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Inactivation of *E. coli O157:H7* by Ohmic Heating at Different Frequencies and Temperatures in Buffer and Pomelo Juice

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The influence of an alternating current (AC) frequency from 50 to 20,000 Hz at 30 V/cm using ohmic heating on the inactivation of *E. coli O157:H7* in buffer peptone water (BPW) and pomelo juice was investigated. Frequency affected bacterial reduction in both BPW and pomelo juice with the same trend, and the most efficient reductions were obtained at 60 and ≥ 500 Hz. Besides, the resistance of *E.coli O157:H7* was investigated. BPW and pomelo juice were treated at different temperatures (62-71 °C) for various times (0-30 s) by ohmic heating (OH) and compared with conventional heating (CH). The technical parameters of OH were an electric field strength of 30 V/cm and frequencies of 60 and 500 Hz. At each temperature, the decimal reduction time (D) and the decimal reduction temperature (z) were determined. Microorganism reduction was significantly higher ($P < 0.05$) with OH than with CH, and there was no significant difference between the juice samples treated by OH at 60 and 500 Hz. For OH at 60 Hz, the D values of *E. coli O157:H7* in pomelo juice were 59.0, 27.3, 10.3, and 3.6 s at temperatures of 60, 62, 65, and 68 °C, with a z value of 6.7 °C. For CH, the D values were 35.4, 14.3, 8.2, and 2.9 s, with a z value of 8.6 °C. From the calculated D and z values, a processing schedule for the pasteurization of pomelo juice can be estimated. Observing the bacterial cells by transmission electron microscopy (TEM) showed considerable changes in the morphology of the bacterial cells with OH, which might be the cause of cell death. These results demonstrated that the electric field obtained with OH yields additional microbial destruction. As a result, the time and temperature required for bacterial inactivation can be reduced, diminishing the negative heat effects of pasteurization on the food products.

1. Introduction

Pomelo (*Citrus maxima)* juice has been known as a rich bioactive resource. The major oxidation reaction in humans may be inhibited by the resource, which is a natural antioxidant (Liu et al., 2012). To assure microbial safety for the juice consumer, the Food and Drug Administration (FDA) has issued a regulation requiring juice processors to achieve a 5-log reduction of the most resistant pathogen. *E. coli O157:H7*, the pathogen in outbreaks associated with the consumption of acidic foods, needs to be controlled. *E. coli O157:H7* is the most common serotype of *enterohemorrhagic E. coli* (EHEC), causing severe diseases in humans worldwide, with a *low infectious dose* and symptoms of serious illness. FDA recommended that if there is no link between a product with specific pathogenic strains, *E. coli O157:H7* should be used as a target microorganism in acid juice (pH ≤ 4.6) (FDA, 2004).

Heat treatment is an important process for inactivating microorganisms for food safety. Conventional heating (CH) supplies heat to food by using the traditional hot-fill process according to the conduction and the convection mechanism. Due to the low conduction of food, the speed of heat transfer is low, and outside layers are overheated. As a result, this process causes loss of nutritional and organoleptic qualities as well as energy waste. Recently, several electro-technologies, such as microwave (Ptak et al., 2019), radio-frequency heating (Bedane et al., 2017), and ohmic heating (OH), have been recognized to have great potential in addressing the drawbacks of CH. Among them, OH, which is suitable for liquid foods, overcomes the disadvantages of CH with milder conditions. OH is a process by which heat is generated when an alternating current (AC) goes through a food mass. The food mass has electrical resistance, so a part of the electrical

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energy is converted into heat energy inside the material (De Alwis and Fryer, 1992). This technology generates heat uniformly and rapidly, resulting in less thermal damage to the food product. Therefore, the product remains of high quality, because of minimal structural, nutritional, and organoleptic changes, and is biological safe (Lee et al., 2013). Although OH has been proposed as a promising food processing technology, it has some technical drawbacks. It is known that electrolysis reactions, namely product burning and corrosion at the electrodes' surface, take place for direct current (DC) and AC at low frequencies (Yongsawatdigul et al., 1995). Lee et al. showed that a high frequency of over 100 Hz can avoid this problem (Lee et al., 2013). The current frequency also affects the heating rate and food quality. Lee et al. (2013) reported that increasing the frequency led to an increase in bacterial destruction but no significant nutrition change with frequencies above 300 Hz. Sastry and Barach (2000), on the other hand, revealed that using low frequencies (50-60 Hz) formed pores in the bacterial membrane, which was the main cause of cell death. Thus, there are contradictory findings regarding the impact of frequency on microorganisms. In addition, the effects of this technique depend on the characteristics of the specific food products. Therefore, the objective of the present study was to investigate the impact of AC frequency and temperature on the inactivation of *E. coli O157:H7* in BPW and pomelo juice.

