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PULSED ELECTRIC FIELDS (PEF) AS AN UNCONVENTIONAL METHOD OF FOOD PRESERVATION

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Technology of pulsed electric fields (PEF) could be an alternative preservation method of food liquids compared to traditional heat pasteurisation where the main purpose is to inactive pathogenic bacteria. The main problem of all novel technologies is their effectiveness in microbial inactivation. The idea of PEF treatment, description of our laboratory PEF generator, factors affecting microbial inactivation during PEF process and effectiveness of that method in food systems were described in the paper. It was concluded that the best effect of food preservation with the use of PEF is achieved upon applying a combination of methods, *i.e.* PEF and high hydrostatic pressure (HHP) and others (according to "hurdle theory"). Different studies indicate that pulsed electric fields could be a useful method in liquid food preservation especially in the context of good organoleptic and functional properties of final products. However, the main assessment criterion of that method is the sufficient improvement of microbiological safety especially in the context of spores inactivation.

INTRODUCTION

The application of pulsed electric fields (PEF) seems to be an alternative and profitable method of food preservation. Most conventional food processing efforts aim towards the reduction or inactivation of microbial populations, which can be achieved through thermal processing (*i.e.* blanching, pasteurization, sterilization) using water, steam, electrical, light or microwave energy as a means for heat transfer [Knorr, 1998]. Non-thermal methods enable the processing of food below temperatures used during thermal pasteurization, hence flavours, essential nutrients and vitamins undergo minimal or no changes [Butz & Tauscher, 2002]. In the past years such preservation methods like aseptic processing, ionizing energy, modified atmospheres, oscillating fields, pulsed electric fields (PEF), microwave energy were observed to become more and more popular [Cardello, 2003]. Many studies indicate microbiological effectiveness of these methods at a good level of sensory characteristics of products [Sitzman, 1995; Knorr, 1998]. A success of a food product depends on both product and consumer factors including these social, cultural, contextual and attitudinal ones. Consumers are afraid of some new technologies more than others. Within this context, the application of novel food processing technologies to commercial foods rises high concern among consumers. The consumers' concern is connected, to the greatest extent, with the addition of bacteriocins, genetic engineering, pulsed X-rays, and irradiation [Cardello, 2003]. The lowest levels of consumers' fear of food preservation concerns old technologies like heat pasteurisation, cold preservation, thermal energy but also some new technologies like radio-frequency heating, microwave radiation, pulsed electric fields, ultrasounds and oscillating magnetic field [Cardello, 2003]. One of these new non-thermal methods of food preservation, *i.e.* pulsed electric fields (PEF), was described in this paper.

PULSED ELECTRIC FIELDS

New technologies of food preservation

The most investigated new preservation technologies are non-thermal inactivation technologies such as high hydrostatic pressure (HHP) and pulsed electric fields (PEF), new packaging systems such as modified atmosphere packaging (MAP) and active packaging, natural antimicrobial compounds and biopreservation. Another investigated inactivation technologies are ionisation radiation, high pressure homogenisation, UV decontamination, pulsed high intensity light, high intensity laser, pulsed white light, high power ultrasound, oscillating magnetic fields, high voltage arc discharge and streamer plasma, but most studies is focused on HHP and PEF [Devlieghere *et al*., 2004].

Idea of PEF treatment

PEF processing involves the application of pulses of high voltage (typically 20-80 kV/cm) to foods placed between 2 electrodes. The effect of PEF is related to the application of high voltage for very short periods of time (in the range of nano- or microseconds). Exposure of bacterial cells to the field changes of the sufficient amplitude affects the electrical properties of the cell membrane, reflected in a decrease in its resistance and an increase in conductance. Consequently,

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permeability of the membrane is altered, which is known as electroporation [Knorr *et al.*, 2001; Heinz *et al.*, 2002]. That method is usually applied to liquid foods like orange juice, liquid whole egg, milk, yoghurt. PEF preservation of liquid food helps also to extend the shelf-life of a product.

PEF generator

Equipment for the PEF treatment usually consists of a pulse generator, a treatment chamber and a system for control and data acquisition. There are many different PEF equipments, but we have described our laboratory PEF generator.

A PEF generator used in our department consists of a cavity adopted from a microwave oven which plays the role of Faraday cage with safety doors. There is a treatment cell placed inside this Faraday cage. We use two different treatment cells: the first is stationary cell, with a volume of 20 mL having the shape of a hollow cylinder, whereas the second flow cell has the shape of a meander cut in fluoropolymer dielectric material and is equipped with the inlet and outlet fittings. The inlet of the flow cell is connected to a peristaltic pump by means of a flexible tubing. Both the stationary and flow cells have the top and bottom made of stainless steel and both have the form of threaded corks screwed into the cells.

The bottom of the cell is grounded to the metal case of the cavity, whereas the top of the cell is connected to the spark gap assembly using a contact plate connected with the latter using a flexible high voltage cable. The contact plate is attached to the dielectric rod which is passing through the cavity top wall and can be moved up and down by the operator. In the up position it touches the grounded contact making the spark gap short to the ground, and when in down position it connects the high voltage potential to the upper cork in the treatment cell allowing the electric pulses produced by spark gap assembly to excite the liquid sample inside the treatment cell.

