Correlated percolation patterns in PEF damaged cellular material

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We present results of numerical and experimental investigation of the electric breakage of a cellular material in pulsed electric fields (PEF). The numerical model simulates the conductive properties of a cellular material by a two-dimensional array of biological cells. The application of an external in the form of the idealised square pulse sequence with a pulse duration \( t_i \) and a pulse repetition time \( \Delta t \) is assumed. The simulation model includes the known mechanisms of temporal and spatial evolution of the conductive properties of different microstructural elements in a tissue. The kinetics of breakage at different values of electric field strength \( E, t_i \) and \( \Delta t \) was studied in experimental investigation. A 5 mm x 55 mm cylindrical slab of apple is taken as a sample. The results of the experimental and numerical studies were compared. We propose the hypothesis for the nature of tissue properties evolution after PEF treatment and consider this phenomena as a correlated percolation, which is governed by two key processes: resealing of cells and moisture transfer processes inside the cellular structure. The breakage kinetics was shown to be very sensitive to the repetition times \( \Delta t \) of the PEF treatment. We observed correlated percolation patterns in a case when \( \Delta t \) exceeds the characteristic time of the processes of moisture transfer and random percolation patterns in other cases. The long-term mode of the pulse repetition times in PEF treatment allows us to visualize experimentally the macroscopic percolation channels in the sample. We observe considerable differences between the damage kinetics at long and short repetition times both for experimental and simulation data.

Keywords: Pulsed electric fields; Computer simulation; Electroporation; Resealing; Moisture transfer; Percolation; Apples

Contents

I Introduction 3

II Materials and experimental methods 4
\hspace{1em} A Materials .......................................................... 4
\hspace{1em} B Experimental methods ........................................ 4

III Description of the simulation model 5
\hspace{1em} A Probability of a single cell damage ......................... 5
\hspace{1em} B Simulation procedure ............................................. 5
\hspace{1em} \hspace{1em} 1 Resistor network model .................................... 5
\hspace{1em} \hspace{1em} 2 Microstructural conductive properties ..................... 6
\hspace{1em} \hspace{1em} 3 Resealing processes ......................................... 7
\hspace{1em} \hspace{1em} 4 Moisture transfer processes ................................. 7
\hspace{1em} \hspace{1em} 5 Finite element analysis ...................................... 7
\hspace{1em} \hspace{1em} 6 Simulated properties and main parameters .................. 7

IV Results and discussion 8
\hspace{1em} A Experimental results ........................................... 8
\hspace{1em} \hspace{1em} 1 Damage kinetics ............................................ 8
\hspace{1em} \hspace{1em} 2 Visualization of damages .................................. 8
\hspace{1em} B Numerical results ................................................ 8
\hspace{1em} \hspace{1em} 1 Simulation of damage kinetics ........................... 8
\hspace{1em} \hspace{1em} 2 Effects of resealing and moisture transfer processes .... 9
Notation

\( A \sim 4d_c^2 \), cross section area of a single cell or a membrane, \( \text{m}^2 \)

\( c = r_c^f / r_c^i = \sigma_c^f / \sigma_c^i \leq 1 \), resistance moisture transfer coefficient

\( C_m \) specific capacity of membrane, \( \text{F} \text{m}^{-2} \)

\( C^* = C_m (\varepsilon_w / \varepsilon_m) / (2\gamma) \)

\( d_m \) membrane thickness, \( \text{m} \)

\( 2d_c \) cell diameter, \( \text{m} \)

\( E \) electric field strength, \( \text{kV} \text{cm}^{-1} \)

\( E^* = 2d_c E / u_o \), normalized electric field strength

\( \Delta F^* = \pi \omega^2 / (kT\gamma) \), reduced critical free energy of pore formation

\( G \) conductivity of the bond in the network, \( \text{Ohm}^{-1} \)

\( k \) Boltzmann constant, \( 1.381 \times 10^{-23} \text{J} \text{K}^{-1} \)

\( L = 2d_c N \), total thickness of a sample (slab of apple), \( \text{m} \)

\( m = r_m^c / r_m^f = \sigma_m^c / \sigma_m^f \), membrane resistance resealing coefficient

\( n \) number of pulses

\( N \times N \) dimensions of a 2D lattice

\( P \) degree of biological tissue damage

\( r \) resistance of the model resistor in the network, \( \text{Ohm} \)

\( r_m \) membrane part of \( r \) resistance, \( \text{Ohm} \)

\( r_c \) cellular part of \( r \) resistance, \( \text{Ohm} \)

\( S(u) \) survival probability function

\( t_i \) pulse duration, \( \mu\text{s} \)

\( dt \) impact time duration, or "elementary" time step \( dt \sim 0.1t_i, \text{s} \)

\( \Delta t \) pulse repetition time, \( \text{ms} \)

\( T \) temperature, \( \text{K} \)

\( u \) transmembrane voltage, \( \text{V} \)

\( u_o \) midpoint of a survival probability function \( S(u) \), \( \text{V} \)

\( u^* = u / u_o \), normalized transmembrane voltage

\( \Delta u \) width of a survival probability function \( S(u) \), \( \text{V} \)

\( \Delta u^* = \Delta u / u_o \), normalized width of a survival probability function \( S(u) \)

\( U \) external voltage, \( \text{V} \)

\( W \) moisture content, \( \% \)

Greek letters

\( \varepsilon_w = 80 \), dielectric constant of water

\( \varepsilon_m = 2 \), dielectric constant of membrane

\( \gamma \) surface tension of membrane, \( \text{N} \text{m}^{-1} \)

\( \lambda \) adjustable relaxation parameter

\( \sigma \) conductivity, \( \text{S} \text{m}^{-1} \)

