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ORIGINAL PAPER

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Killing of microorganisms by pulsed electric fields

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Abstract Lethal effects of pulsed electric fields (PEF) on suspensions of various bacteria, yeast, and spores in buffer solutions and liquid foodstuffs were examined. Living-cell counts of vegetative cell types were reduced by PEF treatment by up to more than four orders of magnitude (> 99.99%). On the other hand, endo- and ascospores were not inactivated or killed to any great extent. The killing of vegetative cell types depends on the electrical field strength of the pulses and on the treatment time (the product of the pulse number and the decay time constant of the pulses). For each cell type, a specific critical electric field strength (E_c) and a specific critical treatment time (t_c) were determined. Above these critical values, the fractions of surviving cells were reduced drastically. The "limits" E_c and t_c depend on the cell characteristics as well as on the type of medium in which the cells are suspended. Especially in acid media living-cell counts were sufficiently decreased at very low energy inputs. In addition to the inactivation of microorganisms, the effect of PEF on food components such as whey proteins, enzymes and vitamins, and on the taste of foodstuffs was studied. The degree of destruction of these food components by PEF was very low or negligible. Moreover, no significant deterioration of the taste of foodstuffs was detected after PEF treatment. Disintegration of cells by PEF treatment in order to harvest intracellular products was also studied. Yeast cells, suspended in buffer solution, were not disintegrated by electric pulses. Hence, PEF treatment is an excellent process for inactivation of microorganisms in acid and in thermosensive media, but not for complete disintegration of microbial cells.

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Introduction

In order to extend the durability of foodstuffs or other perishable media by killing of microorganisms, heat treatment such as pasteurization or the ultra-high temperature (UHT) process is the method predominantly used (Kessler 1988). However, thermal processes cause undesirable side-effects, for example denaturation of proteins, destruction of vitamins and deterioration of the taste (Adams 1991; Oamen et al. 1989; Pagliarini et al. 1990a, b; Heiss and Eichner 1990). For this reason, non-thermal food preservation processes gain in significance (Mertens and Knorr 1992). The pulsed electric fields (PEF) process is one of these non-thermal processes.

Lethal effects of PEF on microorganisms were described first by Doevenspeck (1960, 1961), and a patent was applied for. Sale and Hamilton (1967, 1968) and Hamilton and Sale (1967) respectively systematically analysed the effects of PEF on microorganisms. These authors demonstrated that killing of bacteria and yeasts by PEF depends on the electrical field strength and the treatment time. Thermal effects and electrolytic products could be excluded as causing the killing. According to their calculations, to affect the microorganisms, the electric pulses must induce a membrane potential of more than 1.0 V.

These results were confirmed by the examinations of Hülsheger and co-workers (Hülsheger et al. 1981, 1983; Hülseger and Niemann 1980). Furthermore Hülsheger developed a mathematical model for the calculation of the fraction of cells surviving after PEF treatment. Lethal effects of PEF on microorganisms were also

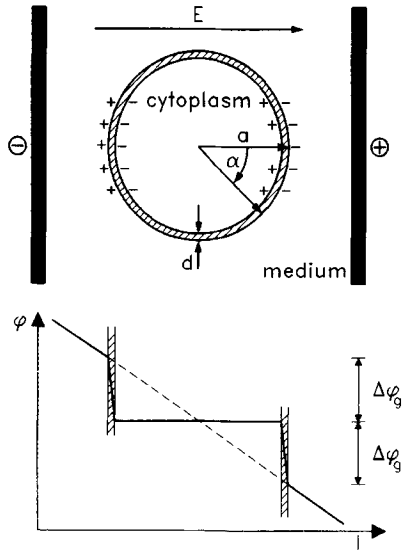

Fig. 1 Induction of a transmembrane potential $\Delta\varphi_g$ in a cell exposed to an external electric field

described by Sakurauchi and Kondo (1980), Jacob et al. (1981), Mizuno and Hori (1988), Mizuno and Hayamizu (1989), Sato et al. (1988), Sato (1989) and Jayaram et al. (1992). All of these authors used artificial media (buffer or salt solutions) and very small batch-treatment vessels of less than 10 ml for their experiments.

On the basis of the patent of Doevenspeck (1984) the company Krupp Maschinentechnik GmbH, Hamburg-Harburg, developed the Elsteril process (Sitzmann and Münch, 1989) and built a laboratory plant that was used for the experiments shown in this paper. The objectives of this research were to study the microbicidal effects of PEF in natural media, especially liquid foodstuffs, and any potential side-effects of these electric pulses on food components and taste. A third aspect was to examine the extent of disintegration of microbial cells by PEF treatment.

