Review paper
UDC 579.67:537.2

THE INFLUENCE OF ELECTRIC FIELD ON MICROBIAL GROWTH

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Abstract

The influence of alternating electric fields of various frequency levels (from 10 to 400 Hz) on the growth of various microorganisms was studied. It was shown, that electric fields with frequencies from 10 to 60 Hz were stimulating microbial growth, while higher frequencies were causing its inhibition. No growth of yeasts and streptococci was detected after 1 hr treatment with 400 Hz frequency electric field.

Such influence of the electric field can be used in food production to prevent the development of undesirable microorganisms inside the equipment, in remote areas or during food processing. This also can be used for continuous sterilization or pasteurization of liquid or viscous food products.

Key words: *Electric field, microbial growth inhibition*.

1. Introduction

Electromagnetic field in all frequency ranges act on living organisms in one way or another. The problem of the influence of the energy fields of different nature on microbial cells is attracting growing attention of researchers. The manifold effects of the interaction of microorganisms with the external fields are described in numerous sources, shows the extraordinary relevance of the development of this direction.

Effect of external fields on the microorganisms is an environmental norm. Consequently, in the process of evolution they had to develop mechanisms of adaptation to such impacts. However, due to the development of technical microbiology and biotechnology the influence of artificial fields on microorganisms is attracting attention of researchers.

High intensity pulsed electric field (PEF) preservation technology is promising and innovative method, based on the ability of such fields to disrupt cell membranes, causing lysis of microbial cells. PEF technology can be considered as a potential alternative to traditional thermal pasteurization processes with the advantages of minimizing sensory and nutritional damage, thus providing fresh-like products.

A major cause of food spoilage is the result of activity of microorganisms. This is one of the important issues of food safety, as it can cause not only huge economic losses, but also threatening people's lives and health. The traditional heat sterilization result in different degrees of damage of some heat-sensitive materials, such as the nutritional content of food products and certain characteristics.

2. History of implementation of electric fields for food preservation

Attempts to explore the use of electricity for food preservation processes were taken since almost from the time that electricity was first made commercially available. It was first applied for the pasteurization of milk using a process known as the Electro-Pure method (Anderson and Finkelstein [1], Fetterman [2] and Getchell [3]). However, this method was in fact a thermal process as the milk was heated up by ohmic resistance (Bendicho *et al*. [4]). Only those researchers who had applied high voltages reported the ability of the process to kill the bacteria "below their thermal death point", the temperature at which a microorganism is inactivated by heat (Beattie [5] and Beattie and Lewis [6]).

In early 1980s a series of papers discussing the sensitivity of various bacteria to PEF and mathematically simulating the effect of the electric fields intensity and treatment time on microbial kills were published (Hülsheger and Niemann [7] and Hülsheger *et al*. [8], [9]). PEF processing involves the influence of pulses of high voltage to foods placed between two electrodes.

Since 1980s PEF treatment, a nonthermal method of processing of foodstuff, has been of increasing interest due to its potential in providing consumers with microbiologically safe and quality foods. In terms of food quality, PEF technology is considered superior to traditional heat treatment of foods because it avoids or greatly reduces the detrimental changes of the sensory and physical properties of foods (Quass [10]) Application of high-voltage electric fields at a certain level for a very short time by PEF not only inhibits pathogenic and spoilage microorganisms but also results in the retention of flavor, aroma, nutrients, and color of foods (Min *et al*. [11]).

2.1 Current state

A typical PEF system consists of the following components: a high-voltage power supply, a pulse generator, a number of energy storage capacitors, treatment chambers (either static or continuous) that house the electrodes, a pump to pass the liquid food through the treatment chambers (if the system is continuous), cooling and heating baths, measurement devices (voltage, current and temperature), and a central process unit to control operations. High field intensities are achieved through storing a large amount of energy from a DC power supply in a capacitor bank, in series with a charging resistor which is then discharged in the form of high voltage pulses (Zhang *et al*. [12]).

Utilization of PEF for inactivation of vegetative cells of microorganisms has been extensively studied for last decades. Successful results were obtained in processing of various liquid products fruit and vegetable juices, milk, liquid eggs, and pea soup (Barbosa-Cánovas *et al*. [13] and [15], Zhang *et al*. [14], Qin *et al.* [16], Selmaa *et al.* [17], Elez-Martínez *et al.* [18], Mosqueda-Melgar *et al*. [19], Shamsi and Sherkat [20] and Malicki*et al*. [21]).

In addition the implementation of PEF has been investigated and reported to be very promising in the recovery and production of commercially interesting and high value metabolites (natural pigments, sugar) from food (Brodelius *et al*. [22], Dörnerburg and Knorr [23], Fincan *et al*. [24] and Eshtiaghi and Knorr [25]), the improvement of fruit an vegetable juices yield in solid-liquid extraction (McLellan *et al*. [26], Knorr [27], Bouzrara and Vorobiev [28], Ade-Omoyawe *et al*. [29] and Rastogi [30]), intensifying diffusion (Jemai *et al*. [31] and El-Beghiti and Vorobiev [32]) and acceleration of mass transport in drying processes (Angersbach and Knorr [33], Rastogi *et al*. [34], Ade-Omoyawe *et al*. [35], [36], Taiwo *et al*. [37] and Teijo *et al*. [38]).