\( \sigma_c \) conductivity of cellular material (barring membrane), \( \text{S} \text{m}^{-1} \)

\( \sigma_m \) conductivity of membrane, \( \text{S} \text{m}^{-1} \)

\( \tau_m \) lifetime of a membrane, \( \text{s} \)

\( \tau_{\infty} \) parameter, lifetime of a membrane at \( T = \infty \), \( \text{s} \)

\( \tau_r \) characteristic time of membrane resealing, \( \text{s} \)

\( \tau_d \) characteristic time of a moisture transfer processes after PEF treatment, \( \text{s} \)

\( \omega \) linear tension of membrane, \( \text{N} \)

Superscripts
Among different nonthermal processing methods used in food technologies, the pulsed electric field (PEF) treatment is one of the most promising. A number of new PEF applications were demonstrated for anti-microbial treatment of liquid foods, e.g., fruit juices, milk etc., (Barbosa-Cánovas, Pothakamury, Palou & Swanson, 1998; Barsotti & Cheftel, 1998; Wouters & Smelt, 1997), and for the cellular tissue materials (Knorr & Angersbach, 1998, Knorr, Geulen, Grahl & Sitzmann, 1994). For years back, the continuous electric field (CEF) treatment was also shown to be good for juice yield intensification and for increasing the product quality in juice production (Bazhal & Vorobiev, 2000; McLellan, Kime & Lind, 1991; Scheglov, Koval, Fuser, Zargarian, Srinivov, Belik et al., 1988), processing of vegetable and plant raw materials (Papchenko, Bologa & Berzoi, 1988; Grishko, Kozin & Chebanu, 1991), processing of foodstuffs (Miyahara, 1985), winemaking (Kalmykova, 1993), and sugar production (Gulyi, Lebovka, Mank, Kupchik, Bazhal, Matvienko et al., 1994; Jemai, 1997). But all these CEF applications were restricted by high and uncontrolled increases in food temperature.

Extension of different PEF applications to nonthermal processing of heterogeneous food materials is limited today by the absence of criteria for choosing optimal parameters of PEF treatment and the unclear mechanism of electric breakdown processes in the cellular systems. Recently, a significant advance in understanding of the nature and mechanisms of electric field influence on different animal, plant, and microbial cells has occurred (Chang, Chassy, Saunders & Sowers, 1992; Weaver & Chizmadzhev, 1996). The strong electric field causes electroporation of cells, increase of their permeability, and, in some cases, disruption of their structural integrity (Zimmermann, 1975). The PEF parameters (field strength $E$, pulse duration $t_i$ and number of pulses $n$) can influence both the degree of membrane destruction or structural alteration and the density of pores in membrane (Rols & Teissie, 1998; Gabriel & Teissie, 1999). The electroporation became very popular because it was found to be an exceptionally practical way of transferring drugs, genetic material (e.g. DNA), or other molecules inside the cells (Chang, Chassy, Saunders & Sowers, 1992; Neumann, Kakorin & Tönsing, 1999). This phenomena is sometimes also called as electropermeabilization.

For complex material, such as tissue, cellular or food material, PEF application results in increase in the electric conductivity and permeability of the whole sample. But the nature of electropermeabilization of complex cellular materials is not yet well understood in all details. The cellular materials are highly heterogeneous and the electrical properties of such systems depend on the electrical properties of single cells as well as on the geometrical and topological properties of materials (Sahimi, 1994). Here the percolation phenomena may play an important role for interpretation of the observed experimental results. Particularly, there is no simple relation between the degree of material damage and its electrical conductivity.

A number of ambiguous and yet unexplained phenomena are observed in this field. For example, long-term changes in the conductivity of a cellular material after its electric field treatment are usually observed. It was reported, for example, that the conductivity of the vegetable tissue can decrease after termination of electric treatment over at least 24 hours (Kulshrestha & Sastry, 1998). The explanation of this phenomenon is not trivial since multiple mechanism can be responsible for time evolution of the conductivity.

One of possible explanations is based on the assumption about existence of a partial membrane rescaling after its breakage in a high electric field. Note, that the electric damage of a cell is itself a rather complex process and there may exist different time scales for the kinetics of pore evolution. At low strength electric field, $E (< 200 \text{ V cm}^{-1})$, and short pulse duration, $t_i (\sim 10^{-5} - 10^{-6} \text{ s})$ the electrical breakdown is spontaneously reversible, and all the damages disappear after the field is switched out (Abidor, Arakelyan, Chernomordik, Chizmadzhev, Pastushenko & Tarasevich,
At moderate PEF treatment \((E = 0.5 \sim 2 \text{kV cm}^{-1}, t_i \sim 10^{-4} \sim 10^{-5} \text{s})\) the integrity of cells drops rapidly, but due to resealing or recovering process some of the cells lose their permeability and the pores may persist in the membrane at larger after PEF application. The resealing process time constant, \(\tau_E\), may be very large, of order \(1 \sim 10^2 \text{s} \text{ at } 25 \degree \text{C}\) (Neumann & Boldt, 1990; Chang & Reese, 1990; Chizmadzhev, Indenbom, Kuzmin, Galichenko, Weaver & Potts, 1998; Neumann, Toensig, Kakorin, Buddle & Frey, 1998; Weaver, Pliuett & Vaughan, 1999; DeBruin & Krassowska, 1999). For vegetable food materials the reported resealing time constant was of order 1 s (Knorr, Heinz, Angersbach & Lee, 2000). The high PEF treatment \((E = 10 \sim 50 \text{kV cm}^{-1}, t_i \sim 10^{-6} \sim 10^{-5} \text{s})\) causes the irreversible damages and this mode of treatment is used for inactivation of microorganisms (Barbosa-Cánovas et al., 1998). The time constant of a resealing process is a complex function of \(E\) and \(t_i\) values and depends on the type of cells or membranes. A number of mechanisms were proposed for explanation of resealing processes, but in a general case the nature of the long-lived permeabilization is still unclear (Saulis, 1997; Teissie & Ramos, 1998; Chizmadzhev et al., 1998; Weaver et al., 1999).