Theory of PEF treatment

When an external electric field (E) is applied to a cell, a transmembrane potential $\Delta\varphi_g$ is induced (Fig. 1). Transmembrane potentials of more than 1 V are believed to induce a permeabilization of the cell membrane (Sale and Hamilton 1968; Hülsheger 1984; Zimmermann et al. 1980, 1988). For a spherical cell of radius a in an electric field, the transmembrane potential between the extracellular and the intracellular surfaces of the cell membrane is given, in the absence of pores, by Eq. 1:

$$\Delta\varphi_g = F \cdot a \cdot E \cdot \cos\alpha \quad (1)$$

where F is a factor determined by the shape (length l and radius a) of the cells:

$$F = \frac{l}{l - 0.67a} \quad (2)$$

Factor $F = 1.5$ for spherical cells ($l = 2a$) and $F = 1$ for rod-shaped cells ($l \gg 2a$): The orientation of the electric field (α) at the poles of the cells is 0° or 180°. Hence $\cos\alpha = 1$ or -1 and only the sign of the transmembrane potential $\Delta\varphi_g$ is affected. For this reason $\Delta\varphi_g$ mainly depends on the electrical field strength and the radius of the cells.

The mechanism of electropermeabilization is not entirely understood. The most widely accepted model, the electromechanical compression of the cell membrane, was proposed by Zimmermann (1986): because of the attraction of opposite charges induced on the inner and outer surfaces of the cell membrane, compression pressures occur resulting in a decrease in membrane thickness. If a critical electrical field strength (E_c) is exceeded, i.e. if the transmembrane potential $\Delta\varphi_g$ rises to about 1 V, the membrane is permeabilized by pore formation. This permeabilization can be reversible or irreversible, depending on the electrical field strength and the treatment time of the pulses applied (Zimmermann 1986).

Materials and methods

Organisms and growth conditions

For PEF treatment, cells of *Escherichia coli*, *Lactobacillus brevis*, *Pseudomonas fluorescens* and *Saccharomyces cerevisiae* were grown in 50 ml of specific medium (see Table 1) by batch cultivation in a 500 ml conical flask on a shaker at 180 rpm and 30°C – except for *E. coli*, which was cultivated at 37 °C. At the end of the exponential growth phase, the cells were harvested by centrifugation at 3000 *g*, washed twice and resuspended in the treatment medium (see Table 2). Endospores of *Bacillus cereus* were enriched by cultivation in 50 ml medium in a 500 ml conical flask for 44 h at 180 rpm and 30°C. *Clostridium tyrobutyricum* endospores were formed by anaerobic cultivation of 50 ml medium in 125-ml injection bottles at 30 °C for 17 days. *Byssochlamys nivea* ascospores were obtained by rinsing the mycelium with dilution buffer after cultivation for 34–36 days on malt-extract agar plates. After PEF treatment the cells were diluted in dilution buffer (8.5 g/l NaCl, 0.3 g/l KH_2PO_4, 0.72 g/l $Na_2HPO_4 \cdot 2H_2O$, 0.1 g/l gelatine; pH 7.0) in steps of 1:10. For determining the living-cell count the diluted cell suspensions were spread on the surface of a petri plate (plate-count technique) with cell-specific media (see Table 1).

Fractions of surviving cells (s) were determined by counting the number of living cells (colony-forming units, cfu) of the untreated and the PEF-treated cell suspensions:

$$s = \frac{\text{living-cell count after PEF treatment (cfu/ml)}}{\text{living-cell count before PEF treatment (cfu/ml)}} \quad (3)$$

Measurement of whey proteins in raw milk

After PEF treatment, raw milk was centrifuged at 60000 *g* (Hereaus Suprafuge 22) for 1 h at 4 °C. The middle skim-milk layer was

Table 1 Microorganisms and cultivation media

Microorganism	Growth medium	Plate count medium
Escherichia coli no. 4517	Nutrient broth (Merck)	Nutrient agar (Merck)
Lactobacillus brevis no. 22/n5 F	MRS bouillon (Merck)	MRS agar (Merck)
Pseudomonas fluorescens	2.5 g/l yeast extract, 5.0 g/l peptone from caseine, 1.0 g/l D(+)-glucose, 1.0 g/l skim-milk powder; pH 6.8	Plate-count skim-milk agar (Merck)
Bacillus cereus ATCC 7064 (endospores)	8.0 g/l nutrient broth (Merck), 5.0 g/l yeast extract, 0.2 g/l $MgCl_2 \cdot 6\ H_2O$, 0.1 g/l $CaCl_2 \cdot 2\ H_2O$, 8.1 mg/l $MnCl_2 \cdot 2\ H_2O$	Nutrient agar (Merck)
Clostridium tyrobutyricum (endospores)	15.0 g/l peptone from casein, 10.0 g/l meat extract, 5.0 g/l yeast extract, 0.5 g/l cysteine, 8.28 g/l $NaOAc \cdot 3\ H_2O$, 7.82 ml/l 50% sodium-lactate solution, 2.5 ml/l 0.1% resazurin solution, pH 6.0	10.0 g/l peptone from caseine, 10.0 g/l meat extract, 3.0 g/l yeast extract, 5.0 g/l NaCl, 0.5 g/l cysteine 5.5 g/l D(+)-glucose $\cdot H_2O$, 7.82 ml/l 50% Sodium-lactate solution 2.5 ml/l 0.1% resazurin solution, 15.0 g/l agar agar, pH 6.0
Saccharomyces cerevisiae	Malt extract bouillon (Merck) or 3.0 g/l yeast extract, 5.0 g/l peptone from caseine, 10.0 g/l D(+)-glucose, 3.0 g/l malt extract, pH 4.8	Malt-extract agar (Merck)
Byssochlamys nivea	Malt-extract agar (Merck)	Malt-extract agar (Merck)

