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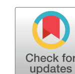
Resistance of *Escherichia coli* O157:H7 ATCC 35150 to ohmic heating as influenced by growth temperature and sodium chloride concentration in salsa



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ABSTRACT

Salsa is a liquid-solid food containing jalapeño and serrano peppers, which result in multistate outbreak in 2008. Storage temperature and sodium chloride (NaCl) concentration of salsa vary depending on the climate, season, and type of product. In this regard, effect of growth conditions, namely, low temperature (15 °C) or NaCl concentration (4.5%) on the resistance of *Escherichia coli* O157:H7 ATCC 35150 to ohmic heating was identified in this study. Cells of *E. coli* O157:H7 ATCC 35150 grown under different growth conditions was inoculated into prepared salsa sample, and then subjected to ohmic heating. Mechanisms of resistance acquisition were identified by transcriptional responses, membrane fatty acid changes and confirmed with propidium iodide (PI) uptake values. Resistance of the pathogen to ohmic heating decreased when growth temperature decreased from 37 °C to 15 °C while increased resistance was observed for this pathogen when grown with 4.5% NaCl. Several heat stress related genes such as *dnaK*, *rpoH*, *grpE*, *groES*, *htpG*, and *htpX* were up-regulated (≥5 fold change) as growth temperature decreasedwhile *groEL*, *dnaK*, *rpoH* were up-regulated when grown with high NaCl concentration in the present study. The ratio of unsaturated fatty acids (USFA) to saturated fatty acids (SFA) of pathogen increased slightly (+0.16) or significantly (+0.79) with increasing NaCl concentration or decreasing temperature, respectively. These results indicate that the cell membrane of the pathogen grown at low temperature was more susceptible to heat than when grown under optimal conditions or high NaCl concentration. Cell membrane damage measured by PI uptake values of the pathogen grown with high NaCl concentration were not significantly different from those of the control ($p > 0.05$), while the values were significantly higher for the pathogen grown at low temperature and subjected to ohmic heating ($p < 0.05$). Based on these results we suggest that resistance of the pathogen grown at low temperature to ohmic heating decreased because of dominant cell membrane damage compared to induced heat stress related genes. The cell membrane damage was dominant by means of an increased ratio of USFA to SFA. On the other hand, pathogen resistance increased when grown in medium of high NaCl concentration because of induced heat stress related genes.

1. Introduction

Biological hazards are one of the major causes of outbreaks involving food products. A variety of pathogens causing foodborne outbreaks have been reported such as *Escherichia coli (E. coli)* O157:H7, *Salmonella* Typhimurium (*S.* Typhimurium), *Listeria monocytogenes (L. monocytogenes)*, and *Campylobacter jejuni* (Jayasena et al., 2015). In particular, *E. coli* O157:H7 may cause hemolytic uremic syndrome with low infective dose (Yang et al., 2013). Thermal treatments have been used widely to control pathogens in foods. Foodborne pathogens subjected to high temperature are easily inactivated because several essential proteins are denatured which contribute to cell death (Nguyen, Corry, & Miles, 2006). However, high temperature thermal treatments could cause food quality losses, and consequently several novel thermal technologies have been introduced to reduce treatment time and temperature. The novel thermal technologies affect food quality to a lesser

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extent by means of rapid and volumetric heating. For example, near-infrared (NIR) heating has been used to control pathogens in ready-to-eat sliced ham as an alternative to convective heating (Ha, Ryu, & Kang, 2012), but only the food surface can be heated by NIR heating. Radio frequency heating and microwave heating also have been used to secure biological safety in food products (Jeong & Kang, 2014; Song & Kang, 2016) even though solid-liquid food is heated non-uniformly by these treatments due to different dielectric properties. Alternatively, food samples can be heated more uniformly by ohmic heating as reported in our previous study (Kim et al., 2016).

Ohmic heating is a novel technology generating heat inside of food using electric current. Electrode corrosion has been a major obstacle when ohmic heating is used for food processing (Pataro et al., 2014). To solve this problem, high frequency or pulse waveforms have been introduced (Kim, Choi, & Kang, 2017; Lee, Ryu, & Kang, 2013). Therefore, research investigations studying inactivation of foodborne pathogens by ohmic heating without incurring electrode corrosion have been reported recently (Lee, Kim, & Kang, 2015). Even though thermal inactivation is the principal mechanism of pathogen decontamination by ohmic heating, a non-thermal effect has also been reported (Park & Kang, 2013). Irreversible pore formation at high temperature by the ohmic heating electric field was observed and reductions of pathogens such as *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* can be accelerated by ohmic heating compared to conventional heating. Moreover, ohmic heating can be used effectively to process solid-liquid samples compared to conventional heating. Solid ingredients are usually heated more slowly than the liquid phase by conventional heating because heat transfer is implemented with conduction and convection. On the other hand, solid and liquid components can be heated simultaneously by ohmic heating. Several research investigations reported that liquid-solid foods such as salsa can be processed effectively by ohmic heating (Kim & Kang, 2017a,b; Lee et al., 2013).