The mechanism of inactivation of microorganisms by PEF is yet to be fully identified and knowledge on microbial inactivation mechanism is essential in order to design and develop more efficient PEF technologies and equipment for effective inactivation of microorganisms in food products (Martin-Belloso *et al*. [39] and [40], Weaver and Chizmadzhev [41] and Fang *et al*. [42]). According to several publications the inactivation of microorganisms is related mainly to the changes in the cell membrane and its electromechanical instability (Jacob *et al*. [43] and Coster and Zimmermann [44]).

When pulsed electric field is applied to the substance placed between two electrodes the pores of the cell membrane are enlarged, which kills the cells and releases their contents into the environment. Cell membrane or plasma membrane is one of the vital parts of a cell that encloses and protects the constituents of a cell. Cell membrane physically separates the contents of the cell from the outside environment and gave the shape to the cell. All cells have pores, which control the flow of nutrients into, and metabolic wastes out of the cell. If these pores become wider and larger, the cytoplasm can leak into the medium, eventually killing the cell. The temperature rise in PEF system is less than 30 °C and therefore the treated material does not attain the pasteurization temperature sufficient to kill pathogens by heat alone. The voltage applied can be between 15000 to 30000 volts, where the lower voltage can kill plant cells while the higher voltage brings about the death of bacterial and fungal cells.

There are several factors affecting microbial inactivation in PEF treatment: type and growth stage of microorganisms, processing parameters and environmental parameters.

The Gram-positive bacteria are more resistant to PEF treatment than Gram-negative ones (Hülsheger *et al*. [9]). Yeasts are more sensitive to electric fields than Gram-positive bacteria due to their larger size, but they may be more resistant than Gram-negative bacteria (Qin *et al*. [16] and Sale and Hamilton [45]). Spores are the most difficult ones to inactivate by PEF treatment and even combination of heat around 60°C with 75 pulses of 60 kV cm^{-1} could not inactivate *B. cereus* spores (Pagan *et al*. [46] and Bendicho *et al.* [47]). Growth stage of microorganisms is another factor to be considered in PEF treatment. Exponential (logarithmic) phase cells are more sensitive to stress than the lag phase or stationary phase cells. Microbial growth in logarithmic phase is characterized by a high number of cells undergoing division, during which the cell membrane is more sensitive to the influence of applied electric fields (Hülsheger *et al*. [9], Gaskova *et al*. [48]).

Among the process parameters, affecting efficiency of PEF treatment the field intensity, pulse wave shape and polarity, treatment time and total specific pulsing energy input should be mentioned (Min *et al*. [49]).

Critical external field intensity required for microbial inactivation is highly dependent on the cell size as well as the field orientation (Heinz *et al*. [50]). Smaller cells require higher field intensity for inactivation. The orientation of the rod-shaped cells along or across the electric field lines also influences the required field intensity (Schoenbach *et al*. [51]).

Pulse wave shape is another factor affecting the microbial inactivation. PEF may be applied in the form of exponentially decaying, square wave, bipolar, or oscillatory pulses. Square pulse waveforms are more lethal and more energy efficient than exponential decaying pulses. Oscillatory decay pulses are the least efficient, because they prevent the cell from being continuously exposed to a high intensity electric field for an extended period of time, thus preventing the cell membrane from irreversible breakdown over a large area (Jeyamkondan *et al*. [52]).

The treatment time and energy input are linearly correlated and increasing of each can result in increased microbial or enzymatic inactivation. The main cause of the inactivation of microorganisms and enzymes by PEF is considered the energy input (Malicki *et al*. [21] and Min *et al*. [53]).

Environmental factors, such as treatment temperature, pH, conductivity and ionic strength of the medium also have significant impact on PEF treatment efficiency.

Some researchers have shown that PEF treatments at moderate temperatures (50 to 60 °C) exhibit additive effects on the inactivation of microorganisms and spores (Dunn and Pearlman [54], Rowan *et al*. [55], Spilimbergo *et al*. [56] and Craven *et al*. [57]).

The combined effects of low pH and organic acids can enhance the inactivation of spoilage microorganisms with the PEF technology. However, as most of organic acids are preservatives, it is possible that the inactivation levels achieved were due to not only the combined effect of pH and PEF treatment but also the chemical properties of the acids.

The effect of pH on the sensitivity of microorganisms to PEF treatment is likely dependent on the target microflora (Pagan *et al*. [58]).

In the media with high electrical conductivities, during PEF treatment electric fields with smaller peaks are generated which do not cause the required killing effects (Barbosa-Canovas *et al*. [59]). An increase in ionic strength of a liquid leads to increased conductivity, resulting in a decreased microbial inactivation level. Thus, the inactivation level of microorganisms increases with decreasing medium conductivity (Jayaram *et al*. [60]). The effectiveness of PEF treatment may be also affected by the type of cations present in the medium (Hülsheger *et al*. [8]).

3. Conclusions

Although PEF preserves the nutritional components of the food, several areas need further research before the technology is applied commercially, and effects of PEF on the chemical and nutritional aspects of foods still must be better understood before it is used in food processing. Nevertheless, there is no doubt, that high voltage pulsed electric fields, may definitely be used for food preservation and pasteurization, to maintain food quality to the maximum level.

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