Table 2 Combinations of microorganisms and media used for pulsed electrical field (PEF) treatments

Microorganism	UHT milk (1.5% fat)	UHT milk (3.5% fat)	Sodium alginate solution (1.5% in deionized water)	Orange juice
	4.5 mS/cm[a]	4.5 mS/cm	2.35 mS/cm	3.3–3.7 mS/cm
E. coli	×	×	×	
L. brevis	×		×	
Ps. fluorescens	×		×	
B. cereus endospores	×			
Cl. tyrobutyricum endospres	×			
S. cerevisiae	×		×	×
B. nivea	×			

[a] Electrical conductivity

filtered (membrane filter 0.45 µm) and the whey proteins analysed by high-performance liquid chromatography on a column of ET 125/8/4 Nucleosil 500–5 C18 PPN (Macherey-Nagel GmbH & Co. KG) according to the appropriate instructions.

Enzyme assays for raw-milk enzymes

Alkaline phosphatase was assayed by the DIN 10337 method. Lactoperoxidase was determined by the method of Sigma (Stellmach 1988). The lipase assay was prepared as described by Castberg et al. (1975) and carried out as published by Versaw et al. (1989).

Disruption of yeast cells

Suspensions of yeast cells (*S. cerevisiae*) in a buffer solution (50 mM TRIS/HCl buffer in deionized water, pH 7.0, diluted about 1.5 times with deionized water to a specific electrical conductivity of 2.5 mS/cm) were treated with up to 20 high-voltage pulses of a charging voltage (U_0) of 14.0 kV and a capacitance of 5.0 µF in the vessel BK-25. For comparison, yeast suspensions were treated with ultrasound for up to 2 min (Branson Sonifier 450, output control 2, sound tube of 5 mm diameter). After treatment, the cell suspensions were centrifuged at 10 700 *g* (Heraeus Biofuge B) for 10 min at room temperature to deposit cells and cell particles. The cell-free lysate

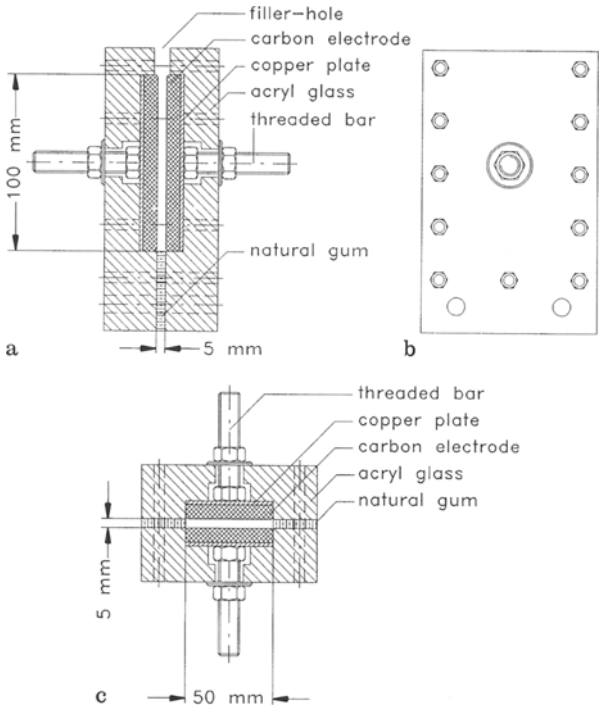

Fig. 2a, b, c Construction of a treatment vessel. The example shows the BV-25 vessel with an electrode area of 50 cm^2 (5 cm × 10 cm) and a distance between the electrodes of 0.5 cm. **a** Longitudinal section, **b** frontal view, **c** cross-section

was assayed for lactate dehydrogenase (EC 1.1.1.27) according to Vassault (1983), for isocitrate dehydrogenase (EC 1.1.1.42) according to Goldberg and Ellis (1983) and 5'-nucleotidase (EC 3.1.3.5) according to van Husen and Gerlach (1984). The protein content of the cell suspensions and the cell lysate was determined by the method of Lowry (1951), and the concentration of RNA and of DNA in the cell lysate was measured by the orcinol reaction (Cooper 1981) and the diphenylamine reaction (Süßmuth et al. 1987) respectively.

Electrical equipment

High electric field pulses were generated by combination of the following components:

1. A high-voltage generator with 5–15 kV d.c. voltage and a pulse frequency of 1–22 Hz.

2. Three capacitors of 0.5 µF, 1.0 µF, and 3.5 µF connected in parallel. Hence the maximum capacity was 5.0 µF.

3. A special high-voltage switch, the Ignitron.

4. A treatment vessel, composed of two plain parallel carbon electrodes of 5 cm × 10 cm = 50 cm^2 area. The distance (d) between the electrodes was adjusted to 0.5 cm (batch vessel BV-25, see Figs. 2 and 3) or 1.2 cm (batch vessel BV-60).

The treatment plant is described by the circuit diagram shown in Fig. 4.