2. Materials and methods

Pomelo fruits (*Citrus maxima*) purchased from farms were pressed with a squeezer (Fujiyama -FJ-400, sieve opening: 1 mm) and kept at -20 °C until the experiments were carried out. The pomelo juice was selected based on a similar degree of total soluble solids, pH, and electrical conductivity values, with 11.0 ± 0.5 °Bx and 4.0 ± 0.2 and 4.0 ± 0.5 mS/cm. The juice was sterilized at 121 °C for 15 min and then cooled.

E. coli O157:H7 (ATCC 43888) was supplied by the American Type Culture Collection (USA). The strain was maintained on tryptic soy agar (TSA) (Sigma-Aldrich, USA) at 4 °C. A single colony of strain cultivated on TSA was grown in 5 mL tryptic soy broth (TSB) (Sigma-Aldrich) incubated at 37 °C for 14 h. The pellets were collected by centrifugation at 4,000 g for 20 min at 4 °C and washed three times with 0.2 % peptone water (PW) and then resuspended in 5 mL of BPW, corresponding to approximately 10^8 – 10^9 CFU/mL.

The OH apparatus consists of a function generator (FG-7005C, Korea) with a frequency of 1 to 10 MHz, an output value of 5 V, a power amplifier (P7000S-Yamaha, Japan) amplifying up to a maximum output of 220 V, a data logger (TR-71WF-T&D, Japan), and an OH chamber. The amplified current went through each side of the electrodes in the heating chamber. The chamber was a Teflon rectangular heating container (2 x 14.5 x 7.5 cm) with a 2 cm distance between the two titanium electrode plates. The temperature of the samples was measured with thermocouples located in the centre of the chamber.

For OH, 0.5 mL of the culture (*E. coli O157:H7*) was added to 50 mL of sterilized BPW/pomelo juice at the target temperature. For CH, 10 mL of sterilized BPW/pomelo juice in the glass tube was heated in a thermostatic water bath (WNE10, Memmert, Germany) operating at 90 °C. When the juice reached the target temperature, 0.1 mL of the culture was added to obtain approximately 106 – 107 CFU/mL. The treated samples by either OH or CH remained at the target temperature for 10-30 s. Then, one mL of the treated samples was transferred into sterile tubes and cooled rapidly to prevent the thermal degradation effect.

To determine the density of the cells, the modified method of Park and Kang (2013) was applied. The sample was 10-fold serially diluted with 0.2 % PW. Then, 0.1 mL of diluted sample was spread-plated onto selective media of eosin methylene blue agar for the enumeration of *E. coli O157:H7*. When the bacterial cells survived at a low density, an extract 1 mL of the treated sample was immediately spread on four plates (about 250 µL per plate to lower the detection limit). After that, all plates were incubated at 37 °C for 24-48 h before counting.

2.1 2.1 Experiment 1: Influence of AC frequency on the inactivation of *E. coli O157:H7*

The electric field strength was fixed at 30 V/cm, and a range of frequencies (50, 60, 70, 100, 500, 1,000, 10,000, and 20,000 Hz) was investigated. The treatments were conducted at 65 °C for 30 s, and then one mL of the treated samples was transferred into sterile tubes and cooled rapidly. The results were evaluated through the density of microorganisms.

2.2 Experiment 2: Influence of temperature on the inactivation of *E. coli O157:H7*

The treatments were examined at 60, 62, 65, 68, and 71 °C for 10, 20, 25, and 30 s at 60 and 500 Hz. The treated samples were transferred into sterile tubes and cooled rapidly. The influence of temperature was evaluated through the density of microorganisms.

