Comparative study of survival of *Escherichia coli* O157:H7 inoculated in pork batter after ohmic cooking and water bath cooking

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A B S T R A C T

In this study, the effects of ohmic cooking (OH) and water bath cooking (WB) on the reduction of *Escherichia coli* O157:H7 inoculated in pork batter with addition of sodium chloride (NaCl) were studied, and the recovery and growth of OH and WB treated *E. coli* O157:H7 were also investigated during storage. The time for samples cooked by OH to reach the targeted endpoint temperature (61, 65, and 72 °C) was shorter than that of WB, and the addition of NaCl dramatically shortened the cooking time of OH treated samples, however, no significant effect was observed by WB. Samples with NaCl and cooked by OH had lower cooking loss than that of WB, but the inactivation effect of *E. coli* O157:H7 by OH was comparable to WB. During storage, the recovery and growth of sublethally injured *E. coli* O157:H7 were slower at 4 °C, and storage at 4 °C for 24 h delayed their recovery at 37 °C from 36 h to 48 h. These results indicated that OH had greater potential in the application of meat batter processing.

1. Introduction

Conventional cooking methods in food processing rely predominantly on the conduction and convection from cooking medium to the insides of foods. When conventional cooking methods are applied in foods, long processing time is required to achieve the targeted temperature, which may cause overcook of the food surface (Shirsat et al., 2004b), and as a result, heterogeneous products are formed. Alternative processing technologies such as radio frequency cooking, microwave cooking, and ohmic cooking (OH) could achieve more homogenous heating, where the whole food is cooked simultaneously (Lee et al., 2015; Tornberg, 2013). Among these technologies, OH has been applied in various food processing stages such as extraction and pasteurization where the electrical resistance of the foods being treated generates energy and dissipates current through the food. It can provide constant cooking rate for single solid or liquid phase food with high energy transfer efficiency (Lee et al., 2015; Llave et al., 2018; Shirsat et al., 2004b; Zell et al., 2009a).

Meat is one of the solid foods consumed by human and is a main protein source in the diet, the possibility of OH applied in meat and meat products has been confirmed by many researchers, including whole meat, meat pieces, even meat batters (Shirsat et al., 2004b; Zell et al., 2009a). The use of OH in meat and meat products has offered a number of advantages such as faster cooking rate, less energy consumption, and safer products (Özkan et al., 2004). Piette et al. (2004) and Shirsat et al. (2004a) demonstrated that it was possible for OH to produce comparable comminuted meat emulsions with conventionally cooked ones. Bozkurt and Icier (2010) reported that OH could be a fast-alternative method for ground beef products. Zell et al. (2009a, 2010a, 2010b) reported that there was a great potential for OH to cook whole beef muscle and whole turkey with comparable qualities to conventionally cooked samples. Dai et al. (2014) also demonstrated that OH could achieve comparable or improved texture profiles of pork with much reduced cooking time. While the use of OH is desirable from the standpoint of product quality, another important consideration for a food product is its safety. There are still inadequate studies on microbial safety of OH treated meat products, primarily due to the complexity of factors that affecting the inactivation effect. Therefore, more studies need to be performed.

The main factors influencing the inactivation effect of meat by OH include cooking time and temperature, voltage gradients, frequency, salt concentration, and fat content (Varghese et al., 2014). In addition, the storage conditions may play an important role in the shelf life of OH treated meat products. *Escherichia coli* O157:H7, a common intestinal

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pathogen in meat and meat products, poses a serious threat to consumer health. Thus, in this study, *E. coli* O157:H7 was used as a model pathogen to investigate the effects of voltage gradients (5, 10, and 15 V/cm), endpoint cooking temperature (61, 65, and 72 °C), and sodium chloride (NaCl) concentration (1.0%, 2.0%, and 3.0%) on cooking loss and *E. coli* O157:H7 reduction in pork batter, and the recovery and growth of *E. coli* O157:H7 after both OH (72 °C, 10 V/cm, 2.0% NaCl) and WB (72 °C, 2.0% NaCl) treatments were also compared during storage at 4, 37, and the combination of 4 and 37 °C.

2. Materials and methods

2.1. Preparation of pork batter

All pork muscles originated from a local abattoir in Beijing, China. The pigs (100 ± 10 kg) were slaughtered at about 6 months of age and stored at 4 °C for 24 h in accordance with European Union regulations (Council Directive of 26 June 1964, 1964). The *femoral biceps* and *Musculi semitendinosus* muscles were taken from the hindquarter of the three carcasses during each experiment. The surface of the pork muscles was disinfected by 70% alcohol to reduce surface bacterial contamination. The surface layer (approximately 1.5 cm) was removed using a sterile knife and the remaining meat was trimmed of visible fat and connective tissue, and cut into approximately $2 \times 2 \times 3 \, cm^3$ cubes under aseptic condition. Then all the samples were cut together into fine batter by a meat mincer (Joyoung Co., Ltd., China). The total viable count (TVC) of the indigenous microbiota was less 3 log CFU/g on plate count agar (PCA) and no *E. coli* O157:H7 was detected before inoculating *E. coli* O157:H7 in pork batter. The samples from the same carcasses were assigned equally into OH and WB groups to eliminate the influence of raw materials. All meat batter was packaged in a sterile plastic container and kept at 4 °C until the inoculation, cooking, and analyses were performed. Each experimental condition was independently repeated three times, yielding a total of 228 samples. The diagram of experiments and sampling methods was presented in Fig. 1.

2.2. Bacterial strain and culture preparation

The strain of *E. coli* O157:H7 (NCTC 12900) was obtained from Shanghai Huiyun Biological Co., Ltd., China. This strain was phenotypically very similar to toxigenic strain *E. coli* O157:H7, i.e., it originated from a verocytotoxigenic strain which lost its ability to produce toxin (Skandamis and Nychas, 2000). The *E. coli* O157:H7 cells of mid- exponential phase were prepared according to the methods in previous study (Tian et al., 2018a). The final cultures were centrifuged at 5000 ×*g* and 4 °C for 10 min, and were washed in 0.85% sterile NaCl solution (pH, 7.2 ± 0.1) twice. The pellets were suspended in 0.85%

sterile NaCl solution to obtain a targeted concentration of 4×10^9 CFU/mL (colony-forming units per milliliter) cell suspension.