Salsa is a liquid-solid food containing jalapeño and serrano peppers (Castro-Rosas et al., 2011). A multistate outbreak in the United States involving more than 1,400 cases was reported in 2008, in which jalapeño and serrano peppers were identified as vehicles of pathogen (Mody et al., 2011). Ma, Zhang, Gerner-Smidt, Tauxe, and Doyle (2010) reported that pathogen can grow in chopped tomatoes, jalapeño peppers, and cilantro when held at 12 °C and above. Because jalapeño and serrano peppers are usually consumed raw in green salads or salsa, adequate decontamination procedures to inactivate bacterial pathogens in salsa is required. Salsa is usually held at room temperature, which varies depending on climate and season, and also has diverse sodium chloride (NaCl) concentration according to the types of product such as hot, medium, chunky, and so forth. Meanwhile, many research studies reported that different environmental conditions can cause many elaborate stress responses in bacterial pathogens (Bergholz, Bowen, Wiedmann, & Boor, 2012; Kim & Rhee, 2016; Lim & Hammer, 2015; Zhang & Griffiths, 2003). The stress response can result in variable bacterial pathogen resistance to thermal (Kim & Rhee, 2016) or non-thermal (Lee, Kim, & Kang, 2019) bactericidal treatments.

Ohmic heating is an efficient technology for inactivating foodborne pathogens in salsa, but to the best of our knowledge, research investigations related to the resistance of foodborne pathogens to ohmic heating grown under different conditions have been limited. In the present study, we investigated the resistances of *E. coli* O157:H7 ATCC 35150 grown under at extreme conditions (4.5% NaCl or 15 °C) to ohmic heating. First, ohmic heating reduction of *E. coli* O157:H7 ATCC 35150 by ohmic heating grown under different conditions was compared in salsa. Secondly, relative expression of heat stress related genes and membrane lipid composition changes were observed to identify the mechanism of the resistance acquisition. Finally, the propidium iodide uptake test was accomplished to verify cell membrane damage resulting from ohmic heating.

2. Materials and methods

2.1. Bacterial cultures and cell suspension

E. coli O157:H7 ATCC 35150 (American Type Culture Collection, Rockville, MD) was obtained from the bacteria culture collection of Seoul National University (Seoul, South Korea). A single colony cultivated from frozen stock on tryptic soy agar (TSA; Difco, Becton, Dickinson, Sparks, MD) was inoculated into 5 ml of tryptic soy broth (TSB; Difco, Becton, Dickinson, Sparks, MD) and incubated in a shaking incubator at 37 °C and 250 rpm for 24 h. To approximate a population of ca. 10^6 CFU/ml of cell suspension in 50 ml TSB, optical density at 600 nm of the cell suspension was determined with a spectrofluorophotometer (Spectramax M2e; Molecular Devices, Sunnyvale, CA) and adjusted to 0.1 with 50 ml TSB amended with 0.5 or 4.5% NaCl. These cell suspensions were incubated under following three growth conditions; 37 °C with 0.5% NaCl (*E*-control), 15 °C with 0.5% NaCl (*E*-15 °C), or 37 °C with 4.5% NaCl (*E*-NaCl).

2.2. Growth kinetics of pathogen

The growth aspect of *E. coli* O157:H7 ATCC 35150 was determined by withdrawing 1 ml of samples at appropriate time intervals. Sampling times intervals were 0.3–1, 2–3, and 3–3.5 h for *E*-control, *E*-NaCl, and *E*-15 °C, respectively. Populations of viable cells were enumerated by plating each sample onto Sorbitol MacConkey (SMAC) agar (Difco). Populations were plotted against time and analyzed by the modified Gompertz equation using GraphPad PRISM (GraphPad Software, Inc., San Diego, CA, USA) as described by (Cho, Lee, Lim, Kwak, & Hwang, 2011; Zwietering, Jongenburger, Rombouts, & Van't Riet, 1990).

$$N = N_0 + C(\exp(-\exp((2.718*u/C)*(M-X)+1))$$

Where N ($\log_{10}$ CFU/ml) is the population of bacteria at time X, N_0 ($\log_{10}$ CFU/ml) is the initial population, C ($\log_{10}$ CFU/ml) is the difference between initial and final cell numbers, u ($\log_{10}$ CFU/h) is the maximum specific growth rate, M (h) is the lag time, and X (h) is the growth time.

