Effect of Frequency and Waveform on Inactivation of *Escherichia coli* O157:H7 and *Salmonella enterica* Serovar Typhimurium in Salsa by Ohmic Heating

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The effect of frequency of alternating current during ohmic heating on electrode corrosion, heating rate, inactivation of food-borne pathogens, and quality of salsa was investigated. The impact of waveform on heating rate was also investigated. Salsa was treated with various frequencies (60 Hz to 20 kHz) and waveforms (sine, square, and sawtooth) at a constant electric field strength of 12.5 V/cm. Electrode corrosion did not occur when the frequency exceeded 1 kHz. The heating rate of the sample was dependent on frequency up to 500 Hz, but there was no significant difference ($P > 0.05$) in the heating rate when the frequency was increased above 1 kHz. The electrical conductivity of the sample increased with a rise in the frequency. At a frequency of 60 Hz, the square wave produced a lower heating rate than that of sine and sawtooth waves. The heating rate between waveforms was not significantly ($P > 0.05$) different when the frequency was > 500 Hz. As the frequency increased, the treatment time required to reduce *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium to below the detection limit (1 log CFU/g) decreased without affecting product quality. These results suggest that ohmic heating can be effectively used to pasteurize salsa and that the effect of inactivation is dependent on frequency and electrical conductivity rather than waveform.

Ohmic heating is a highly attractive technology by which internal heating as a result of electrical resistance is rapidly induced when passing an alternating current through a food product (1). This technology can provide a uniform temperature distribution with the absence of a cold spot in the product, because both liquid and solid phases can be heated simultaneously (2). Therefore, it is ideally suited to thermal processing of solid-liquid food mixtures with little structural, nutritional, or organoleptic changes and as well as those that are microbiologically safe and can be successfully treated using a short processing times (3). Commercial ohmic sterilization has been developed for particulate and multiphase foods based on applied research of ohmic heating (4–6), and it has been widely used in blanching, evaporation, dehydration, fermentation, extraction, and pasteurization (7).

Although viewed as a promising food processing technology, ohmic heating has some technical limitations. Most ohmic heating systems have been used at an alternating current frequency of 50 to 60 Hz. One constraint of low alternating current frequency in ohmic heating is that electrolytic reactions can take place at the electrode surface, leading to product burning and corrosion of electrodes (8, 9). To prevent undesirable electrochemical reactions between electrodes and solid or viscous liquid products, increasing the frequency or changing the waveform of alternating current has been suggested (10). There have been some efforts to clarify the impact of frequency and waveform of alternating current on ohmic heating rates of solid or semisolid foods (11–15). However, there has been no comprehensive research on the effects of frequency on electrode corrosion, electrical conductivity and microbial inactivation in solid-liquid food mixtures. Also, thus far, the ohmic heating rate for different waveforms (sine, square, and sawtooth waves) > 60 Hz was not been studied. Therefore, it is necessary to examine the effect of frequency and waveform on the heating rate of food products in order to ensure uniform commercial

sterilization when applying ohmic heating. Salsa was selected as a model system for investigating the effects of ohmic heating technology on a solid-liquid food mixture. Because of increasing tolerance of food-borne pathogens to low-pH food products, salsa presents a potential public health hazard (16). A large multistate outbreak with more than 1,400 cases of *Salmonella* spp. infections associated with multiple raw produce items such as jalapeño peppers and tomatoes occurred in the United States in July 2008 (17). The implicated foods were often eaten in the form of salsa. During this outbreak, two clusters of 47 and 33 ill persons were significantly associated with eating salsa. Restaurant salsas have been contaminated with *Escherichia coli* in Mexico and Texas (18). In the United States 20 salsa-associated outbreaks were reported to the Centers for Disease Control and Prevention (CDC) between 1998 and 2008. Thermal processing of food products is the most widely used method of food sterilization, but constraints on the number of particulates and the size of particulates still limits the widespread use of conventional heating (19).

The objectives of the present study were to examine the impact of various frequencies and waveforms of alternating current on the heating rate of salsa. The effects of ohmic heating for inactivating *E. coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in salsa, as well as electrode corrosion, electrical conductivity, and quality of salsa, including pH, color, vitamin C, and lycopene, with various electric field frequencies were investigated.