2.3. Inoculation of the pork batter samples

Pork batter was weighed aseptically into 190 g portion and placed into sterile plastic bag. 10 mL of cell suspension containing approximately 4×10^9 CFU/mL was added to each 190 g portion, resulting in approximately 2×10^8 CFU/g of *E. coli* O157:H7 in pork batter. Although, this high inoculation level of *E. coli* O157:H7 was most unlikely to occur in naturally contaminated meat, it was selected in order to compare the reduction effect obtained by the different treatments (Masana et al., 2015). No other additives were added except NaCl (1.0%, 2.0%, and 3.0%) and inoculated *E. coli* O157:H7 suspension. Each packaged sample was thoroughly mixed by gloved hands for 2 min to ensure homogenous distribution of *E. coli* O157:H7 in the batter and was kept at 4 °C for a maximum of 2 h until cooked.

2.4. Experimental design

2.4.1. Water bath cooking
WB was performed using a thermostatic water bath (Ke Xi Instrument Co., Ltd., China). In brief, approximately 170 ± 5 g of pork batter was stuffed into a specially designed glass bottle (diameter, 4.2 cm; length, 10 cm) and cooked in an 80 °C water bath. The geometrical core temperature of the sample was measured continuously using a temperature probe (Industrial Temperature Sensors Ltd., China) and recorded at 60 s interval. The samples with different NaCl concentrations (0%, 1.0%, 2.0%, and 3.0%) were cooked until the core temperature reached 61, 65, and 72 °C, respectively. After cooking, the samples were cooled to 4 °C in an ice-water bath for the subsequent bacterial enumeration and storage analysis.

2.4.2. Ohmic cooking
OH was conducted with a specifically designed batch cooking system, which was described in detail by Dai et al. (2013). About 170 ± 5 g of pork batter was assigned to the cooking cell (diameter, 4.2 cm; length, 10 cm) with each end clamped with foodstuff-grade stainless electrodes, and the electrodes should be in full contact with the samples to ensure a smooth flow of current. During cooking, the temperature and current of the samples at geometrical core were recorded by the data acquisition system continuously. Firstly, three different voltage gradients (5, 10, and 15 V/cm) were used to cook the samples without NaCl to different endpoint temperatures (61, 65, and 72 °C). Then samples with different NaCl concentrations (1.0%, 2.0%, and 3.0%) were cooked to 61, 65, and 72 °C by 10 V/cm, respectively. The 10 V/cm was selected according to the results of cooking time and
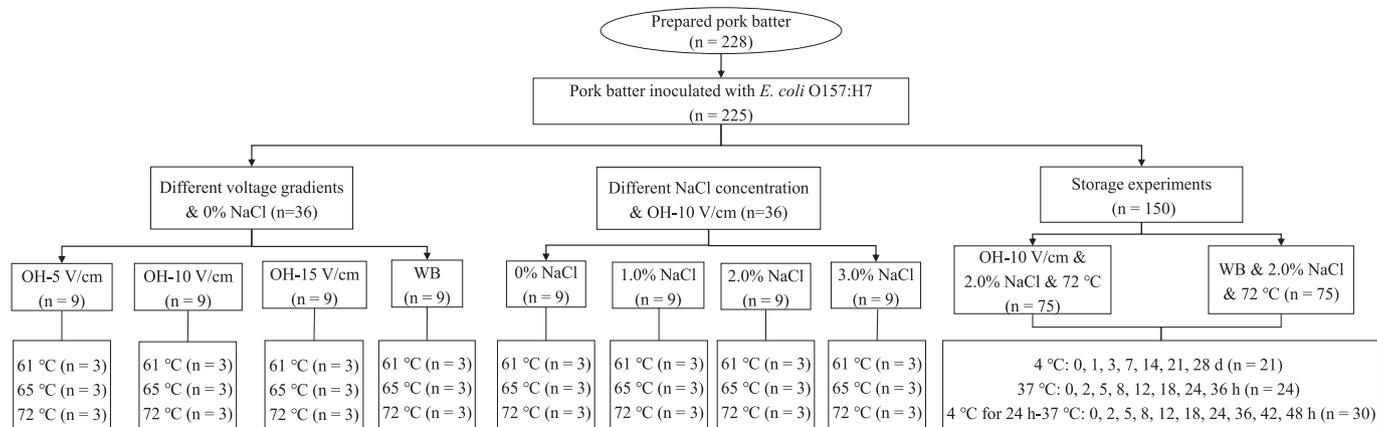

