Inactivation of *Listeria monocytogenes* in Recirculated Brine for Chilling Thermally Processed Bacon Using an Electrochemical Treatment System

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ABSTRACT: An electrochemical treatment system consisting of a pulsed electrical power supply and an electrical treatment chamber was designed and evaluated for inactivation *Listeria monocytogenes* in recirculated brine for chilling processed bacons. The brine was tested under different currents and temperatures. An average D-value of 1.61 min in the storage tank could be achieved at 7 mA/cm^3 current with the fresh brine (t = 0 h). For the spent brine (t = 20 h), the D-value was 2.5 min in the treatment chamber at 35 mA/cm^3. The average D-values in the treatment chamber were approximately 2.5 min at all three temperatures (4, 0, –8 8C) at 35 mA/cm^3.

Key words: *Listeria monocytogenes*, brine, electrochemical treatment

Introduction

Listeria, ALONG WITH *Salmonella* AND TOXOPLASMA, ARE responsible for 1500 deaths each year (Mead and others 1999). Since the early 1980s, food transmission has been recognized as a major source of human listerosis (Schuchat and others 1991). This pathogen poses a serious threat to public health and the economy. The latest multistate outbreak of listeriosis, which claimed 21 lives, caused 30 million lbs of hot dogs to be recalled and cost the industry over $80 million (CDC 1999; Kuhn 1999), further illustrates the seriousness of this problem. Bacterial recontamination of thermally processed or cooked poultry and meat products is directly linked to the safety of ready-to-eat food products. In many cases, bacteria may survive, grow, and spread in chilling brine in which these foods are processed. The USDA regulations require that brines used to chill processed meat products must be discarded after a specific time (USDA, 2000). Extending the life of brine could dramatically reduce the usage of salt and water as well as the costs associated with waste brine discharge. This presents a challenge for food processors to effectively control *L. monocytogenes* in brine while reducing the cost of the chilling process.

Several methods have been investigated for their applications in *Listeria* intervention, such as organic acids (Podolak and others 1996; Zeitoun and Debevere 1991), trisodium phosphate (Deledesma and others 1996; Somers and others 1994), nitrite (Schlyter and others 1993), microfiltration (Hart and others 1988), and high voltage pulsed electrical fields (Qin and others 1995).

Low-voltage electricity has been applied to saline solution to generate electrochemically activated (ECA) solution (Li and others 1995; Yang and others 1999) or electrolyzed oxidizing (EO) solution (Venkitanarayanan and others 1999a) to control or eliminate pathogens like *Salmonella*, *Listeria*, *Campylobacter* or *E. coli*. Unlike pulsed electric field which involves very high voltage electricity, the electricity used in low voltage treatments is usually < 100 V. This low voltage is much safer in 9 to 14% salt content brine. Low voltage can minimize or eliminate the generation of unwanted, sometimes harmful byproducts that can be a problem for high voltage pulsed electric field (Lubicki and Jayaram, 1997). Low voltage electricity is also more economic due to low equipment cost and low power consumption.

However, no reports have been found to show the effectiveness of low-voltage electrical method in controlling *L. monocytogenes* in brine. The objectives of this research were: to design and evaluate a laboratory-scale electrochemical treatment system on inactivation of *L. monocytogenes* in recirculated brine, to determine the effects of current level and temperature of brine on the destruction of *L. monocytogenes*, and to determine the generation of free chlorine, pH and conductivity changes with different treatments. These objectives would provide the information for adopting this technique in the food processing industry as well as for designing a large-scale system that could be used in a processing plant.

Materials and Methods

Brine

Brine was obtained from a food processing plant before being used for chilling (the fresh or the t = 0 h brine) and after 20 h of operation (the spent or the t = 20 h brine). The brine was used to chill bacons at 8 °C with a salt concentration approximately 14%. The samples were stored at –12 ± 1 °C until use.

Bacterial Culture

Bacterial culture, *Listeria monocytogenes* (101M, sera type 4B), from Food and Drug Administration (FDA) was confirmed by Oxford agar and FDA identification procedures (Hitchins 1998). A fresh bacteria culture revived from cryogenic vial was centrifuged with a VSMC-13 mini-centrifuge (Shelton Scientific, Shelton, Conn,, U.S.A.) at 12,000 g and resuspended in phosphate buffer saline (PBS, 0.05 M, pH 7.4)

to approximately 10^9 CFU/ml.