2.3. Sample preparation and inoculation

Pasteurized salsa (pH 3.7), purchased at a local grocery store (Seoul, South Korea) and stored at room temperature (22 ± 1 °C), was used in this experiment. The salsa contained no chemical preservatives and included tomatoes, jalapeño peppers, onions, garlic, and distilled vinegar. Fifty grams of each sample were put into the ohmic heating chamber.

Cells of *E. coli* O157:H7 ATCC 35150 grown to early stationary phase under the three different growth conditions were collected by centrifugation at 4,000 × *g* for 20 min at 4 °C. Pellets were resuspended in 9 ml of 0.2% peptone water (PW; Bacto, Becton, Dickinson, Sparks, MD), and the suspended pellets contained 10^{9-10} CFU/ml numbers of cells. The cell suspension was inoculated into each prepared sample before treatment. The volume of inoculum was adjusted to attain a final bacterial population of 10^6–10^7 CFU/g.

2.4. Ohmic heating treatment

Inoculated samples were subjected to pulsed ohmic heating (0.05 duty ratio, 500 Hz) with fixed electric strength (7.0 Vrms/cm). Ohmic heating treatments were carried out in a previous described apparatus (Kim & Kang, 2017a). The ohmic heating system consisted of a function generator (catalog number 33210A; Agilent Technologies, Palo Alto, CA), a precision power amplifier (catalog number 4510; NF Corp., Yokohama, Japan), a two-channel digital-storage oscilloscope (catalog number TDS2001C; Tektronix, Inc., Beaverton, CO), a data logger

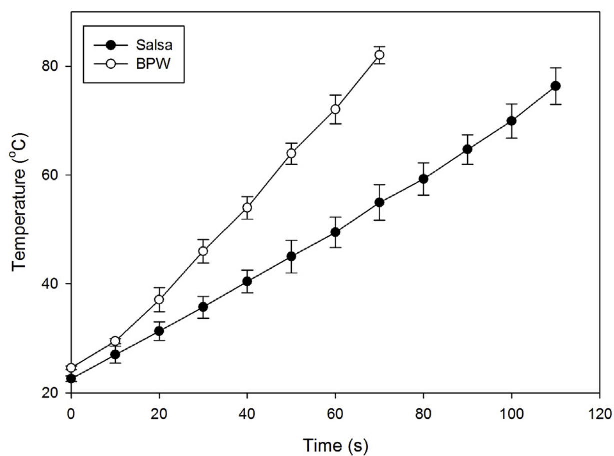

Fig. 1. Temperature history of salsa and buffered peptone water (BPW) subjected to ohmic heating.

(catalog number 34970A; Agilent Technologies), and an ohmic heating chamber. The function generator produced various waveforms at frequencies from 1 mHz to 10 MHz and a maximum output level of 5 V. The signals generated through the power amplifier were amplified up to a maximum output of 141 V alternating current (AC) by the power amplifier. The expanded signals were delivered to each of two titanium electrodes. The two-channel digital storage oscilloscope was used to measure signals, including waveform, frequency, voltage, and current. K-type thermocouples were inserted at the center of the ohmic heating chamber and temperatures were recorded at 0.6-s intervals by a data logger. The distance between the two electrodes was 4 cm, and the cross-sectional area was 60 cm^2. Temperature increase of buffered peptone water and salsa subjected to ohmic heating was observed (Fig. 1). Samples were taken after each treatment and populations of surviving microorganisms were enumerated.

2.5. Bacterial enumeration

For microbial enumeration, each treated 50 ml sample was immediately transferred into a sterile stomacher bag (Labplas, Inc., Sainte-Julie, Quebec, Canada) containing 100 ml of sterile 0.2% PW and homogenized for 2 min using a stomacher (Easy Mix; AES Chemunex, Rennes, France). After homogenization, 1 ml samples were 10-fold serially diluted with 9 ml of sterile 0.2% PW and 0.1 ml of stomached or diluted samples were spread plated onto Sorbitol MacConkey (SMAC) agar (Difco). All plates were incubated at 37 °C for 24 h before counting colonies characteristic of the pathogen. Phenol red agar base with 1% sorbitol (SPRAB; Difco) was used to recover injured cells of *E. coli* O157:H7 (Lee and Baek, 2008). After incubation at 37 °C for 24 h, typical white colonies characteristic of *E. coli* O157:H7 were enumerated. Randomly selected isolates from SPRAB plates were subjected to serological confirmation as *E. coli* O157:H7 (RIM, *E. coli* O157:H7 latex agglutination test; Remel, Lenexa, KS), because SPRAB is not typically used as a selective agar for enumerating *E. coli* O157:H7.

