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Influence of Certain Pulsed Electric Field Conditions on the Growth of *Lactobacillus acidophilus* LA-K

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Abstract

Pulsed Electric Field (PEF) processing involves the application of pulses of electricity, for less than one second to fluid products placed between two electrodes. *Lactobacillus acidophilus* is an important probiotic bacterium used for the production of fermented dairy foods. The objective was to study the influence of electric pulse period, electric field strength, and electric pulse width on the growth of *Lactobacillus acidophilus* LA-K. *Lactobacillus acidophilus* LA-K, suspended in sterile peptone water was treated using an OSU-4 PEF processor. The treatments were pulse periods of 10,000, 20,000 and 30,000 µs electric field strength of 5, 15 and 25 kV/cm, and pulse widths of 3, 6 and 9 µs. Growth was determined hourly for 16 hours of anaerobic incubation at 37°C. Pulse period had a significant (p=0.0017) influence on the growth. There were no significant differences among the control, 30,000 µs and 20,000 µs. The growth of *Lactobacillus acidophilus* subjected to the pulse period of 10,000 µs was significantly lower than the growth of the control, and the growth when subjected to 30,000 µs. Electric field strength had a significant (p<0.0001) influence on the growth. Growth subjected to 15 and 25 kV/cm was significantly lower than the control and 5 kV/cm. There were no significant differences between the control and 5 kV/cm. There were no significant differences between the growth, when *Lactobacillus acidophilus* was subjected to 15 and 25 kV/cm. Bipolar pulse width effect had a significant (p<0.0001) influence on the growth. Growth of the control was significantly higher than the growth of *Lactobacillus acidophilus* subjected at any of the bipolar pulse widths studied. There were no significant differences in growth among the three different bipolar pulse widths. Electric field strength significantly influenced growth of *Lactobacillus acidophilus* LA-K. Bipolar pulse width and pulse period slowed log stage growth of *Lactobacillus acidophilus* LA-K. Slower growth of adjunct bacteria can sometimes be good in the manufacture of cultured dairy foods, as it results in controlled release of bacterial enzymes for improved flavor and texture development.

Keywords: Non thermal; Probiotic; Acidophilus; PEF

Introduction

Temperature has been used to kill microorganisms (pasteurization), grow microorganisms (incubation) and preserve microorganisms (freezing). Temperature is widely used in food processing to kill the pathogens, to result in a safe product for consumption. High temperatures kill pathogens in food, but have several disadvantages on the food product, including loss of heat sensitive components. Example: loss of heat sensitive vitamins, denaturation of some proteins and induction of cooked flavors, which is a sensory defect. To avoid these disadvantages in a food product and yet kill the pathogens, there is increased interest in non thermal processing. Pulsed Electric Field (PEF) is one of the non thermal hurdle technologies gaining interest.

Pulsed electric field involves passage of pulses of electricity to fluid food that flow between two electrodes. The destruction of pathogens is achieved by irreversible electroporation. Destruction of pathogens in liquid media using PEF has been reported earlier.

Pothakamury et al. [1] obtained a 5 log reductions in *Escherichia coli* counts by applying 60 pulses, 16 kV/cm and 300 µs. These results were compared with those obtained by using heat (82.2°C, 171 s), which produced 5 log reductions, but caused degradation of organoleptic and nutritive characteristics. Qin et al. [2] achieved more than a 6 log cycle reduction in *E. coli,* using electric field intensity of 36 kV/cm and 50 pulses PEF treatment. The temperature of the chamber in this study was maintained below 40°C during the PEF treatment, which is lower than the temperature of commercial pasteurization (70-90°C) for milk. Grahl and Markl [3] reported the influence of pulse number in microbial inactivation of *E. coli*. They were able to reduce the populations of *E. coli* in UHT milk by 1, 2 and 3 log cycles when 5, 10, and 15 pulses at 22 kV/cm were applied. The killing effect of PEF on *Escherichia coli* ATCC 8739 suspended in an orange juice and milk beverage was studied by Rivas et al. [4]. Bipolar square pulse widths with a pulse width of 2.5 µs, electric field strengths from 15 to 40 kV/cm, and treatment times from 0 to 700 µs were applied in this study. They found a maximum of 3.83 log reductions with 15 kV/cm and 700 µs, and no significant differences at the electric field strength range from 25-40 kV/cm.

