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Acid Tolerance of *Lactobacillus acidophilus* LA-K as Influenced by Various Pulsed Electric Field Conditions

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ABSTRACT

Pulsed electric field (PEF) processing involves the application of pulses of voltage for less than one second to fluid foods placed between two electrodes. *Lactobacillus acidophilus* is an important probiotic bacterium used for the production of fermented dairy products. Acid tolerance is an important probiotic characteristic. The influence of PEF on acid tolerance of *Lactobacillus acidophilus* is not known. Objective of this study was to elucidate the influence of certain PEF conditions on the acid tolerance of *Lactobacillus acidophilus* LA-K. Freshly thawed *Lactobacillus acidophilus* LA-K was suspended in sterile peptone 0.1% w/v distilled water and treated in a pilot plant PEF system. The treatments were pulse width (3, 6 and 9 µs), pulse period (10,000; 20,000 and 30,000 µs) and voltage (5, 15 and 25 kV/cm). Control was run through PEF system at 60 mL/min without receiving any pulsed electric field condition. Data were analyzed using the PROC GLM of the Statistical Analysis Systems (SAS). Differences of least square means were used to determine significant differences at $P < 0.05$. The control and the three different bipolar pulse widths studied were significantly different from each other. The acid tolerance of the control was significantly the highest, followed by the acid tolerance of the culture subjected to 3 µs and 6 µs. The acid tolerance of culture subjected to 9 µs was the lowest. The acid tolerance of the control was significantly the highest, followed by the acid tolerances subjected to the pulse period of 30,000 and 20,000 µs. The acid tolerance of culture subjected to pulse period 10,000 µs was significantly the lowest. The acid tolerance of the control was significantly the highest followed by the acid tolerances of culture subjected to electric field strength of 5 and 15 kV/cm. The acid tolerance of culture subjected to 25 kV/cm was significantly the lowest. Acid tolerance of *Lactobacillus acidophilus* LA-K lowered by increasing pulse widths and voltages but lowering pulse periods.

Keywords: *Lactobacillus acidophilus*; Pulsed Electric Field; Probiotic

1. Introduction

This High intensity pulsed electric field (PEF) processing involves the application of pulses of high voltage (typically 20 - 80 kV/cm) for short time periods (less than 1 second) to fluid foods places between two electrodes [1]. Application of PEF is restricted to foods products that can withstand high electric fields, have low electrical conductivity, and do not contain or form bubbles (e.g., liquid foods as milk or fruit juices) [2]. PEF technology is considered better than heat treatment of foods because it achieves high microbial inactivation, avoids or greatly reduces detrimental changes in the sensory and physical properties of food, and inactivates some enzymes [3]. Pulsed electric field is more energy efficient than thermal pasteurization. This nonthermal technology would add only \$0.03 - \$0.07 to final food costs [4]. Several theories have been proposed to explain microbial inactivation by PEF. The most commonly accepted theory is electroporation, which is the phenomenon in which the lipid bilayer and proteins of cell membrane exposed to high intensity electric field pulses are temporarily destabilized [5].

Lactobacillus acidophilus is a bacterium with several health benefits, including enhancement of immune system, reduction of various types of diarrhea in humans, alleviation of Crohn's disease, lower cholesterol, improve symptoms of lactose intolerance, and balancing of intestinal microflora through the growth modulation of bacteria present in the gastrointestinal tract [6]. *Lactobacillus acidophilus* is used extensively for the production of fermented dairy products and is increasingly applied in the area of health improvement, as probiotics, in the form of yogurts and dietary supplements.

Several PEF process factors namely electric field strength, pulse waveshape, treatment time and treatment temperature influence microbial inactivation [7]. Influence of PEF on acid tolerance of *Lactobacillus acidophilus* is not known. The objective was to study the influence of pulsed width, pulse period and kV on the acid tolerance *Lactobacillus acidophilus* LA-K.