Electrochemical Treatment System

A lab-scale electrochemical treatment system was constructed in the laboratory (Figure 1). Brine was pumped through the treatment chamber and back to the Nalgene 8-L storage tank using a Little Giant Model 1 submersible pump (Little Giant Pump Co., Oklahoma City, Okla., U.S.A.). Temperature of the brine in the storage tank was controlled using a PolyScience Heated/Refrigerated Circulator (Model 07690, PolyScience, Niles, Ill., U.S.A.). The temperature of the brine was monitored with a microprocessor thermometer (Model HH23, Omega Engineering, Inc., Stamford, Conn., U.S.A.). A Lab Stirrer (Model LR 400C, Yamato Scientific Co., Ltd. Tokyo, Japan) set at 1,000 rpm was used to homogenize the brine in the storage tank.

The treatment chamber (concentric cylinder) was made of a stainless steel cylinder, 2.54 cm in dia and 30.5 cm in length, which also served as the cathode. A platinum wire, 99.9% purity (Omega Engineering, Inc., Stamford, Conn., U.S.A.), 0.8 mm in dia and 35 cm in length, was placed at the center of the treatment cylinder and served as the anode. Two rubber stoppers were placed at each end of the chamber to seal the treatment chamber. The effective volume of the treatment chamber was 141.9 cm^3. A power supply (Model ATE 150-7DM, Kepco, Inc., Flushing, N.Y., U.S.A.) that can provide a maximum voltage of 150 V and a maximum current of 10 A was used. A 100 Hz, 50% duty cycle pulsed square wave was generated by triggering the power supply with a function generator constructed in the laboratory. The function generator could produce pulsed square wave signals at 100 Hz with 3 constant current levels: 1, 2.5, and 5 A, corresponding to 7, 17.5, and 35 mA/cm^3. The output of the generator was monitored by a digital oscilloscope (Model TDS210, Tektronix, Inc., Beverton, Ore., U.S.A.).

Electrochemical Treatment Procedures

Six liters of brine were placed into the storage tank and inoculated with *L. monocytogenes* to an initial population of approximately 10^6 CFU/ml. The brine was pumped through the treatment chamber at a flow rate of 0.95 L/min with a residence time of approximately 10 sec. Current level and brine temperature were investigated for their effects on inactivation of *L. monocytogenes* in the brine. Three current levels, 7, 17.5, and 35 mA/cm^3, were tested with –8 °C brine. At 35 mA/cm^3 current level, 3 brine temperatures (4, 0, and –8 °C) were also studied. The total treatment time was 60 min and 35 ml of brine was taken at 0, 5, 10, 15, 20, 25, 30, 45, and 60 min from the storage tank and the outflow of the treatment chamber.

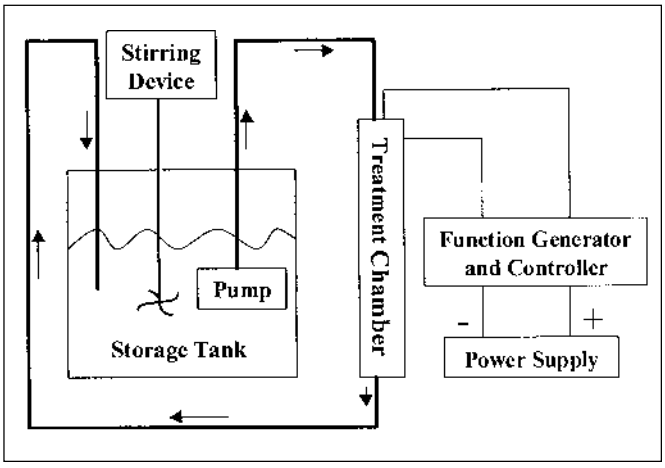

Figure 1—Schematic diagram of electrochemical treatment system for recirculated brine

Sample Analysis

The pH and conductivity of all samples were measured using an Accumet® pH/Ion/Conductivity meter (Model 50, Fisher Scientific, Pittsburgh, Pa., U.S.A.). The absorbence at 500 nm was measured by a Spectronic 20 Genesys spectrophotometer (Spectronic Instruments, Inc., Rochester, N.Y., U.S.A.). Free chlorine levels were determined by an Ion Specific Meter (Model HI 93711, Hanna Instruments, Woonsocket, R.I.,U.S.A.).