2.6. RNA extraction and real-time RT-PCR analysis

One ml *of E. coli* O157:H7 ATCC 35150 grown under the three different conditions was collected by centrifugation at 4,000 ×g for 20 min at 4 °C. Supernatants were discarded, and the cell pellets were resuspended in 1 ml phosphate-buffered saline (PBS; Corning, pH 7.4). Total RNA was extracted in centrifuge tube using the miRNeasy Mini Kit (Qiagen Inc., Hilden, Germany) according to the manufacturer's protocol. Extracted total RNA was subjected to reverse transcription using QuantiTect Reverse Transcription Kit (Qiagen Inc.).

Real-time reverse transcription polymerase chain reaction (real-time RT-PCR) was performed using a Real-time PCR thermocycler (Exicycler 96 Real-Time Quantitative Thermal Block; Bioneer, Daejeon, Korea) with conditions comprised of pretreatment at 50 °C for 2 min and initial denaturation at 95 °C for 10 min and 40 cycles of denaturation, annealing, and extension at 95 °C for 15 s, 63 °C for 1 min, and 72 °C for 30 s, respectively. Primers used in the present study are listed in Table S1. Relative expression was assayed with each 25 µl reaction mixture containing 12.5 µl of SYBR Select Master Mix (Applied Biosystems, Carlsbad, CA, USA), 2.5 µl of 0.2 µM of each primer, 2 µl of reverse-transcribed cDNA, and 5.5 µl of diethyl pyrocarbonate (DEPC) treated water. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the reference gene. Relative gene expression levels were determined using the $2^{-\triangle\triangle C_T}$ method (Livak & Schmittgen, 2001). Gene expressions of *E*-control were used as a control.

2.7. Membrane lipid composition

Membrane fatty acid profiles of *E. coli* O157:H7 ATCC 35150 grown under the three growth conditions were analyzed. The cells were collected by centrifugation at 4,000 × *g* for 20 min at 4 °C after incubation to early stationary phase. Each pellet was subjected to fatty acid extraction as described in MIDI Technical note no. 101 (7). Mixtures of hexane and methyl *tert*-butyl ether were used to extract the fatty acid methyl esters (FAMEs). FAMEs in the upper phase were analyzed with an Agilent gas chromatograph (model 7890A, Agilent Technologies, Santa Clara, CA, USA) equipped with a split-capillary injector and a flame ionization detector (Garcés & Mancha, 1993). Separations were obtained using a DB-23 column (60 mm × 0.25 mm I. d., 0.25 µm, Agilent Technologies). The injector temperature was set at 250 °C, the column oven at 50 °C for 1 min, followed by an increase at a rate of 15 °C/min to 130 °C, 8 °C/min to 170 °C, and 2 °C/min to 215 °C, which was held for 10 min. Hydrogen, air, and helium were used as the carrier gas, and the flow rate was set to 35 ml/min, 350 ml/min, and 35 ml/min, respectively. The detector temperature was held at 280 °C. Supelco 37 component FAME mix (Supelco, Inc., PA, USA) was used for analyzing fatty acid profiles. Relative contents (%) of total saturated fatty acid (SFA) and unsaturated fatty acid (USFA) were represented and ratios were calculated by dividing the relative content of USFA to SFA (USFA/SFA).

2.8. Propidium iodide uptake test

The fluorescent dye propidium iodide (PI; Sigma-Aldrich, P4170) was used to determine cell membrane damage. The PI uptake test was conducted using a slight modification of the method described previously (Kim & Kang, 2017a). Inoculated buffered peptone water (Difco, Sparks, MD, pH 7.2) was subjected to pulsed ohmic heating (0.05 duty ratio, 500 Hz) with fixed electric strength (13.4 Vrms/cm). Untreated and treated buffered peptone water were centrifuged at 10,000 × *g* for 10 min. Supernatants were discarded, and the cell pellets were resuspended in 1 ml PBS (Corning, pH 7.4) to an optical density at 680 nm of approximately 0.1 with a spectrofluorophotometer (Spectramax M2e; CA). PI was added to a final concentration of 2.9 µM and incubated for 10 min. After incubation, samples were centrifuged under the same conditions. The final cell pellets were resuspended in 1 ml PBS and fluorescence was measured with the spectrofluorophotometer at an excitation wavelength of 493 nm and an emission wavelength of 630 nm. Fluorescence data obtained for untreated cells were subtracted from all treated values and then normalized for OD_{680}.

