor carrier into potato tissue. The innovation in the study of PEF treated potato slices lies in the adaptation of the industrial used geometry of the samples for potato chips, the special surface/volume ratio, which influences the effect of PEF. Improvement of drying efficiency due to PEF was additionally analyzed. It was detected that water loss of PEF treated potato slices after baking in a convection oven increased with the process conditions intensity. Concerning oil uptake during deep fat frying, it could be revealed that PEF application on potato strips leads to a reduction of fat content, more effective than hot water blanching. This observation offers new possibilities to produce low-fat French fries without the use of special coatings or thermal pre-treatments.

Thus, PEF treatment might provide a potential to be implemented in potato chips or French fries processing in order to provide a non-thermal method for structural modifications of food matrix with simultaneously avoiding heat induced nutritional and sensorial degradations.

Acknowledgments

This work has been supported by an EU- integrated Project NovelQ “Novel Processing Methods for the Production and Distribution of High-Quality and Safe Food”, FP6-CT-2006-015710, Priority 5 ‘Food Quality and Safety’.

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Innovative Food Science and Emerging Technologies xxx (2011) xxx–xxx

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Impact of PEF treatment on quality parameters of white asparagus (*Asparagus officinalis L.*)

A. Janositz *, J. Semrau, D. Knorr

Technical University of Berlin, Institute of Food Technology and Food Chemistry, Germany
Technical University of Berlin, Department of Food Biotechnology and Food Process Engineering, Germany

ARTICLE INFO

Article history:
Received 18 November 2010
Accepted 10 February 2011
Available online xxxx

Keywords:
Pulsed Electric Fields (PEF)
Asparagus
Storage
Lignin
Dry solids

ABSTRACT

Pulsed Electric Field (PEF) application on white asparagus (*Asparagus officinalis L.*) was exercised to examine influence of electroporation on spear characteristics as composition and texture. PEF treated spears showed altered storage behaviour, which was noticed by increased mass transfer as higher water loss as well as the decrease of the Maillard reaction substrate glucose. Cell disintegration measurement revealed significant influence of electric field orientation on electroporation. Since the anisotropy of asparagus tissue, PEF processing in longitudinal direction of the spear axis resulted in 9.06% higher cell membrane permeabilization than treatment in transverse direction. Furthermore, total solids inclusive lignin content were measured to obtain textural improvements of asparagus spears. It could be shown that dry weight as well as the amount of lignin was reduced after PEF implementation. Lignin degradation (−2.4%) might be attributed to the PEF induced interference of electrostatic dipole–dipole interactions between lignin and cellulose and subsequent delignification.

Industrial relevance: Since three decades the technology of Pulsed Electric Fields (PEF) received considerable relevance in food — and bioengineering as well as in medicine. Besides the use of PEF to inactivate microorganisms main focus is put on the disintegration of biological cell material to enhance mass transfer in drying or extraction processes. Although the effects of PEF on various plant cell materials are well studied only scarce knowledge exists concerning the impact of PEF on white asparagus. In the study undertaken the PEF-induced changes in asparagus texture and composition were examined. The investigations shall help to reduce unfavored intra- and extracellular components to gain food safety as well as softer spear texture.