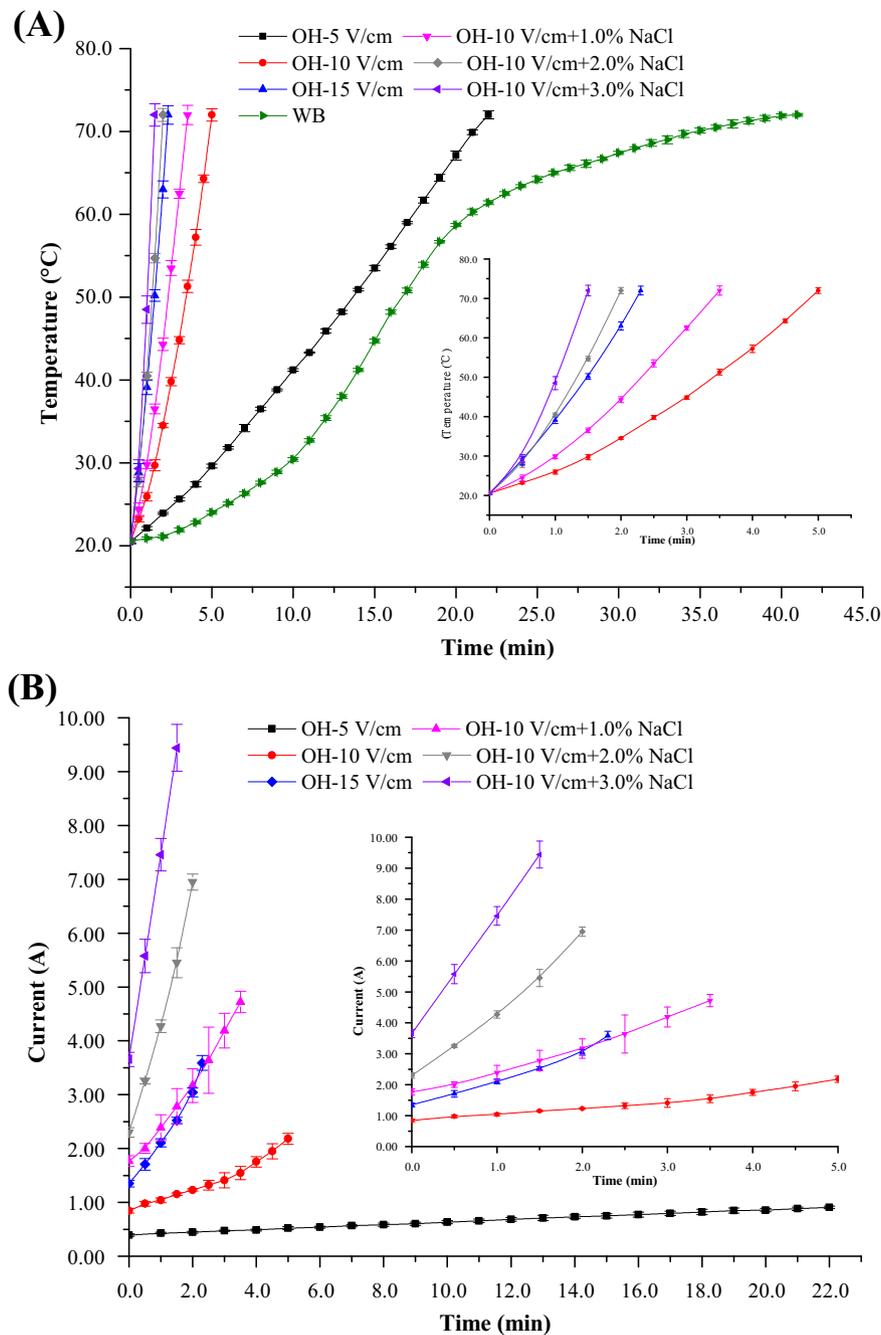Fig. 1. Diagram of experiment and sampling methods.

Fig. 2. The profiles of temperature and current of pork batter during ohmic cooking and water bath cooking. Values were means ± standard deviation of three replicates.

cooking loss. After cooking, samples were cooled to 4 °C in an ice-water bath. The cooking cell was washed with detergent followed by disinfection using 70% ethanol for 10 min, and was washed with sterile deionized water five times, then dried under aseptic condition before each cooking.

2.4.3. Measurements of recovery and growth of the sublethally injured E. coli O157:H7

After cooling, the samples deriving from OH (72 °C, 10 V/cm, 2.0% NaCl) and WB (72 °C, 2.0% NaCl) were vacuum packaged (0.10 MPa). As shown in Fig. 1, the samples were stored at 4 °C for 28 days in a refrigerator, 37 °C for 36 h in a constant temperature incubator, and 4 °C for 24 h and then 37 °C for the next 48 h. The samples were taken randomly according to the sampling interval described in Fig. 1. In

order to ensure that the *E. coli* O157:H7 detected on TAL and IMSA were the ones inoculated in pork batter at each time point during storage, the samples without inoculation of *E. coli* O157:H7 were also cooked and stored with the same protocol as described in inoculated samples.

2.5. Enumeration of E. coli O157:H7

The pork batter (25 g) was aseptically weighed and homogenized for 1 min in a sterile bag with 225 mL sterile 0.85% NaCl solution by a slapping homogenizer (IUL Masticator, Spain). The initial 1/10 (w/v) dilution was diluted successively according to Hong et al. (2012). The microbial counting was performed by spreading 100 μL of an appropriate sample dilution onto the media. PCA (tryptone 5.0 g/L, yeast

extract powder 2.5 g/L, glucose 1.0 g/L, agar 15.0 g/L, pH 7.0 ± 0.2, 02-035A, Beijing AoBoXing Bio-Technology Co., Ltd), thin agar layer (TAL) method, and Improved-MacConkey sorbitol agar (IMSA, peptone 20.0 g/L, sodium chloride 5.0 g/L, neutral red 0.03 g/L, agar 15.0 g/L, sorbitol 10 g/L, bile salts no.3 1.5 g/L, crystal violet 0.001 g/L, cefo-taxime 12.5 µg/L, potassium tellurate 25.0 mg/L, pH 7.0 ± 0.2, 02–328, Beijing AoBoXing Bio-Technology Co., Ltd) were used to de-termine TVC, total *E. coli* O157:H7 cells, and non-injured *E. coli* O157:H7 cells, respectively. All of them were incubated at 37 ± 1 °C for 48 h. Each dilution was carried out in duplicate. The storage ex-periments were ended when the TVC exceeded the initial colony forming unit before cooking (2×10^8 CFU/g).

To enumerate the total *E. coli* O157:H7 cells, including both injured and non-injured cells, TAL was used according to Kang and Fung (2000), which was made by overlaying 14 mL (7 mL/7 mL; two times overlay) of nonselective medium tryptone soy agar (TSA, tryptone 15.0 g/L, soytone 5.0 g/L, sodium chloride 5.0 g/L, agar 15.0 g/L, pH 7.3 ± 0.2, T8650, Solarbio Life Science Co., Ltd) onto 25 mL of pre-poured and solidified selective medium IMSA in a petri dish (9.0 cm diameter). On TAL, the injured *E. coli* O157:H7 could resuscitate and grow on the top TSA, and then the selective agents from IMSA could diffuse to the top TSA, the resuscitated *E. coli* O157:H7 could produce a typical reaction, and other microorganism could be inhibited by the selective agents.