PI value = (fluorescence value of treated cells – fluorescence value of untreated cells)/(OD_{680}*10)

2.9. Statistical analysis

All experiments were replicated three times. Membrane fatty acid and PI uptake data were analyzed by the analysis of variance procedure of the Statistical Analysis System (version 9.3, SAS Institute, Cary, NC) and mean values were separated using Duncan's multiple-range test. Significant differences in the processing treatments were determined at a significance level of $\alpha = 0.05$.

3. Results

3.1. Growth kinetics of E. coli O157:H7 ATCC 35150 cultured under different conditions

Growth conditions of low temperature or high NaCl concentration influenced the growth kinetics of *E. coli* O157:H7 ATCC 35150 (Table S2). The difference between initial and final cell numbers (C) decreased with added NaCl or decreased growth temperature. C values ($\log_{10}$ CFU/ml) were 3.12, 1.91, and 2.52 for *E*-control, *E*-NaCl, and *E*-15 °C, respectively. Increasing NaCl concentration did not influence the maximum specific growth rate value (u) while this value decreased with decreasing temperature. The u values ($\log_{10}$ CFU/h) were 1.14, 1.29, and 0.17 for *E*-control, *E*-NaCl, and *E*-15 °C, respectively. Lag time (M) increased with added NaCl or decreasing temperature. The M values (h) were 0.51, 3.09, and 14.3 for *E*-control, *E*-NaCl, and *E*-15 °C, respectively.

3.2. Reduction of E. coli O157:H7 ATCC 35150 grown under different conditions

Growth conditions had a significant effect on the inactivation of *E. coli* O157:H7 ATCC 35150 by ohmic heating in salsa (Fig. 2). Reduction levels increased as treatment time increased regardless of growth conditions and enumeration media. When enumerated on selective media (Fig. 2A), reduction levels (log CFU/g) of *E*-15 °C were not significantly different ($p > 0.05$) from those of *E*-control while reduction levels of *E*-NaCl were lower than *E*-control after 110 s treatment. For example, the reductions after 120 s ohmic heating treatment were 4.37, 4.02, and 2.23 for *E*-control, *E*-15 °C, and *E*-NaCl, respectively. When enumerated on resuscitation media (Fig. 2B), reduction levels of *E*-15 °C were higher than the *E*-control while reduction levels of *E*-NaCl were lower than the control after 110s treatment. For instance, the reductions following 120 s ohmic heating treatment were 2.68, 3.01, and 1.60 for *E*-control, *E*-15 °C, and *E*-NaCl, respectively. Initial cell counts (log CFU/g) were 7.91, 8.44, and 7.59 for *E*-control, *E*-15 °C, and *E*-NaCl, respectively, when enumerated on selective media. Initial cell counts (log CFU/g) were higher when enumerated on resuscitation media resulting in 8.17, 8.57, and 8.26 for *E*-control, *E*-15 °C, and *E*-NaCl, respectively.

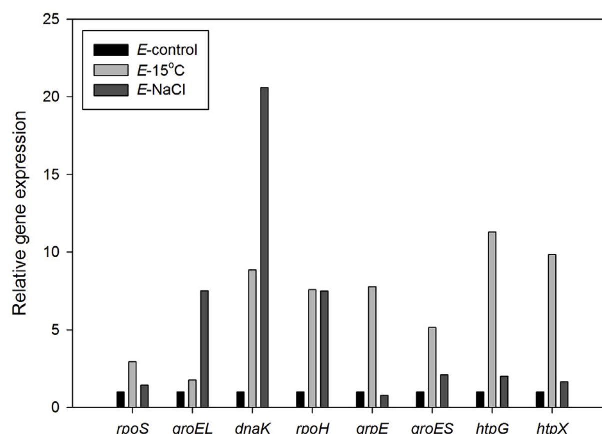

Fig. 3. Relative expression levels of *rpoS*, *groEL*, *dnaK*, *rpoH*, *grpE*, *groES*, *htpG*, and *htpX* of *E. coli* O157:H7 ATCC 35150 cultured at different conditions. Cell suspension incubated at 37 °C with 0.5% NaCl, 15 °C with 0.5% NaCl, or 37 °C with 4.5% NaCl represented as *E*-control, *E*-15 °C, and *E*-NaCl, respectively.