2.2 2.3 Determination of the D and z value

The destruction of *E. coli* was estimated with a first-order model (Opstal *et al.*, 2005). The survivors of *E. coli O157:H7* were plotted against heating times at each constant temperature. The following linear primary model

was used to model the thermal destruction of *E. coli:* log (N) = log (N_o) + s.t, where N is the number of cells (CFU/mL) that survived after heating at time t, N_o is the number of cells (CFU/mL) that were alive at time t = 0, s is the slope of the survival curve, and t = treatment time (s) at a specified temperature. The decimal reduction times (D values) were calculated from a plot of the logarithm of the survived microorganism curves versus heating time. The D values were determined at each temperature by taking the negative inverse of the relevant s value. The decimal reduction temperature *(*z value) was determined by taking the negative inverse slope of the plot of log (D) versus temperature.

2.4 Experiment 3: Determination of the structural changes in *E. coli O157:H7*

To investigate the structural changes in *E. coli O157:H7***,** transmission electron microscopy (TEM) analysis (JEOL JEM-1010, USA) was performed. TEM analysis revealed the cell morphology of *E. coli O157:H7* according to the method of Lee et al. (2012). BPW containing *E. coli O157:H7* was heated at 65 °C for 30 s for both OH and CH.

All experiments were in triplicate, with mean +/- standard deviation reported. The significant differences in the mean values were assessed with a one-way analysis of variance (ANOVA) test at a significance level of $P \leq 0.05$.

3. Results and discussion

3.1 Influence of AC frequency on the inactivation of *E. coli O157:H7* in pomelo juice and BPW

Pomelo juice is a complex mixture of numerous components, such as flavonoids, alkaloids, steroids, terpenoids, and saponins (Oikeh et al., 2016), and can cause microorganism cell death. Therefore, besides pomelo juice, BPW with a neutral environment was used as a control to evaluate the influence of frequency on the inactivation of *E. coli O157:H7* (Figure 1). Overall, the destruction of the microorganisms gradually increased from 50 to 500 Hz ($P \leq 0.05$), but there was no significant effect with higher frequencies than 500 Hz ($P \geq 0.05$), particularly at 60 Hz, the highest destruction efficiency. In addition, the survival rate of *E. coli O157:H7* in BPW was higher than that in pomelo juice. The impact of the frequency on the pathogen showed the same trend for both pomelo juice and BPW.

Many previous studies also demonstrated that fruit juices with low pH increased the efficiency of *E. coli O157:H7* inactivation because this microorganism became more sensitive to thermal treatment in acid conditions (Opstal et al., 2005). Contradictory findings have been produced regarding the influence of frequency on microbial inactivity. Lee et al. (2012) indicated that an increase in frequency from 60 to 500 Hz increased cell inactivation. In contrast, Sastry and Barach (2000) suggested that low frequencies (50-60 Hz), causing cell walls to puncture and rupture and therefore intracellular constituents to leak, result in increased lethality of bacteria. The results of our study show a combination of both these trends, with the highest efficiency at 60 Hz and >= 500 Hz. According to Shawki and Gaballah (2015), a frequency from 50 to 500 Hz combined with a low voltage (20 to 160 V/cm) has the effect of changing the membrane conductivity, causing the membrane to break. A higher frequency means more oscillations, increasing the impact between ions and resulting in increased efficiency in killing cells. In particular, at 60 Hz, Schumann resonance occurs (Montiel and Bardasano, 2003), with fast oscillating ions causing the largest change in the cell membrane of microorganisms (Llave et al., 2018). On the other hand, at a high frequency (> 500 Hz), the charge moves and reverses direction quickly, so it has not enough time to attain the threshold electricity breaking the membrane. At the high-frequency range (towards the radio frequency), charged particles with strong fluctuations and large frictions produce much energy. This energy transforms into the vibrational energy of large biological molecules, disrupting the covalent bonds in DNA or proteins and so causing cell death (Hassan and Ramaswamy, 2014).