2.6. Cooking loss

The measurement of cooking loss was carried out according to the previous study (Tian et al., 2016). The cooking loss was calculated according to the weights of pork batter before cooking (W1) and after cooking (W2, which was determined after the pork batter being cooled to approximately 25 °C), and the equation was as follows:

$$\text{Cooking loss } (\%) = \frac{(\text{W1} - \text{W2})}{\text{W1}} \times 100\%$$

2.7. Statistical analysis

Post Hoc (equal variances assumed by Duncan) multiple comparison test was carried out during one-way analysis of variance (ANOVA), and independent-samples *t*-test was performed to analyze the data between OH and WB treated samples using SPSSv16.0 software, and the differ-ences were considered significant at $P < 0.05$. The values in all figures and tables were given as means ± standard deviation of the three re-plicates.

3. Result and discussion

3.1. Effect of voltage gradients and NaCl concentrations on cooking temperature and current

As shown in Fig. 2, the higher voltage gradient resulted in higher cooking rate and higher current of pork batter. As a result, when the samples were cooked to 61 °C, the cooking time required by OH-5 V/cm, OH-10 V/cm, and OH-15 V/cm was 17.80, 4.25, and 1.93 min, to 65 °C was 19.21, 4.55, and 2.05 min, and to 72 °C was 22.00, 5.00, and 2.30 min, respectively (data not shown). However, the samples cooked by WB took 21.50, 26.00, and 41.00 min to reach the core temperature of 61, 65, and 72 °C, respectively. Therefore, the cooking time required by OH was much shorter than that of WB. In the meantime, the tem-perature increased quickly with the increasing cooking time during OH, which was due to the increasing electrical conductivity of pork batter with increasing temperature (Piette et al., 2004; Zell et al., 2009b). This phenomenon was explained that high temperature was more conducive to the movement of ions than low temperature (Guo et al., 2017). In this study, 5 V/cm had lower cooking rate than 10 and 15 V/cm, and could not markedly evidence the faster cooking characteristic of OH. Al-though 15 V/cm had the highest cooking rate, the samples treated by 15 V/cm had the highest cooking loss. Therefore, OH at 10 V/cm was selected to investigate the effect of NaCl concentrations on cooking rate, cooking loss, and inactivation effect of *E. coli* O157:H7.

In OH, the formation of current depended on the movement of electrons, therefore, the salt or other electrolytic ingredients were es-sential to transfer the heat energy in the products. As expected, the temperature and current increased quickly with the increasing NaCl concentrations during OH (Fig. 2). For example, the cooking time was shortened from 5.00 min to 3.50, 2.00, and 1.50 min with addition of 1.0%, 2.0%, and 3.0% NaCl at 72 °C, respectively. However, NaCl concentration had no significant effect on the changes in temperature of WB treated samples (data not shown), which was closely related to the cooking principle based on the temperature gradient between water bath and samples. The present results were similar to Shirsat et al. (2004b) who found that the electrical conductivity of meat batter was proportional to the level of NaCl added at concentrations of up to 3.8% when the proportions of all other ingredients were held constant. Zell et al. (2009b) also found that the addition of NaCl and phosphate could cause a significant increase in electrical conductivity of beef, which in turn led to an increase in cooking rate by OH.

3.2. Effect of voltage gradients and NaCl concentrations on cooking loss

Cooking loss was an important parameter for meat processing, which determined the yield of meat products. As shown in Table 1, the

Table 1
Cooking loss of pork batter by ohmic cooking and water bath cooking.

Treatments	Cooking loss (%)			P value
	61 °C	65 °C	72 °C	
OH-5 V/cm	7.88 ± 0.60[Ag]	11.45 ± 0.98[Be]	15.84 ± 0.91[Ce]	8.03e−05
OH-10 V/cm	6.16 ± 0.41[Aef]	9.05 ± 0.84[Bcd]	14.49 ± 0.93[Ce]	3.04e−05
OH-15 V/cm	7.21 ± 0.56[Afg]	10.01 ± 1.06[Bd]	17.9 ± 0.99[Cf]	1.70e−05
OH-10 V/cm + 1.0% NaCl	2.45 ± 0.74[Aab]	3.29 ± 0.99[Aa]	4.95 ± 0.51[Ba]	1.91e−02
OH-10 V/cm + 2.0% NaCl	2.65 ± 0.58[Aab]	3.20 ± 0.56[Aa]	4.56 ± 0.68[Ba]	2.17e−2
OH-10 V/cm + 3.0% NaCl	2.16 ± 0.46[Aa]	3.03 ± 0.49[Aa]	4.31 ± 1.01[Ba]	2.52e−2
WB	9.65 ± 0.83[Ah]	13.15 ± 1.09[Bf]	15.48 ± 1.17[Ce]	1.38e−3
WB + 1.0% NaCl	5.87 ± 0.94[Ade]	8.97 ± 0.71[Bcd]	11.54 ± 1.42[Cd]	1.88e−3
WB + 2.0% NaCl	4.81 ± 1.14[Acd]	7.87 ± 0.46[Bc]	9.42 ± 0.46[Cc]	8.42e−4
WB + 3.0% NaCl	3.56 ± 0.87[Abc]	5.79 ± 0.33[Bb]	6.78 ± 0.27[Bb]	1.25e−2
P value	2.51e−10	1.24e−12	5.92e−14	