3.3. Heat stress related gene expression of E. coli O157:H7 ATCC 35150 grown under different conditions

Growth conditions had a significant effect on the heat stress related gene expression of *E. coli* O157:H7 ATCC 35150 (Fig. 3). Some heat stress related genes were induced by adding NaCl or decreasing temperature in the growth medium. In particular, *dnaK, rpoH, grpE, groES, htpG*, and *htpX* were up-regulated (≥5 fold change) for *E*-15 °C. The relative gene expression levels were 11.3, 9.8, 8.86, 7.78, 7.59, and 5.15 for *htpG*, *htpX*, *danK*, *grpE*, *rpoH*, and *groES*, respectively (Table S3). On the other hand, *groEL, dnaK*, and *rpoH* were up-regulated (≥5 fold change) for *E*-NaCl. The relative gene expression levels were 20.6, 7.52 and 7.48 for *dnaK*, *groEL*, and *rpoH*, respectively (Table S3).

3.4. Membrane lipid composition of E. coli O157:H7 ATCC 35150 grown under different conditions

Growth conditions had a significant effect on the membrane lipid composition of *E. coli* O157:H7 ATCC 35150 (Table 1 and Fig. S1). Both low temperature and high NaCl concentration conditions increased ratio of USFA. Even though growth conditions has no significant effect

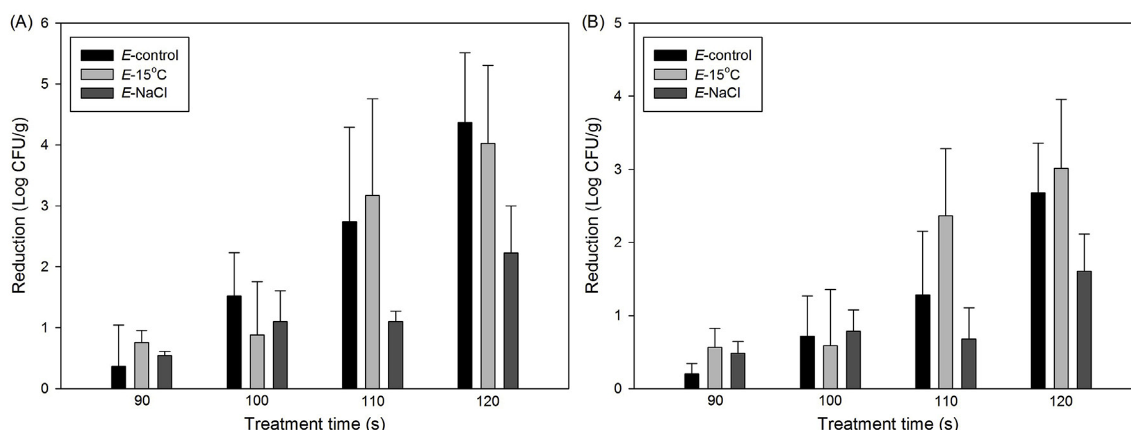

Fig. 2. Reduction of *E. coli* O157:H7 ATCC 35150 subjected to ohmic heating in salsa after growth under different conditions and enumerated on Sorbitol MacConkey agar (A) or Phenol red agar base with 1% sorbitol (B). Cell suspension incubated at 37 °C with 0.5% NaCl, 15 °C with 0.5% NaCl, or 37 °C with 4.5% NaCl represented as *E*-control, *E*-15 °C, or *E*-NaCl, respectively.

Table 1
Total saturated fatty acid (SFA) and unsaturated fatty acid (USFA) of *E. coli* O157:H7 ATCC 35150 after subjection to different cultuing conditions. Cell suspension incubated at 37 °C with 0.5% NaCl, 15 °C with 0.5% NaCl, or 37 °C with 4.5% NaCl represented as *E*-control, *E*-15 °C, and *E*-NaCl, respectively.

	E-control	*E*-NaCl	*E*-15 °C
Total USFA	6.76 ± 0.13 A[a]	18.50 ± 2.79 B	46.16 ± 1.33 C
Total SFA	93.24 ± 0.13 C	81.50 ± 2.79 B	53.84 ± 1.33 A
Ratio[b]	0.07 ± 0.00 A	0.23 ± 0.04 B	0.86 ± 0.05 C

[a] Values in the same row followed by the same letter are not significantly different ($p > 0.05$).
[b] Relative content (%) of total saturated fatty acid (SFA) and unsaturated fatty acid (USFA) were represented and ratio were calculated by dividing the relative content of USFA to SFA (USFA/SFA).

on the relative content of lauric acid (C12:0), myristic acid (C14:0), stearic acid (C18:0), heneicosanoic acid methyl ester (C21:0), or beheric acid methyl ester (C22:0), the relative content of palmitoleic acid (16:1) increased by adding NaCl or decreasing temperature in the growth medium while that of palmitic acid (16:0) decreased. As a result, the relative ratio (%) of USFA was the highest for *E*-15 °C (46.16) followed by *E*-NaCl (18.50) and *E*-control (6.76), and ratios of USFA to SFA were 0.07, 0.23, and 0.86 for *E*-control, *E*-NaCl, and *E*-15 °C, respectively.