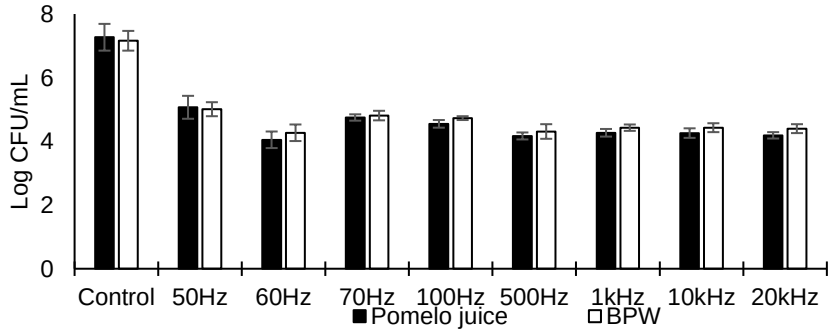

Figure 1: Survival of E. coli O157:H7 in pomelo juice and BPW at different frequencies.

3.2 Influence of temperature (OH and CH) on the inactivation of *E. coli O157:H7*

The inactivation process was investigated with OH and compared with CH. The viability of *E. coli O157:H7* in pomelo juice after OH and CH is shown in Figure 2a - c. The population for the juice samples at different holding temperatures (60, 62, 65, 68, and 71 °C) was determined. In general, the reduction in population size increased with increasing treatment temperature and time. At the same treatment condition, the bactericidal effect by OH was higher than that by CH. For example, with OH, at 65°C for 30 s, the bacterial density reduced by 3.1 and 3.0 log with treatment conditions of 60 and 500 Hz, whereas with CH, the log reduction was 2.2. The number of viable *E. coli O157:H7* in BPW is illustrated in Figure 2d - f. The reduction in pathogen showed the same trend as in the juice. The survival of cells after OH and CH was higher in BPW (pH 7.2) than in pomelo juice (pH 4.0). In general, OH reduced one to three logs more than CH at the same conditions for both BPW and pomelo juice.