Values were means ± standard deviation of three replicates. Values in the same row with different capital letters (A–C) were significantly different ($P < 0.05$), and values in the same column with different lowercase letters (a–h) were significantly different ($P < 0.05$).
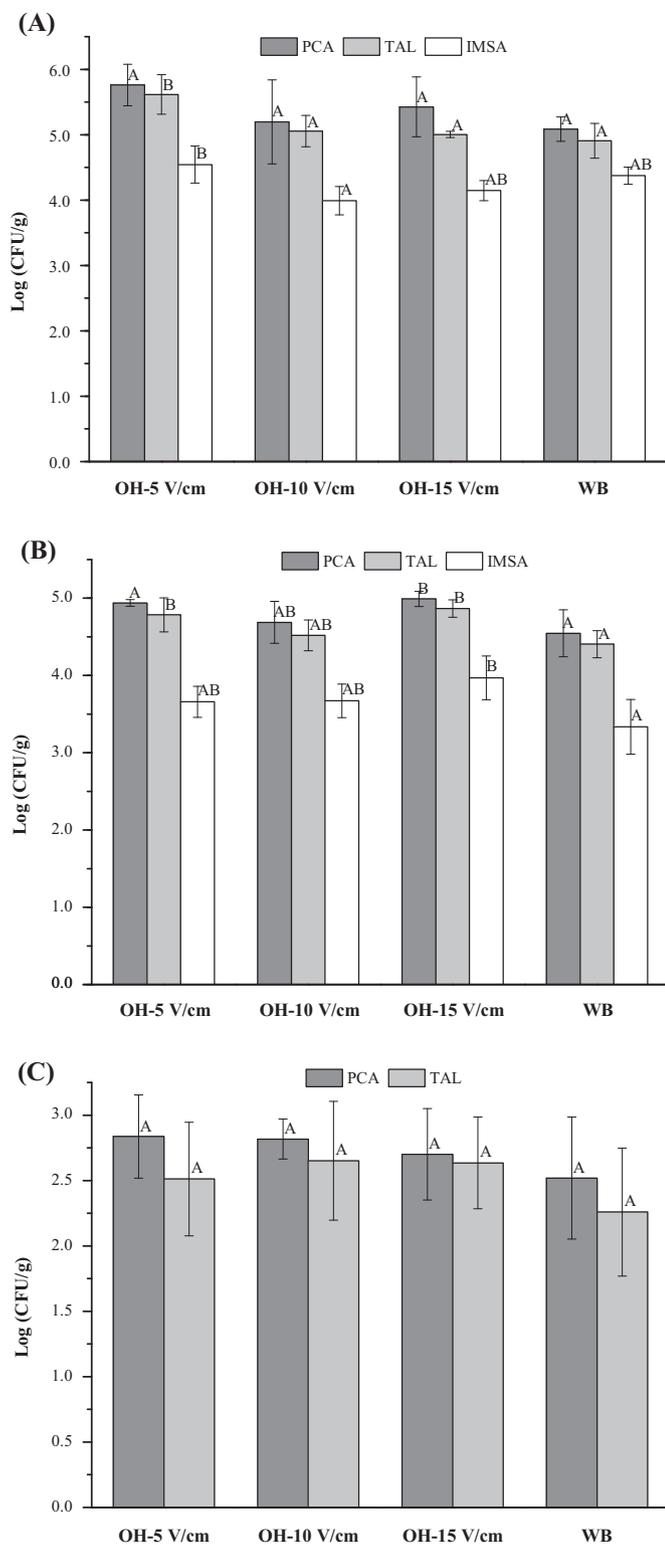
(A)

(B)

(C)

Fig. 3. Survival of *E. coli* O157:H7 in pork batter during ohmic cooking and water bath cooking. (A) At 61 °C; (B) at 65 °C; (C) at 72 °C. Values were means ± standard deviation of three replicates. Values with different capital letters (A–B) were significantly different on the same plate (*P* < 0.05).

cooking loss of samples cooked by OH (5, 10, and 15 V/cm) was comparatively lower than that of WB at 61 and 65 °C without addition of NaCl (*P* = 0.005). This might be due to the higher cooking rate of OH, where shorter heat exposure could not denature the proteins of meat extensively (Kılıç et al., 2014), resulting in a lower cooking loss. A

(A)

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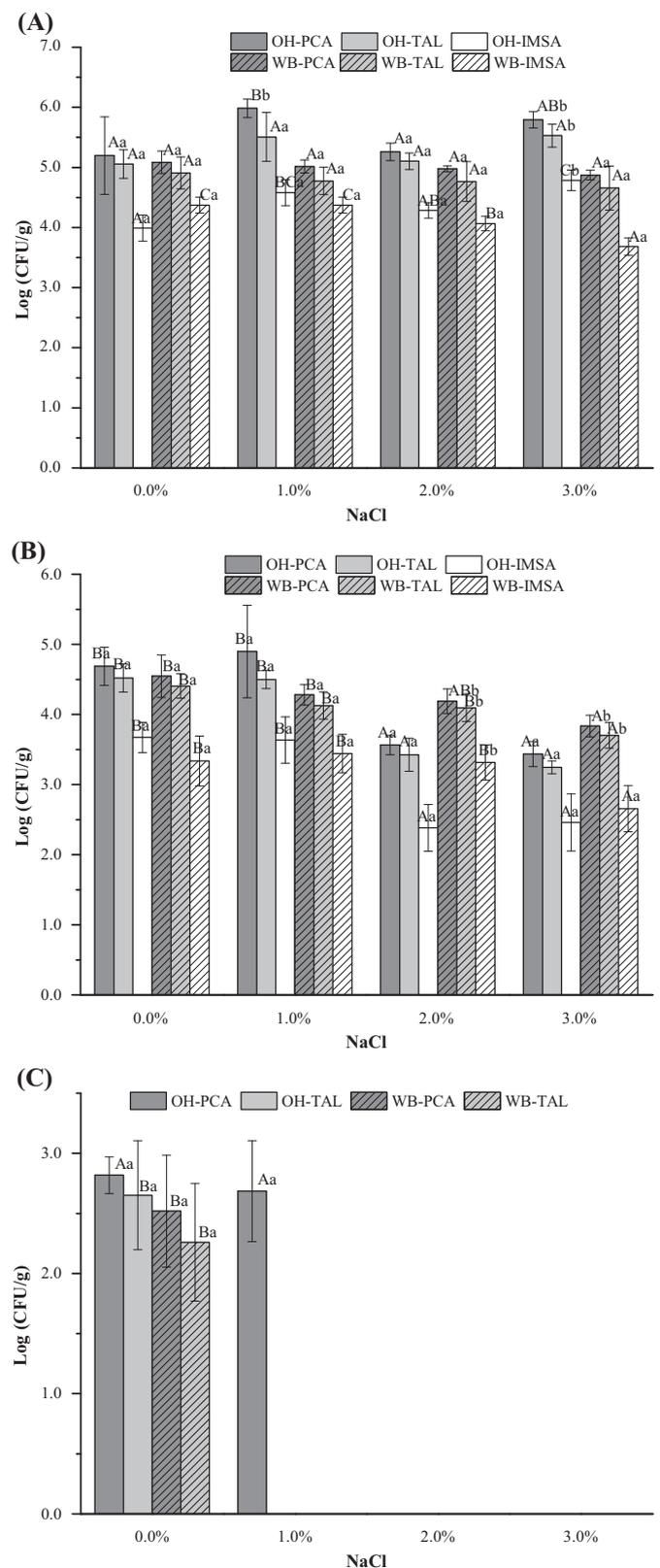
Fig. 4. Survival of *E. coli* O157:H7 in pork batter with different NaCl concentrations during ohmic cooking and water bath cooking. (A) At 61 °C; (B) at 65 °C; (C) at 72 °C. Values were means ± standard deviation of three replicates. Values with different capital letters (A–C) were significantly different on the same plate of OH or WB treated samples (*P* < 0.05), and values with different lowercase letters (a–b) were significantly different on the same plate between OH and WB treated samples (*P* < 0.05).