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FIG 1 Schematic diagram of high frequency ohmic heating system at Seoul National University (Seoul, South Korea).

MATERIALS AND METHODS

Bacterial strains and cell suspension. Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, and ATCC 43890) and *S. Typhimurium* (ATCC 19585, ATCC 43971, and DT 104) were obtained from the Food Science and Human Nutrition culture collection at Seoul National University (Seoul, South Korea) for the present study. All strains of *E. coli* O157:H7 and *S. Typhimurium* were cultured individually in 5 ml of tryptic soy broth at 37°C for 24 h, collected by centrifugation at $4,000 \times g$ for 20 min at 4°C, and washed three times with buffered peptone water (BPW; Difco, Sparks, MD). The final pellets were resuspended in BPW, corresponding to ca. 10^8 to 10^9 CFU/ml. To inoculate salsa, suspended pellets of the three strains of both species were combined to construct a mixed culture cocktail. These cell suspensions were used in subsequent experiments.

Sample preparation and inoculation. Pasteurized tomato-based salsa (pH 4.16) was purchased at a local supermarket (Seoul, South Korea). The salsa contained no chemical preservatives and included tomatoes, jalapeño peppers, onions, garlic, and distilled vinegar. For inoculation, 0.2 ml of the mixed culture cocktail (*E. coli* O157:H7 and *S. Typhimurium*) was added to 25 g of salsa at room temperature ($22 \pm 1^\circ\text{C}$) and mixed with a spatula for 2 min. The final cell concentration was 10^7 to 10^8 CFU/g. Inoculated salsa samples were then immediately subjected to ohmic heating.

Experimental apparatus. The ohmic heating system (Fig. 1) consisted of a function generator (33210A; Agilent Technologies, Palo Alto, CA), a precision power amplifier (4510; NF Corp., Yokohama, Japan), a two-channel digital storage oscilloscope (TDS2001C; Tektronix, Inc., Beaverton, CO), a data logger (34790A; Agilent Technologies), and an ohmic heating chamber. The function generator produced various wave forms at frequencies from 1 mHz to 10 MHz and a maximum output level of 5 V. The amplifier coupled with a function generator could deliver 1 kW of power and boost signals in the range of 45 Hz to 20 kHz to a maximum output of 141 V AC. An amplified output was connected to each side of titanium electrodes in the ohmic heating chamber. When operating the function generator and the amplifier output, signals (frequency, voltage, and current at the sample) were measured with a two-channel digital storage oscilloscope. The ohmic heating chamber was composed of two titanium plate electrodes in contact with the sample and K-type thermocouples inserted at the center of a rectangular (2 by 15 by 6 cm) Pyrex glass container of 0.5-cm thickness. The distance between the two electrodes was 2 cm, and the cross-sectional area was 60 cm^2 . Temperatures were recorded at 1-s intervals by a data logger linked to a computer.

Ohmic heating treatment. For the ohmic heating treatment, 25 g of inoculated salsa was placed in the ohmic heating chamber. In all experi-

ments, the electric field strength was fixed at 12.5 V/cm. Seven frequencies (60, 100, 300, 500, 1,000, 10,000, and 20,000 Hz) and three wave forms (sine, square, and sawtooth) were applied to each sample and heated to 90°C. At selected time intervals, the 25-g treated samples were immediately transferred into sterile stomacher bags (Labphas, Inc., Sainte-Julie, Quebec, Canada) containing 225 ml of BPW and homogenized for 2 min with a stomacher (Easy Mix; AES Chemunex, Rennes, France). After homogenization, 1-ml aliquots of sample were 10-fold serially diluted in 9 ml of BPW, and 0.1 ml of sample or diluent was spread plated onto each selective medium. *E. coli* O157:H7 and *S. Typhimurium* were enumerated on sorbitol MacConkey agar (Difco) and xylose lysine desoxycholate agar (Difco), respectively. When low levels of surviving cells were anticipated, 250 μl of sample was spread plated onto each of four plates. All plates were incubated at 37°C for 24 to 48 h before counting.