By switching over the Ignitron to position "b", the capacitor charge is discharged through the treatment chamber of resistance R_K, thus resulting in an exponentially decaying high-voltage pulse, $u_K(t)$. In addition, the circuit diagram considers the inductance (L) and the resistance of the lead-in wires (R_V).

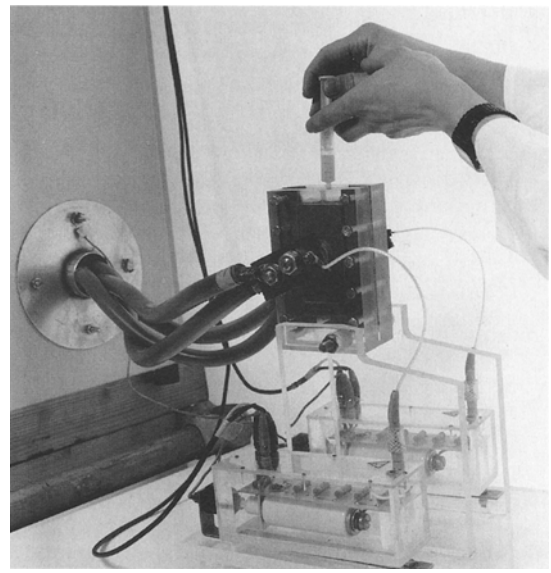

Fig. 3 Sampling from the batch vessel BV-25 by a sterilized 5-ml syringe

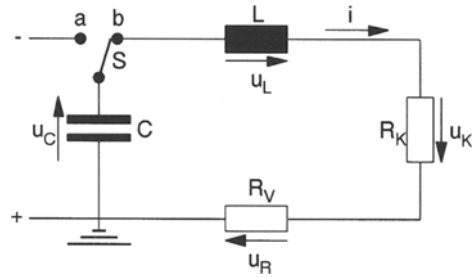

Fig. 4 Circuit diagram of a pulsed electrical field (PEF) plant

On the basis of this circuit diagram the time-dependent voltage course is described by the rules of Kirchhoff. The equations for calculating the voltage drop at the four components of the circuit diagram are:

$$u_L = L\frac{di}{dt} \tag{4}$$

$$u_K = R_K \cdot i \tag{5}$$

$$u_C = \frac{q_C}{C} \tag{6}$$

$$u_R = R_V \cdot i \tag{7}$$

where: q_C = charge of the capacitor and:

$$u_L + u_K + u_R + u_C = 0 \tag{8}$$

The resonant circuit can be described by the following homogeneous differential equation of the second order:

$$u_K \cdot \frac{1}{L \cdot C} + \frac{du_K}{dt} \cdot \frac{R_K + R_V}{L} + \frac{d^2u_K}{dt^2} = 0 \tag{9}$$

On condition that

$$R_K + R_V \geq 2\sqrt{\frac{L}{C}},$$

the voltage in the resonant circuit drops aperiodically. This condition was fulfilled within all experiments shown in this paper.

The solution of the differential equation solved for the starting conditions $u_K(0) = 0$ and $u_C(0) = -U_0$, where U_0 is the charging voltage of the capacitor, runs as follows (Hülsheger 1984; Steinau 1992; Stürken 1989):

$$u_K(t) = \frac{(R_K + R_V)U_0}{2L \cdot \psi} \cdot e^{\frac{R_K + R_V}{2L}} \cdot \sinh(\psi \cdot t) \tag{10}$$

where

$$\psi = \sqrt{\frac{(R_K + R_V)^2}{4L^2} - \frac{1}{L \cdot C}}$$

The inductance of the resonant circuit is calculated by the rising time t_a of the high-voltage pulses. The rising time must be determined from the oscillographic recordings of the pulses. The inductance of the system is calculated according to the study of Steinau (1992) as follows:

$$L = \frac{(R_K + R_V)t_a \cdot \chi}{\ln\left(\frac{1+\chi}{1-\chi}\right)} \tag{11}$$

where

$$\chi = \sqrt{1 - \left(1 - \frac{U_{max}(R_K + R_V)}{U_0 \cdot R_K}\right)^2 \left(\frac{e}{e-2}\right)^2}$$

and: U_{max} = maximum voltage at the treatment vessel.

Owing to the inductance of the resonant circuit the maximum voltage U_{max} at the treatment vessel possibly differs widely from the charging voltage U_0 of the capacitor. For $u_K(t = t_a)U_{max}$ results in:

$$U_{max} = U_0 \cdot \frac{1}{\sqrt{\xi}} \cdot \left(\frac{2}{\xi}(1 - \sqrt{1-\xi}) - 1\right)^{\frac{1}{2\sqrt{1-\xi}}} \cdot \frac{2R_K}{R_K + R_V} \tag{12}$$

where

$$\xi = \frac{4L}{(R_K + R_V)^2 \cdot C}$$

Equation (12) is difficult to handle and, in order to calculate the maximum voltage at the treatment vessel (U_{max}), it can be replaced by the following approximation formula, which was developed by Steinau (1992). This formula differs less than 1.8% from the precise Eq. 12:

$$U_{max} = U_0\left(\frac{R_K}{R_K + R_V} - \frac{2(e-2)}{e} \cdot \frac{R_K}{(R_K + R_V)^2} \cdot \sqrt{\frac{L}{C}}\right) \tag{13}$$

The resulting maximum electrical field strength (E) is described by:

$$E = \frac{U_{max}}{d} \tag{14}$$

where d is the distance between the electrodes. The duration of the electric pulse is characterized by the decay time constant (τ):

$$\tau = (R_K + R_V)C \tag{15}$$

τ depends on the capacitance (C) of the capacitors, the resistance of the system (R_V) and the resistance of the treatment vessel (R_K):

$$R_K = \frac{d}{\kappa \cdot A} \tag{16}$$

The resistance of the treatment vessel is determined by the area of the electrodes (A), the distance between them (d) and the specific electrical conductivity of the medium in the vessel (κ, see Table 2).