2. Materials and Methods

2.1. Experimental Design

Control and Pulsed Electric Field (PEF) treatment samples were inoculated with *Lactobacillus acidophilus* LA-K (F-DVS LA-K, Chr. Hansen's Laboratory, Milwaukee, WI, USA). The treatments were pulse width (3, 6, and 9 µs), pulse period (10,000 µs, 20,000 µs, and 30,000 µs), electric field strength (5, 10, and 15 kV/cm). Control was run through the PEF equipment at 60 mL/min without receiving any pulsed electric field treatment. Acid tolerances were determined in the control and PEF treatment samples. Acid tolerance was evaluated at 0, 5, 10 and 15 minutes of incubation. The experimental design was a repeated measure design. Three replications were conducted.

2.2. Control and PEF Treatment Samples Preparation

Control and PEF treatment samples for the acid tolerance analyses 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). *Lactobacillus acidophilus* LA-K in control and PEF treatment samples for protease analysis was inoculated at 10% (v/v).

2.3. Acid Tolerance Test

The acid tolerance of *Lactobacillus acidophilus* LA-K was conducted as described earlier [8] with slight modifications. Control and PEF treated samples were inoculated (10% [v/v]) into acidified MRS broth (Criterion™, Hardy Diagnostics, Santa Maria, CA) previously adjusted to pH 2.0 with 1N HCl. The acidified MRS broth mixtures were incubated in a water bath at 37˚C for 15 minutes. One milliliter samples were taken at various times (0, 5, 10, and 15 min), serially 10-fold diluted in peptone water, and plated in duplicate onto MRS agar (Difco, Detroit, MI). The plates were incubated at 37˚C for 24 h under anaerobic condition before enumeration.

2.4. Statistical Analysis

Data were analyzed using the General Linear Model (PROC GLM) of the Statistical Analysis Systems (SAS). Differences of least square means were used to determine significant differences at P < 0.05 for main effects (pulse width, pulse period, voltage) and interaction effects (pulse width * time, pulse period * time, voltage * time). Data are presented as mean ± standard error of means. Significant differences were determined at $\alpha = 0.05$.

3. Results and Discussion

3.1. Pulse Width

The acid tolerance at different bipolar pulse widths over the four time points of 0, 5, 10 and 15 minutes are shown in **Figure 1**. Various pulse widths applied are shown in **Table 1**. Bipolar pulse width * minute interaction effect was significant (P < 0.0001) (**Table 2**). From minutes 0 to 15 there were significant differences between the control and the different bipolar pulse widths. At minute 0, among the three different bipolar pulse widths, the acid tolerance of culture subjected to bipolar pulse widths of 3 µs was significantly higher than 6 µs and 9 µs. At minute 5 the acid tolerances of culture subjected to bipolar pulse widths of 3 µs and 6 µs were significantly higher compared to 9 µs. The acid tolerance of culture subjected to 3 µs was significantly the highest at minute 10 followed by 6 µs and 9 µs consecutively. There were no significant differences among the three different bipolar pulse widths at 15 minutes. Bipolar pulse width effect had a significant (P < 0.0001) influence on the acid tolerance (**Table 2**). The control and the three different bipolar pulse widths studied were significantly different from each other (**Table 3**). The acid tolerance of the control

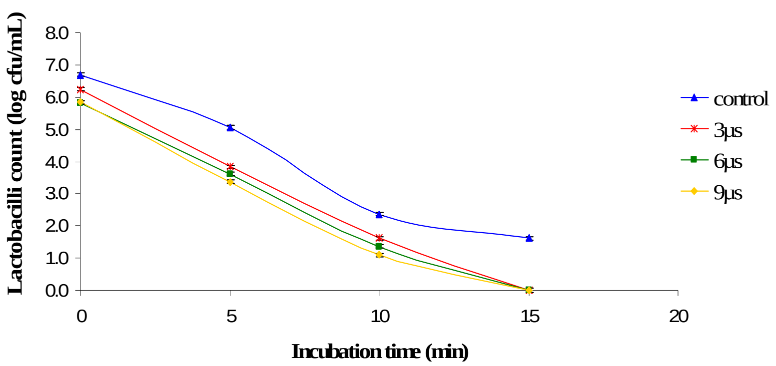

Figure 1. Pulse width influence on acid tolerance of LA-K.