1. Introduction

From the sixteenth century until now, white and green asparagus has been gaining global popularity. Over the past decade, global asparagus production has risen substantially, to around one million tonnes. The steady consumer demand for asparagus can be seen as a result of the distinctive flavour devoid of fat or sodium and of the availability of antioxidants, folic acids, and dietary fibers, which constitute the high nutritional value (Steinmetz & Potter, 1996). Near antioxidant activity, texture is an important quality factor of the white asparagus. During postharvest storage, asparagus texture deteriorates rapidly and endures hardening processes, causing quality degradation. Fibrous texture strongly reflects the amount of product loss during peeling, which increases with the extent of hardening and can affect half the product (Clore, Carter, & Drake, 1976). The degree of hardening is related to biochemical modifications of the cell wall composition and can be affected by lignification (Salunkhe & Desai, 1984). Lignin, a phenolic biopolymer, is mainly located in cell wall and crosslinked with different plant polysaccharides, causing mechanical strength to cell wall and tissue (Hepler, Fosket, & Newcomb, 1970; Krogmann, 1973). To reduce post harvest losses, it is required to process fresh asparagus quickly or preserve it by canning, pickling, freezing and drying. During treatment with heat, it has to be regarded, that acrylamide formation can occur as a result of Maillard reaction between reduced sugars and amino acids (Stadler et al., 2002). Acrylamide, a toxin that has been found in various heat-processed foods, is considered to be a health risk to humans caused by its mutagenic and carcinogenic potential (Dearfield, Douglas, Ehling, Moore, Sega & Brunsick, 1995; International Agency for Research on Cancer [IARC], 1994). Due to the high amount of asparagine (11,000–94,000 mg/kg) in asparagus and the availability of glucose and fructose, asparagus processing as roasting and frying, which include high temperatures (≥200 °C) and result in low moisture products has

* Corresponding author at: TU Berlin, Department of Food Biotechnology and Food Process Engineering, Koenigin-Luise-Str. 22, D-14195 Berlin, Germany. Tel.: +49 30 314 71411.
E-mail address: annajanositz@yahoo.de (A. Janositz).

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doi:10.1016/j.ifset.2011.02.003

become a critical view concerning human health aspects (Hurst, Boulton, & Lill, 1998). An elevated level of acrylamide (143 μg/kg) was found in roasted asparagus (Friedman, 2003) and it intensified the relevance of developing gentle food technologies, to assist or replace the currently common thermal processing.

The application of Pulsed Electric Fields (PEF) on biological cell material results in permeabilization of the cell membrane. The external electrical field affects the cell in form of short repeated pulses (μs–ms) of a high voltage (kV), which induce either temporary or permanent pores in cell membrane. Temporary pores can be formed when the electric field is removed and the induced pores in cell membrane are small related to the total membrane area. Thus, the resealing of membrane pores remains the cell vitality. Irreversible pores are formed at higher treatment intensities, which induce stable pores in cell membrane and cause lethal cell damage (Zimmermann, Pilwat, & Riemann, 1974). Reversible permeabilization is a useful tool to induce stress reactions on plant systems and stimulate the generation of secondary cell metabolites (Guderjan, Toepfl, Angersbach, & Knorr, 2005; Ye, Huang, Chen, & Zhong, 2004). Irreversible PEF treatment can be applied to inactivate microorganisms (Barbosa-Cánovas, Góngora-Nieto, Pothakamury, & Swanson, 1999; Heinz, Toepfl, & Knorr, 2003; Jaeger, Schulz, Karapetkov, & Knorr, 2009) or to facilitate mass transfer and improve the diffusion of intra and extra cellular liquids. Increased extractions efficiency of juice and cell compounds from food plants could be shown by several researchers (Eshtiaghi & Knorr, 2000; Schilling et al., 2008; Yin, Han, & Han, 2006). The improvement of drying rates with resulting reduction of drying time and temperature was reported after application of PEF on vegetables (Ade-Omowaye, Rastogi, Angersbach, & Knorr, 2001; Lebovka, Shynkaryk, & Vorobiev, 2007; Taiwo, Angersbach, & Knorr, 2002). Based on the enhanced mass transfer after PEF processing, it could be demonstrated that PEF cause not only higher release of intra molecular content but also improve the uptake of low molecular substances into the tissue. An increased infusion into PEF treated potato slices was shown for glucoseoxidase to assist the removal of reducing sugars and for sodium chloride to examine the potential of PEF to insert flavour carrier into the food matrix (Jaeger, Janositz, & Knorr, 2010; Janositz et al., submitted for publication).