Another cause of the long-term changes in the conductivity may be related with the different transport phenomena in a structured cellular material (Aguilera & Stanley, 1999), e.g. diffusional motion, osmotic flow and redistribution of moisture inside the sample, which can be enhanced by PEF application. We can estimate the time constant of the diffusion processes inside the cellular material as \(\tau_d \sim d^2/(6D) \approx 1 \text{s at } 25 \degree \text{C}\), where \(d_c \sim 10^{-4} \text{m}\) is a radius of cell, and \(D \sim 10^{-9} \text{m}^2 \text{s}^{-1}\) (Gekkas, 1992) is an effective diffusion coefficient for the moisture inside a cellular material.

The aim of this study is to elucidate the mechanism of PEF treatment and long-term changes in the conductivity of cellular materials. We consider the damage of a biological tissue in the electric field as a correlated percolation phenomena, which is governed by the resealing and moisture transfer processes. The developed simulation model includes the known mechanisms of temporal and spatial evolution of the conductive properties of microstructural elements (cell membranes, tissue frameworks, etc.). The results of the experimental and numerical studies are compared and possible scenarios of the tissue conductive properties evolution after PEF treatment are discussed.

II. MATERIALS AND EXPERIMENTAL METHODS

A. Materials

Freshly harvested apples of the Golden Delicious variety were selected for investigation and stored at 4\degree\text{C} until required. A moisture content of apples \(W\) was within 80-85%. Typical high resolution scanning electron micrograph of the apple sample is presented in Fig. 1. Images were obtained on the instrument XL30 ESEM-FEG (Philips, \(V=15 \text{kV}, P=3.5 \text{Torr}\)). The initial specific conductivity of samples (before treatment) was within the interval \(\sigma^i = 0.033 \sim 0.007 \text{S m}^{-1}\). The final specific conductivities (after treatment) depend upon the mode of treatment and they were within the range of \(\sigma^f = 0.035 \sim 0.070 \text{S m}^{-1}\). The specific conductivity of the apple juice extracted from the sample apples was \(\sigma_j = 0.22 \sim 0.05 \text{S m}^{-1}\).

B. Experimental methods

The conductivities were measured by contacting electrode method with an LCR Meter HP 4284A (Hewlett Packard, 38 mm guarded/guard Electrode-A HP 16451B) for thin apple slice samples at a frequency of 100 Hz and with a Conductimetre HI8820N (Hanna Instruments, Portugal) for the apple juice samples at a frequency of 1000 Hz (these frequencies were selected as optimal in order to remove the influence of the polarizing effects on electrodes and inside the samples).

Figure 2 is a schematic representation of the experimental pulsed electric field treatment set-up. A high voltage pulse generator, 1500V-15A (Service Electronique UTC, France) allowed to vary \(t_i\) within the interval of 10 \sim 1000 \text{µs} \text{ with precision } \pm 2 \text{µs}, \Delta t\) within the interval of 1 \sim 100 ms \text{ with precision } \pm 0.1 \text{ms} \text{ and } n\) within the interval of 1 \sim 100000.

Pulse protocols and all the output data (current, voltage, impedance and temperature) were controlled using a data logger and a special software HPVEE v.4.01 (Hewlett-Packard) adapted by Service Electronique UTC, France. The temperature was recorded in the on-line mode by a thermocouple THERMOOAX type 2 (AB 25 NN, \pm 0.1\degree\text{C}).

The thin apple slabs (of thickness 5 \sim 0.2 mm and of diameter 55 \sim 0.5 mm) were used as samples in the present investigation. The freshly cut cells at the outer boundary of a sample cause the initial time dependence of the conductivity. During the period about 200 s (Fig. 3) the conductivity achieves about 90% of its stationary value. So, in all our experiments we always skipped this transition time before the PEF treatment. Usually the sample
III. DESCRIPTION OF THE SIMULATION MODEL

A. Probability of a single cell damage

Weaver & Chismadzhev (1996) gave the comprehensive discussion of different models of the membrane rupture. The model that seems to be most reasonable from the physical point of view, is the, so called, transient aqueous pore model, in which the average membrane lifetime $\tau_m$ can be estimated with the help of the following expression:

$$\tau_m(u) = \tau_\infty \exp(\Delta F^*/(1 + u^2C^*)),$$

where $\tau_\infty$ is a parameter (equals to a lifetime of a membrane at infinite temperature, $T = \infty$), $\Delta F^* = \pi \omega^2/(kT\gamma)$ is a reduced critical free energy of pore formation, $k = 1.381 \times 10^{-23}$ J K$^{-1}$ is the Boltzmann constant, $\omega$ is a linear tension of membrane, $N$, $\gamma$ is a surface tension of membrane, $N \text{ m}^{-1}$, $u$ is a transmembrane voltage, $V$, $C^* = C_m(\varepsilon_w/\varepsilon_m - 1)/(2\gamma)$, $C_m$ is a specific capacity of membrane, $F \text{ m}^{-2}$, $\varepsilon_w = 80$ is a dielectric constant of water, $\varepsilon_m = 2$ is a dielectric constant of membrane.