3.5. PI uptake levels of E. coli O157:H7 ATCC 35150 grown under different conditions

Decreasing growth temperature had a significant effect on the PI uptake levels of *E. coli* O157:H7 ATCC 35150 subjected to ohmic heating (Table 2). PI uptake values increased as treatment time increased regardless of growth conditions. Even though significant differences were not observed for *E. coli* O157:H7 subjected to ohmic heating for 40 s and 50s ($p > 0.05$), PI uptake values of *E*-15 °C were significantly higher than that of *E*-control or *E*-NaCl when subjected to ohmic heating for 60 s or 70s ($p < 0.05$). For example, the values after 70 s ohmic heating treatment were 58.3, 51.4, and 78.6 for *E*-control, *E*-NaCl, and *E*-15 °C, respectively. In contrast to decreasing temperature, adding NaCl to the growth medium had no significant effect on PI uptake value.

4. Discussion

Growth conditions of decreasing temperature or increasing NaCl concentration influenced the resistances of *E. coli* O157:H7 ATCC 35150 to ohmic heating in the present study. At first, resistance of the pathogen decreased as growth temperature decreased from 37 °C to 15 °C. This result is in harmony with the previous research investigations reporting decreased heat resistance of foodborne pathogens grown at low temperatures. Yang, Khoo, Zheng, Chung, and Yuk (2014) identified that *Salmonella* Enteritidis grown at relatively low temperatures (10 °C and 25 °C) exhibited lower heat resistance than cells grown at relatively high temperature (37 °C and 42 °C). Leenanon and Drake (2001) and Zhang and Griffiths (2003) also reported that thermal tolerance of *E. coli* O157:H7 appears to be lower under refrigeration temperatures. In contrast to the effect of decreased temperature, the resistance of *E. coli* O157:H7 ATCC 35150 to ohmic heating increased as NaCl concentration in the growth medium increased from 0.5% to 4.5% in the present study. Jørgensen, Stephens, and Knøchel (1995) also reported that *Listeria monocytogenes* grown in medium of high NaCl concentration showed greater thermotolerance than this pathogen grown in normal medium. However, to the best of our knowledge, the reason for decreased or increased heat resistance relative to growth temperature or NaCl concentration is remains uncertain. In the present study, it was assumed that heat shock response and alternation in membrane lipid composition of pathogen contribute the resistance decrease or increase.

Bacterial heat shock response is involved not only in heat shock stress, but is also involved in many unfavorable environmental conditions (Ban, Kang, & Yoon, 2015). It is well known that induced heat shock proteins play a significant role in the cross protection of bacteria (Zhang & Griffiths, 2003). In this regard, we identified the genetic response to the unfavorable conditions of low temperature (15 °C) or high NaCl concentration (4.5%) in the present study. Several heat stress related genes were activated by decreasing growth temperature or increasing NaCl concentration. By increasing the NaCl concentration, genes encoding heat shock proteins such as *groEL* and *dnaK* were induced, which is in accordance with previous research investigations (Duché, Trémoulet, Glaser, & Labadie, 2002; Solheim et al., 2014). Additionally, *rpoH*, which regulates heat shock response, was also induced in the present study. These results coincide with our finding that resistance of *E. coli* O157:H7 ATCC 35150 to ohmic heating increased after this bacterium was grown in medium of high NaCl concentration. However, the resistance of *E. coli* O157:H7 ATCC 35150 to ohmic heating decreased after growing at low temperatures even though heat stress related genes such as *dnaK*, *rpoH*, *grpE*, *groES*, *htpG*, and *htpX* were induced with decreasing growth temperature. From these results we assumed that there must be another factor influencing the resistance of the pathogen to ohmic heating. Because the cell membrane is a major bactericidal target of thermal treatment, we paid attention to changes in the cell membrane relative to growth conditions.

