

## Antimicrobial Effects

J Environ Sci (China). 2004;16(2):348-52.

### **Sterilization of Escherichia coli cells by the application of pulsed magnetic field.**

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The inactivation of microorganisms by pulsed magnetic field was studied. It was improved that the application of electromagnetic pulses evidently causes a lethal effect on *E. coli* cells suspended in phosphate buffer solution  $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$  (0.334/0.867 mmol/L). Experimental results indicated that the survivability ( $N/N_0$ ; where  $N_0$  and  $N$  are the number of cells survived per milliliter before and after electromagnetic pulses application, respectively) of *E. coli* decreased with magnetic field intensity  $B$  and treatment time  $t$ . It was also found that the medium temperatures, the frequencies of pulse  $f$ , and the initial bacterial cell concentrations have determinate influences in destruction of *E. coli* cells by the application of magnetic pulses. The application of an magnetic intensity  $B = 160$  mT at pulses frequency  $f = 62$  kHz and treatment time  $t = 16$  h result in a considerable destruction levels of *E. coli* cells ( $N/N_0 = 10^{-4}$ ). Possible mechanisms involved in sterilization of the magnetic field treatment were discussed. In order to shorten the treatment time, many groups of parallel inductive coil were used. The practicability test showed that the treatment time was shortened to 4 h with the application of three groups of parallel coil when the survivability of *E. coli* cells was less than 0.01%; and the power consumption was about 0.2 kWh/m<sup>3</sup>.

J Bone Joint Surg Br. 2003 May;85(4):588-93.

### **Electromagnetic augmentation of antibiotic efficacy in infection of orthopaedic implants.**

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Infection of orthopaedic implants is a significant problem, with increased antibiotic resistance of adherent 'biofilm' bacteria causing difficulties in treatment. We have

investigated the in vitro effect of a pulsed electromagnetic field (PEMF) on the efficacy of antibiotics in the treatment of infection of implants. Five-day biofilms of *Staphylococcus epidermidis* were grown on the tips of stainless-steel pegs. They were exposed for 12 hours to varying concentrations of gentamicin or vancomycin in microtitre trays at 37 degrees C and 5% CO<sub>2</sub>. The test group were exposed to a PEMF. The control tray was not exposed to a PEMF. After exposure to antibiotic the pegs were incubated overnight, before standard plating onto blood agar for colony counting. Exposure to a PEMF increased the effectiveness of gentamicin against the five-day biofilms of *Staphylococcus epidermidis*. In three of five experiments there was reduction of at least 50% in the minimum biofilm inhibitory concentration. In a fourth experiment there was a two-log difference in colony count at 160 mg/l of gentamicin. Analysis of variance (ANOVA) confirmed an effect by a PEMF on the efficacy of gentamicin

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### **Inactivation of *Salmonella* Typhimurium in orange juice containing antimicrobial agents by pulsed electric field.**

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Combinations of different hurdles, including moderately high temperatures (<60 degrees C), antimicrobial compounds, and pulsed electric field (PEF) treatment, to reduce *Salmonella* in pasteurized and freshly squeezed orange juices (with and without pulp) were explored. Populations of *Salmonella* Typhimurium were found to decrease with an increase in pulse number and treatment temperature. At a field strength of 90 kV/cm, a pulse number of 20, and a temperature of 45 degrees C, PEF treatment did not have a notable effect on cell viability or injury. At and above 46 degrees C, however, cell death and injury were greatly increased. *Salmonella* numbers were reduced by 5.9 log cycles in freshly squeezed orange juice (without pulp) treated at 90 kV/cm, 50 pulses, and 55 degrees C. When PEF treatment was carried out in the presence of nisin (100 U/ml of orange juice), lysozyme (2,400 U/ml), or a mixture of nisin (27.5 U/ml) and lysozyme (690 U/ml), cell viability loss was increased by an additional 0.04 to 2.75 log cycles. The combination of nisin and lysozyme had a more pronounced bactericidal effect than did either nisin or lysozyme alone. An additional *Salmonella* count reduction of at least 1.37 log cycles was achieved when the two antimicrobial agents were used in combination. No significant difference ( $P > 0.05$ ) in cell death was attained by lowering the pH value; only cell injury increased. Inactivation by PEF was significantly more extensive ( $P < 0.05$ ) in pasteurized orange juice than in freshly squeezed orange juice under the same treatment conditions. This increase might be due to the effect of the chemical composition of the juices.

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### **Inactivation of *Listeria innocua* in liquid whole egg by pulsed electric fields**

**and nisin.**

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Consumer demand for fresh-like products with little or no degradation of nutritional and organoleptic properties has led to the study of new technologies in food preservation. Pulsed electric fields (PEF) is a nonthermal preservation method used to inactivate microorganisms mainly in liquid foods. Microorganisms in the presence of PEF suffer cell membrane damage. Nisin is a natural antimicrobial known to disrupt cell membrane integrity. Thus the combination of PEF and nisin represents a hurdle for the survival of *Listeria innocua* in liquid whole egg (LWE). *L. innocua* suspended in LWE was subjected to two different treatments: PEF and PEF followed by exposure to nisin. The selected frequency and pulse duration for PEF was 3.5 Hz and 2 micros, respectively. Electric field intensities of 30, 40 and 50 kV/cm were used. The number of pulses applied to the LWE was 10.6, 21.3 and 32. The highest extent of microbial inactivation with PEF was 3.5 log cycles (U) for an electric field intensity of 50 kV/cm and 32 pulses. Treatment of LWE by PEF was conducted at low temperatures, 36 degrees C being the highest. Exposure of *L. innocua* to nisin following the PEF treatment exhibited an additive effect on the inactivation of the microorganism. Moreover, a synergistic effect was observed as the electric field intensity, number of pulses and nisin concentration increased. *L. innocua* exposed to 10 IU nisin/ml after PEF exhibited a decrease in population of 4.1 U for an electric field intensity of 50 kV/cm and 32 pulses. Exposure of *L. innocua* to 100 IU nisin/ml following PEF resulted in 5.5 U for an electric field intensity of 50 kV/cm and 32 pulses. The model developed for the inactivation of *L. innocua* by PEF and followed by exposure to nisin proved to be accurate ( $p = 0.05$ ) when used to model the inactivation of the microorganism by PEF in LWE with 1.2 or 37 IU nisin/ml. The presence of 37 IU nisin/ml in LWE during the PEF treatment for an electric field intensity of 50 kV/cm and 32 pulses resulted in a decrease in the population of *L. innocua* of 4.4 U.