The objective of this study was to investigate the effects of PEF on asparagus to find a pre-treatment method to prior thermal treatments or an alternative to heat processing for the production of better quality asparagus. Lignin content of PEF treated asparagus was analyzed to clarify impact of PEF on the biopolymer lignin, gaining improved macroscopic characteristics of the spears. The influence of PEF on the asparagus matrix concerning the release of reduced sugars was investigated to identify the potential of PEF on asparagus for the removal of Maillard reaction substrates. In addition, post permeabilization changes like water loss and colour changes of PEF treated spears were studied, identifying the effect of PEF on storage behaviour.

2. Material and methods

2.1. Sampling

White Asparagus *(Asparagus officinalis)* was obtained from Beelitz/Germany and experiments were performed within 4 to 24 h after harvest. Samples were stored in a refrigerator at 4 °C. Before treatment 10 g of the spear base section (circular area = circa 7 cm²) was cut and divided vertically. PEF treatment was performed with one-half of the spear base; the other half was used as a reference. After treatment, samples were either directly analyzed, stored at 4 °C in the dark or freeze dried (Freeze Dryer Modulys; Erwardy High Vacuum International; West Sussex/England) to perform further studies. Dry solids and water evaporation were analyzed on days 0, 4 and 6. Sugar content was analyzed on days 0 and 4. The direct effect of PEF on lignin content was analyzed immediately after PEF treatment.

2.2. Experimental set-up and electric field pulses protocol

PEF treatment was applied with exponential pulses. The cuboid batch treatment chamber included two parallel-plate electrodes with a size of 20 × 7 cm and an electrode gap of 3 cm (420 ml in volume). The treatment chamber was connected to a capacitor bank of four DP 30560 (GA, San Diego, USA), 15 kV, and 2 μF in series has been used to achieve a total capacity of 0.5 μF and was charged using an ALE802 (Lambda-Emi, Neptune, USA), 40 kV power supply. The applied PEF treatment intensities for asparagus were: output voltage: 15,000 V, electrode gap: $d = 3$ cm; electric field strength: $E = 5$ kV/cm; pulse number: $n = 20$; pulse duration: $\tau = 400$ μs and frequency: $f = 2$ Hz. The specific energy per pulse was 337.5 J. The energy input per treatment was 16.0714 kJ/kg.

2.3. Determination of sugar content (D-glucose, D-fructose)

PEF treated asparagus samples were washed in tap water (500 ml) and freeze dried subsequently or stored before freeze drying. The freeze dried samples were homogenized in an Ultra-Turrax (T 25 digital Ultra-turrax, IKA laboratory technology, Germany) at 15,000 rpm for 3 min at room temperature. Distilled water was added to the mixture in a ratio 1:1. 5 ml Carrez I solution (3.60 g K4[Fe(CN)6] × 3H2O (potassium hexacyanoferrate/100 ml) and 5 ml Carrez II solution (7.20 g ZnSO4 × 7 H2O (zinc sulfate hepta hydrate/100 ml) were added to asparagus mash (pH = 7.0–7.5). In a volumetric flask 0.3 ml n-Octanol was added to the sample and shook until foam was dissolved. Filtration was performed after addition of distilled water to the mark of 250 ml. Sugar content was analysed spectrophotometrically (Kontron 25/Germany) at 334 nm wavelength.

2.4. Determination of cell vitality through impedance measurement

Cell disintegration index (CDI) was analyzed after Angersbach, Heinz, and Knorr (1999). The method based on the frequency depending conductivity of intact and permeabilized tissue. The cell disintegration index CDI analysis was carried out via impedance measurement equipment (Biotronix GmbH, A. Angersbach, Hennigsdorf, Germany).

CDI was calculated by following equation:

$$CDI = 1 - b\frac{\left(K_h' - K_l'\right)}{K_h - K_l} \qquad b = \frac{K\boldsymbol{h}}{K'\boldsymbol{h}} \quad 0 \le CDI \le 1 \tag{1}$$

where K_l and K'_l indicate the electrical conductivity of untreated and treated cell materials in a low-frequency field (1–5 kHz), respectively; and K_h and K'_h indicate the electrical conductivity of untreated and treated materials in a high-frequency field (3–50 MHz).