Electrical conductivity measurement. Electrical conductivity of samples was determined from voltage and current data (20) and calculated as follows (equation 1):

$$\sigma = \frac{L}{AR} \quad (1)$$

where σ is the electrical conductivity (S/m), L is the distance between electrodes (m), A is the cross-sectional area of the electrodes (m^2), and R is the resistance of the sample (Ω).

Analysis of electrode corrosion. Scanning electron microscopy (SEM) was performed to observe changes of electrode surfaces due to electrode corrosion. Surfaces of the electrodes after ohmic heating treatment were examined using a field-emission SEM (SUPRA 55VP; Carl Zeiss, Jena, Germany). Concentrations of Ti (from the titanium electrodes) migrating into the salsa sample were taken as measures of electrode corrosion. In each experimental run, once ohmic heating was completed, a 1-g sample was collected into a polypropylene sample bottle and then stabilized by adding 30 ml of concentrated nitric acid (60% [vol/vol]). An unheated sample taken from the ohmic heating chamber was used as a blank. Quantitative analyses of the metal ions were performed by an inductively coupled plasma-mass spectrometer (820-MS; Varian, Australia).

Color and pH measurement. Color was measured using a Minolta colorimeter (CR400; Minolta Co., Osaka, Japan). Color values for L^* , a^* , and b^* were recorded to evaluate color changes of salsa subjected to ohmic heating at different frequencies. Measurements were taken from treated and untreated uninoculated samples taken at three different locations, and averaged. L^* , a^* , and b^* values indicate color lightness, redness, and yellowness of the sample, respectively. The pH was measured with a pH meter (Seven Multi 8603; Mettler Toledo, Greifensee, Switzerland).

FIG 2 SEM micrographs of titanium electrode. (A) Untreated electrode. (B to D) Electrode after ohmic heating treatment with frequencies of 60 Hz (B), 100 Hz (C), and 1 kHz (D).

Lycopene and vitamin C measurement. The total lycopene content was measured spectrophotometrically according to the method performed by Davis et al. (21). This method determines the content of lycopene and other derivatives such as hydroxylycopene and lycopene epoxides. Salsa samples (0.6 g) were extracted with a mixture of 5 ml of 95% ethanol, 5 ml of 0.05% butylhydroxytoluene in acetone, and 10 ml of hexane on ice for 15 min. After 3 ml of distilled water was added to the sample extract, the mixture was shaken and separated into two layers. The absorbance of the upper hexane layer was measured spectrophotometrically using a Beckman DU series 68 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA) with 1-cm square cuvettes at 503 nm against a hexane blank. The amounts of lycopene in salsa were then estimated using the absorbance at 503 nm and the sample weight (22, 23).

Ascorbic acid concentration in salsa was determined using high-performance liquid chromatography (HPLC; Ultimate 3000; Dionex, Sunnyvale, CA) equipped with an autosampler and an UV detector set at 265 nm. A reversed-phase C_{18} column (5- μ m particle size, 4.6-mm diameter, 250-mm length; Dionex) was used to separate the ascorbic acid using 50 mM potassium phosphate buffer (pH 7.2) and acetonitrile (95:5 [vol/vol]) as a mobile phase. The mobile phase was filtered using a 0.45- μ m-pore-size membrane filter (Micron Separations, Inc., Westboro, MA) and degassed via vacuum before being applied to the column. A flow rate of 0.5 ml/min was used, and the retention time was 3.7 min. A standard calibration curve was obtained by using L-ascorbic acid (Sigma Chemical Co., St. Louis, MO) in concentrations ranging from 5 to 80 mg/100 ml. The samples (5 g) were homogenized with 10 ml of 5% (62.5 mM) metaphosphoric acid with a stomacher at medium speed for 1 min. The homogenate was centrifuged at $12,000 \times g$ for 10 min at 5°C. The supernatant was vacuum filtered through Whatman no. 1 paper and then passed through a Millipore 0.45- μ m-pore-size membrane. A 10- μ l portion of supernatant was injected into the column using the HPLC autosampler.

Statistical analysis. All experiments were duplicate plated and replicated three times. All data were analyzed by using the analysis of variance procedure of the Statistical Analysis System (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 $P = 0.05$.