Effect of PEF on many other pathogens has also been studied. Fernandez-Molina et al. [5] reported 2.6 and 2.7 log reductions for different microorganisms such as *Listeria innocua* in raw skim milk (0.2% milk fat) and *Pseudomonas fluorescens* with 2 µs, 100 pulses and 50 kV/cm at room temperature. They achieved 3.5 log reductions using 29 kV/cm, 4 µs, and 71.3 µs of total treatment time. Sobrino-López et al. [6] reached a 4.5 log reduction in *Staphylococcus aureus* by applying 150 bipolar pulses of 8 µs, each at 35 kV/cm. They found bipolar pulses to be more effective than monopolar pulses.

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Although there is extensive literature on use of PEF conditions to kill pathogens, there is scanty literature on PEF conditions to modulate growth of desirable bacteria. Just as high temperatures are used to kill pathogens and mild temperatures are used to grow culture bacteria, it is not known if pulsed electric field can have a similar effect. The hypothesis was whether certain "mild" pulse electric field conditions can modulate the growth of the probiotic bacterium *Lactobacillus acidophilus* LA-K, without killing it. Cultured food such as yogurt and cheese contain bacterial cultures of at least a million cfu's/g. These modulated growths of culture bacteria are needed in cultured food products in various applications, such as enhanced textures and flavors during cheese ripening, and milder sourness in yogurt with enhanced flavors. The objective was to study the influence of electric pulse period, electric field strength and electric pulse width on the growth of *Lactobacillus acidophilus* LA-K.

Materials and Methods

Sample preparation and treatment

Samples for the growth were prepared by inoculating 1% (v/v) of *Lactobacillus acidophilus* (F-DVS LA-K, Chr. Hansen's Laboratory, Milwaukee, WI, USA) in peptone water (0.1% wt/v) at room temperature (21°C). The OSU-4 pulsed electric field processor (The Ohio State University, Columbus, OH) was used to generate the pulsed electric field conditions. The treatments were pulse periods of 10,000 µs, 20,000 µs, and 30,000 µs, electric field strengths of 5, 10, and 15 kV/cm, and electric pulse widths of 3, 6, and 9 µs. Control was run through the PEF equipment at 60 mL/min, without receiving any pulsed electric field treatment. Growth was evaluated hourly over 16 hours of incubation. The experimental design was a repeated measure design. Three replications were conducted.

Growth

Growth of *Lactobacillus acidophilus* LA-K was determined according to Loghavi et al. [7]. The growth was monitored by measuring the optical density at 600 nm (OD 600) through an UV-Vis Spectrophotometer (Nicolet Evolution 100, Thermo Scientific; Madison, WI, USA). Control and PEF treated samples were inoculated (10% v/v) into MRS broth (Criterion™, Hardy Diagnostics, Santa Maria, CA), which was previously autoclaved at 121°C for 15 min with pH 6.5 ± 0.2. The inoculated MRS broth had an initial OD 600 of 0.200 ± 0.005, and was incubated under anaerobic conditions at 37°C for 16 hours. The OD values were collected hourly. The spectrophotometer was calibrated by using MRS broth as blank. An average of two values per treatment was taken, i.e. two cuvettes per treatment. An estimate of bacterial counts (CFU/mL) was calculated from OD 600 readings, using a standard curve (Figure 1).

Statistical analysis

An analysis of the data was done using PROC GLM of the Statistical Analysis Systems. Differences of least square means were used to determine significant differences at $P<0.05$ for main effects and interaction effects. Data are presented as mean ± standard error of means. Significant differences were determined at α=0.05.

Results and Discussion

Pulse period effect on growth

The OD values at different pulse periods over the growth period of 16 hours are shown in figure 2. Various treatments applied are in table 1. Pulse period*hour interaction effect was not significant (p=1.0000) (Table 2). Pulse period had a significant (p=0.0017) influence on the growth curve (Table 2). According to table 3, there were no significant differences among the control, 30,000 µs and 20,000 µs. The growth curve subjected to the pulse period of 10,000 µs was significantly lower than the growth curve of control and the growth curve subjected at 30,000 µs.