The CDI varies between 0 for intact cells and 1 for total disintegration. Cylindrical pieces were cut out of the asparagus spear and placed into a plastic test tube. The electrode area of the measuring cell was 2 cm². The gap was adjusted to 1.0 cm.

2.5. Colour determination

Colour of PEF treated and untreated asparagus samples was measured photometrically using a colorimeter (CR-200 Minolta, Japan). L*a*b*-values are a numerical classification of the colours of the different concentrates. L* represents the lightness of the sample and ranges from black = 0 to white = 100, a* indicates sample position between red (+) and green (−) and b* its position between yellow (+) and blue (−).

2.6. Determination of water content and dry weight

PEF treated and untreated asparagus samples (1–2 g) were dried to constant weight in a drying oven at 105 °C for 5 h. After cooling in a

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dehydrator output weight was determined and dry weight/water content was calculated by following equations:

$$DW[\%] = \frac{DW}{(WC + DW)} \times 100 \quad (2)$$

Water Content WC = initial weight – output weight [g]

Dry Weight DW = output weight – tara [g].

2.7. Analysis of lignin

2.7.1. Qualitative

Phloroglucin, a benzentriol (1,3,5-Trihydroxybenzol, Merck, Darmstadt/Germany), was solubilized in a mixture of ethanol/water (1:1) (w = 5%). For the qualitative detection of lignin, phloroglucin solution was applied to the asparagus sample and one drop of hydrochloric acid was added to turn the contained lignin red.

Pictures were recorded by using a light microscope (Nikon Eclipse E400) equipped with a digital camera (JVC, TK-10070E).

2.7.2. Quantitative

Lignin content determination is based on Association of Official Analytical Chemists [AOAC] methods (1984) according to the procedures of Goering and Van Soest (1970). The analysis includes the detection of ADF (Acid Detergent Fiber) and ADL (Acid Detergent Lignin). The freeze dried samples were homogenized in an Ultra-Turrax (T 25 digital ultra-turrax, IKA laboratory technology, Germany) at 24,000 rpm for 5 min at room temperature. Detection of ADF content: 100 ml of acid detergent dissolution (20 g of N-trimethyl-ammonium bromide) was soluted in sulphuric acid (c: 1/2 H_2SO_4 = 1 mol/l) and added with 0.5 ml Octanol. 1 g of grounded sample was weighted out and mixed with the solution and boiled for 60 min. After boiling, content of the glass beaker was vacuum-filtrated through a filter crucible and washed afterwards with 250 ml hot water and acetone. Filter crucible was dried over night in a drying oven at 100 °C and weighted out after cooling in a dehydrator. The content of ADF was determined by the formula:

$$ADF = \frac{(m2 - m1) * 100}{E} \quad (3)$$

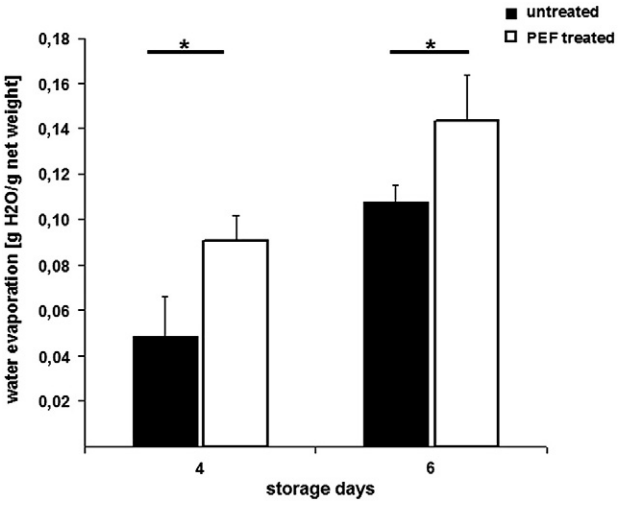

Fig. 1. Water evaporation of PEF treated (E = 5 kV/cm, n = 20) and untreated asparagus after 4 and 6 days of storage. Day 0 showed no water evaporation. Statistical significance (*P < 0.05, **P < 0.01, and ***P < 0.001).