RESULTS

Analysis of electrode corrosion related to applied frequencies. SEM micrographs of electrode surfaces treated with frequencies

ranging from 60 Hz to 1 kHz of sine wave are shown in Fig. 2. Electrode fouling, the formation of a film of food particles on the electrode, occurred in the low-frequency range (60 and 100 Hz). Surface corrosion was more severe at 60 Hz. As the frequency increased, electrode fouling diminished. When conducting experiments on the samples at 1 kHz and higher, corrosion on the electrode surface was no longer visible.

The concentration of titanium ions in the sample following ohmic heating at different frequencies was measured (Table 1). When 60 Hz was applied to the sample, the migrated concentration of titanium ions was highest (4.91 mg/kg) among all treatments. Increasing frequency resulted in lower concentrations of titanium ions in the sample. The concentration of titanium ions fell below the detection limit (1 ppb) at higher frequency levels (above 300 Hz) and in the blank sample.

Temperature curves of salsa at different frequencies. The heating rate of salsa during ohmic heating at various frequencies from 60 Hz to 20 kHz of the sine wave and a constant voltage gradient of 12.5 V/cm is shown in Fig. 3. At the same frequency, the temperature increased with increasing treatment times. Heating times decreased as a result of higher heating rates resulting from higher applied frequencies. The heating rate of salsa was dependent on frequency up to 500 Hz, but there was no significant

TABLE 1 Concentration of titanium ions migrated into salsa after ohmic heating treatment at different frequencies

Frequency (Hz)	Mean concn of titanium (mg/kg) $\pm$ SD
0	ND ^a
60	4.91 $\pm$ 0.88
100	0.16 $\pm$ 0.15
300	ND
500	ND
1,000	ND
10,000	ND
20,000	ND

^a ND, below detection limit (1 ppb).

FIG 3 Temperature curves of salsa during ohmic heating at frequencies of 60 Hz (●), 100 Hz (○), 300 Hz (▼), 500 Hz (△), 1 kHz (■), 10 kHz (□), and 20 kHz (◆).

effect on the heating rate in the range from 1 kHz to 20 kHz ($P > 0.05$). Salsa increased from room temperature (20.0°C) to 90.9°C when exposed to frequencies ranging from 1 to 20 kHz for 50 s. For the same treatment time, the temperature of salsa did not exceed 60°C at 60 Hz.

Effect of frequency on electrical properties of salsa. The electrical conductivity curves for salsa subjected to various frequencies from 60 Hz to 20 kHz of sine wave are presented in Fig. 4. At the same frequency, the electrical conductivity of the sample increased linearly with time. As the frequency increased from 60 to 500 Hz, the electrical conductivity of the sample increased. However, no significant difference in electrical conductivity of the sam-

ple was observed in the range from 1 to 20 kHz ($P > 0.05$). The electrical conductivity of the sample varied slightly at 60 and 100 Hz, although the temperature of the sample increased to 90°C (until 84 s). In the high-frequency range (1 to 20 kHz), the electrical conductivity of the sample was higher than at the low-frequency range (below 500 Hz).

Temperature curves of salsa at different waveforms. The comparison of the thermal histories of salsa subjected to ohmic heating with sine, square, and sawtooth waves at 60 Hz, 500 Hz, and 20 kHz is shown in Table 2. The heating rate for the square wave was significantly lower than for sine and sawtooth waves at 60 Hz. A significant difference in temperature of the sample was

FIG 4 Electrical conductivity of salsa during ohmic heating at frequencies of 60 Hz (●), 100 Hz (○), 300 Hz (▼), 500 Hz (△), 1 kHz (■), 10 kHz (□), and 20 kHz (◆).