Electric field strength influence on growth

The OD values at different electric field strengths over the growth period of 16 hours are shown in figure 3. Treatments applied are shown in table 4. Voltage*hour interaction effect was not significant (p=0.2706) (Table 5). Voltage had a significant (p<0.0001) influence on the growth curve (Table 5). The LA-K subjected to 15 and 25 kV/cm had significantly lower growth than the control and 5 kV/cm (Table 6). There were no significant differences between the control and 5 kV/cm. Furthermore, there were no significant differences between the growth curves at 15 and 25 kV/cm.

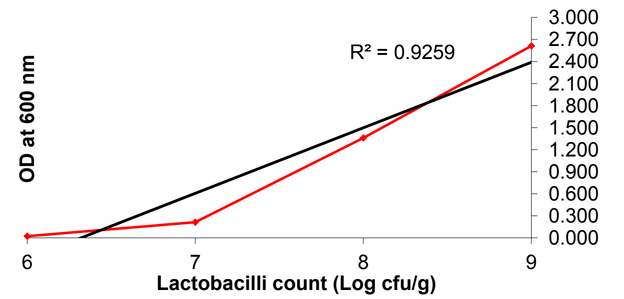

Figure 1: Standard curve for growth of LA-K in MRS broth.

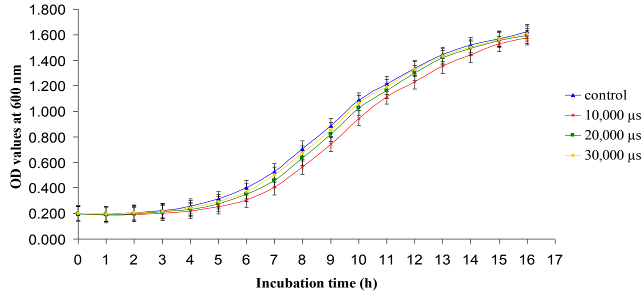

Figure 2: Pulse period effect on growth of LA-K.

Treatment parameter	Condition
Bipolar pulse width (µs)	3
Electric field strength (kV/cm)	25
Pulse period (µs)	10,000; 20,000; 30,000
Delay time (µs)	20
Flow rate (mL/min)	60

Table 1: Pulsed Electric Field (PEF) treatment conditions applied during the study of the influence of various pulse periods on *Lactobacillus acidophilus* LA-K.

	Growth
Source	**MS Pr>F**
Pulse period	0.070<0.0017
Hour	4.840<0.0001
Pulse period*hour	0.002<1.0000
Error	0.013

Table 2: Mean Square (MS) and Pr>F of pulse period, hour and their interaction for growth characteristics, bile tolerance and protease activity.

Pulse width influence on growth

The OD at different bipolar pulse widths over the growth curve period of 16 hours are shown in figure 4. Various treatments applied are in table 7. Bipolar pulse width*hour interaction effect was significant (p=0.0155) (Table 8). From hours 5 to 10, there were significant differences between the control and the different bipolar pulse widths. Bipolar pulse width effect had a significant (p<0.0001) influence on the growth curve (Table 8). The growth curve of the control was significantly higher than the growth curves subjected at any of the bipolar pulse widths studied. There were no significant differences among the three different bipolar pulse widths (Table 9). The logarithmic phase of Lactobacillus LA-K in control was reached faster, than when treated at different bipolar pulse widths. The slope in the growth curve of the control was higher than the slope of the curve of the different bipolar pulse widths. From hours 5 to 10, the control was an average of OD 0.300 higher than the different bipolar pulse widths.

According to Hülsheger et al. [8], bacterial cells in the stationary and lag growth are more resistant to PEF treatments than exponentially growing cells. The reason of this is because microbial growth in logarithmic phase is characterized by a high proportion of cells undergoing division, during which the cell membrane is more susceptible to the applied electric field.

