Inactivation of *Listeria* in Recirculated Chilling Brine Using Flow-Through Electrolyzing Treatment

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Abstract. Listeria *contamination of cooked meat and poultry products during chilling process presents a food safety problem and the food industry needs effective method to inactive* Listeria *in chilling brine. The objectives of this research were to design and construct a pilot-plant-scale electrolyzing treatment chamber, to evaluate the antibacterial efficacy of the electrolyzing system, and to determine the effect of electrolyzing treatment on the physical and chemical properties of chilling brine. 150 liters of used bacon brine was innoculoated with* Listeria innocua *and recirculated continuously for 4 d at –4°C within a portable brine chiller. 3 A DC powered electrolyzing treatment system was connected into the loop of the brine recirculation. Listeria was reduced by 6 log CFU/ml in 12 h. As the electrolyzing treatment time increased, the concentration of free chlorine, total chlorine and ORP in the brine increased, but the pH and salt concentration of the brine kept unchanged. ORP linearly increased first and then become stable at 640 mV. The color of bacon brine become darker at the beginning and then turned to clear, possibly due to the reaction between some organic materials and chlorine. The results of the pilot-plant-scale experiments showed that the electrolyzing treatment system was very effective to inactive Listeria and aerobic microorganisms in recirculated chilling brine. This technology can provide the food processing industry a more effective method to control Listeria in chilling brine for ensuring food safety.*

Keywords. Brine chiller, *Listeria*, Foodborne pathogen, Food safety, Electrolyzing treatment

Introduction

Listeriosis is an infection caused by *Listeria monocytogenes*. Even though most people do not suffer clinical symptoms from listeriosis, pregnant women and their neonates, elderly people and immunocompromised individuals are considered to be at the highest risk. The Center for Disease Control and Prevention (CDC) estimated that up to 2,500 cases of listeriosis, resulting in 500 deaths, occur annually in the United States (Mead et al., 1999).

Almost all listeriosis is foodborne. One survey of *L. monocytogenes* shows that the overall prevalence in ready-to-eat foods collected from retail markets at Maryland and northern California was 1.82% (Gombas et al., 2003). *L. monocytogenes* is considered ubiquitous in environment and has been isolated from a wide variety of foods, including dairy products, meat and poultry products, vegetables, seafood and other products.

Meat and poultry products, such as hot dog and bacon, need a cooling process after they are cooked or smoked. Because brine has a much lower freezing point than water, it is widely used by the food industry as a cooling medium. *L. monocytogenes* can survive in a variety of hostile environmental conditions, such as particularly elevated osmolarity (10% NaCl) (McClure et al., 1989) and reduced temperature (-0.1°C) or freezing (Walker et al., 1990). Cooked poultry and meat products may get microbial recontamination during chilling, therefore, there is a potential food safety hazard during chilling process.

The USDA Food Safety and Inspection Service (FSIS) recommends that "brine solution that is reused to chill raw or heat-treated, raw but not fully cooked product (e.g., smoked bacon) should be reconditioned in a manner to prevent the brine solution from becoming contaminated and adulterating the product" in its Sanitation Performance Standards Compliance Guide (USDA, 2003). When the brine solution is used without reconditioning for one shift or longer, the FSIS further recommends that the solution should be discarded at the following specified intervals: 24 h for brine solution with minimum 5% salt and maintained at 4°C or lower; 1 week for brine solution with minimum 9% salt and maintained at –2°C or lower; and 4 weeks for brine solution with minimum 20% salt and maintained at –12°C or lower. In addition, FSIS also recommends maintaining a free chlorine concentration of 1-5 ppm in the reused brine solution (USDA, 2003).

Many food processors use chlorine or other chemicals to treat the chilling brine. However levels of dissolved and suspended solids in the solution exhibit high chemical demand. Additionally, the chemical residuals and disinfection by-products are another two shortages of the chemical treatment. Ultrafiltration and ultraviolet (UV) are alternatives for the food processors to treat their chilling brine. Ultrafiltration is effective to eliminate the microbes in the brine, but only applicable for brine solutions with low solid concentration. In a typical brine chill loop, a fixed quantity of fluid is continuously recirculated over the heat-treated meat product, collected in a small tank, rechilled and then returned to the contact area for reuse. Because the ultrafiltration speed is relatively slow, the ultrafilter cannot be used in the recirculating loop. UV light is effective for clear water and is easy to mount into the chilling brine circulating loop, but UV light cannot penetrate a solution with high turbidity, high concentration salt or heavy solid content.