The energy delivered to the spark gap assembly can vary continuously by altering the voltage. The maximum energy is as high as 100 Joule which corresponds to a discharge voltage of 30 kV in the 0.25 microfarad capacitor. The capacitor is charged to the prescribed level from a voltage multiplier fed by a switch-mode power supply unit.

The electronic circuitry along with microprocessor controller is housed within the L shaped metal box the main volume of which is occupied by the discharge capacitor. There is also a special tool provided for handling the cells inside the cavity. This tool is made of stainless steel in the form of big scissors which are grounded *via* a flexible cable connected to the metal enclosure.

The output pulse is of exponential type, which corresponds to the discharge of a capacitor C through a resistor R with time constant RC. In fact, most of liquid foodstuff in electric field behave as resistive load. The effective time of the discharge corresponds to 3RC and is measured by an electronic gate recording the effective time duration of the current flow through the cell.

The entire PEF process is controlled by a computer. The software enables the operator to chose a voltage level of 0-30 kV with increments of 100 V, the number of pulses 1-100, repetition period of the pulses 1-10 sec, and to estimate the effective duration time of pulses in the range of 1-400 µs, the electric field strength and power density per sample volume. Our laboratory generator of PEF has 16 protection levels against electrical shock, which makes working with this equipment as safe as it is possible.

Factors affecting microbial inactivation

Factors affecting microbial inactivation described below have been provided by San-Martin *et al*. [2003]. According to these authors, the microbial inactivation is determined by three main factors, *i.e.* electrical treatment, microorganism and suspending medium.

Factors dependent on the electrical treatment are PEF pulse waveshape, electric field strength and treatment time. The correlation between the microbial inactivation and electric field strength is simply: the higher the electric field strength, the higher the inactivation achieved [Grahl & Märkl, 1996]. Treatment time is equal to the number of pulses applied times the pulse width. In general, the microbial inactivation increases with an increasing number of pulses, but the significant heating of products is likely to occur.

Factors dependent on the microorganism are cell size, growth stage, microbial concentration and the presence of spores. Larger microbial cells will require less intense field strengths to undergo an equivalent inactivation as compared to smaller cells. Cells in the exponential growth phase are more sensitive to PEF treatment than the cells in lag or stationary phase [Alvarez, 2000]. Although PEF treatment is rather not an effective method for spore inactivation there are some research indicating that spores may be inactivated by PEF and that the type of pulse may also play a key role [Marquez, 1997].

Factors dependent on the suspending medium are temperature, pH, ionic strength, conductivity and medium composition. To achieve the same amount of inactivation, lower electric field strengths are needed at higher temperatures. The effects of pH, ionic strength and conductivity should be taken into consideration when selecting a suspending medium, but these aspects need to be further investigated. There is no general agreement about medium composition and PEF treatment, nevertheless certain components of food, such as protein or lipids, may have a protective effect over microorganisms [Barsotti & Cheftel, 1999].

Effectiveness of PEF treatment in food preservation

The effectiveness of PEF treatment depends on many previously described factors. Authors report the effectiveness of PEF for specified processing conditions. Below there are described some examples for that aspect of food preservation.

The inactivation of *Escherichia coli* by PEF was studied in liquid, solid and semisolid foods or model systems by Manas *et al*. [2001]. They found that agitation of the inoculated liquid samples (16 mmol/L sodium phosphate buffer) during pulse processing resulted in efficient microbial inactivation – 5 log cycles at 33 kV/cm and about 25^{o}C after 261 µs of cumulated pulses.

The highest extent of *Listeria innocua* inactivation in liquid whole egg (LWE) was 3.5 log cycles for an electric field

TABLE 1. Parameters of PEF and effectiveness of microbial inactivation in different liquid food products [Vega-Mercado, 1997].

Product	PEF parameters				Effectiveness of microbial inactivation	
	Electric field strength E (kV/cm)	Total time t (μs)	Number of pulses n (-)	Temperature of product after PEF treatment T (^{o}C)	Microflora	log cycles D (or description)
Orange juice	6.7	20	5	45-50	*Saccharomyces cerevisiae*	5 D
Milk	28.6	100	23	42.8	*Escherichia coli*	3 D
Milk	36.7	100	40	63	*Salmonella dubin*	3 D
Milk	22	20	20	45-50	*Lactobacillus brevis*	4.6 D
Yoghurt	23-38	100	20	63	*Lactobacillus bulgaricus Streptococcus thermophilus*	2 D
Liquid whole egg	25.8	4	100	37	*Escherichia coli*	6 D
Skim milk	45	1.8-6	64	35	*Escherichia coli*	2 D
Fluid food	12-25	1-100	25	45-55	Natural	Shelf-life extended from 3 to 7 days