Table 1. PEF treatment conditions applied during the study of the influence of various pulse widths on LA-K.

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 2. Mean square (MS) and Pr > F of pulse width, minute and their interaction for acid tolerance.

	Acid tolerance	
Source	MS	Pr > F
Pulse width	4.436	<0.0001
Minute	78.519	<0.0001
Pulse width* minute	0.169	<0.0001
Error	0.009	

Table 3. Least square means for acid tolerance as influenced by pulse width.

Acid tolerance	
Treatment	LS Mean
Control	3.933[A]
3 µs	2.924[B]
6 µs	2.754[C]
9 µs	2.575[D]

was significantly the highest, followed by the acid tolerance of culture subjected to 3 µs and 6 µs. The acid tolerance of culture subjected to 9 µs was the lowest (**Table 3**). The majority of studies have used exponential decay pulses when studying microbial inactivation by PEF. However, square wave pulses are more energy and lethally efficient as well as more accurate for calculation of treatment time at a given electric field strength [9]. The bipolar pulse widths applied in this study were square wave pulses. According to Qin *et al*., [10] bipolar pulse widths are more lethal than monopolar pulses because PEF causes a movement of charged molecules in the cell membranes of microorganisms, and reversal in the orientation or polarity of the electric field causes a corresponding change in the direction of charged molecules.

3.2. Pulse Width

The acid tolerance at different pulse periods over the four time points of 0, 5, 10 and 15 minutes are shown in **Figure 2**. Various pulse periods applied are shown in **Table 4**. Pulse period * minute interaction effect was significant ($P < 0.0001$) (**Table 5**). At minute 0 there was a significant difference between the control and 10,000 µs. From minutes 5 to 15 there were significant differences between the control and the different pulse periods. From minutes 0 to 15 there were no significant differences between the acid tolerance of culture subjected to 20,000 µs and 30,000 µs. From minutes 0 to 10 the acid tolerance of culture subjected to pulse periods of 10,000 µs was significantly lower than the acid tolerance of culture subjected to pulse periods of 30,000 µs. At minute 15 there were no significant differences among the three different pulse periods. Pulse period had a significant ($P < 0.0001$) influence on the acid tolerance (**Table 5**). The control and the three different pulse periods evaluated were significantly different from each other (**Table 6**). The acid tolerance of the control was significantly the highest, followed by the acid tolerances of culture subjected to 30,000 µs and 20,000 µs consecutively. The

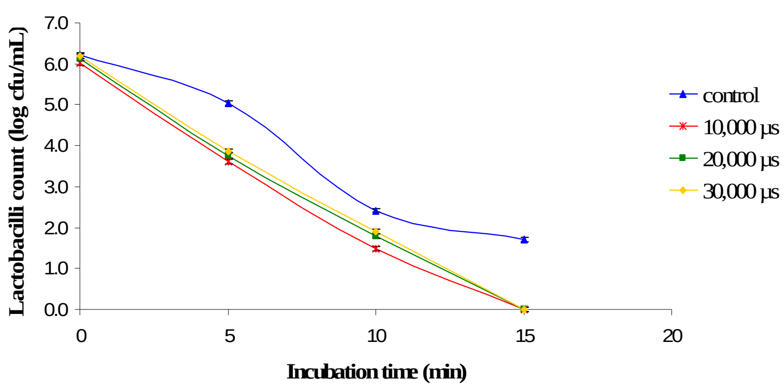

Figure 2. Pulse period effect on acid tolerance of LA-K.

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

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 5. Mean square (MS) and Pr > F of pulse period, minute and their interaction for acid tolerance.

	Acid tolerance	
Source	MS	Pr > F
Pulse period	2.827	<0.0001
Minute	75.104	<0.0001
Pulse width* minute	0.383	<0.0001
Error	0.007	

Table 6. Least square means for acid tolerance as influenced by pulse period.