Electrolyzed water (EW) or electrochemically activated solution (ECA) generated by electrolysis of salt solution (0.1%-15% NaCl) has been studied to inactivate pathogenic bacteria on vegetables (Izumi, 1999; Bari et al., 2003; Yang et al., 2003), chicken carcasses (Li et al., 1995; Yang et al., 1999), and other different solid surfaces (Venkitanarayanan et al., 1999; Park, 2002). EW includes anode water and cathode water, which are generated near the anode and cathode (separated by a diaphragm), respectively (Yang et al., 1999; Yamanaka et al., 1999; Bari et al., 2003; Kim et al., 2000). The antibacterial effect of EW mainly depends on the anode solution. Because the anode solution normally has a low pH (< 2.7) and high oxidation reduction

potential (ORP) (>1100 mV), the EW also is named as electrolyzed acidic water or electrolyzed oxidizing water.

Ye et al. (2001) developed a lab scale flow-through electrolyzing chamber to inactivate *L. monocytogenes* in recirculated chilling brine. An average D value of 2.5 min was obtained on 6 L of 20 h used bacon brine with 4 A power supply. The development and evaluation of a large scale flow-through electrolyzing treatment system is needed for industrial applications. Therefore, the objectives of this research were (1) to design and construct a pilot-plant-scale electrolyzing treatment chamber, (2) to evaluate the antibacterial efficacy of the electrolyzing system for used bacon chilling brine, and (3) to determine the effect of electrolyzing treatment on the physical and chemical properties of the chilling brine.

Materials and Methods

Brine

Used bacon brine (after 48 h of chilling recirculation) was obtained from a food processing plant. The concentration of NaCl and total suspended solid in the brine solution was 9.5% and 0.5%, respectively. The brine solution was stored in a 4°C cooler until use.

Culture preparation and inoculation

Listeria innocua (ATCC 33090) was obtained from USDA ARS Research Laboratory (Fayetteville, AR). *L. innocua* was stored at 4°C in brain heart infusion (BHI)(Difco, Detroit, MI), then was subcultured twice in BHI at 37°C for 24 h to obtain a bacterial culture with a cell number of 10^8 CFU/ml before being used.

Electrolyzing treatment system

A pilot-plant-scale electrolyzing treatment chamber was designed and constructed (Fig. 1). Seven parallel titanium plates (7 cm × 30.5 cm) were placed in a plastic chamber to serve as anodes and cathodes. The distance between each two plates was 64 mm. The chamber was connected into the recirculation loop of a portable brine chiller (ALKAR, Lodi, WI) that consisted of a product chiller module and a heat exchanger module (Fig. 2). After being filtered, the brine was first pumped from the collection tank of the chiller module, passed through the electrolyzing chamber, then was chilled in the heat exchanger and sprayed in the chiller. Finally the brine was cumulated into the collection tank and thus a recirculation loop ended. The recirculation rate of the brine was adjustable in a range of 0-189 L/min. A power supply (Model ATE 150-7DM, Kepco, Inc., Flushing, NY) that can provide a maximum voltage of 150 V and a maximum direct current of 10 A was connected to the electrolyzing chamber(s). The brine temperature in the collection tank was monitored using a data logger-computer system (Model 34907A, Agilent Technologies, Inc., Palo Alto, CA).

Electrolyzing treatment

The electrolyzing treatment system was set up in the Poultry Processing Pilot Plant at University of Arkansas. 150 L of brine was used in the chiller, recirculating through the electrolyzing treatment system at a rate of 151 L/min. When the brine temperature was reduced to and kept at –4 ± 1° C, the brine was directly inoculated with *L. innocua* at a level of 4.57 log CFU/ml. After 5 min of recirculation, 4 L of inoculated brine was transferred from the chiller collection tank into a sterilized 6-L plastic bottle with a lid to serve as control sample. The bottle was placed in the

collection tank and immersed in the chilled brine. Then, the electrolyzing chamber was powered with a 3 A DC to start the electrolyzing process.