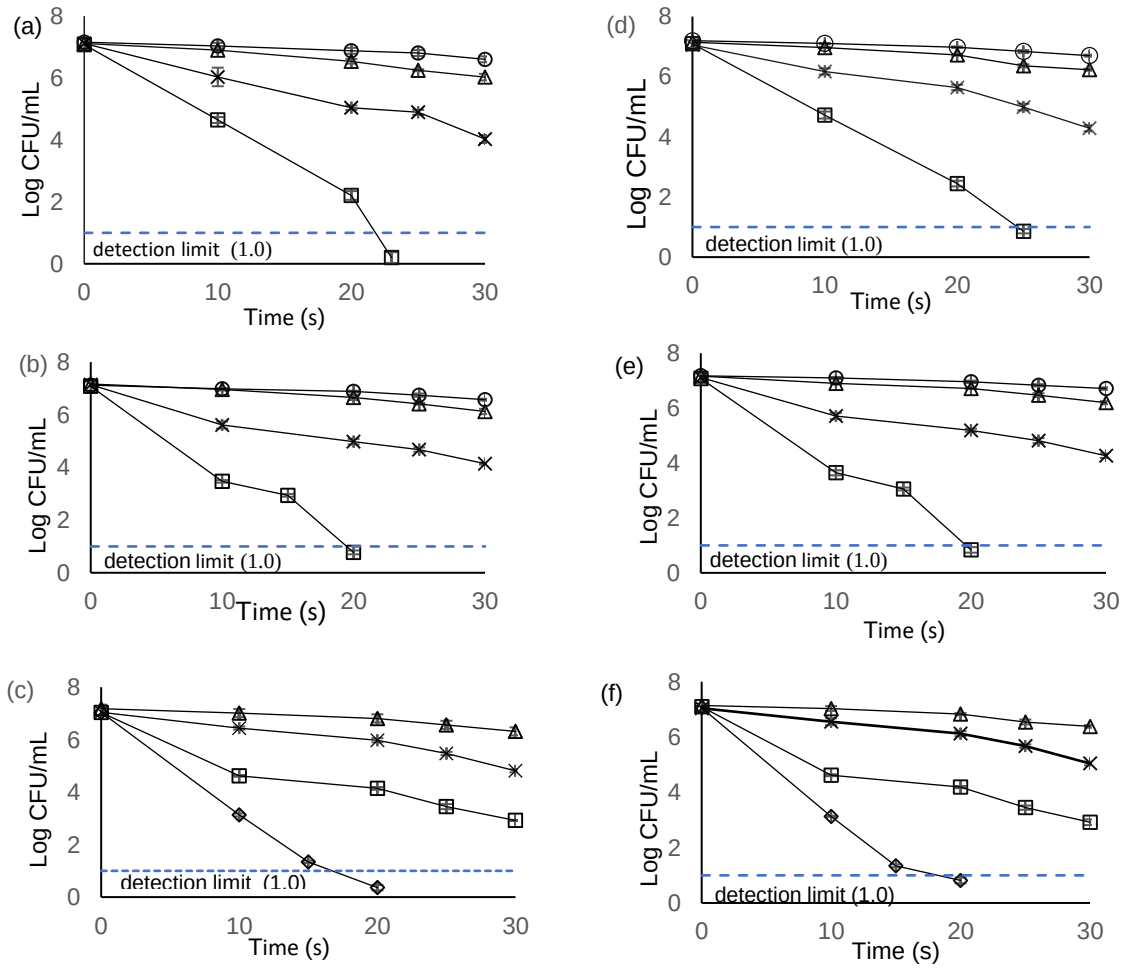

Figure 2: Survival curve of E. coli O157:H7 after heating in pomelo juice (a) 60 Hz,(b) 500 Hz, (c) CH) and BPW (d) 60 Hz, (e) 500 Hz, (f) CH (○ 60 °C, ∆ 62 °C, x 65 °C, □ 68 °C, ◊ 71 °C*).*

3.3 Kinetic parameters of *E. coli O157:H7* in OH and CH

The difference in resistance of *E. coli O157:H7* between OH and CH, as evaluated by the inactivation kinetic parameters, is summarized in Table 1. The inactivation began at 60 °C for OH and at 62 °C for CH. The low temperature of 60 °C can stimulate the growth of *E. coli O157:H7*, thereby contributing to increased heat resistance within the tolerable temperature range of the microorganism (Pereira et al., 2007). The D and z values in the samples treated with OH were lower than those in the samples treated with CH ($P < 0.05$). Therefore, the presence of the electrical current affected the mortality rate and the mortality temperature of *E. coli O157:H7*.

Previous studies have indicated that for *E. coli O157:H7* at 62 °C in white grape juice, the D value was 94.2 s with a z value of 9.2 °C, and at 62, 64, and 66 °C in apple juice these values were 36, 18, and 12 s with a z

value of 9.5 °C by CH (Enache, 2015). In the present study, the D values of *E. coli O157:H7* conventionally heated at 62, 65, 68, and 71 °C were 39.3, 15.6, 8.5, and 3.1 s in BPW and 35.4, 14.3, 8.2, 2.9 s in pomelo juice. Compared with the values obtained in previous studies, our values show some variation in the thermal resistance of the microorganism in food. Several researchers demonstrated that differences in environmental factors, such as pH, soluble solid content, and food composition, influence the heat resistance of *E. coli O157:H7* (Opstal et al., 2005). Therefore, the difference between the composition of the pomelo juice used in this study and the other food may explain the difference. Several researchers compared the influence of OH and CH on the heat resistance of *E. coli O157:H7* in fruit juice. They concluded that pathogen inactivation by OH was more efficient than that by CH because of the thermal effect, with an additional killing effect caused by the electric current (Park and Kang, 2013). In the present study, CH was also less effective for *E. coli O157:H7* reduction than OH. By reducing the time and temperature required for the inactivation of microorganisms, the use of OH in the food industry can diminish the negative heat effects of pasteurization on the food products mentioned.

Table 1: D and z values of *E. coli O157:H7* in OH and CH.