Table 2
The recovery and growth of sublethally injured *E. coli* O157:H7 in pork batter followed by ohmic cooking and water bath cooking during storage at 4 °C.

Treatments	Bacteria types	Colony counts (log CFU/g)						
		0 d	1 d	3 d	7 d	14 d	21 d	28 d
OH	Total viable counts	ND	2.19 ± 0.20[Aa]	3.22 ± 0.24[Bay]	4.38 ± 0.28[Dby]	4.10 ± 0.13[Day]	4.07 ± 0.09[Day]	3.71 ± 0.12[Cay]
	Total *E. coli* cells	ND	ND	3.04 ± 0.27[Aay]	4.28 ± 0.24[Bby]	4.00 ± 0.10[Bay]	3.85 ± 0.07[Bay]	3.56 ± 0.13[Aay]
	Non-injured *E. coli* cells	ND	ND	2.38 ± 0.33[Aax]	3.77 ± 0.13[Dbx]	3.47 ± 0.20[CDbx]	3.27 ± 0.20[BCbx]	3.06 ± 0.11[Bbx]
WB	Total viable counts	ND	ND	3.02 ± 0.14[Aay]	3.39 ± 0.25[Aay]	3.86 ± 0.28[Aay]	3.56 ± 0.24[Aay]	3.37 ± 0.25[Aay]
	Total *E. coli* cells	ND	ND	2.66 ± 0.22[Aax]	3.16 ± 0.32[ABaxy]	3.77 ± 0.13[Bay]	3.39 ± 0.35[ABay]	3.27 ± 0.09[ABay]
	Non-injured *E. coli* cells	ND	ND	ND	2.62 ± 0.39[ABax]	3.01 ± 0.09[Bax]	2.59 ± 0.26[Aax]	2.36 ± 0.09[Aax]

Total viable counts: PCA. Total *E. coli* cells: TAL. Non-injured *E. coli* cells: IMSA. Values were means ± standard deviation of three replicates. Values in the same row with different capital letters (A–D) were significantly different ($P < 0.05$), values in the same column with different lowercase letters (a–b) were significantly different on the same plate between OH and WB treated samples ($P < 0.05$), and values in the same column with different lowercase letters (x–y) were significantly different on the different plate of the same samples ($P < 0.05$). ND: The minimum level of detection was 2 log CFU/g.

lower cooking loss is associated with higher juiciness, and it is one of the major advantages of rapid OH compared with conventional cooking methods (Zell et al., 2010b). Compared with samples cooked to 61 and 65 °C, samples with endpoint temperature of 72 °C had higher cooking loss, which might be due to the longer cooking time and the higher cooking temperature, causing more loss by the release of juice inside samples (Li et al., 2018; Zell et al., 2010b). However, the cooking loss of samples treated by 15 V/cm was significantly higher than that of 5 V/cm, 10 V/cm, and WB, but there was no significant difference observed among 5 V/cm, 10 V/cm, and WB treated samples at 72 °C. This reason might be that the higher cooking rate of 15 V/cm caused the sharp contraction of myofibrils, which squeezed out more juice in pork batter, and more specific research needs to be performed to elucidate this phenomenon.

As shown in Table 1, the cooking loss of pork batter decreased significantly with addition of NaCl in both OH and WB treatments compared with samples without NaCl. The cooking loss values of OH treated samples (4.31%–4.95%) were significantly lower than that of WB treated samples (6.78%–11.54%) with NaCl with endpoint temperature of 61, 65, and 72 °C, which indicated that the combination of NaCl and OH resulted in a better water-holding capacity than that of WB. Mukherjee et al. (2010) reported that NaCl and phosphate increased the water-holding capacity of beef, and the cooking loss was lower in NaCl/phosphate beef samples (< 1%) compared with other formulations and the control group (7.4% to 15.9%) at 60 or 65 °C. The cooking loss of WB treated samples decreased with the increased NaCl concentrations, but there was no significant difference observed in OH treated samples with NaCl concentrations ranging from 1.0% to 3.0%.

3.3. Effect of voltage gradients and NaCl concentrations on inactivation of E. coli O157:H7

The initial population of *E. coli* O157:H7 inoculated in pork batter were 8.26 ± 0.05, 8.18 ± 0.08, and 8.08 ± 0.17 log CFU/g on PCA, TAL, and IMSA, respectively, with no significant difference ($P = 0.121$). Values obtained on IMSA were similar to those observed on PCA and TAL, indicating that the major colonies obtained on PCA and TAL were *E. coli* O157:H7. As shown in Fig. 3, there was a tendency to obtain lower counts on IMSA than on PCA and TAL for the same treatment. This phenomenon indicated the existence of sublethally injured cells during both OH and WB treatments, and the use of selective medium to enumerate *E. coli* O157:H7 might lead to an underestimation of the real risk, i.e. the sublethally injured cells could recover viability when conditions were conducive to their growth during storage (Masana et al., 2015). The inactivation of *E. coli* O157:H7 increased with the increasing temperature regardless of the cooking methods. Reductions in *E. coli* O157:H7 ranged from 2.44–3.15, 2.49–3.23, and 3.54–4.12 log CFU/g on PCA, TAL, and IMSA at 61 °C, respectively. As expected, the survival of *E. coli* O157:H7 was only 2.65–2.90 and 2.40–2.79 log

CFU/g on PCA and TAL, and was not detected on IMSA at 72 °C. Moreover, OH at 5, 10, and 15 V/cm had comparable inactivation effects to WB, even with a shorter cooking time. This result might be related to the additional electrical effects of OH on *E. coli* O157:H7, where the applied electric field caused damage to the cell membrane of *E. coli* O157:H7 and formation of pores on the membrane surface, eventually led to breakdown of cells (Lee et al., 2012; Yoon et al., 2002).