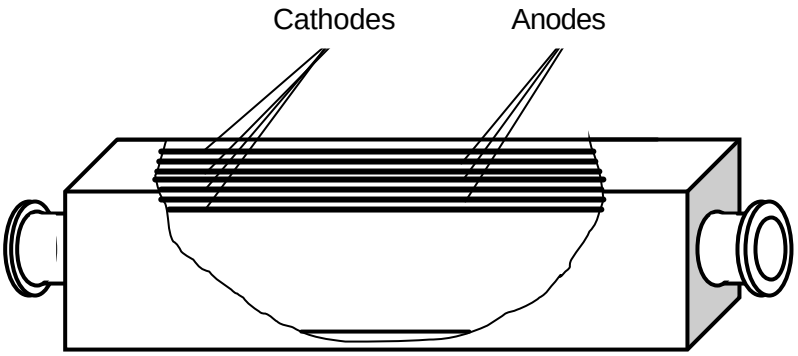

Figure 1 The structure of electrolyzing treatment chamber. Anodes and cathodes were interleaved titanium plates (7 cm × 30.5 cm) closely spaced (64 mm) in a plastic chamber.

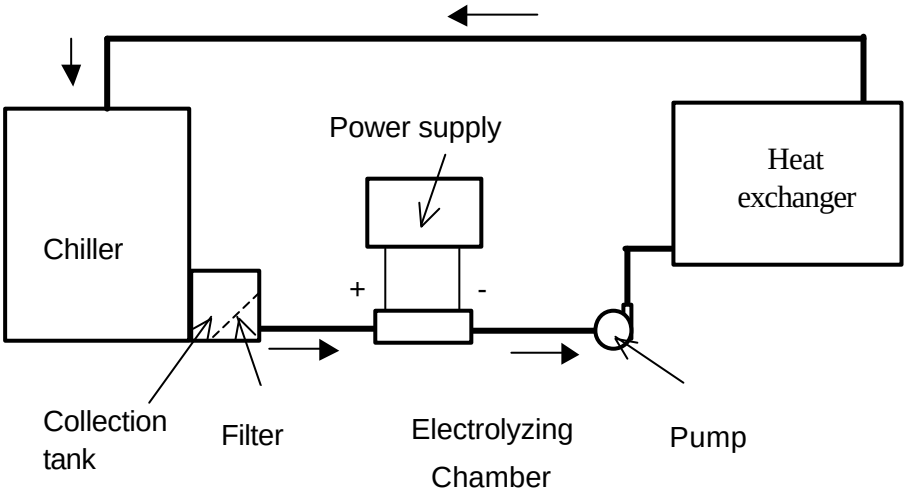

Figure 2 The schematic diagram of the pilot-plant-scale electrolyzing treatment system. Electrolyzing chamber was connected into the brine recirculation loop of an ALKAR portable brine chiller.

Experimental design

Test 1: one electrolyzing chamber was used. No *L. innocua* was inoculated and the chiller system was running continuously for 6 d. Samples were taken at 0, 9, 23, 38, 49, 62, 72, 85, 99, 107, 124, and 148 h and only total aerobic microorganisms were tested.

Test 2: two chambers were used in series. *L. innocua* was inoculated and the chiller system was running continuously for 4 d. Samples were taken at 0, 12, 23, 43, 70, and 94 h and both total aerobic microorganisms and *L. innocua* were examined.

Sampling and brine property measurement

At each scheduled sampling time, 6 15-ml-brine samples were taken from the collection tank, and 6 15-ml-brine control samples were taken from the control bottle. One brine sample was used to measure pH, ORP (Oxidation Reduction Potential), and free and total chlorine concentrations using a pH meter (Model 250A, Orion Research, Inc., Boston, MA), ORP meter (Model SP21, Thermo Orion, Beverly, MA), and chlorine meter (Model HI93711, Hanna Instruments, Woonsocket, RI). Other 5 samples were sent to the research laboratory for microbial test.