Many foodborne pathogens can survive under unfavorable conditions by altering membrane fluidity (Beales, 2004). Regulating the ratio of USFA to SFA is one way to retain membrane fluidity by *E. coli* O157:H7 (Yoon, Lee, Lee, Kim, & Choi, 2015). In the present study, the ratio of USFA to SFA increased slightly (+0.16) or significantly (+0.79) with increasing NaCl concentration or decreasing temperatures, respectively. Changes in fatty acid composition by decreasing growth temperatures were reported previously. Casadei, Manas, Niven, Needs, and Mackey (2002) reported that the fluidity index (ratio of USFA to SFA) increased as growth temperature decreased in the range of 45 °C–10 °C. It is crucial for bacteria to increase membrane fluidity by altering the ratio of USFA to SFA because it enables membrane proteins to continue to pump ions, take up nutrients, and perform respiration (Berry & Foegeding, 1997). In contrast to the effect of growth temperature, the major change in response to high NaCl concentration occurs in the head group of lipids (Beales, 2004). Generally, the

Table 2
PI uptake value of *E. coli* O157:H7 ATCC 35150 subjected to ohmic heating after growth under different culturing conditions. Cell suspension incubated at 37 °C with 0.5% NaCl, 15 °C with 0.5% NaCl, or 37 °C with 4.5% NaCl represented as *E*-control, *E*-15 °C, and *E*-NaCl, respectively[a].

	40 s	50 s	60 s	70 s
E-control	5.2 ± 0.7 Aa	23.6 ± 10.9 Aa	43.1 ± 5.4 Ab	58.3 ± 11.6 ABb
E-NaCl	7.2 ± 2.9 Aa	26.2 ± 11.2 Ab	41.2 ± 4.3 Abc	51.4 ± 11.0 Ac
E-15 °C	4.7 ± 2.8 Aa	28.1 ± 8.00 Ab	66.9 ± 4.5 Bc	78.6 ± 8.80 Bc

Mean values ± standard deviation.
Values in the same column followed by the same upper case letter are not significantly different ($p > 0.05$).
Values in the same row followed by the same lower case letter are not significantly different ($p > 0.05$).

proportion of anionic phospholipids and/or glycolipids increases to preserve membrane lipids in the proper bilayer phase when a_w decreases by means of high NaCl concentration (Russell et al., 1995). This is why the ratio of USFA to SFA increased only slightly (+0.16) by increasing NaCl concentration in the present study. We postulated that altered membrane fluidity to allow survival and growth under unfavorable conditions would affect membrane damage by thermal treatment.

In the present study, PI uptake values of *E. coli* O157:H7, which represent cell membrane damage of this pathogen, were not significantly influenced by increasing NaCl concentration at all treatment times ($p > 0.05$). This result indicates that the cell membrane changes induced by increasing NaCl concentration were not significant enough influence cell membrane damage by ohmic heating. On the other hand, PI uptake values of the pathogen increased significantly ($p < 0.05$) with decreasing growth temperature when subjected to ohmic heating for 60 s and 70 s. Increased membrane fluidity by means of the changed USFA to SFA ratio may have influenced the cell membrane damage by ohmic heating. Even though thermal inactivation is the major component of ohmic heating, a non-thermal effect also has been reported (Park & Kang, 2013). In particular, cell membrane pore formation was revealed as important in non-thermal effect in the study of Park and Kang (2013). Therefore, increased membrane fluidity associated with decreasing growth temperature not only made the membrane more susceptible to thermal damage but also more vulnerable to the non-thermal effect of ohmic heating.

In conclusion, several heat stress related genes were up-regulated when *E. coli* O157:H7 ATCC 35150 was grown at low temperature (15 °C) or with high NaCl concentration (4.5%). Meanwhile, the ratio of USFA to SFA in the cell membrane increased significantly or slightly for the pathogen grown at low temperature or with high NaCl concentration, respectively. As a result, cell membrane damages were significantly higher for the pathogen grown at low temperature ($p < 0.05$) while no significant differences were observed for the pathogen grown at high NaCl concentration ($p > 0.05$). For the pathogen grown at low temperature, the effect of increased cell membrane damage was dominant compared to heat stress related gene expression. On the other hand, for the pathogen grown with high NaCl concentration, the effect of heat stress related gene expression was significant while additional cell membrane damage was not observed. Consequently, resistance of *E. coli* O157:H7 ATCC 35150 to ohmic heating increased when grown with high NaCl concentration while the resistance decreased for the pathogen grown at low temperature. Because storage temperature and NaCl concentration of salsa vary depending on the climate, season and type of product, respectively, processing conditions for ohmic heating should be determined carefully considering the growth conditions of *E. coli* O157:H7 ATCC 35150.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2019.03.037.

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