Then the survival probability for a membrane (as a whole cell) during the impact period of $dt$ may be estimated as

$$S(u) = \exp(-dt/\tau_m(u)),$$

Taking Eq. (1) into account, we can rewrite Eq. (3) in the following convenient dimensionless form

$$S(u^*) = \exp\left(-\ln 2/\exp a((1 - (1 - u^2)/(a\Delta u^* \ln 2))^{-1} - 1)\right),$$

where $u^* = u/u_o$, $\Delta u^* = \Delta u/u_o$, $u_o = \sqrt{(\Delta F^*/a - 1)/C^*}$, $\Delta u = u_o/((1 - a/\Delta F^*)a \ln 2)$, and $a = \ln(dt/(\tau_\infty \ln 2))$ is a parameter.

There is no first principle basis for correct estimations of the different parameters in Eqs. (1)-(3) (Weaver & Chismadzhev, 1996), so the numerical values obtained from fitting of $\tau_m(u)$ to experimental data are used as a rule. For example, Lebedeva (1987) presented the following estimations for the lipid membranes: $\tau_\infty \approx 3.7 \times 10^{-7}$ s, $\omega \approx 1.69 \times 10^{-11}$ N, $\gamma \approx 2 \times 10^{-3}$ N m$^{-1}$, $C_m \approx 3.5 \times 10^{-3}$ F m$^{-2}$ at $25 \, ^\circ C$. From these estimations we can obtain the following parameters for Eq. (3): $u_o \approx 1.52$ V, $\Delta u^* \approx 1.07$ and $a \approx 1.36$ (at $dt = 1 \, \mu$s) and $u_o \approx 0.71$ V, $\Delta u^* \approx 0.26$ V, and $a \approx 5.97$ (at $dt = 100 \, \mu$s). But for real cellular systems the parameters of Eq. (3) are not clear-cut and they would depend on physical properties, type and quality of raw materials as well as on the value of $dt$. Yet, for definiteness in the following computation we always use the parameters of $\tau_\infty$, $u_o$ and $\Delta u^*$ estimated on the basis of aforecited data of Lebedeva (1987).

The example of the survival curve $S(u^*)$ is shown in Fig. 3. We see that $S(u^*)$ is a kind of probability transition function and $u^* = 1$ ($u = u_o$) corresponds to the midpoint, where $S(u) = 1/2$. In fact, the value of $u_o$ may serve as an estimate for the critical value of transmembrane voltage, which causes the abrupt decrease of the membrane lifetime. Here we define the width of this transition function $\Delta u^*$ by drawing a tangent straight line to a curve $S(u^*)$ in the midpoint $u^* = 1$ as it is shown in the Fig. 3 (see dotted line). The dashed line at this Figure corresponds to the normalised density distribution function $S'/S'_{\text{max}}$, where $S' = dS/du^*$.

B. Simulation procedure

1. Resistor network model

We simulate the conductive structure of a cellular material as a two-dimensional array of cells located at the nodes of a simple square lattice. The lattice has a size $N^2$ with periodic boundary conditions in the $x$ direction in order to reduce the finite size scaling effects (Watanabe, 1995). The boundary conditions for $y$ direction are as follows: at $y = 0$ and $y = N + 1$ we put two electrodes with a constant potential difference $U$ (Fig. 3). So the mean drop of potential per cell is equal to $u = U/(N + 1)$ and the reduced voltage on membrane in Eq. (3) is defined as $u^* = U/(u_o(N + 1))$. Then mean strength of the electric field along $y$-axis is equal to

$$E = U/(2d_c(N + 1)) = u^*u_o/(2d_c),$$

(4)
where $2d_c$ is a cell diameter.

From this equation we obtain, for example, $E = u^* u_o / 2d_c = 50u^* \text{ V cm}^{-1}$ at $u_o = 1 \text{ V}$ and $d_c = 100 \mu\text{m}$. We can introduce also the normalised field strength defined as $E^* = 2d_c E / u_o \equiv u^*$. As it was mentioned above, the exact value of $u_o$ unknown. So we can chose the $u_o$ parameter from the condition of best fitting to the observed experimental data. In our simulation we use $N = 250$ and the total sample thickness is $L = 2d_c N \approx 5 \text{ cm}$ when $d_c \approx 100 \mu\text{m}$.

2. Microstructural conductive properties

We suppose that each node is connected with neighbouring nodes through four conducting resistors, which simulate the conductive properties of the cellular media microstructural elements. The resistance of such resistors is determined by the two constituent parts

$$r = r_m + r_c,$$

which correspond to the membrane ($r_m$) and cellular ($r_c$) medium contributions, respectively. Here, membrane contribution includes the effective conductive properties of the different membranes in the cellular structure (mainly plasmatic and tonoplast membrane). Cellular medium contribution reflects both intra- and extra-cellular conductive properties of cellular materials. Intra-cellular contribution includes the effective conductive properties of the cytoplasm with its organelles (occupies about 10% of the cell volume), and the vacuole (about 80% of the cell volume). Extra-cellular contribution includes the apparent conductive properties of the rigid cell wall (occupies about 10% of the total volume and its main structural element is cellulose), of pores and intercellular spaces filled with air etc., (account for around 20-25% of the total volume in apple, see Aguilera & Stanley (1999)).

We estimate the resistance values of $r_m$ and $r_c$ in Eq. (5) as

$$r_m = d_m / (\sigma_m A),$$

and

$$r_c = d_c / (\sigma_c A),$$

where $d_m$ is the thickness of membrane, $A$ is a mean cross-section area of a single cell, $\sigma_m$ and $\sigma_c$ are the conductivities of the membranes and cellular material (barring membranes), respectively.