TABLE 2 Temperature of salsa after various ohmic heating treatments using different frequencies and waveforms

Treatment period(s)	Mean temp (°C) ± SD ^a								
	60 Hz			500 Hz			20 kHz		
	Sine	Square	Sawtooth	Sine	Square	Sawtooth	Sine	Square	Sawtooth
0	20.69 ± 0.56 ^A	20.66 ± 0.30 ^A	19.89 ± 0.26 ^A	19.48 ± 0.30 ^A	19.21 ± 0.20 ^A	19.47 ± 0.22 ^A	21.91 ± 0.23 ^A	21.76 ± 0.61 ^A	22.47 ± 0.62 ^A
5	22.47 ± 0.48 ^A	22.30 ± 0.62 ^A	22.31 ± 0.78 ^A	21.50 ± 0.69 ^A	20.99 ± 0.23 ^A	21.28 ± 0.29 ^A	24.23 ± 0.50 ^A	24.16 ± 0.02 ^A	23.58 ± 1.05 ^A
10	26.02 ± 0.31 ^A	25.36 ± 0.91 ^A	26.17 ± 1.40 ^A	25.15 ± 0.73 ^A	24.20 ± 0.59 ^A	25.08 ± 0.32 ^A	29.09 ± 0.64 ^A	29.18 ± 0.04 ^A	28.24 ± 0.75 ^A
15	30.29 ± 0.23 ^{AB}	28.66 ± 0.98 ^A	30.79 ± 1.41 ^B	30.01 ± 0.83 ^A	29.22 ± 0.61 ^A	29.96 ± 0.30 ^A	34.68 ± 0.64 ^A	34.72 ± 0.06 ^A	34.02 ± 0.67 ^A
20	34.86 ± 0.10 ^B	32.28 ± 1.02 ^A	35.76 ± 1.39 ^B	35.40 ± 0.48 ^A	34.82 ± 0.56 ^A	35.43 ± 0.38 ^A	41.03 ± 0.66 ^A	40.82 ± 0.20 ^A	40.35 ± 0.66 ^A
25	39.77 ± 0.17 ^B	36.12 ± 1.11 ^A	41.06 ± 1.38 ^B	42.66 ± 0.30 ^A	41.16 ± 0.54 ^A	41.48 ± 0.44 ^A	47.93 ± 0.70 ^A	47.41 ± 0.43 ^A	47.31 ± 0.68 ^A
30	44.88 ± 0.37 ^B	40.24 ± 1.21 ^A	46.52 ± 1.26 ^B	49.31 ± 0.38 ^A	48.11 ± 0.51 ^A	48.15 ± 0.64 ^A	55.17 ± 0.68 ^A	54.41 ± 0.73 ^A	54.75 ± 0.52 ^A
35	49.91 ± 0.60 ^B	44.41 ± 1.28 ^A	52.01 ± 1.27 ^C	56.37 ± 0.55 ^A	55.52 ± 0.66 ^A	55.30 ± 0.86 ^A	63.16 ± 0.79 ^A	62.44 ± 0.78 ^A	62.73 ± 0.57 ^A
40	54.58 ± 0.84 ^B	48.62 ± 1.30 ^A	57.16 ± 1.22 ^C	64.33 ± 0.76 ^A	63.77 ± 0.92 ^A	63.37 ± 1.12 ^A	71.84 ± 0.84 ^A	71.29 ± 0.87 ^A	71.45 ± 0.60 ^A
45	59.16 ± 0.94 ^B	52.72 ± 1.36 ^A	62.37 ± 1.09 ^C	72.81 ± 0.97 ^A	72.81 ± 1.24 ^A	72.05 ± 1.46 ^A	80.85 ± 0.95 ^A	80.45 ± 1.05 ^A	80.67 ± 0.77 ^A
50	63.89 ± 1.11 ^B	56.66 ± 1.46 ^A	67.76 ± 1.07 ^C	82.10 ± 1.42 ^A	82.67 ± 1.60 ^A	82.14 ± 1.88 ^A	90.89 ± 1.10 ^A	91.01 ± 1.43 ^A	90.45 ± 0.25 ^A
55	68.43 ± 1.18 ^B	60.44 ± 1.30 ^A	73.21 ± 1.07 ^C	92.05 ± 1.49 ^A	92.94 ± 1.10 ^A	92.16 ± 0.75 ^A	–	–	–
60	72.90 ± 1.32 ^B	64.25 ± 1.20 ^A	79.32 ± 1.04 ^C	–	–	–	–	–	–
65	77.01 ± 1.38 ^B	68.02 ± 1.03 ^A	84.47 ± 1.06 ^C	–	–	–	–	–	–
70	81.11 ± 1.05 ^B	71.95 ± 1.15 ^A	88.80 ± 0.31 ^C	–	–	–	–	–	–
75	85.09 ± 0.69 ^B	75.69 ± 1.03 ^A	92.54 ± 0.73 ^C	–	–	–	–	–	–
80	89.14 ± 0.50 ^B	79.23 ± 1.56 ^A	–	–	–	–	–	–	–
85	92.40 ± 0.13 ^B	82.61 ± 1.10 ^A	–	–	–	–	–	–	–
90	–	85.89 ± 0.52 ^A	–	–	–	–	–	–	–
95	–	90.26 ± 1.05 ^A	–	–	–	–	–	–	–