Sample brine was serially (1:10) diluted with PBS (0.05 M, pH 7.4), and 0.1 ml of each dilution was plated onto Oxford agar (Difco Laboratories, Detroit, Mich., U.S.A.). *Listeria monocytogenes* colonies were counted after incubating at 37 °C for 72 h.

D-value is the time needed in min to achieve a 1-log reduction of *L. monocytogenes* under the electrochemical treatment at a given current level. Since this was a dynamic system, not all data showed linear relationships between the number of *L. monocytogenes* and treatment time on a semi-log plot. Therefore, average D-values were determined based on two points, *Listeria* numbers at the t = 0 and t = 60 min or using the time when the 6 log reduction of *Listeria monocytogenes* occurred to compare the effectiveness of different treatments.

Results and Discussion

Effect of Electric Current

Both the fresh and the spent brine were tested in the system. The average D-values of *L. monocytogenes* for the fresh brine were ≤ 0.79 min in the storage tank and ≤ 0.03 min in the treatment chamber at all current and temperature combinations except current level 7 mA/cm^3 at –8 °C (Table 1). With current level 7 mA/cm^3 at –8 °C, the average D-value was 1.61 min in the storage tank, and 0.81 min in the treatment chamber. Temperature made almost no difference on killing *L. monocytogenes* in the fresh brine at a current level of 35 mA/cm^3.

Inactivation of *L. monocytogenes* was much slower in the spent brine under the same treatments. The inactivation of *L. monocytogenes* at –8 °C for the spent brine with a current level of 35 mA/cm^3 was much faster than 7 and 17.5 mA/cm^3 (Figure 2). An average D-value of 2.5 min was obtained in the treatment chamber at a current level of 35 mA/cm^3 (Table 1). However, D-values increased to 9.4 and 61.2 min at current levels of 17.5 and 7 mA/cm^3. The reduction of *L. monocytogenes* in the storage tank showed a similar trend but lagged behind the treatment chamber at corresponding current levels. The average D-value was 5.0 min at a current level of 35 mA/cm^3, while the average D-values increased to 11.8 and 77.9 min at the current levels of 17.5 and 7 mA/cm^3, respectively. These results clearly indicated that higher current levels had a greater killing effect on the *L. monocytogenes* population in the brine.

Free chlorine was found by many researchers to be a key antibacterial mechanism in electrolyzed solution (Ma and others 2000; Venkitanarayanan and others 1999a). In the brine, the chlorine generation occurred at the anode (plati-

Table 1-Electrochemical inactivation of *L. monocytogenes* in the fresh and the spent brine under different treatments

	Average D-value (min)									
	35 mA/cm³ at 4 °C		35 mA/cm³ at 0 °C		35 mA/cm³ at –8 °C		17.5 mA/cm³ at –8 °C		7 mA/cm³ at –8 °C	
	fresh	spent	fresh	spent	fresh	spent	fresh	spent	fresh	spent
Storage Tank	0.81	3.3	0.80	4.3	0.80	5.0	0.79	11.8	1.61	77.9
Treatment Chamber	0.03	2.5	0.03	2.5	0.03	2.5	0.03	9.4	0.81	61.2