The electric pulses were recorded by a two-channel storage oscilloscope (Philips 3020/40).

The energy input (W) into the material treated in the chamber is calculated by:

$$W = \frac{U_0^2 \cdot C}{2} \tag{17}$$

The temperature increase (ΔT) resulting from the energy inputs for batch process is:

$$\Delta T = \frac{Wn}{V \cdot \rho \cdot c} \tag{18}$$

and for continuous processing:

$$\Delta T = \frac{W \cdot f}{\dot{V} \cdot \rho \cdot c} \tag{19}$$

where V is the volume of the treatment chamber, $\dot{V}$ the flow rate, n the number of pulses applied, f the frequency of the electric pulses applied, ρ the density weight of the medium and c the specific heat capacity of the medium.

In the process decribed in this paper, the generator voltage U_0 was 15.0 kV and the capacity of the capacitors was 5.0 µF. Therefore, the energy input was maximum 562.5 J/high-voltage pulse. Using the 25-ml batch vessel (BV-25), the temperature increase of hydrous solution (i.e. milk, juices etc.), where $\rho = 1\ kg/m^3$ and $c = 4\ kJ/kg^{-1}\ K^{-1}$), was about 5.6 K/pulse. Hence it was possible to heat up the medium to temperatures where lethal effects of PEF treatment are overlapped by thermal inactivation.

In the experiments presented in this paper, the temperature of the medium did not exceed 45–50°C. Within the batch experiments, the heat input was largely absorbed by the batch vessel. On the other hand, the pulses were applied in series and the medium was cooled down in between these series of pulses.

Results

The dependence of the fractions of surviving cells (s) of the yeast *S. cerevisiae* and of *E. coli* on the applied electric field strength is shown in Figs. 5 and 6 respectively. Within each series of tests, the number of electric pulses and hence the treatment time were held constant. For each cell type, a specific critical electrical field strength (*S. cerevisiae*: $E_c = 4.7$ kV/cm; *E. coli*: $E_c = 13.7$ kV/cm) was detected. This threshold value is defined as point of intersection of the straight survival curves with $s = 1$. Owing to the comparatively greater diameter of the yeast cells of *S. cerevisiae* their E_c value is much lower than those of the smaller bacterial cells (see Eq. 1). Above E_c, living-cell counts of the microorganisms were reduced by more than four orders of magnitude, for example from 1×10^7 cfu/ml to fewer than 1×10^3 cfu/ml.

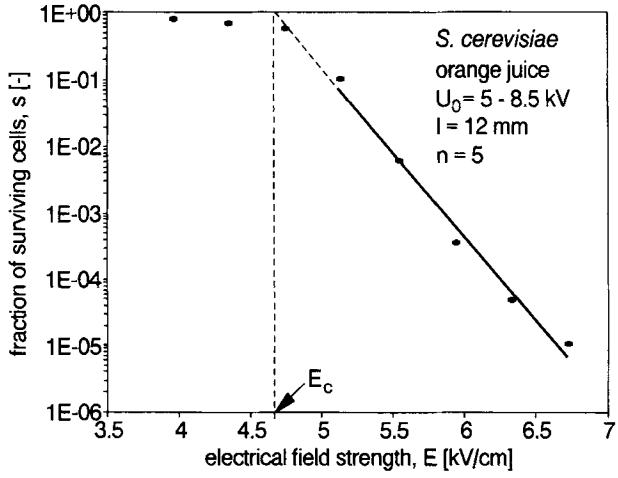

Fig. 5 Dependence of the fractions of surviving cells of *S. cerevisiae* suspended in orange juice on the electrical field strength (experiments using batch vessel BV-60)

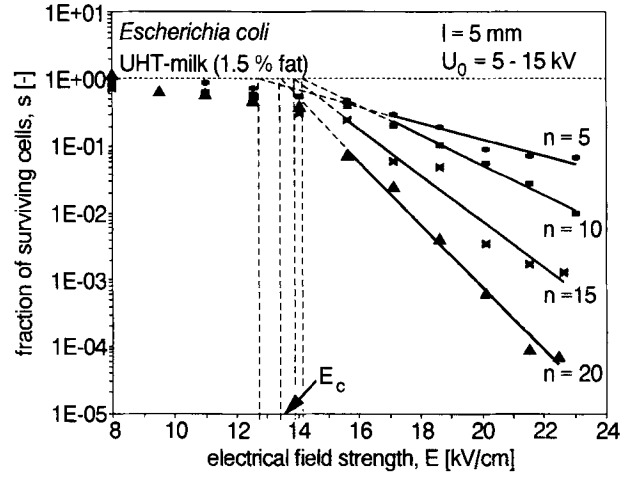

Fig. 6 Dependence of the fractions of surviving cells of *E. coli* suspended in UHT milk (1.5% fat content) on the electrical field strength (experiments using batch vessel BV-25)

The E_c value of *E. coli* almost does not depend on the number of pulses, i.e. the treatment time within each series of experiment.