Salt concentration of the samples was measured by titrating the brine with silver nitrate solution ($AgNO_3$) in triplicate and using sodium chromate ($Na_2CrO_4 \cdot 4H_2O$) as the indicator (Fritz and Schenk, 1974). A colorimeter (Minolta Corporation, Ramsey, NJ) was used to measure the color of bacon brine. The brine was measured for *L*, *a*, and *b* values with triplicates.

Microbial enumeration

The total aerobic microorganisms were tested using direct plating method with Trypticase Soy Agar (EM Science, Gibbs Town, NY). For each sample, all the 5 tubes of brine were plated, each with triplicates. After incubated at 37°C for 24 h, the colonies on plates were counted and their means were reported as the final result.

A three-tube MPN method was used to test the *L. innocua* concentration in bacon brine. Firstly a serial decimal dilution (to 10^{-6}) was made using PBS (0.05 M, pH 7.4), and then 0.5 ml of each dilution was enriched in 4.5 ml UVM I broth (Oxiod, Basingstoke, Hampshire, UK) in triplicate at 37°C for 24 h. To confirm the growth of *L. innocua*, 0.2 ml of enriched UVM I broth from each tube was plated on OXFORD agar (Oxiod, Basingstoke, Hampshire, UK). After being incubated for 48 h at 37°C, positive *L. innocua* in the tube would be confirmed if black CFU(s) grew on one or more correspondent plates.

Results and Discussion

Microbial inactivation

The change of total aerobic microorganisms in the brine during electrolyzing process is shown in Fig. 3. In the first 23 h of the one-chamber-electrolyzing process, the total aerobic organisms in the brine linearly decreased from 4.4 to 2.1 log CFU/ml at a rate of 0.1 log CFU/ml per h, then in the following 125 h, it slowly but continuously decreased to 0.7 log CFU/ml. While for the two-chamber-electrolyzing process, the total aerobic organisms had a linear reduction from 4.6 to 1.5 log CFU/ml at a rate of 0.13 CFU/ml per h, then, similarly as the one-chamber process, it slowly decreased to 1.2 log CFU/ml in the following 76 h. Comparatively, the total aerobic microorganisms in the control sample decreased from 4.6 to 3.6 log CFU/ml in 94 h at the same rate of about 0.01 log CFU/ml per h. The results showed that the two-chamber-electrolyzing treatment was more effective than the one-chamber-electrolyzing treatment.

The result on *L. innocua* is presented in Fig. 4. In the first 12 h of the two-chamber-electrolyzing treatment, *L. innocua* sharply decreased from 580,000 CFU/ml to 0.36 MPN/ml at a reduction rate of 0.5 log CFU/ml per h. While in the control brine, the *L. innocua* decreased from 580,000 CFU/ml to 930 MPN/ml in the first 43 h. The results indicate that *L. innocua* could be effectively inactivated by the electrolyzing treatment.

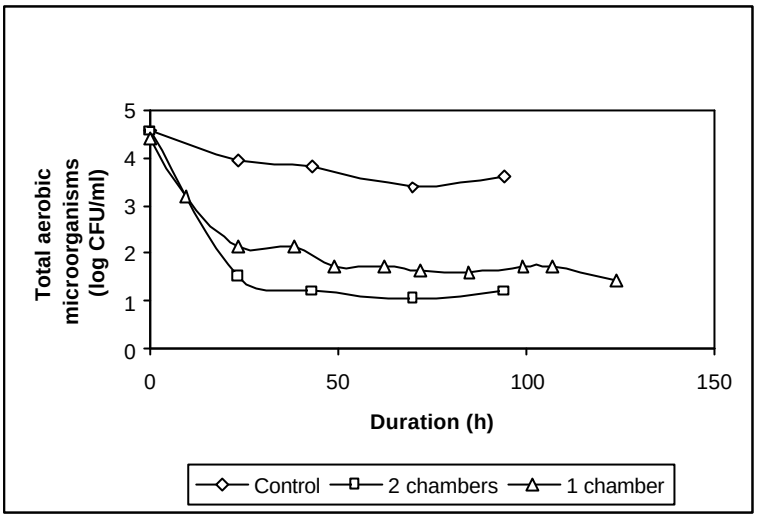

Figure 3 The survival of aerobic microbes in the bacon brine electrolyzed with different number of electrolyzing chambers. The total aerobic microorganisms were reduced by 3.1, 2.3, and 0.4 log CFU/ml at 23 h when 2, 1, and no electrolyzing chamber was used.