At the initial stage of simulation we suppose that all the cells are intact and corresponding resistors in the model are equal to

$$r^i = r_m^i + r_c^i.$$

In this case the effective conductivity of the whole sample $\sigma$ may be calculated as

$$\sigma = \sigma^i = \frac{d_m + d_c}{r A} = \frac{d_m + d_c}{d_m / \sigma_m^i + d_c / \sigma_c^i} \approx \frac{\sigma_m^i d_c / d_m}{1 + \sigma_m^i d_c / \sigma_c^i d_m},$$

where we take into account that $d_m \ll d_c$.

If the potentials in all the nodes are known, $u_{x,y}$, then we can easily determine the transmembrane voltages $u$ at all membranes in a system. Consequently, we can determine with the help of Eq. (3) which of membranes will destroy after the PEF treatment. The conductivity of these membranes after PEF breakage increases considerably ($\sigma_m^i \Rightarrow \sigma_m^d \Rightarrow \infty$), and so $r_m^i \Rightarrow r_m^d \approx 0$.

Figure 6(a) presents the case, when the potential difference in the vertical ($y$) direction $|u_{x,y} - u_{x,y+1}|$ exceeds some critical value and, as a result, two cells in the vertical direction are damaged. Note, that at all accounts we always observe for this model the simultaneous damage of two cells, because two adjacent cells suffer equal voltage loading. In this case, we should make the following interchange of the resistors

$$r^i \Rightarrow r^d \Rightarrow r(t) = r_m(t) + r_c(t),$$

as it is shown at the Fig. 6(a). The similar case for horizontal ($x$) direction is presented in Fig. 6(b). Here $r^d$ corresponds to resistance of damaged cell, and its time evolution may be found with the help of Eqs. (11)-(10).
3. Resealing processes

The model accounts for the possibility of temporal electropermeabilization as follows. If any membrane is damaged then it begins to reseal immediately, and we suppose, that this resealing results in increasing of the $r_m$ as

$$r_m(t) \simeq r_m^f(1 - e^{-t/\tau_r})$$  \hspace{1cm} (11)

where $r_m^f = d_m/(\sigma_m^f A)$, $\sigma_m^f$ is a final conductivity of a membrane after the complete resealing, and $\tau_r$ is a time constant of resealing process.

We define the membrane resealing coefficient as

$$m = r_m/r_m^f = \sigma_m^f / \sigma_m^i \leq 1$$  \hspace{1cm} (12)

4. Moisture transfer processes

The moisture transfer processes at different hierarchical levels, such as diffusional migration, osmotic flow and redistribution of moisture inside the sample (Aguilera & Stanley, 1999), enhance as a result of PEF application. The new conducting channels arise inside the sample and this causes the temporal decreasing of $r_c$ value. We approximate this evolution as

$$r_c(t) = r_c^i - (r_c^i - r_c^f)(1 - e^{-t/\tau_d})$$  \hspace{1cm} (13)

where $r_c^f = d_c/(\sigma_c^f A)$, $\sigma_c^f$ is a final conductivity of a cellular material after completion of moisture transfer process in the sample, and $\tau_d$ is a time constant of this process.

For the quantitative description of the moisture transfer processes contribution to the change in cellular material conductivity we introduce the resistance moisture transfer coefficient $c$ defined as

$$c = r_c^f / r_c^i = \sigma_c^f / \sigma_c^i \leq 1$$  \hspace{1cm} (14)

5. Finite element analysis

The simulation of temporal evolution of the system requires a knowledge of the potential distribution in the lattice. This distribution can be obtained numerically by solving (Lebovka & Mank, 1992) the discretized version of Laplace’s equation on a lattice with given boundary potentials. For this purpose we have used the successve relaxation scheme (Press et al., 1997). We update the chosen site potential $u_{x,y}^n$ at the n-th relaxation step according to the following equation

$$u_{x,y}^n = u_{x,y}^{n-1} + \lambda \left( \frac{G_1 u_{x,y+1}^{n-1} + G_2 u_{x-1,y}^{n-1} + G_3 u_{x,y-1}^{n-1} + G_4 u_{x+1,y}^{n-1}}{G_1 + G_2 + G_3 + G_4} - u_{x,y}^{n-1} \right)$$  \hspace{1cm} (15)

where $\lambda$ is an adjustable relaxation parameter and $G_1 \div G_4$ are the conductivities of the bonds which connect the chosen site $x,y$ with all its neighbours (Fig. 7).

The iteration procedure over all sites in the lattice is continued until the maximum of the relative difference between potentials in two successive iterations, $u_{x,y}^n / u_{x,y}^{n-1} - 1$, converges to a small value $\delta$ ($= 10^{-3}$).

6. Simulated properties and main parameters

The effective media conductivity $\sigma$ was calculated on the basis of $r(x,y)$ values by applying a highly efficient Frank & Lobb (1988) algorithm. The total damage degree $P$ was estimated as the membrane damage degree with the help of the following relation

$$P = \left( 1 - \frac{1}{4N^2} \sum_{x,y=1}^{N} \frac{r_m(x,y)}{r_m^i} \right)$$  \hspace{1cm} (16)
Note that $P = 1$ when all cells are damaged ($r_m(x, y) = r^d_m = 0$) and $P = 0$ when all cells are intact ($r_m(x, y) = r^i_m$).

We assume the pulse application of external electric field in the form of an idealised square pulse sequence with a pulse duration $t_i$, and a pulse repetition time $\Delta t$. In order to increase the accuracy of calculation we introduce the "elementary" time step $dt$ which is much smaller then the pulse duration $t_i$. In this work we put $dt = 0.1 t_i$.