In agreement with the results of Hülsheger (1981, 1984), above E_c, the fraction of surviving cells (s) follows the equation below:

$$\log s = B_E(E - E_c) \tag{20}$$

where B_E is the coefficient of regression of the straight survival curves.

Table 3 gives a survey of the results for other microorganisms and media. The E_c value is about in the same range for the bacteria examined here. What is remarkable is the discrepancy between the coefficients of regression (B_E) of *E. coli* treated with 5 high-voltage pulses and with 20 pulses suspended in different media. At 5 pulses B_E, which describes the gradient of the straight survival curves, is much lower in solutions of alginate than in UHT-milk of 1.5% fat content. Furthermore, at 20 pulses, B_E is lower in milk with a fat content of 1.5% than in milk with a fat content of 3.5%. Thus, the fat particles of the milk seem to protect the bacteria against the electric pulses.

The dependence of the fractions of surviving cells (s) of *E. coli* cells and spores on treatment time (t) is shown in Fig. 7 and in Fig. 8 respectively, where t is defined by the following equation:

$$t = n \cdot \tau \tag{21}$$

Within each series of tests the electrical field strength (E) was adjusted to a constant value above E_c.

Figure 7 shows a critical treatment time (t_c) of about 46 µs for *E. coli*. t_c is defined as as point of intersection of the straight survival curves with $s = 1$. Above t_c, a decrease of living cell counts for up to more than four orders of magnitude was measured.

Table 3 Dependence of the fraction of surviving cells of different microorganisms on the electrical field strength under PEF treatment. r coefficent of correlation of the half logarithmic regression of the electrical field strength and the logarithm of the fraction of surviving cells (log s), E_c critical electrical field strength, B_E regression coefficient, n number of pulses applied

Microorganism	Medium	n	E_c (kV/cm)	B_E (cm/kV)	r
E. coli	Solution of sodium-alginate	5	14.0	− 0.401	0.991
	UHT milk (1.5% fat)	5	12.7	− 0.123	0.974
	UHT milk (1.5% fat)	10	14.2	− 0.218	0.994
	UHT milk (1.5% fat)	15	13.9	− 0.347	0.974
	UHT milk (1.5% fat)	20	13.5	− 0.475	0.994
	UHT milk (3.5% fat)	20	11.9	− 0.307	0.996
L. brevis	Solution of sodium-alginate	5	12.1	− 0.348	0.989
	UHT milk (1.5% fat)	20	12.6	− 0.467	0.996
P. fluorescens	Solution of sodium-alginate	5	11.5	− 0.440	0.990
	UHT milk (1.5% fat)	20	10.7	− 0.331	0.993
S. cerevisiae	Solution of sodium-alginate	5	5.4	− 1.949	0.983
	UHT milk (1.5% fat)	5	4.7	− 2.464	0.983
	Orange juice	5	4.7	− 2.547	0.992

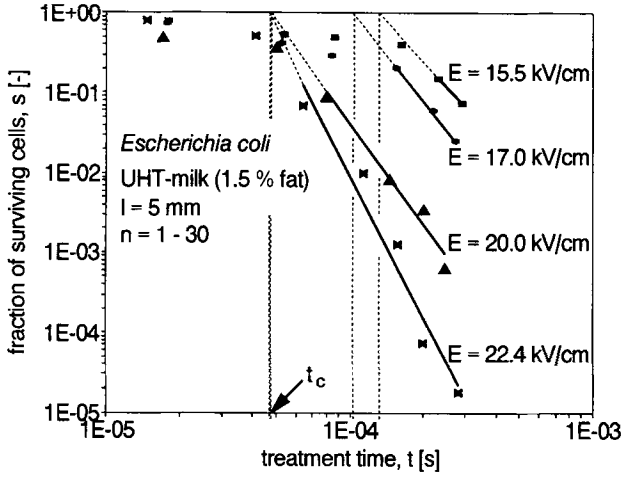

Fig. 7 Dependence of the fractions of surviving cells of *E. coli* on the treatment time of PEF treatment (experiments using batch vessel BV-25)

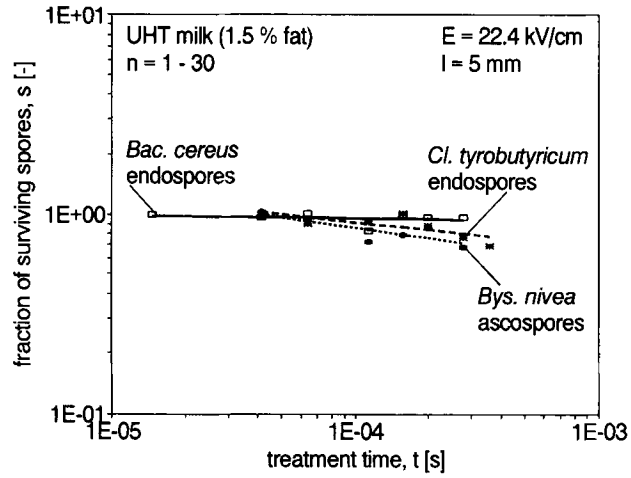

Fig. 8 Inactivation of endospores of *B. cereus* and *C. tyrobutyricum* and of ascospores of *B. nivea* (experiments using batch vessel BV-25)