Acid tolerance	
Treatment	LS Mean
Control	3.852^{A}
10,000 μs	2.782^{D}
20,000 μs	2.916^{C}
30,000 μs	2.993^{B}

acid tolerance of culture subjected to 10,000 μs was significantly the lowest. In this study *Lactobacillus acidophilus* LA-K subjected to any of the different pulse widths and pulse periods studied did not survive after 15 minutes at pH 2.0. In a study carried out by Pereira and Gibson [8] it was shown that the viability of *Lactobacillus pentosus* (B) and *Streptococcus thermophilus* DSM 20617 was lost in less than 15 minutes at pH 2.0. They also found that *Lactobacillus fermentum* KC5b, *Lactobacillus delbrueckii* JCM 1002, and *Lactobacillus acidophilus johnsonii* were the most acid tolerant strains by retaining around 100% viability for up to 2 hours at pH 2.0.

3.3. Electric Field Strength

The acid tolerance at different electric field strengths over the four time points of 0, 5, 10 and 15 minutes are shown in **Figure 3**. Various electric field strengths applied are shown in **Table 7**. Electric field strengths * minute interaction effect was significant (P < 0.0001) (**Table 8**). From minutes 0 to 10 the acid tolerance of the control along with the acid tolerance of culture subjected to 5 kV/cm were significantly higher than the acid tolerances of culture subjected to 15 kV/cm and 25 kV/cm. Moreover, at this same time interval, the acid tolerance of culture subjected at 25 kV/cm was significantly the lowest, followed by the acid tolerance of culture subjected to 15 kV/cm. At minute 15 the acid tolerance of the control was significantly the highest compared to the other electric field strengths, followed by the acid tolerance of culture subjected to 5 kV/cm. At this same minute there were no significant differences between the acid tolerances of culture subjected to 15 kV/cm and 25 kV/cm. Electric field strength had a significant (P < 0.0001) influence on the acid tolerance (**Table 8**). The control and the three different electric field strengths studied were significantly different from each other (**Table 9**). The acid tolerance of the control was significantly the highest followed by the acid tolerances of culture

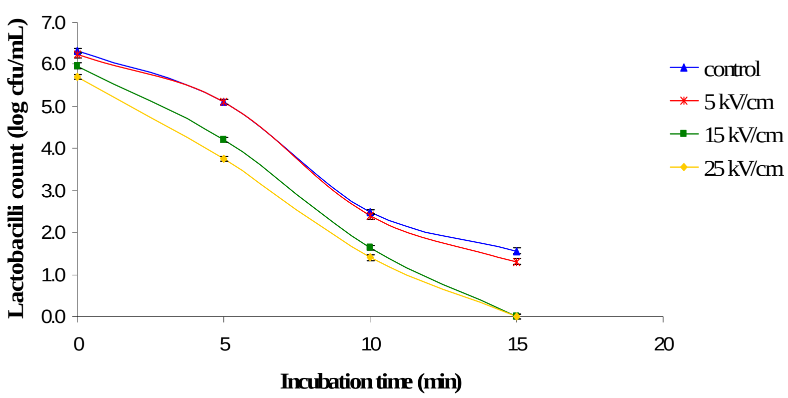

Figure 3. Electric field strength influence on acid tolerance of LA-K.

Table 7. Pulsed electric field (PEF) treatment conditions applied during the study of the influence of various electric field strengths on *Lactobacillus acidophilus* LA-K.

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 8. Mean square (MS) and Pr > F of electric field strength, minute and their interaction for acid tolerance.

	Acid tolerance	
Source	MS	Pr > F
Electric field strength	3.981	<0.0001
Minute	70.209	<0.0001
Electric field strength* minute	0.198	<0.0001
Error	0.012	

Table 9. Least square means for acid tolerance as influenced by voltage.

Acid tolerance	
Treatment	LS Mean
Control	3.871[A]
5 kV/cm	3.756[B]
15 kV/cm	2.952[C]
25 kV/cm	2.716[D]

subjected to 5 kV/cm. The acid tolerance of culture subjected to 25 kV/cm was the lowest followed by the acid tolerance of culture subjected to 15 kV/cm. Different lactobacilli strains were studied for their ability to grow on MRS broth at pH 2.0 [11]. In this study it was found that *L. acidophilus* ATCC 4962, *L. casei* ASCC 290 and *L. casei* ASCC 292 were the most acid tolerant strains with more than 10^7 cfu/mL after incubation for 2 hours at pH 2.0, while *L. casei* ASCC 1520, *L. casei* ASCC 1521, *L. casei* ASCC 279, *L. casei* 15820 and *L. casei* CSCC 2607 were the least acid tolerant with only 10^4 total cfu/mL after the 2 hours of incubation. They also found that strains of *L. acidophilus* showed greater acid tolerance over the entire incubation period, and their counts decreased by 2.66 to 4.38 log cycles, compared with 3.16 and 6.20 log cycles for *L. casei.*