intensity of 50 kV/cm, 32 pulses and total time duration of 64 μs [Calderon-Miranda, 1999]. The presence of 37 IU nisin/mL in LWE under the same PEF conditions resulted in a better effectiveness of *Listeria innocua* inactivation (4.4 log cycles). It points to better results achieved upon the application of combined methods for food preservation, which was confirmed in the next studies where liquid whole egg (LWE) with 0.15% addition of citric acid and after PEF treatment (E=30 kV/cm, t=489 ms, W=6331 J/mL) had the shelf-life of 20 days at 4^{o}C. The same LWE but with 0.50% addition of citric acid and after a slightly different PEF treatment (E=30 kV/cm, t=55 ms, W=357 J/mL) was characterized by 30-day shelf-life at a temperature of 4^{o}C [Gongora-Nieto *et al.*, 2003]. The above examples indicate that a skilful combination of preservation methods results in a safer product with extended shelf-life. Although some studies have concluded that PEF preserves the nutritional components of the food, effects of PEF on the chemical and nutritional aspects of specific foods should be better recognized [Qin *et al.*, 1995].

The effectiveness of PEF treatment on different microorganisms and food systems was shown in Table 1 [Vega-Mercado, 1997]. The maximum microbial reduction of the presented examples was 6 log cycles for *Escherichia coli* in liquid whole egg. We have also evaluated the efficiency of pulsed electric field (PEF) against *Escherichia coli* contaminating the liquid whole egg (LWE) in our studies [Malicki *et al.*, 2004]. The samples of LWE were inoculated with the test bacteria and subsequently treated for 30 μs by the different number of pulses (20-180) of PEF (32.89 kV x cm^{-1}). The application of PEF resulted in a statistically significant reduction of the test microorganisms, proportional to the number of pulses used. Depending on the studied strain, the treatment with 150-160 PEF pulses was required to obtain the reduction of initial bacteria level by 4 log units. Considering the obtained results, PEF seems to be an effective technique that improves the microbiological status of LWE. Its industrial application is, therefore, highly advisable. Moreover, we have studied functional and rheological properties of LWE after the PEF treatment [Oziembłowski *et al.*, 2005]. LWE was obtained from eggs of 27-week-old layer hens, Tetra SL. Parameters of the treatment were chosen according to earlier experimental results where a significant reduction of microflora was observed at 32.89 kV/cm using 20, 60, 100 pulses. It was concluded that the functional properties of liquid whole egg after PEF were not worse than those of the control sample. Furthermore, foam ability and emulsifying capacity of LWE after PEF were significantly better with an increasing number of impulses compared to the control sample. Viscosity of LWE at a shear rate 250 (1/s) was higher after the PEF treatment: 112 mPa s (20 pulses) and 106 mPa s (100 pulses). For the control sample, the apparent viscosity was 102 mPa s. The results obtained indicate that pulsed electric fields could be useful in the preservation of the liquid whole egg, especially in the context of functional and rheological properties. It is obvious, however, that the sufficient improvement of microbiological safety was the main assessment criterion of that method.

CONCLUSIONS

Pulsed electric fields as a new preservation method has its opportunities and drawbacks. Negative aspects of the PEF treatment include the following facts: spores are not sensitive to PEF treatment, upscaling of PEF equipment is still under development, the method is rather limited to liquid products and its efficiency depends on the electrical conductivity of food [Devlieghere *et al.*, 2004]. Nevertheless, the PEF method affords a number of opportunities, including:

high retention of nutrients and vitamins in products after the PEF treatment, continuity of the PEF process, high organoleptic quality of final products, application to acid foods as spores will not germinate in acid foods [Devlieghere *et al.*, 2004]. The PEF technology is much more effective when used with other preservation methods, like high hydrostatic pressure (*"hurdle theory"*), however the effect of a combination with other preservation methods to inactivate spores is still under investigation.

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PULSACYJNE POLA ELEKTRYCZNE JAKO NIEKONWENCJONALNA METODA UTRWALANIA ŻYWNOŚCI

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Technologia pulsacyjnych pól elektrycznych (PEF) może być alternatywną metodą utrwalania płynnej żywności w stosunku do tradycyjnej termicznej pasteryzacji, której głównym celem jest inaktywacja patogennej mikroflory. Głównym problemem wszystkich nowych technologii jest ich efektywność inaktywacji drobnoustrojów. W pracy przedstawiono główne idee dotyczące oddziaływań PEF, opisano generator PEF będący w posiadaniu naszej katedry, omówiono czynniki wpływające na inaktywacje drobnoustrojów podczas procesu PEF jak również efektywność tej metody w odniesieniu do produktów żywnościowych. Stwierdzono, że najlepszą skuteczność utrwalania żywności za pomocą PEF można uzyskać stosując metody kombinowane, np. PEF oraz technikę wysokich ciśnień (HHP) lub inne (zgodnie z teorią „płotków"). Różne badania wskazują, że pulsacyjne pola elektryczne mogą być użyteczną metodą utrwalania płynnych produktów żywnościowych szczególnie w aspekcie dobrych właściwości organoleptycznych i funkcjonalnych finalnych produktów. Niemniej jednak, głównym kryterium oceny tej metody jest podniesienie bezpieczeństwa mikrobiologicznego szczególnie w odniesieniu do inaktywacji przetrwalników.