We use in our calculations the following values of parameters: $d_m = 5 \times 10^{-9}$ m, $d_c = 10^{-4}$ m, $\sigma_m^i = 3 \times 10^{-7}$ S m$^{-1}$ (Kotnik, Miklavcic & Slivnik, 1998), $\sigma_i^i = 0.1$ S m$^{-1}$ (approximately corresponds to the conductivity of the absolutely damaged cellular material), and treat $m$, $c$, $\tau_r$ and $\tau_d$ as adjustable variables.

With this sets of parameters we can adjust the experimentally observed parameters $\sigma^i$ and $\sigma^f$ (see Section (II A)). For example, in the case when $c = 0.1$, we obtain $\sigma^i \simeq 3.75 \times 10^{-3}$ S m$^{-1}$ (Eq. 9), and $\sigma^f \simeq 0.100$ S m$^{-1}$ at $m = 0$ (i.e., when resealing is absent) and $\sigma^f \simeq 1.07 \times 10^{-2}$ S m$^{-1}$ at $m = 0.5$.

The example of the simulated kinetics of a breakdown is presented in Fig. 8. During the period of pulse action we observe destruction of a system and increase of $P$ and $\sigma$. In the interpulse period the system begins to reseal and we can observe the partial decrease of $P$ and $\sigma$.

IV. RESULTS AND DISCUSSION

A. Experimental results

1. Damage kinetics

Figure 10 presents the examples of the experimental curves of apple slabs relative conductivity $\sigma^f / \sigma^i$ versus time $t$ dependencies at different values of the electric field strength $E$ and pulse protocols: $t_i = 1$ ms, $n = 1 - 15$, $\Delta t = 60$ s. After each pulse application we observe a rather long-time resealing-like behaviour of $\sigma^f / \sigma^i$ values during the period of order 10 s. So, in each case we measured the equilibrium values of $\sigma^f / \sigma^i$ at time $t_m \approx 10$ s after each PEF pulse. The results of measurement at two pulse protocols $t_i = 1$ ms, $n = 1 - 15$, $\Delta t = 60$ s (protocol I), and $\Delta t = 10$ ms (protocol II) and different values of the electric field strength $E = 200$ V cm$^{-1}$ and $E = 500$ V cm$^{-1}$ are presented in Fig. 11. We see, that there exist significant difference between kinetics of $\sigma^f / \sigma^i$ for these two pulse protocols. As we have demonstrated before (Lebovka et al, 2000) the pulse repetition time in the interval of $\Delta t = 1 - 100$ ms does not influence the $\sigma^f / \sigma^i$ versus $n$ dependencies essentially. We have enlarged significantly the pulse repetition time in the protocol I ($\Delta t = 60$ s) which resulted in the significant elevation of the conductivity curves to compare with those obtained for the protocol II.

2. Visualization of damages

Figure 11 shows the photographs that illustrate the macroscopic structure changes of the apple slabs after PEF treatment using protocols I(a) and II(b). The dark (brown) spots on the slabs treated using the protocol I seems to correspond the formation of the moisture-saturated and more conductive channels in the cellular material, which are absent in the case of the protocol II. The visually observed behaviour reveals the different modes of cellular material breakage. The only difference between protocols I and II is the pulse repetition time $\Delta t$. The considerable increasing of $\Delta t$ in the case of protocol II allows us to visualize the existence of certain long-time and large scale moisture transfer processes.

B. Numerical results

1. Simulation of damage kinetics

Figure 12(a) represents some examples of the simulated $\sigma^f / \sigma^i$ kinetic curves after application of $n$ ($n = 1 - 15$) pulses of $t_i = 1$ ms duration for two different pulse repetition times $\Delta t = 60$ s (solid lines, protocol I) and $\Delta t = 10$ ms (dashed lines, protocol II). We have used in these calculations the following values of parameters: $m = 0.1$, $c = 0.1$, $\tau_r = 10$ s, $\tau_d = 0.5$ s and $E^* = 0.65$. For protocol I we have $\Delta t > \tau_r, \tau_d$, which means that the resealing and mass transfer processes are finished during the time interval $\Delta t$ and we observe the typical transition process. For protocol II we have $\Delta t \ll \tau_r, \tau_d$, and in this case the system is in transient state during the PEF treatment. For this case we observe the increase of the relative conductivity during the time period of order $\tau_d$ resulting from moisture transfer processes and subsequent decrease of this value due to resealing processes.
Figure 12(b) depicts the \((\sigma^f/\sigma^i)_{t_m}\) versus \(n\) dependencies for the above-mentioned protocols. The calculated values of \((\sigma^f/\sigma^i)_{t_m}\) were "measured" at time \(t_m\) after \(n\)-th PEF pulse application, and this procedure corresponds to the real experimental measurement procedure described earlier in Section IV A 1. So, we can consider the Fig. 12(b) as a simulated analogue of Fig. 11. The kinetics of \((\sigma^f/\sigma^i)\) for the protocol I is represented in Fig. 12(b) by a dashed-dotted line. It is evident from these data that increase of the pulse repetition time \(\Delta t\) leads to the elevation of \((\sigma^f/\sigma^i)_{t_m}\) versus \(n\) dependencies in accordance with the experimental observations as discussed in Section IV A 1.

The examples of simulated breakage patterns for two different pulse repetition times \(\Delta t = 60\) s (protocol I) and \(\Delta t = 10\) ms (protocol II) are shown in Figs. 13(a) and (b) respectively. Here, each pattern displays only those cells, which were broken after the \(n\)-th pulse. We can see that the long repetition time pulse protocol I results in more extended and spatially correlated damage patterns (Fig. 13(a)). Dark clusters of the damaged cells show clearly the existence of collective percolation phenomena, which are typical for the electrical breakage of inhomogeneous materials (Sahimi, 1994). The short repetition time pulse protocol II results in more rare and uncorrelated damage patterns (Fig. 13(b)).