num wire) when the electrical current was passing through the solution. Platinum wire was chosen as the anode because it is resistant to alkali hydroxides and acids which could be produced during the electrolysis of chiller brine (Cotton and Wilkinson 1972). Since high concentration of free chlorine normally generated at the anode (Ma and others 2000), the high resistance of platinum wire to oxidation and acids would prevent corrosion on the electrode and generation of metallic by-products. According to Faraday's Law, it was expected that higher currents would result in higher chlorine concentration. The results clearly showed that higher current level resulted in higher free chlorine concentrations in the storage tank and in the treatment chamber with both the fresh and the spent brine (Figure 3). Free chlorine in the storage tank for the fresh brine was 800 ppm for current level 35 mA/cm³, 266 ppm for 17.5 mA/cm³, and 59.5 ppm for 7 mA/cm³ after 60 min of treatment. But for the spent brine, the free chlorine concentrations were much lower, 12.10 ppm for 35 mA/cm³, 1.93 ppm for 17.5 mA/cm³, and only 0.90 ppm for 7 mA/cm³ after 60 min. Organic compounds in the spent brine could have dramatically decreased the solubility of Cl_2 or directly reacted with free chlorine in the solution, therefore decreased the free chlorine concentration in the brine. Chlorine concentrations showed similar trends in the treatment chamber for the fresh and the spent brine, but were consistently higher than that in the storage tank under all treatments. This could be the main reason for the faster killing rate in the treatment chamber.

Effect of Brine Temperature

The effects of brine temperature on the inactivation of *L. monocytogenes* as well as free chlorine generation were also studied. As mentioned before, temperature made no difference on killing *L. monocytogenes* at a current level of 35 mA/cm³ in the fresh brine. But as shown in Figure 4, temperature did have an impact on the inactivation of *L .monocytogenes* in the spent brine. The average D-values in the treatment chamber were approximately 2.5 min at all 3 temperatures (4, 0, and –8 °C) with a current level of 35 mA/cm³ (Table 1). However, in the storage tank the D-value was higher than that in the treatment chamber and depended on the temperature of the brine, 3.3 min for 4 °C, 4.3 min for 0 °C, and 5.0

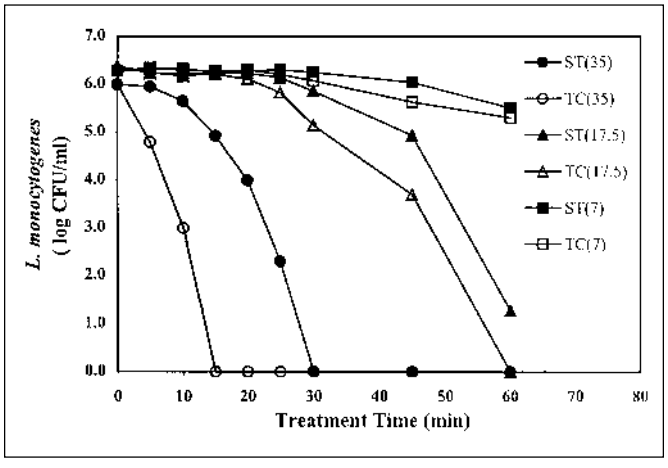

Figure 2—Effect of current levels on inactivation of *L. monocytogenes* in recirculated spent brine with electrochemical treatment system at –8 °C. Symbols: ST(35), storage tank at 35 mA/cm³; TC(35), treatment chamber at 35 mA/cm³; ST(17.5), storage tank at 17.5 mA/cm³; TC(17.5), treatment chamber at 17.5 mA/cm³; ST(7), storage tank at 7 mA/cm³; TC(7), treatment chamber at 7 mA/cm³.