Inactivation of *E. coli* O157:H7 inoculated in pork batter with NaCl are presented in Fig. 4. Changes in the NaCl concentration of pork batter had an impact on the survival of *E. coli* O157:H7. At endpoint temperature of 61 °C, the counts of samples with 1.0% NaCl were significantly higher on PCA and IMSA than those without NaCl during OH, this phenomenon might be attributed to the protective effect of low concentration NaCl on the survival of *E. coli* O157:H7 and enhance the heat resistance of *E. coli* O157:H7 during OH. In addition, the counts of samples with 3.0% NaCl were significantly higher on IMSA than others during OH, which might be due to the fact that high concentration NaCl resulted in a higher cooking rate and shorter cooking time, as a result, *E. coli* O157:H7 could not be inactivated effectively. However, there was no significant difference observed on TAL. The different trends among the plates could be attributed to the different proportion of sublethally injured cells. At endpoint temperature of 72 °C, the counts of samples with 2.0% and 3.0% NaCl were below the detection limit on the three plates, and only 2.69 log CFU/g was observed on PCA with 1.0% NaCl during OH. In the meantime, the counts in WB treated samples were all below the detection limit on the three plates. The better inactivation effect for samples with addition of 2.0% and 3.0% NaCl might be due to the fact that increased NaCl concentration resulted in the higher osmotic pressure, as a result, *E. coli* O157:H7 were inactivated more readily. The similar results were reported by the previous researchers (Tuncan and Martin, 2010), who suggested that the effect of salts (NaCl and KCl) on thermal inactivation of *Staphylococcus aureus* MF-31 was mainly related to reduced water activity and increased osmotic pressure of the heating media.

3.4. Effect of temperature on the recovery and growth of E. coli O157:H7

The adaptability of *E. coli* O157:H7 to high concentration salt and low temperatures is very important for its survival and possible growth in meat products during storage. Considering the effective inactivation effect of OH-10 V/cm, and combining the results of cooking time and cooking loss, samples with 2.0% NaCl had a significant shorter cooking time and comparable cooking loss to 1.0% NaCl sample during OH, and 2.0% NaCl was closer to the usual salt content in meat products. Therefore, OH-10 V/cm and WB treated samples with 2.0% NaCl were selected to investigate the recovery and growth of the surviving *E. coli* O157:H7 during storage. In this study, the *E. coli* O157:H7 was not detected in samples without inoculating *E. coli* O157:H7, which

Table 3
The recovery and growth of sublethally injured *E. coli* O157:H7 in pork batter followed by ohmic cooking and water bath cooking during storage at 37 °C and a combination of 4 °C for 24 h followed by 37 °C.

Treatments	Bacteria types	Colony counts (log CFU/g)									
		0 h	2 h	5 h	8 h	12 h	18 h	24 h	36 h	42 h	48 h
OH	Total viable counts	ND	2.73 ± 0.17^{Ab}	3.37 ± 0.06^{Bcy}	4.13 ± 0.13^{Ccy}	4.79 ± 0.02^{Ddz}	5.63 ± 0.43^{Eby}	6.80 ± 0.06^{Fby}	8.14 ± 0.05^{Gcy}		
	Total *E. coli* cells	ND	ND	3.24 ± 0.21^{Aby}	4.02 ± 0.05^{Bcy}	4.61 ± 0.04^{Bby}	5.55 ± 0.11^{Cby}	6.73 ± 0.11^{Dby}	8.01 ± 0.13^{Ecxy}		
	Uninjured *E. coli* cells	ND	ND	2.00 ± 0.30^{Aax}	3.34 ± 0.23^{Bcx}	3.89 ± 0.06^{Bbx}	4.78 ± 0.09^{Cbx}	6.09 ± 0.16^{Dcx}	7.90 ± 0.08^{Ecx}		
WB	Total viable counts	ND	2.29 ± 0.26^{Aab}	2.88 ± 0.20^{Aby}	3.93 ± 0.06^{Bcy}	4.55 ± 0.06^{Bcy}	5.61 ± 0.06^{Bcy}	6.58 ± 0.19^{Dby}	7.95 ± 0.09^{Eby}		
	Total *E. coli* cells	ND	ND	2.88 ± 0.04^{Aby}	3.80 ± 0.09^{BCcy}	4.45 ± 0.22^{Cby}	5.53 ± 0.12^{Dby}	6.51 ± 0.15^{Eby}	7.67 ± 0.06^{Fby}		
	Uninjured *E. coli* cells	ND	ND	2.16 ± 0.15^{Aax}	2.77 ± 0.18^{Abx}	3.59 ± 0.15^{Bbx}	4.90 ± 0.08^{Cbx}	5.60 ± 0.20^{Dbx}	7.19 ± 0.10^{Ebx}		
OH-4 °C for 24 h	Total viable counts	ND	2.22 ± 0.24^{Aab}	3.20 ± 0.11^{Bbcx}	3.39 ± 0.18^{Bby}	3.86 ± 0.06^{Cby}	4.27 ± 0.34^{Day}	5.65 ± 0.17^{Eay}	7.00 ± 0.08^{Fay}	7.94 ± 0.02^{Ghy}	8.10 ± 0.11^{Gax}
	Total *E. coli* cells	ND	ND	3.07 ± 0.16^{Abx}	3.23 ± 0.09^{Aby}	$3.82 \pm 0.06^{A Bay}$	4.28 ± 0.05^{Bay}	5.57 ± 0.22^{Cay}	6.88 ± 0.09^{Day}	7.84 ± 0.02^{Eay}	8.06 ± 0.15^{Eax}
	Uninjured *E. coli* cells	ND	ND	ND	2.29 ± 0.26^{Aax}	2.69 ± 0.30^{Aax}	3.55 ± 0.23^{Bax}	4.51 ± 0.23^{Cax}	6.31 ± 0.06^{Dax}	7.06 ± 0.26^{Eax}	7.64 ± 0.39^{Eax}
WB-4 °C for 24 h	Total viable counts	ND	ND	2.38 ± 0.33^{Aa}	$3.11 \pm 0.10^{A Bay}$	3.71 ± 0.13^{BCay}	4.19 ± 0.03^{Cay}	5.44 ± 0.03^{Day}	6.97 ± 0.08^{Eay}	7.80 ± 0.01^{EFay}	8.18 ± 0.06^{Fax}
	Total *E. coli* cells	ND	ND	ND	2.91 ± 0.04^{Aay}	3.63 ± 0.15^{Aay}	4.06 ± 0.04^{Aay}	5.36 ± 0.14^{Bay}	6.82 ± 0.09^{Cay}	7.79 ± 0.05^{CDay}	8.14 ± 0.20^{Dax}
	Uninjured *E. coli* cells	ND	ND	ND	2.26 ± 0.24^{Aax}	2.75 ± 0.13^{ABax}	3.36 ± 0.13^{Bax}	4.43 ± 0.18^{Cax}	6.24 ± 0.06^{Dax}	7.53 ± 0.13^{Eax}	8.07 ± 0.10^{Eax}