^a Values in the same row for the same frequency that are followed by the same superscript uppercase letter are not significantly different ($P > 0.05$). –, temperature of salsa above 93°C.

observed starting at 20 s. The mean time taken to reach 90°C was 95 s for the 60 Hz square wave, while the 60-Hz sine wave and the 60-Hz sawtooth wave took 82 and 75 s, respectively. As the frequency increased, the differences in heating rates between waveforms decreased. There was no significant difference in heating rates between the waveforms from 500 Hz to 20 kHz. The sine and sawtooth waveforms had similar heating rates at all frequencies except for 60 Hz.

Effect of frequency on inactivation of microorganisms in salsa. The survival of *E. coli* O157:H7 and *S. Typhimurium* in salsa during ohmic heating is depicted in Fig. 5. As the sine wave frequency increased from 60 Hz to 20 kHz, the surviving populations of both pathogens decreased more effectively. The levels of surviving cells of both pathogens were reduced to below the detection limit (1 log CFU/g) within 50 s when treated at frequencies of 1, 10, and 20 kHz. At 500 Hz, the levels of *E. coli* O157:H7 were reduced by 3.50 log CFU/ml after 50 s and to below the detection limit after 54 s of treatment. The cell numbers of *S. Typhimurium* experienced a significant reduction of 3.85 log CFU/ml after 50 s and an >6.47-log reduction to below the detection limit after 54 s of treatment. At 300 Hz, the numbers of *E. coli* O157:H7 and *S. Typhimurium* were reduced to below the detection limit after 57 s of treatment. The levels of *E. coli* O157:H7 and *S. Typhimurium* in the sample were greatly reduced to undetectable levels when treated by 100 and 60 Hz after 71 and 82 s, respectively. These results indicate that higher frequencies increase the inactivation efficacy of ohmic heating in salsa.

Influence of frequency during ohmic heating on color and pH of salsa. The color and pH values of salsa after ohmic heating treatment with sine wave frequencies ranging from 60 Hz to 20 kHz are summarized in Table 3. The L*, a*, and b* values of ohmic heating-treated samples were not significantly different ($P > 0.05$)

from that of nontreated samples. There was no significant pH difference between untreated and treated salsa. The pH value of untreated salsa was 4.16 ± 0.01 , while the pH of treated salsa was ca. 4.15 ± 0.01 . Thus, ohmic heating at various frequencies did not affect the color and pH value of salsa ($P > 0.05$).

Effect of frequency during ohmic heating on lycopene and vitamin C of salsa. The lycopene content of untreated salsa was 105.04 ± 0.85 mg/kg, whereas salsa treated with ohmic heating at 60 Hz to 20 kHz had a lycopene content ranging from 106.43 ± 1.61 to 107.86 ± 1.21 mg/kg (Table 3). There were no statistically significant differences in lycopene content between untreated and treated salsa ($P > 0.05$). The ascorbic acid content of untreated salsa was 15% higher than that of the salsa subjected to ohmic heating at a frequency of 60 Hz and 19% higher than that of salsa treated at a frequency of 100 Hz ($P < 0.05$), respectively. However, there were no significant differences in ascorbic acid content between salsa treated at frequencies >300 Hz and that of nontreated salsa ($P > 0.05$).