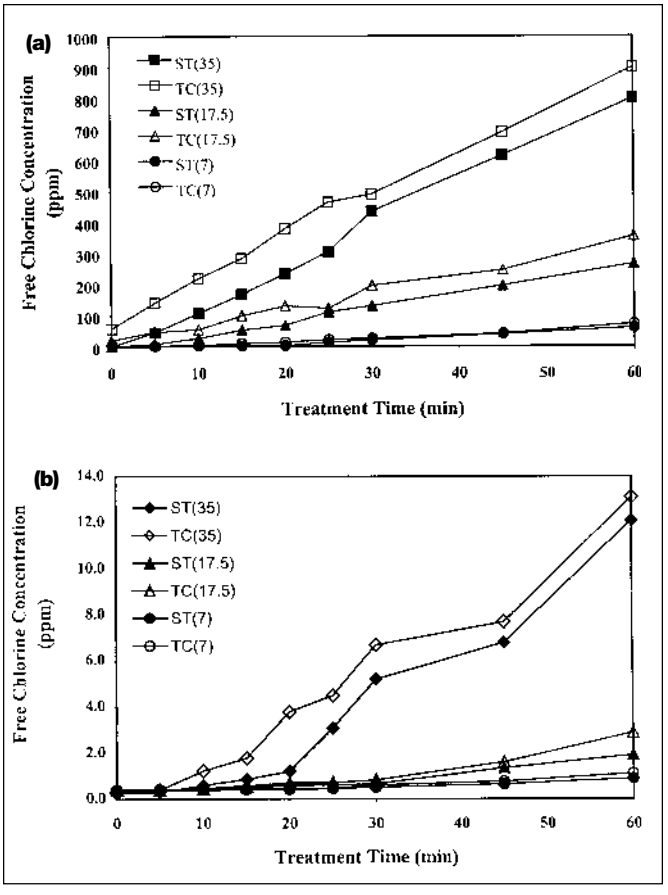

Figure 3—Generation of free chlorine in the electrochemical treatment of brine at different current levels with brine temperature of –8 °C: A. the fresh brine; B. the spent brine. Symbols: ST(35), storage tank at 35 mA/cm³; TC(35), treatment chamber at 35 mA/cm³; ST(17.5), storage tank at 17.5 mA/cm³; TC(17.5), treatment chamber at 17.5 mA/cm³; ST(7), storage tank at 7 mA/cm³; TC(7), treatment chamber at 7 mA/cm³.

Table 2—pH, conductivity, and absorbence at 500nm (Ab_{500}) of brine after 60 min of electrochemical treatment with different current-temperature combinations

		Fresh Brine						Spent Brine					
Current	Temperature	pH		Conductivity (mS/cm)		Ab_{500}		pH		Conductivity (mS/cm)		Ab_{500}	
(mA/cm3)	(°C)	0 m	60 m	0m	60 m	0 m	60 m	0 m	60 m	0 m	60 m	0 m	60 m
35	4	7.10	8.63	126.4	127.3	0.003	0.005	50.9	5.13	114.6	110.7	0.277	0.465
	0	7.13	8.40	127.4	128.8	0.002	0.003	5.20	5.51	115.6	115.8	0.273	0.574
	–8	7.54	8.34	127.6	127.8	0.000	0.001	5.09	5.24	136.4	136.0	0.268	0.621
17.5	–8	7.33	8.05	127.7	127.9	0.004	0.004	5.11	5.26	116.6	123.3	0.259	0.405
7	–8	7.27	7.86	126.4	126.6	0.002	0.003	5.12	5.23	128.5	125.4	0.321	0.404

min for –8 °C.

Even though free chlorine concentrations increased with time in the storage tank and treatment chamber, there was no apparent correlation between free chlorine concentration and brine temperature. This indicated that the current level was the major factor on the generation of free chlorine and inactivation of *L. monocytogenes* during the electrochemical treatment. All chemical reaction rates were temperature dependent, generally the lower the temperature the slower the reaction rate. Since the main killing effect was the oxidation-reduction reaction between the free chlorine and bacteria cell membrane (Venkitanarayanan and others 1999a; Len and others 2000), the differences in inactivation rates showed in the storage tank among the 3 temperatures for the spent brine could simply be due to a slower killing rate at a lower temperature. In the treatment chamber, however, the free chlorine concentration could be high enough to kill all the bacteria cells passing though the chamber after certain treatment time (15 min in this case) regardless of the temperature.

Change in Brine Properties

The changes in pH, conductivity, and 500 nm absorbance of the brine were presented in Table 2. The pH only increased slightly with 60 min of treatment at all current-temperature combinations for the spent brine, but a much bigger increase of pH was observed for the fresh brine. The change in pH ranged from 0.04 to 0.31 units with an initial pH at about 5.0 for the spent brine and 0.59 to 1.53 units with an initial pH at about 7.0 for the fresh brine. Contacting with food products during chilling has introduced food residues into the spent brine which could have helped decrease the overall pH and made the brine more resistant to pH change. For the fresh brine, the higher the electrical current or temperature, the greater the pH increase. But there was no clear trend for the spent brine.