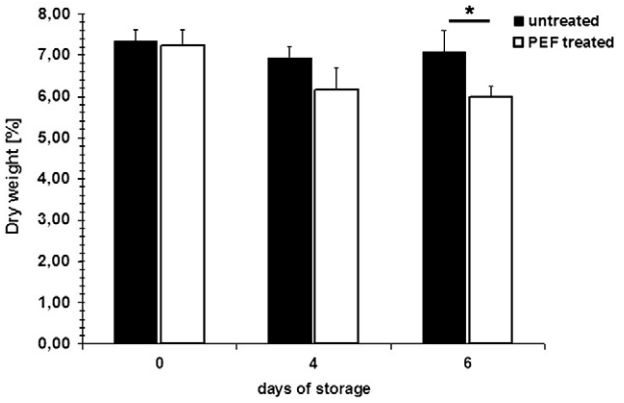

Fig. 2. Dry weight content of PEF treated (E = 5 kV/cm, n = 20) and untreated asparagus after 0, 4 and 6 days of storage. Statistical significance (*P < 0.05, **P < 0.01, and ***P < 0.001).

where *m*1 indicates the mass of the filter crucible [g], *m*2 indicates the mass of the filter crucible and ADF [g] and *E* notifies the initial weight [g].

The filter residue can be used for the detection of raw lignin = ADL (Acid Detergent Lignin).

2.7.2.1. Determination of ADF content. Filter crucible with residue of ADF analysis was weighted out and placed in a glass beaker. Crucible content was covered with 72% sulphuric acid, which was cooled to 15 °C. Over a period of three hours, sulphuric acid was refilled and mixture was stirred hourly at a temperature of 20–23 °C. Suction, hot water washing, drying and weighting were performed subsequently. After incineration of organic substances the specimen was weighted again. The annealing loss equates the amount of raw lignin. The experiments were performed in duplicates and replicated five times for statistical purposes.

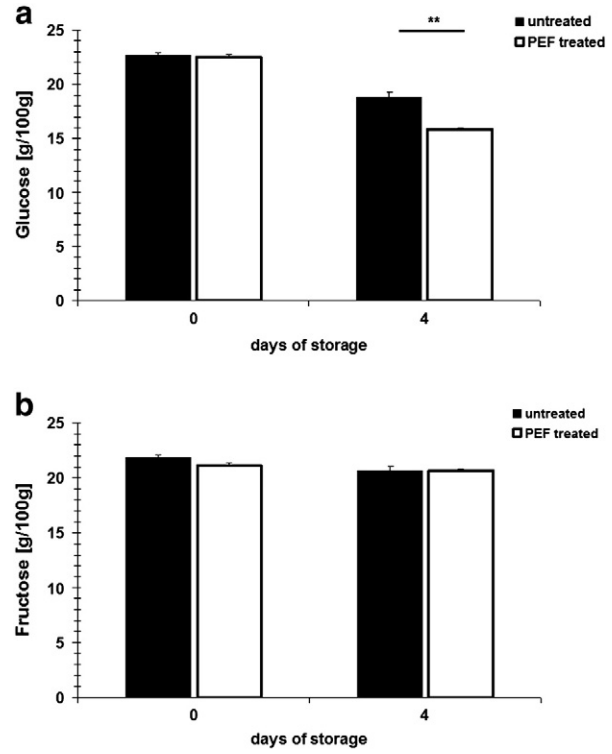

Fig. 3. Glucose (a) and fructose (b) content of PEF treated (E = 5 kV/cm, n = 20) and untreated asparagus after 0 and 4 days of storage. Statistical significance (*P < 0.05, **P < 0.01, and ***P < 0.001).