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### **Effects of pulsed electric field processing and storage on the quality and stability of single-strength orange juice.**

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The effects of pulsed electric field (PEF) processing on microorganisms in orange juice and on the flavor and color of the juice during storage for 112 days at 4 and 22 degrees C were investigated. Single-strength orange juice was PEF processed at an electric field

strength of 35 kV/cm for 59 micros and placed into sterilized glass bottles in a sanitary glove box. PEF-processed orange juice was microbiologically stable at 4 and 22 degrees C for 112 days. PEF processing resulted in significant increases in the hydrocarbons D-limonene, alpha-pinene, myrcene, and valencene ( $P < \text{or} = 0.05$ ) but did not have any effect on octanal, decanal, ethyl butyrate, and linalool. The levels of hydrocarbon compounds did not change at 4 and 22 degrees C in 112 days. Octanal, decanal, ethyl butyrate, and linalool levels significantly decreased in 14 days at 4 degrees C and in 2 days at 22 degrees C. The decrease in these compounds did not have a significant effect on the sensory quality of the orange juice ( $P > \text{or} = 0.05$ ). The microorganisms in PEF-processed orange juice, along with the flavor and color of the juice, remained stable at 4 degrees C for 112 days.

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### **Inactivation of *Saccharomyces cerevisiae* suspended in orange juice using high-intensity pulsed electric fields.**

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*Saccharomyces cerevisiae* is often associated with the spoilage of fruit juices. The purpose of this study was to evaluate the effect of high-intensity pulsed electric field (HIPEF) treatment on the survival of *S. cerevisiae* suspended in orange juice. Commercial heat-sterilized orange juice was inoculated with *S. cerevisiae* (CECT 1319) (10(8) CFU/ml) and then treated by HIPEFs. The effects of HIPEF parameters (electric field strength, treatment time, pulse polarity, frequency, and pulse width) were evaluated and compared to those of heat pasteurization (90 degrees C/min). In all of the HIPEF experiments, the temperature was kept below 39 degrees C. *S. cerevisiae* cell damage induced by HIPEF treatment was observed by electron microscopy. HIPEF treatment was effective for the inactivation of *S. cerevisiae* in orange juice at pasteurization levels. A maximum inactivation of a 5.1-log (CFU per milliliter) reduction was achieved after exposure of *S. cerevisiae* to HIPEFs for 1,000 micros (4-micros pulse width) at 35 kV/cm and 200 Hz in bipolar mode. Inactivation increased as both the field strength and treatment time increased. For the same electric field strength and treatment time, inactivation decreased when the frequency and pulse width were increased. Electric pulses applied in the bipolar mode were more effective than those in the monopolar mode for destroying *S. cerevisiae*. HIPEF processing inactivated *S. cerevisiae* in orange juice, and the extent of inactivation was similar to that obtained during thermal pasteurization. HIPEF treatments caused membrane damage and had a profound effect on the intracellular organization of *S. cerevisiae*.

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### **Effects of combined exposure of *Micrococcus luteus* to nisin and pulsed**

**electric fields.**

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Death and injury following exposure of *Micrococcus luteus* to nisin and pulsed electric field (PEF) treatment were investigated in phosphate buffer (pH 6.8, sigma = 4.8 ms/cm at 20 degrees C). Four types of experiment were carried out, a single treatment with nisin (100 IU/ml at 20 degrees C for 2 h), a single PEF treatment, a PEF treatment followed by incubation with nisin (as before) and addition of nisin to the bacterial suspension prior to the PEF treatment. The application of nisin clearly enhanced the lethal effect of PEF treatment. The bactericidal effect of nisin reduced viable counts by 1.4 log<sub>10</sub> units. Treatment with PEF (50 pulses at 33 kV/cm) resulted in a reduction of 2.4 log<sub>10</sub> units. PEF treatment followed by nisin caused a reduction of 5.2 log<sub>10</sub> units in comparison with a 4.9 log<sub>10</sub> units reduction obtained with nisin followed by PEF. Injury of surviving cells was investigated using media with different concentrations of salt. Sublethally damaged cells of *M. luteus* could not be detected by this means, following PEF treatment.

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**Biological effects of magnetic fields: studies with microorganisms.**

Moore R.

Five bacteria and one yeast were grown in magnetic fields of 50-900 gauss with frequencies of 0-0.3 HZ and square, triangular, or sine waveform. Growth of these microorganisms could be stimulated or inhibited depending upon the field strength and frequency of the pulsed magnetic field. Spore germination and mutation frequency were unaffected by the magnetic fields used in this study.