During the electrochemical treatment, chlorine (hypochlorous acid) was generated at the anode while hydrogen and sodium hydroxide were produced at the cathode. The pH at the anode was reported to be around 2 and at the cathode about 12 in poultry chiller solution with 1% sodium chloride added (Ma and others 2000). Even though the brine from these 2 electrodes mixed quickly in the treatment system, the pH in the closer surrounding to the electrodes would still be low at the anode and high at the cathode. Reports (Leyer and Johnson 1997; Venkitanarayanan and others 1999b) indicated that low pH may sensitize the outer membrane of bacteria cell to facilitate the entry of hypochlorous acid, therefore enhance the germicidal effect of free chlorine. The optimum pH for *Listeria* growth was generally in the range of 6 to 8 with pH below 4.1 and above 9.6 being bactericidal (Jay 1992). Therefore, it may be speculated that extreme pH values at the anode and the cathode alone could also contribute to the inactivation of *Listeria*.

The conductivity of the brine basically remained unchanged during the electrochemical treatment with an average change of only 2.86 mS/cm for the spent brine and 0.58 mS/cm for the fresh brine (Table 2). A high concentration of sodium chloride (14%) in the brine minimized any change in conductivity due to the electrochemical treatment.

There was no change in absorbance at 500 nm of the fresh brine after 60 min electrical treatment as shown in Table 2. However, the color of the spent brine was darker after 60 min of treatment as demonstrated by the absorbance increase at 500 nm for all treatments. Complex organic compounds in the brine could be oxidized by hypochlorous acid and changed the absorbance. It has been reported that organic compounds could potentially decrease the effectiveness of hypochlorous acid in inactivating bacteria (Kotula and others 1997). It was the consumption of hypochlorous acid through these reactions in the brine that could decrease the effectiveness of free chlorine in destroying bacteria. Apparently, higher current levels were causing faster absorbance change in the solution because of their high rate of free chlorine generation. With current level increasing from 7 to 35 mA/cm^3, absorbance at 500 nm increased from 0.083 to 0.353 after 60 min of treatment at –8 °C. The changes of absorbance (35 mA/cm^3 current level) at 3 different temperatures (4, 0, –8 °C) were ranging from 0.188 to 0.353 after 60 min of treatment, there was no apparent trend can be observed.

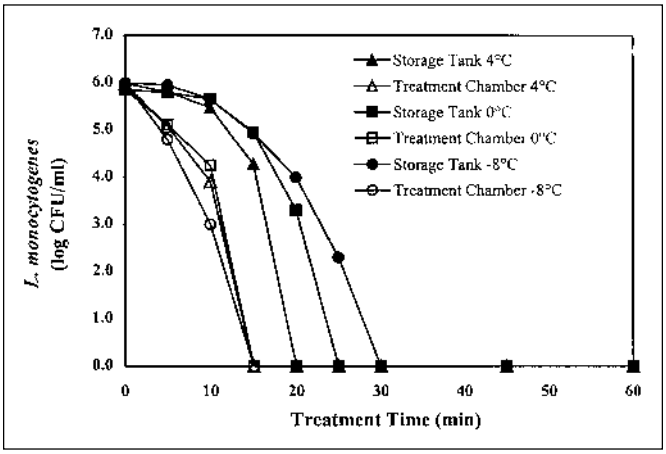

Figure 4—Effect of brine temperature on inactivation of *L. monocytogenes* in the electrochemical treatment of the spent brine at a current level of 35 mA/cm^3

Conclusions

THIS UNIQUE DESIGN OF THE LAB-SCALE ELECTROCHEMICAL treatment system allows a safe, continuous inline treatment of the brine. *L. monocytogenes* could be effectively controlled or eliminated without interrupting the chilling operation. When compared with an offline alternative, the treatment system would also make the implementation simple, without extra brine, storage tanks, or pumping equipment. The treatment system proved to be a very effective way to inactivate *L. monocytogenes* in brine. This system took full advantage of the pulsed electrical treatment and the NaCl-containing brine. Two important features of the system are no additional NaCl needs to be added and the separation of ECA from the 2 electrodes is not required. The ?fresh? free chlorine generated around the anode could be immediately used to inactivate *L. monocytogenes* in the brine. Therefore, the electrochemical treatment system can be used as a simple yet effective inline treatment method to inactivate *L. monocytogenes* in brine.

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