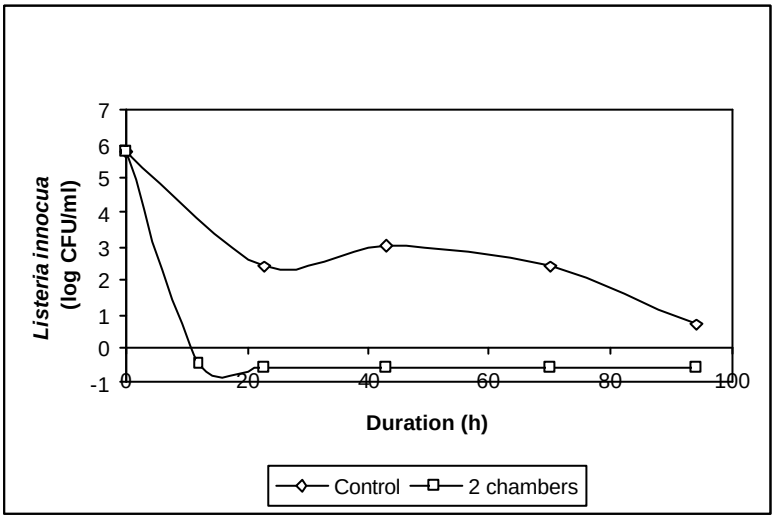

Figure 4 The survival of *Listeria innocua* in the electrolyzed bacon brine. *L. innocua* had a 6 log MPN/ml reduction at 12 h in the electrolyzed brine, whereas the control brine only had a 2 log decrease.

Chlorine production

The main product of the brine electrolysis is chlorine gas (Cl_2). In water, Cl_2 mainly exist as HOCl and OCl^- (White, 1999). The unreacted mixture of Cl_2, HClO, and OCl^- is called free chlorine, which depletes through oxidation-reduction reactions with a variety of inorganic and organic materials. Furthernore, it can react with ammonia and amino acids to form N-chloro

compounds. The total amount of this form of reacted chlorine is termed combined chlorine. Total chlorine is the sum of free and combined chlorine (Oomori et al., 2000). The free chlorine produced during electrolysis is believed to be the main bactericidal agent (Len et al., 2002; Yang et al., 2003).

The free and total chlorine content in the brine is shown in Fig. 5. The free chlorine concentration of one-chamber process and two-chamber process had no significant difference during the whole electrolyzing process. The accumulation rate of total chlorine was much quicker than that of free chlorine; and the total chlorine level of two-chamber process was higher than the one-chamber process.

The total chlorine in both the one-chamber and the two-chamber process had two different accumulation rates. In the first 23 h, which is the correspondent "linear decrease period" of total aerobic microorganisms for both the one-chamber and the two-chamber cases, the total chlorine accumulated much slower, while it increased much quicker after 23 h. The bactericidal efficacy of HClO is due to the relative ease with which it can penetrate cell walls (White, 1999).