Total viable counts: PCA. Total *E. coli* cells: TAL. Non-injured *E. coli* cells: IMSA. Values were means ± standard deviation of three replicates. Values in the same row with different capital letters (A–G) were significantly different ($P < 0.05$), values in the same column with different lowercase letters (a–d) were significantly different on the same plate among OH and WB treated samples ($P < 0.05$), and values in the same column with different lowercase letters (x–z) were significantly different on the different plate of the same samples ($P < 0.05$). ND: The minimum level of detection was 2 log CFU/g.

indicated that the counts on TAL and PCA corresponded the *E. coli* O157:H7 inoculated in pork batter during the whole storage at 4 °C and 37 °C (data not shown). As presented in Table 2, the counts on TAL and PCA were not significantly different except OH treated samples at day 1 and WB treated samples at day 3, meaning that the counts on TAL corresponded primarily to *E. coli* O157:H7 during the whole storage period. These results indicated that the indigenous microbiota had little effect on the recovery and growth of *E. coli* O157:H7 during storage at 4 °C for 28 days. The counts of *E. coli* O157:H7 were < 6 log CFU/g on the three plates at the end of storage at 4 °C, and the counts on IMSA were lower than PCA and TAL. This phenomenon might be due to the fact that the sublethally injured *E. coli* O157:H7 could not recover on IMSA completely. This result was similar to the findings of previous study on the recovery of sublethally injured *E. coli* O157:H7 treated with OH in nutrient broth at 4 °C (Tian et al., 2018b). However, the counts on IMSA in OH treated samples were higher than those of WB from day 7 to day 28, but no significant difference was observed on PCA and TAL between OH and WB treated samples from day 14 to day 28, which meant that there were more sublethally injured *E. coli* O157:H7 cells repaired in OH treated samples than those of WB. These data suggested that OH followed by low temperature storage added a potential hurdle for bacteria growth in pork batter, but due to the higher recovery of sublethally injured *E. coli* O157:H7 cells in OH treated samples, the microbial safety of OH treated ones still needs to be taken seriously during storage.

As shown in Table 3, after 2 h of recovery at 37 °C, there were 2.73 and 2.29 log CFU/g observed on PCA in OH and WB treated samples, and no colony was observed on TAL and IMSA. The significant recovery and growth of *E. coli* O157:H7 occurred from 2 to 5 h. Finally, the counts of *E. coli* O157:H7 on IMSA were not significantly different from PCA and TAL at 36 h (with the counts of > 8 log CFU/g), which meant that the recovery of sublethally injured *E. coli* O157:H7 was completed.

In the present study, OH and WB treated pork batter were stored at 4 °C for 24 h, and then stored at 37 °C. As expected, the storage at 4 °C for 24 h delayed the recovery and growth of *E. coli* O157:H7 in pork batter in the subsequent storage at 37 °C, and the count on IMSA was still below the detection limit until 5 h of recovery. After 36 h of recovery, the counts were only 7.00 and 6.97 log CFU/g on PCA, 6.88 and 6.82 log CFU/g on TAL, and 6.31 and 6.24 log CFU/g on IMSA for OH and WB treated samples, respectively, which were significantly lower than those stored at 37 °C all the time. Finally, the counts were > 8 log CFU/g on PCA and TAL at 48 h of recovery, and the counts on PCA, TAL, and IMSA were not significantly different between OH and WB treated samples at 48 h. Overall, additional 12 h was required to achieve the complete recovery for *E. coli* O157:H7 after the samples were stored at 4 °C for 24 h. This result indicated that 4 °C for 24 h followed by 37 °C was effective in inhibiting the recovery of *E. coli* O157:H7. These results were similar to the previous study. Zhao et al. (2009) reported that the shelf life of pulsed electric field (PEF) treated green tea infusions without cold treatments was only 4 days at 37 °C, but it was extended to > 90 days when 7 days of cold storage treatment was employed following PEF processing. Accordingly, the storage temperature following OH played an important role in bacterial recovery, this result could provide reference for other researchers and food industries to apply OH more effectively.

4. Conclusion

In summary, pork batter cooked to the same endpoint temperature, the time required by OH was much shorter than WB. OH-10 V/cm and OH-15 V/cm had a better inactivation effect of *E. coli* O157:H7 with shorter cooking time compared with OH-5 V/cm. Increased NaCl concentration decreased the cooking time by OH, but no significant effect was observed by WB. In the meantime, the addition of NaCl decreased the cooking loss of both OH and WB treated samples, and 2.0% and 3.0% NaCl achieved a better inactivation effect on *E. coli* O157:H7.

Sublethally injured *E. coli* O157:H7 could not recover completely after OH (72 °C, 10 V/cm, 2.0% NaCl) and WB (72 °C, 2.0% NaCl) treatments during storage at 4 °C for 28 days, but they recovered more quickly in OH treated samples than WB treated ones. In addition, the samples stored at 4 °C for 24 h significantly delayed the recovery of sublethally injured *E. coli* O157:H7 at 37 °C. These results indicated that OH had a great potential in pork batter processing with shorter cooking time, but more attention should be given to the sublethally injured bacteria and their recovery during storage.

Acknowledgments

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