2. Effects of resealing and moisture transfer processes

The influence of \(m\) and \(c\) parameters on the "measured" values of \((\sigma^f/\sigma^i)_{t_m}\) after the application of \(n = 15\) pulses \((\tau_d = 0.5\) s, \(E^* = 0.65\)) are demonstrated in Figs. 14(a) and (b), respectively. Here, Fig. 14(a) presents \((\sigma^f/\sigma^i)_{t_m}\) versus \(\Delta t\) dependencies for the case of \(c = 0.1\) and \(\tau_r = 10\) s, and Fig. 14(b) presents the analogue dependencies for the case of \(\tau_r = \infty\) (i.e., when resealing is absent). All these dependencies display characteristic dispersion behaviour in the range where \(\Delta t \sim \tau_r\), and in all the cases we observe increase of \((\sigma^f/\sigma^i)_{t_m}\) with \(\Delta t\) increase.

The results for the simulated kinetics of damage degree \(P\) and relative conductivity \(\sigma^f/\sigma^i\) for different values of \(\tau_d\) are presented in Fig. 15(a) \((\tau_r = 10\) s\) and Fig. 15(b) \((\tau_r = \infty)\). These calculations were performed for the following values of parameters: \(m = 0.05\), \(c = 0.1\) s and \(E^* = 0.65\). The data show that resealing influences significantly the \(\sigma^f/\sigma^i\) versus \(t\) dependencies and this effect is the mostly pronounced at large values of \(\tau_d\). At small \(\tau_d\) values (< 5 s), the \(\sigma^f/\sigma^i(t)\) curves show the well pronounced maximum, which is practically absent on \(P(t)\) curves (Fig. 15(a)). The interesting finding is that there is no direct proportionality between the damage degree \(P\) and relative conductivity \(\sigma^f/\sigma^i\). We can observe the obvious decrease of \(\sigma^f/\sigma^i\) even in the case when \(P(t)\) practically does not change. This behaviour reflects the fact that \(\sigma^f/\sigma^i\) in the percolation phenomena depends not only on damage degree \(P\) but also on the spatial distribution of the damaged cells over the system (Sahimi, 1994).

V. DISCUSSION

The main hypothesis of the present work is that the electric field damage of a biological tissue is a phenomena of correlated percolation. This phenomena is governed by the two key processes: resealing of cells and moisture transfer inside of cellular structure. In the simulation model we try to use the minimal number of parameters in order to imitate only the main feature of this very complex phenomena, which comprises different processes at various micro and macro-hierarchical levels of membranes, cells, tissue structure, etc. We have found a considerable difference between the damage kinetics at long-term and short-term repetition times \(\Delta t\) (see Fig. 10(a) for experimental data, and Fig. 12(a) for simulation data).

In the case of long-term repetition times \(\Delta t \gg \tau_d\) a damage process in a system has a correlated character. The origin of this behaviour is the following. For heterogeneous tissue structure in external electric field the largest gradient of field strengths arise near the already damaged cells. So the new cells (after the next PEF pulse) are destroyed mainly near the previously damaged cells. In this case the damage processes are spatially correlated and we really observe this character of damage distribution at the simulated patterns (see Fig. 13(a)). In the case of short-term repetition times \(\Delta t \ll \tau_d\) a damage process in a system has a random character. Cells are also destroyed after each PEF pulse, but their damage is latent and is "invisible" for the rest of the system. So, in this case the damage processes are spatially random (see Fig. 13(a)).

Unfortunately, we are presently unable to make more strict comparison between the theoretical and experimental data as far as we have no precise data for the sets of parameters used in the present model. From the technological point of view, it is preferable to use such mode of PEF treatment that allows to achieve more homogeneous damage of a cellular material and the maximal degree of damage. These conditions are subject for optimization through variation of the PEF treatment mode, but we also need here data on model parameters.
We should mention also some restrictions of the present model. This model does not take into account rather important details concerning the structure of a cellular material, and particularly, the large scale spatial fluctuations of electrophysical properties inside the sample. These fluctuations become experimentally visualized after application of PEF with the long-term repetition times $\Delta t$ (see Fig. 11(a)) as the large scale percolative channels. For this reason we do not discuss here the experimentally observed effects of electric field strength $E$, because this behaviour may be extremely sensitive to the above-mentioned spatial fluctuations. Moreover, our model was developed only for two-dimensional systems. It allows us to simulate rather large-scale and realistic systems and avoid the well-known finite size scaling effects (Watanabe, 1995). But three-dimensional simulation is, of course, more realistic. All these restrictions should be overcome in future models in order to attain more profound understanding of tissue damage kinetics.

VI. CONCLUSION

The breakage of a biological tissue under the PEF treatment may be described as a correlated percolation phenomena, which is controlled by two key processes: resealing of the cells and moisture transfer inside of cellular structure. The breakage kinetics is very sensitive to the repetition times $\Delta t$ of PEF treatment. We observe correlated percolation patterns for the case when $\Delta t$ exceeds the time of moisture transfer processes $\tau_d$ and in the other case, when $\Delta t < \tau_d$, the random percolation patterns are observed. The long-term mode of pulse repetition times in PEF treatment allows us to visualize the macroscopic percolation channels in the sample.

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FIG. 1. Typical high resolution scanning electron micrograph of the apple sample. The "WET" chamber mode was employed that allowed observation of hydrated apple specimens in their natural state.

FIG. 2. Schematic representation of the experimental set-up used in the study of pulsed electric field treatment of apple slices.

FIG. 3. The example of the initial apple slab conductivity changes caused by the freshly cut boundary regions. The dashed line shows the transition time which always is skipped before the PEF treatment.