Above t_c, the fraction of surviving cells follows the equation below (compare to Hülsheger 1981, 1984):

$$\log s = B_t \cdot \frac{t}{t_c} \tag{22}$$

where B_t is the coefficient of regression of the straight survival curves.

t_c depends on the electrical field strength applied. If an electrical field strength is chosen that is only slightly above E_c of the microorganism, the value obtained for t_c is much higher than in experiments where the electrical field strength is significantly higher than E_c. With *E. coli*, for the series with $E = 20$ kV/cm and $E = 22.4$ kV/cm the time t_c value determined was identical. At an electrical field strength what is more than 1.5 times higher than E_c, t_c seems to be constant. Consequently, for determination of t_c an electrical field strength should be selected that is more than 1.5 times higher than E_c.

The extent of inactivation of endospores of *C. tyrobutyricum* by high-voltage pulses was negligible, nor were any lethal effects detected on endospores of *B. cereus* or ascospores of *B. nivea* (see Fig. 8). Therefore, no critical treatment time or critical electrical field strength could be determined.

Table 4 shows a survey of the dependence of the fraction of vegetative bacterial cells surviving on the treatment time. Data for the yeast *S. cerevisiae* are not listed in Table 4, because t_c could not be determined exactly, but was below 5 µs.

As shown for E_c in Table 3, the higher the fat content of the medium, the better the bacteria are protected against the electrical field pulses: t_c is much lower in solutions of sodium alginate than in milk and at an electrical field strength of $E = 22.4$ kV/cm, B_t is lower in milk with a fat content of 1.5% than in milk with a fat content of 3.5%.

In consideration of the two critical values E_c and t_c and of the correlation described in Eq. 24, Hülsheger (1981, 1984) stated the following empirical equation for the calculation of the surviving fraction, which is confirmed by the results presented in this paper:

$$s = \left(\frac{t}{t_c}\right)^{\frac{E-E_c}{k}} \tag{23}$$

Table 4 Dependence of the fraction of surviving cells of different microorganisms on the time (t) of PEF treatment. r coefficent of correlation of the double-logarithmic regression of the log$t$ and log$s$. t_c critical treatment time, B_t regression coefficient

Microorganism	Medium	E [kV/cm]	t_c [µs]	B_t	r
E. coli	Solutions of sodium-alginate	24.8	4.5	− 3.575	0.988
	UHT milk (1.5% fat)	15.5	130.2	− 3.253	1.000
	UHT milk (1.5% fat)	17.0	104.2	− 3.806	1.000
	UHT milk (1.5% fat)	20.0	46.3	− 4.149	0.986
	UHT milk (1.5% fat)	22.4	45.7	− 5.968	0.975
	UHT milk (3.5% fat)	22.4	30.0	− 4.016	0.999
L. brevis	Solutions of sodium-alginate	24.8	10.9	− 4.950	0.996
	UHT milk (1.5% fat)	22.4	46.1	− 7.274	0.988
P. fluorescens	Solutions of sodium-alginate	24.8	0.40	− 2.483	0.976
	UHT milk (1.5% fat)	22.4	19.8	− 3.589	0.971

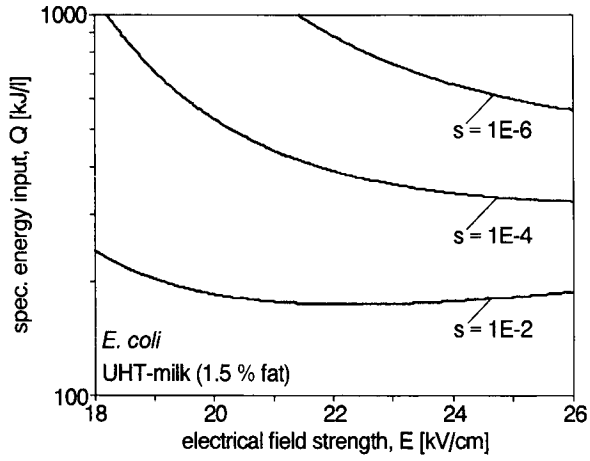

Fig. 9 Specific energy input necessary to achieve given fractions of surviving cells as a function of the electrical field strength