4. Conclusion

Bipolar pulse width and pulse period significantly lowered acid tolerance. Voltage significantly influenced acid tolerance. Acid tolerance of the control LA-K was significantly higher than the acid tolerance of LA-K subjected to any of the three voltages studied. The highest the voltage applied resulted in the lowest the acid tolerance of LA-K. Acid tolerance of LA-K lowered by increasing pulse widths and voltages but lowering pulse periods.

REFERENCES

[1] G. V. Barbosa-Cánovas, M. M. Góngora Nieto, U. R. Pothakamury and B. G. Swanson, "Preservation of Foods with Pulsed Electric Fields," Academic Press, San Diego, 1999.

[2] M. L. Calderon-Miranda, G. V. Barbosa-Cánovas and B. G. Swanson, "Transmission Electron Microscopy of *Listeria innocua* Treated by Pulsed Electric Fields and Nisin in Skimmed Milk," International Journal of Food Microbiology, Vol. 51, No. 1, 1999, pp. 31-38. doi:10.1016/S0168-1605(99)00071-9

[3] D. W. Quass, "Pulsed Electric Field Processing in the Food Industry," A Status Report on PEF. Palo Alto, Electric Power Research Institute, Palo Alto, 1997.

[4] R. Ramaswamy, T. Jin, V. M. Balasubramaniam and H. Zhang, "Pulsed Electric Field Processing. Fact Sheet for Food Processors," Department of Food Science and Technology, The Ohio State University, Extension Service. 2008. http://ohioline.osu.edu/fse-fact/pdf/0002.pdf

[5] D. C. Chang, B. M. Chassy, J. A. Saunders and A. E. Sowers, "Guide to Electroporation and Electrofusion," Academic Press, San Diego, 1992.

[6] M. E. Sanders, "Considerations for Use of *Probiotic bacteria* to Modulate Human Health," *Journal of Nutrition*, Vol. 130, No. 2, 2000, pp. 384S-390S.

[7] B. L. Qin, G. V. Barbosa-Cánovas, B. G. Swanson and P. D. Pedrow, "Inactivating Microorganism Using a Pulsed Electric Field Continuous Treatment System," *IEEE Transactions on Industry Applications*, Vol. 34, No. 1, 1998, pp. 43-49. doi:10.1109/28.658715

[8] D. I. A. Pereira and G. R. Gibson, "Cholesterol Assimilation by Lactic Acid Bacteria and Bifidobacteria Isolated from the Human Gut," *Applied and Environmental Microbiology*, Vol. 68, No. 9, 2002, pp. 4689-4693. doi:10.1128/AEM.68.9.4689-4693.2002

[9] P. C. Wouters, I. Alvarez and J. Raso, "Critical Factors Determining Inactivation Kinetics by Pulsed Electric Food Processing," *Trends in Food Science and Technology*, Vol. 12, No. 3-4, 2001, pp. 112-121. doi:10.1016/S0924-2244(01)00067-X

[10] B. L. Qin, Q. Zhang and G. V. Barbosa-Cánovas, "Inac-

tivation of Microorganisms by Pulsed Electric Fields of Different Voltage Waveforms," *IEEE Transactions on Dielectrics and Electrical Insulation*, Vol. 6, No. 1, 1994, pp. 1047-1057. doi:10.1109/94.368658

[11] M. T. Liong and N. P. Shah, "Acid and Bile Tolerance and Cholesterol Removal Ability of *Lactobacilli strains*," *Journal of Dairy Science*, Vol. 88, No. 1, 2005, pp. 55-56. doi:10.3168/jds.S0022-0302(05)72662-X