FIG. 4. Survival probability for a cell \( S \) versus normalised transmembrane voltage \( u^* = u/u_o \) calculated using Eq. (3) at \( \Delta u^* = 0.26 \), \( \tau_{\infty} = 3.7 \times 10^{-7} \) s, and \( dt = 100 \) µs. Here, the dotted straight line is a tangent line to a curve \( S(u^*) \) in the midpoint \( u^* = 1 \) (is shown by the small circle), and the dashed line corresponds to the normalised density distribution function \( S'/S'_{\max} \), where \( S' = dS/du^* \).

FIG. 5. The two-dimensional model of the cellular material structure. Each cell is represented by a node with four conducting bonds. Here \( d_c \) is a mean radius of a cell, \( d_m \) is a membrane thickness, \( L = 2d_c N \) is the total thickness of a sample, and \( U \) is an external voltage applied to the sample.

FIG. 6. This explains the procedure of the resistors interchange when the cells are damaged in vertical (a) or horizontal (b) directions. Here resistances \( r_i \) and \( r_d \) correspond to the intact and damaged cells and are defined by the Eq. (8) and Eq. (10), respectively.

FIG. 7. The calculation of the chosen site potential \( u_{x,y}^p \) according to successive relaxation scheme. For this particular case the central intact cell is surrounded by three intact and one damaged cells. The bond conductivities are given by \( G_1 = (r(x,y) + r(x,y+1))^{-1} = (2r^i)^{-1} \), \( G_2 = (r(x,y) + r(x-1,y))^{-1} = (2r^i)^{-1} \), \( G_3 = (r(x,y) + r(x,y-1))^{-1} = (2r^i)^{-1} \), and \( G_4 = (r(x,y) + r(x+1,y))^{-1} = (r^i + r^d)^{-1} \), respectively.

FIG. 8. The example of the simulated breakdown kinetics: degree of breakdown \( P \) and effective conductivity \( \sigma \) of the system versus number of pulses \( n \). Here \( t_i = 1 \) ms is a pulse duration, \( \Delta t = 5t_i = 5 \times 10^{-3} \) is a pulse repetition time, \( \tau_r = 10^{-2} \) s is a resealing time, and \( \tau_d \) is a mass transfer process time. The calculations are performed at \( E^* = 0.75 \), \( m = 1 \) (a case of complete resealing) and \( c = 0.1 \). Here, all the parameters are chosen only with the purpose of clear illustration of the work of algorithm.

FIG. 9. Examples of relative conductivity \( \sigma^f/\sigma^i \) of apple slabs versus time \( t \) dependencies at different values of the electric field strength \( E = 500 \) V cm\(^{-1} \) and \( E = 200 \) V cm\(^{-1} \) and pulse protocols: \( t_i = 1 \) ms, \( n = 1 - 15 \), \( \Delta t = 60 \) s.

FIG. 10. Relative conductivity \( \sigma^f/\sigma^i \) of apple slabs versus number of pulses \( n \) at different values of the electric field strength \( E = 200 \) V cm\(^{-1} \) and \( E = 500 \) V cm\(^{-1} \) and two pulse protocols: \( t_i = 1 \) ms, \( n = 1 - 15 \), \( \Delta t = 60 \) s (protocol I), and \( \Delta t = 10 \) ms (protocol II). In all the cases the value of \( \sigma^f/\sigma^i \) was measured at time \( t_m = 10s \) after the end of PEF treatment. All the experiments were repeated five times. The error bars represent standard data deviations.

FIG. 11. Photographs which illustrate the structure changes of the apple slabs after PEF treatment at \( E = 500 \) V cm\(^{-1} \), \( t_i = 1 \) ms, \( n = 10 \) and different pulse repetition time \( \Delta t = 60 \) s (a, protocol I), and \( \Delta t = 10 \) ms (b, protocol II).
FIG. 12. Calculated curves of relative conductivity $\sigma_f/\sigma_i$ versus time $t$ (a) and number of pulses $n$ (b) for two different pulse repetition times $\Delta t = 60$ s (protocol I) and $\Delta t = 10$ ms (protocol II). The following values parameters were used in these calculations: $t_i = 1$ ms, $m = 0.1$, $c = 0.1$, $\tau_r = 10$ s, $\tau_d = 0.5$ s, $n = 1 - 15$ and $E^* = 0.65$.

FIG. 13. Simulated breakage patterns for two different pulse repetition times $\Delta t = 60$ s (a, protocol I) and $\Delta t = 10$ ms (b, protocol II). We have used the following values of parameters in these calculations: $t_i = 1$, $m = 0.1$, $c = 0.1$, $\tau_r = 10$ s, $\tau_d = 0.5$ s and $E^* = 0.65$. In each pattern we display only those cells, which were broken after the $n$-th pulse.

FIG. 14. Calculated $(\sigma_f/\sigma_i)_{tm} \to \infty$ versus $\Delta t$ dependencies at different values of $m$ (a, $C = 0.1$ and $\tau_r = 10$ s) and $c$ (b, $\tau_r = \infty$, i.e., when the resealing is absent). All calculation were performed at $\tau_d = 0.5$ s. The relevant repetition time when $\Delta t \sim \tau_r$ is shown by dashed line.

FIG. 15. The simulated kinetics of damage degree $P$ and relative conductivity $\sigma_f/\sigma_i$ for different values of $\tau_d$, $\tau_r = 10$ s (a) and $\tau_r = \infty$ (b). Arrows show the direction of $\tau_d$ increase. All the calculations were performed for the following values of parameters $m = 0.05$, $c = 0.1$ s and $E^* = 0.65$. 

13
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