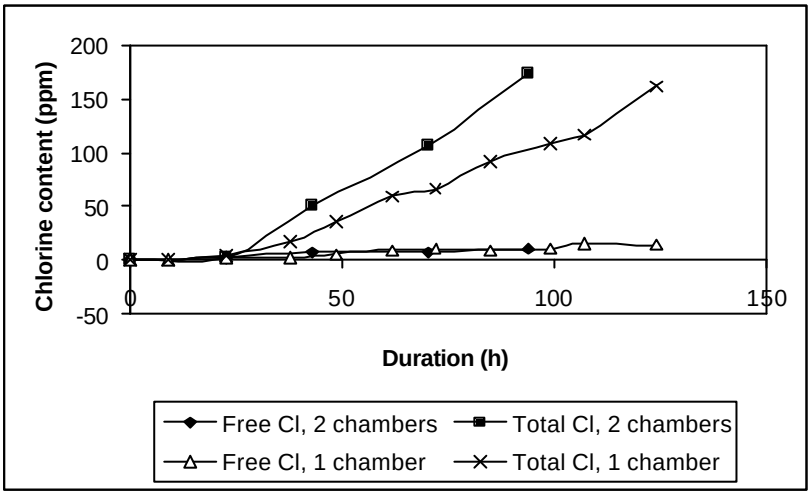

Figure 5 The free and total chlorine concentrations in the bacon brine electrolyzed with different number of electrolyzing chambers. During the "linear decrease period" of both *L. innocua* (0-12 h) and total aerobic microorganisms (0-23 h), the free Cl was in the range of 0-1.6 ppm and the total Cl was in the range of 0-3.6 ppm.

ORP, pH, NaCl, and color change

ORP value characterizes the relative state of an electrochemical system for gaining or losing electrons. Compared with the linear increase trend of chlorine during the whole electrolyzing process, the ORP of the brine also built up at a linear rate during the first 70 h, then the ORP trend had a stable phase in the range of 600-650 mV (Fig. 6). The total chlorine had a higher increase rate in two-chamber electrolysis than in one-chamber electrolysis, while the increasing rates of ORP were almost same when electrolyzed with different number of chambers. The change in pH was not significant. During the electrolyzing process, the pH of the bacon brine kept between 6.3-6.7. The NaCl concentration of the brine solution was very stable.

During the electrolyzing process, the bacon brine slowly became darker and then changed back to original color and finally became clear. We conducted a laboratory-scale test using platinum and titanium electrodes in the bacon brine. The color of bacon brine changed to darker under both cases, indicating that the electrode material was not the cause. It is speculated that some

organic materials in the bacon brine reacted with chlorine during electrolyzing treatment, which resulted in the darker color. As the organic materials were dissolved with chlorine and precipitated, the brine became clear.

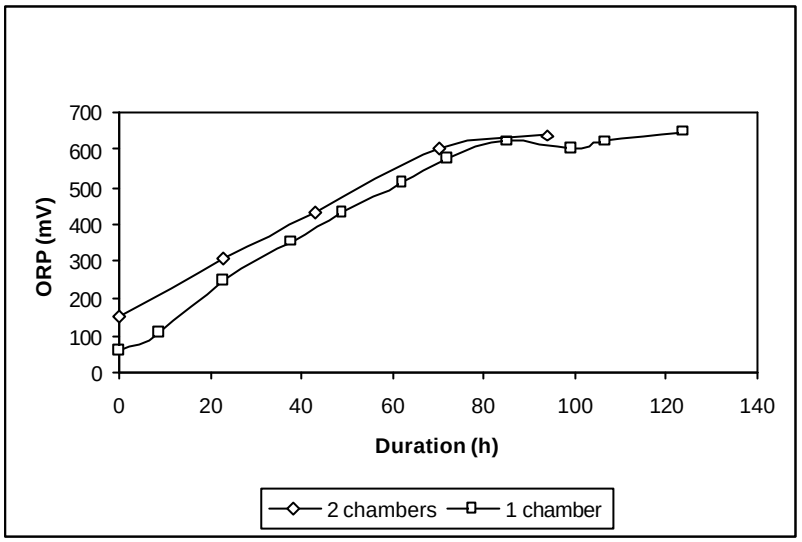

Figure 6 The ORP of the bacon brine electrolyzed with different number of electrolyzing chambers. After linearly increase, the ORP value kept stable in the range of 600-650 mV.

Conclusions

The results of pilot-plant-scale experiments showed that the electrolyzing treatment system was very effective to inactive *Listeria* and aerobic microorganisms in recirculated chilling brine. As the electrolyzing treatment time increased, the concentration of free chlorine, total chlorine and ORP in the brine increased, but the pH and salt concentration of the brine kept stable. The color of bacon brine become darker at the beginning and then returned to clear, possibly due to the reaction between some organic materials and chlorine.

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