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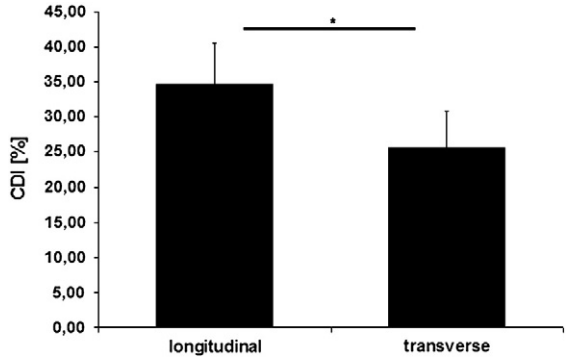

Fig. 4. Cell disintegration index of PEF treated asparagus (E = 5 kV/cm, n = 20) with electrode orientation in longitudinal or diagonal path direction. Statistical significance (*P<0.05, **P<0.01, and ***P<0.001).

3. Results and discussion

3.1. Effect of PEF on mass transfer

3.1.1. Cell liquid and dry solids

It is known that PEF exposure on plant material leads to an enhanced mass transfer rate via permeabilization of the cell membrane (Ade-Omowaye et al., 2001; Lebovka et al., 2007). A trend towards the facilitated release of inner cell liquid was noticed by the higher water loss of PEF treated asparagus during the storage period of six days. Fig. 1 presents the water evaporation of PEF treated (E = 5 kV/cm, n = 20) and untreated asparagus during short-time storage on days 4 and 6. On day 4, the release of cell liquid from PEF treated tissues amounted twice as high as for the untreated sample. Additionally, the water evaporation increased with storage time and both samples showed similar rise within the two days. Although higher release of cell liquid from electroporated asparagus tissue indicates advantages during drying processes in food industry as shown in shorter drying times and lower drying temperatures (Tedjo, 2003), increased water loss after harvest constitutes a problem due to product shrivelling and the loss of glossy appearance. To reduce moisture loss, constant cooling of the spears should be provided as well as holding the produce in plastic liners. Otherwise, water loss is not always a detriment. Mechanical damage of the product in handling and processing can be reduced due to the loss of turgidity (Suslow, 2000).

The measurement of dry weight indicated an influence of PEF on asparagus solids. As shown in Fig. 2, dry matter of untreated asparagus remained constant over the period of storage, whereas dry solid content of PEF processed asparagus decreased within the six days by 1.23%. Explanations for the increased reduction of solids after PEF processing might be found in the enhanced release of intracellular substances as sugars (see Section 3.1.2), enzymes and antioxidants (Bazhal, Lebovka, & Vorobiev, 2001; Eshtiaghi & Knorr, 2000; Van Loey, Verachtert, & Hendrickx, 2002). Although experiments were performed without additional mechanical compression of the tissue, PEF-induced cell membrane permeabilization could cause sufficient cell destruction to transfer particularly low molecular substances out of the cell. Furthermore, the release of endogenous enzymes as hydrolases after PEF treatment as well as microbial infection of the spears could result in increased degradation of dry weight during the period of storage.

3.1.2. Reducing sugars (D-glucose, D-fructose)

As represented in Fig. 3a no alteration of glucose level was found for untreated and PEF treated asparagus directly after treatment. However, on the fourth day of storage, both samples showed significant reduction of glucose content. PEF treated samples amounted 3% less glucose than the reference.

The lowering in glucose content during storage could be attributed to respiratory processes, microbial load and/or the enhanced release of endogenous enzymes due to PEF-induced cell disintegration causing degradation of glucose (Bisson, Jones, & Robbins, 1926). As demonstrated in Fig. 3b, only minimal changes in fructose content due to PEF treatment were observed, but no further decrease of fructose in untreated as well as in PEF treated asparagus was noticed.

3.2. Effect of PEF direction on cell disintegration index (CDI)

The enhanced diffusion can be regulated by the degree of cell disintegration. Higher CDI results in higher release of cell content, causing better diffusion of low molecular substances (Jaeger et al., 2010; Janositz et al., submitted for publication). Fig. 4 presents the CDI of PEF processed (E = 5 kV/cm, n = 20) asparagus, treated and measured in longitudinal and diagonal direction. CDI increases by 9.06% when orientating the electrodes longitudinally relative to the major axis of the tissue. This observation demonstrates that the direction of the electric field has a significant influence on the effectiveness of PEF treatment. Asparagus tissue can be seen as distinctly anisotropic, since the cells have a diameter of approximately 20 μm, but a length of up to 100 μm (Gassner, Hohmann, & Deutschmann, 1989). Electrical conduction along the length of cell, filled with rich ionic intracellular liquid, is thus easier than conduction between the cells in the less conductive extracellular matrix and the non-conductive cell membrane.