Conclusion

Electric field strength significantly influenced growth of *Lactobacillus acidophilus* LA-K. Growth of the control LA-K and LA-K subjected to 5 kV/cm were higher than growth of LA-K subjected to 15 and 25 kV/cm. Bipolar pulse width and pulse period slowed log stage growth of *Lactobacillus acidophilus* LA-K. Slower growth of adjunct bacteria can sometimes be good in the manufacture of cultured dairy foods, as it results in controlled release of bacterial enzymes for improved flavor and texture development.

	Growth
Treatment	**LS Mean**
Control	0.808 ^A^
10,000 µs	0.733 ^B^
20,000 µs	0.772 ^AB^
30,000 µs	0.792 ^A^

LS Mean with same letter are not significantly different (*p*<0.05)

Table 3: Least square means for growth characteristics, bile tolerance and acid tolerance, as influenced by pulse period.

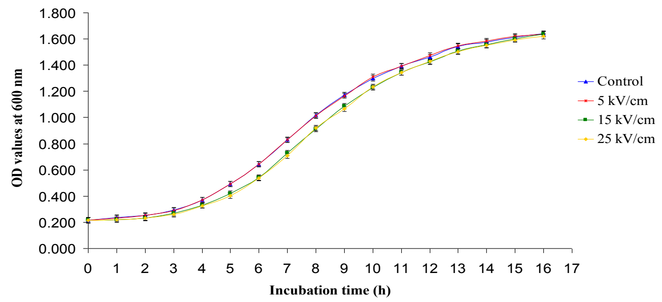

Figure 3: Electric Field Strength influence on LA-K.

Treatment parameter	Condition
Bipolar pulse width (µs)	3
Electric field strength (kV/cm)	5; 15; 25
Pulse period (µs)	30,000
Delay time (µs)	20
Flow rate (mL/min)	60

Table 4: Pulsed Electric Field (PEF) treatment conditions applied during the study of the influence of various electric field strengths on *Lactobacillus acidophilus* LA-K.

	Growth
Source	**MS Pr>F**
Voltage	0.044<0.0001
Hour	3.698<0.0001
Voltage*hour	0.001<0.2706
Error	0.001

Table 5: Mean Square (MS) and Pr>F of voltage, hour and their interaction for growth characteristics, bile tolerance and protease activity.

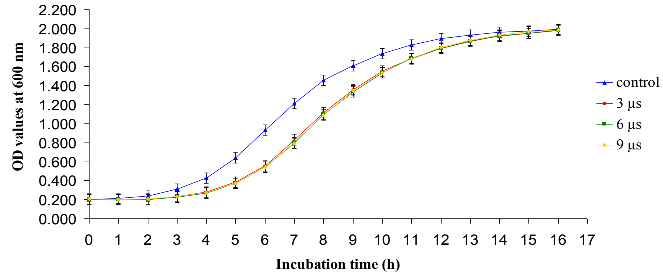

Figure 4: Pulse width influence on growth of LA-K.

	Growth
Treatment	**LS Mean**
Control	0.945 ^A^
5 kV/cm	0.946 ^A^
15 kV/cm	0.897 ^B^
25 kV/cm	0.892 ^B^

LS Mean with same letter are not significantly different (*p*<0.05)

Table 6: Least square means for growth characteristics, bile tolerance and acid tolerance, as influenced by voltage.

Treatment parameter	Condition
Bipolar pulse width (µs)	3,6,9
Electric field strength (kV/cm)	25
Pulse period (µs)	10,000
Delay time (µs)	20
Flow rate (mL/min)	60

Table 7: Pulsed Electric Field (PEF) treatment conditions applied during the study of the influence of various pulse widths on *Lactobacillus acidophilus* LA-K.

	Growth
Source	**MS Pr>F**
Pulse width	0.278<0.0001
Hour	6.384<0.0001
Pulse width*hour	0.015<0.0155
Error	0.009

Table 8: Mean Square (MS) and Pr>F of pulse width, hour and their interaction for growth characteristics, bile tolerance and protease activity.

	Growth
Treatment	**LS Mean**
Control	1.210 ^A^
3 µs	1.066 ^B^
6 µs	1.059 ^B^
9 µs	1.062 ^B^

LS Mean with same letter are not significantly different (*p*<0.05)

Table 9: Least square means for growth characteristics, bile tolerance and acid tolerance, as influenced by pulse width.

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