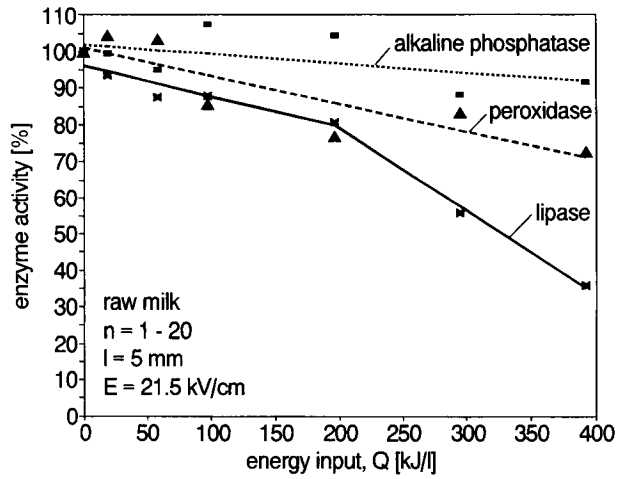

Fig. 10 Effect of PEF treatment on milk enzymes (experiments using batch vessel BV-25)

where k is a constant factor:

$$k = \frac{E - E_c}{B_{t(E)}} = \frac{\log \frac{t}{t_c}}{B_{E(t)}} \tag{24}$$

An important factor for those applying the PEF process is the specific energy input (Q) necessary to produce a given fraction of surviving cells of a defined cell type in a defined medium and a specified treatment plant:

$$Q = \frac{W \cdot n}{V} \tag{25}$$

By transformation and combination of the Eqs. 23 and 25, is described by the following equation:

$$\log Q = \log\left(\frac{E^2 \cdot l \cdot t_c}{2(R_K + R_V) \cdot A \cdot v^2}\right) + \log s \cdot \frac{k}{E - E_c} \tag{26}$$

where

$$v = \frac{U_{max}}{U_0}$$

Equation 26 and Eq. 23 are exclusively valid for $E > E_c$ and $t > t_c$. By inserting a given fraction of surviving cells Q can be determined as a function of the electric field strength (see Fig. 9).

Effects of PEF on food ingredients

PEF-induced destruction of food components was only established at high energy inputs ($Q > 200$ kJ/l) for the enzyme lipase (see Fig. 10) and vitamin C (ascorbic acid) in milk (maximum extent of destruction: 90%, data not shown). Other food components that were analysed (alkaline phosphatase, peroxidase, vitamin A, and whey proteins), did not show any large-scale inactivation. Sensory evaluations of milk and orange juice indicated that the taste of these foodstuffs does not deteriorate significantly following electric pulse treatment. Therefore, PEF is a very careful process as far as food components are concerned.

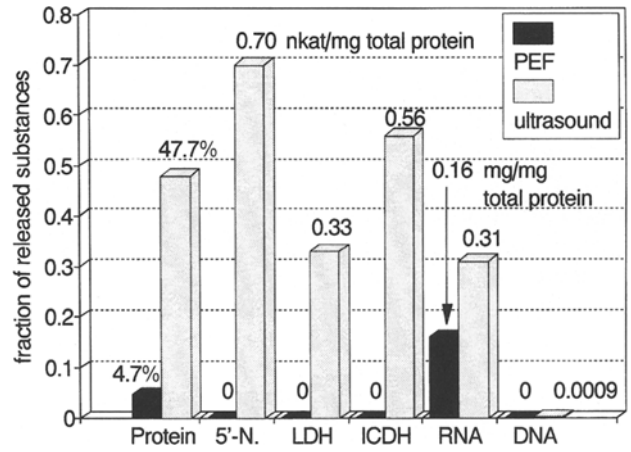

Fig. 11 Disintegration of yeast cells by PEF treatment in comparison to ultrasonic treatment

Disintegration of yeast cells

Disintegration of yeast cells (*S. cerevisiae*) by PEF treatment was not detectable to any great extent (see Fig. 11). The protein concentration in the cell lysate after high-voltage pulsing was ten times lower than after ultrasonic treatment. Activities of the enzymes 5'-nucleotidase, lactate dehydrogenase, and isocitrate dehydrogenase were not released by PEF treatment, although they were released by ultrasonic processing. Only a release of RNA and ribonucleotides was

measured after high-voltage treatment – about 50% of the concentration releasable by utrasonic treatment. Scanning electron micrographs did not show any destruction or damage of the surface of the yeast cells after electropulsing. Hence, the PEF process is not suitable for the disintegration of microbial cells.

Discussion

The results show that PEF treatment has an effect only on vegetative cells. Microbial spores (endospores and ascospores) were not inactivated by the electric pulses. For that reason, it is impossible to sterilize media by PEF processing. On the other hand, thermosensitive food ingredients such as proteins and vitamins are not destroyed to any great extent by PEF applications. Consequently this process might be suitable for the reduction of microbial cell counts in media containing thermosensitive components, which are difficult to pasteurize by (conventional) heat processing.

In correlation with the theory of PEF treatment (see Eq. 1), microbial cells of large diameter, e.g. yeast cells, are killed at lower electric field strength than cells of small diameter, such as typical bacterial cells. Hence, the reduction of living-cell counts by PEF treatment in media containing primarily yeast cells, i.e. fruit juices or other acid media, will be of interest, because in this case sufficient lethal effects are already produced by very low energy input.

The experiments on the disintegration of yeast cells illustrate that the cells did not burst as a result of the PEF applications. Only small cell components were released from the cells. These results confirm the theory of electroporation, i.e. that the electric pulses result in the formation of small pores in the cell membrane.

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