3.3. Colour determination

Colour coordinates of PEF processed and unprocessed asparagus were determined to investigate impact of PEF on sensory qualities directly after treatment and during storage of 0, 4 and 6 days. By comparing the samples, markedly colour differences were noticed. In agreement with visual observation, instrumental light measurement showed higher browning of PEF treated asparagus samples compared to untreated samples, which was indicated by lower L* values (Table 1). Analysis of a* and b* measurements showed yellow tint of PEF treated or green tint of untreated asparagus, respectively. At all samples intensified darkening, indicated by higher b* values, was observed during storage period. The more pronounced darkening of the PEF treated samples might be referred to the higher release of polyphenol oxidase (PPO) after cell membrane permeabilization. An addition of ascorbic acid could help to prevent the oxidation of phenolic compounds and thus the browning of the samples (Mayer & Harel, 1979; Vamos-Vigyazo, 1981).

Table 1
*A**, *b** and light values of PEF treated (E = 5 kV/cm, n = 20) and untreated asparagus after 0, 4 and 6 days of storage.

	L* value			A* value			B* value		
	Day 0	Day 4	Day 6	Day 0	Day 4	Day 6	Day 0	Day 4	Day 6
Untreated	82.96 (±0.43)	83.08 (±0.97)	82.93 (±2.04)	−1.30 (±0.14)	−1.47 (±0.09)	−1.51 (±0.26)	5.22 (±0.56)	6.33 (±0.48)	6.97 (±0.96)
PEF treated	78.98 (±1.96)	78.73 (±1.60)	82.09 (±2.33)	−1.51 (±0.19)	−1.22 (±0.24)	−1.68 (±0.29)	4.81 (±0.68)	4.73 (±0.71)	6.08 (±1.19)

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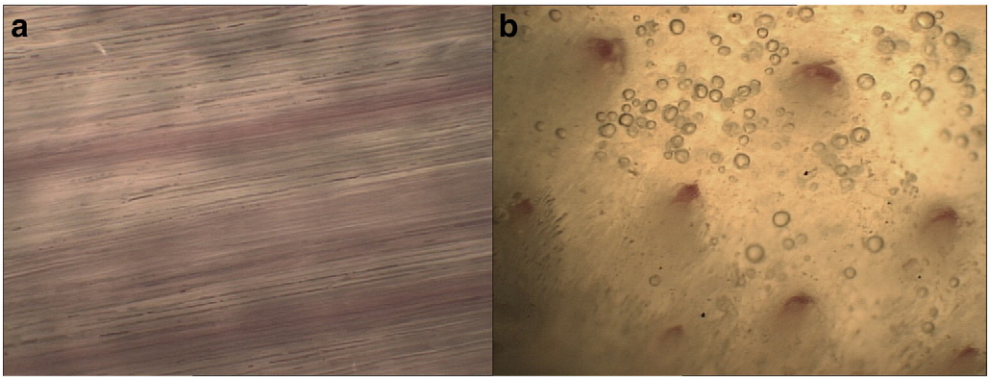

Fig. 5. Cross section of asparagus spear (a) and longitudinal cut of asparagus pod (b) performed after reaction with phloroglucin to visualize lignin.

3.4. Effect of PEF on lignin content

3.4.1. Qualitative

In Fig. 5 asparagus tissue with red stained lignin is shown. The chemical reaction with phloroglucin and sulphuric acid was performed to visualize the distribution of lignin in the spear. It is seen that lignin is particularly abundant in the pod (a) and located in longitudinal direction of the spear. This can be clarified by viewing cross-sectional imaging (b). Lignin deposition was noticed as compact and bundled grown in asparagus tissue.