	Temp (°C)	OH (60 Hz), D (s)	z (°C)	OH (500 Hz), D (s)	z (°C)	CH D (s)	z (°C)
Pomelo juice	60	$59.0^{a} \pm 2.4$	6.7	$58.4^{a} \pm 0.5$	6.5	-	8.6
	62	$27.3^{a} \pm 1.4$		$29.3^{a} \pm 1.8$		$35.4^{b} \pm 1.6$	
	65	$10.3^{a} \pm 0.4$		$10.7^{a} \pm 0.2$		$14.3^{b} \pm 0.4$	
	68	$3.6^{a} \pm 0.1$		$3.3^{b} \pm 0.0$		$8.2^{c} \pm 0.1$	
	71	-		-		2.9 ± 0.1	
BPW	60	$62.1^{a} \pm 0.8$	6.8	$65.4^{a} \pm 1.9$	6.2	-	8.4
	62	$31.9^{a} \pm 1.3$		$33.1^{a} \pm 2.7$		$39.3^{b} \pm 2.2$	
	65	$11.4^{a} \pm 0.5$		$11.3^{a} \pm 0.7$		$15.6^{b} \pm 0.4$	
	68	$4.1^{a} \pm 0.1$		$3.4^{b} \pm 0.1$		$8.5^{c} \pm 0.1$	
	71	-		-		3.1 ± 0.1	

Note: The letters a, b, c on the same row represent significant differences ($p < 0.05$).

3.4 Transmission electron microscopy analysis

Analysis of the TEM specimens revealed the external morphology of *E. coli O157:H7* cells (Figure 3). CH and OH were carried out at 65 °C for 30 s in BPW (pH 7.2).

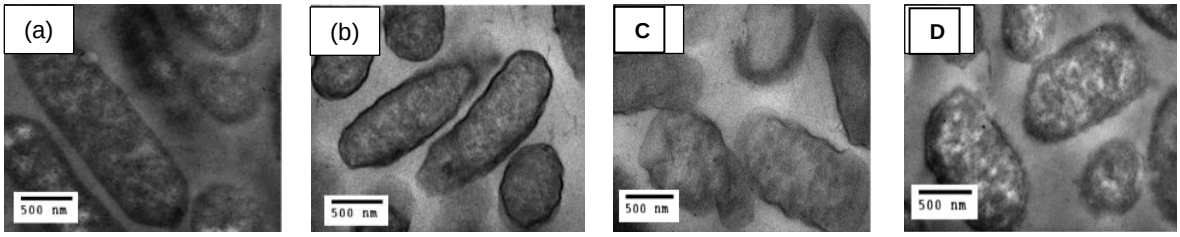

Figure 3: TEM micrographs of (a)non-treated (b)CH, OH (c) 60 Hz (d) 500Hz E. coli O157:H7 cells.

A little morphological change in the cell membrane was observed in CH cells (Figure 3b) with a slightly wrinkled membrane. There were more intense changes after OH than after CH, with gradually enlarged periplasmic space, degenerated intracellular substances, and uneven cell walls compared with conventionally heated cells. For example, the membrane space of OH cells was more damaged at 60 Hz (Figure 3c) than at 500 Hz. The intracellular variability of OH cells was greater at 500 Hz (Figure 3D) than at 60 Hz, but the cell membrane was not broken in both conditions. The increased cell death rate in the presence of thermo-electricity factors in OH was similar to that in the studies of Park and Kang (2013) and Lee et al. (2012). Park and Kang (2013) reported that membrane rupture and leakage of intracellular contents caused cell death in OH, while Lee et al. (2012) showed that OH cells have more space between the cell wall and the membrane than conventionally heated cells and irregular changes in the cell wall. Hence, the mechanism by which OH inactivates bacteria depends on various process parameters and food products.

4. Conclusions

In summary, the results showed that the frequencies that significantly influenced the lethal activity of *E. coli O157:H7* were 60 Hz and >= 500Hz at 30 V/cm. Compared with that by CH, the inactivation efficiency by OH was more than 1-3 log larger. The D and z values of *E. coli O157:H7* for BPW and pomelo were lower with OH than with CH. Determining the parameters helps to calculate the real-time temperature history of a process. Taken together, our results demonstrated that the electric effect of OH was a crucial factor in reducing the times and temperatures of the treatment process by enhancing the levels of inactivation of *E. coli O157:H7*. Therefore, OH is a promising alternative technology for controlling food-borne pathogens, allowing the processor to obtain good products in terms of microbiological, nutritional, and organoleptic quality. In addition to frequency, the electric field strength is also a system parameter of OH. In this study, the electric field strength was fixed at 30 V/cm; the effects of electric field strength will be investigated in our next study.

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