3.4.2. Quantitative

The application of PEF has an influence on lignin content in asparagus. As represented in Fig. 6, the amount of raw lignin decreased from 12.6% (±0.08) in untreated asparagus samples to 10.2% (±0.34) in the PEF treated asparagus base section. The behaviour of macromolecules exposed to an intense electric field is not well understood (Neumann, 1986). Lignin, a complex phenolic polymer, is seen as highly resistant to biodegradation (Crawford, 1981). Its chemical structure is branched and the macromolecule is bonded with various lignin cross-links and also linkaged between lignin and polysaccharides as cellulose and hemicellulose (Eriksson, Goring, & Lindgren, 1980). Application of PEF may be able to enhance separation of cellulosic material from lignin. High voltage pulses may be effective to break intermolecular and intramolecular bonds within or between the cellulose, hemicellulose, and lignin (Navapanich & Giorgi, 2008). Explanation for the breakage could be that cellulose microfibrills contain large number of hydroxyl groups on the surface causing interactive force attraction with the hydroxyl and methoxyl groups of coumaryl, coniferyl, and sinapyl alcohols from lignin (Houtman & Atalla, 1995). These findings indicate that the dominant force connecting lignin and cellulose is caused by electrostatic dipole–dipole interactions. Subsequent delignification can occur when the covalent bonds are cleaved resulting in solubilization of polymer fragments (Goring, 1971).

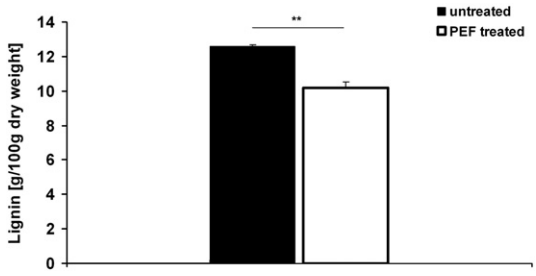

Fig. 6. Amount of Acid Detergent Lignin (= raw lignin) of PEF treated (E = 5 kV/cm, n = 20) and untreated asparagus. Statistical significance (*P<0.05, **P<0.01, and ***P<0.001).

4. Conclusions

The findings suggest that PEF application on asparagus presents a promising tool to improve macroscopic characteristics of the spears. Enhanced mass transfer of PEF treated tissue was noticed by higher moisture loss of PEF treated spears during post-harvest storage. Concerning the removal of glucose, increased mass transfer could not be confirmed. Nevertheless, a higher decrease of glucose after PEF treatment was observed after storage. A pronounced decrease of the Maillard reaction substrates glucose and fructose could be shown for PEF treated potato slices in laboratory as well as in technical scale (Jaeger et al., 2010; Janositz et al., submitted for publication). These observations mark the different influences of PEF on different food matrices.

Colour determinations showed darker spears with higher a* and b* values. Raised water reduction as well as intensified browning should be minimized by fast process control and the addition of citric or ascorbic acid. The measurement of the cell disintegration index indicated the anisotropy of asparagus tissue. PEF processing in longitudinal direction of the major axis caused higher cell rupture than PEF application in transverse direction. Furthermore, influence of PEF on dry solids including lignin was studied. A trend towards the decrease of dry weight content of PEF produced asparagus within the period of storage was noticed. Reduction on lignin content was found after PEF treatment. This effect might be attributed to the PEF induced leakage of lignin–cellulose bonds, enabling the start of delignification to gain softer texture of the spear. The research findings shall help to include PEF technology in asparagus processing as a method prior to heat treatment especially for the production of roasted and fried asparagus. Future investigations on asparagus tissue shall deal with the influence of longitudinal and transverse direction of the electric field concerning the different ability to enhance mass transfer processes as well as to alter dry solid and lignin content.

Acknowledgments

This work has been supported by an EU-founded Integrated Project NovelQ “Novel Processing Methods for the Production and Distribution of High-Quality and Safe Food”, FP6-CT-2006-015710, Priority 5 ‘Food Quality and Safety’.

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