DISCUSSION

The objectives of the present study were to examine the effect of frequency of alternating current during ohmic heating on electrode corrosion, heating rate, inactivation of food-borne pathogens, and quality of salsa. The impact of waveforms of alternating current on the ohmic heating rate of salsa was also investigated. As the frequency increased, electrode fouling and migration of titanium ions decreased. At low frequencies (60 to 100 Hz), the formation of adherent surface films on electrodes and some blue and violet coloration were observed. Simultaneously, migration of titanium ions from electrodes into the surrounding salsa occurred. Titanium is a major component of the electrode. An important consideration in ohmic heating is the diffusion of electrode cor-

FIG 5 (A and B) Survival curves for *E. coli* O157:H7 (A) and *S. Typhimurium* (B) in salsa treated with frequencies of 60 Hz (●), 100 Hz (○), 300 Hz (▼), 500 Hz (△), 1 kHz (■), 10 kHz (□), and 20 kHz (◆).

rosion products into the food during processing. These results imply that electrochemical reactions occurred at the electrode surface, leading to product burning and the corrosion of electrodes. The electrolytic reactions and electrode corrosion during ohmic heating can be described based on theory in electrical circuit analysis. When voltage is applied to a pair of electrodes, the electrons in the electrolyte are transferred to the electrode surface, resulting in an increase in the thickness of ions (24). This layer is often called the electrical double layer capacitor since it behaves as a capacitor (25, 34). The current is utilized to charge the double layer until the threshold voltage equals that of the charging current, which does not produce any chemical reactions or charge transfer at this stage (26). Once the capacitor is fully charged above the threshold voltage, faradaic current flows, and electrochemical reactions occur at the electrode surface (8). Under conditions such as low alternating frequencies, a chain of chemical reactions involving mass transport of electroactive species to the electrode occur and accelerate electrode corrosion (27). On the other hand, with high alternating current frequencies (>1 kHz) the rapid movement of electric

charge periodically reverses direction supplied to the electrode. Consequently, the capacitor can no longer attain the threshold voltage because there was insufficient time to fully charge the double-layer capacitor (24). Only the charging current will flow at the electrode surface and electrochemical faradaic reactions will not start (25). Based on this theory it has been shown that electrode corrosion can be limited by applying high frequencies.

Increasing the frequency of alternating current not only reduces electrode corrosion but also increases the ohmic heating rate of salsa. Frequency can be one of the key parameters for rapid heating by increasing electrical conductivity of salsa. The heat generation of ohmic heating (Q) is given by the equation $Q = kE^2$, where k is the electrical conductivity of the sample, and E is the electric field strength. From this equation, the heat generated is increased by increasing the electrical conductivity and the electric field strength. In the present study, electric field strength was fixed at 12.5 V/cm to only examine the impact of frequency during ohmic heating. Our results agree with the equation: more heat was generated as a result of higher electrical conductivity of salsa re-

TABLE 3 Color values, pH, and nutritional content of treated and untreated salsa subject to ohmic heating at different frequencies

Frequency (Hz)	Mean $\pm$ SD ^a					
	pH	Color ^b			Concn	
		L*	a*	b*	Ascorbic acid (mg/100 g)	Lycopene (mg/kg)
0	4.16 $\pm$ 0.01 ^A	31.39 $\pm$ 0.46 ^A	16.85 $\pm$ 0.89 ^A	25.01 $\pm$ 0.83 ^A	16.53 $\pm$ 1.26 ^A	105.04 $\pm$ 0.85 ^A
60	4.16 $\pm$ 0.01 ^A	31.85 $\pm$ 0.59 ^A	17.12 $\pm$ 1.16 ^A	25.00 $\pm$ 0.75 ^A	14.08 $\pm$ 0.99 ^B	107.77 $\pm$ 3.06 ^A
100	4.15 $\pm$ 0.01 ^A	31.61 $\pm$ 0.63 ^A	17.07 $\pm$ 0.69 ^A	24.42 $\pm$ 0.49 ^A	13.42 $\pm$ 0.22 ^B	107.86 $\pm$ 1.21 ^A
300	4.17 $\pm$ 0.00 ^A	31.42 $\pm$ 0.35 ^A	17.03 $\pm$ 0.05 ^A	24.69 $\pm$ 0.44 ^A	14.62 $\pm$ 1.69 ^{AB}	107.15 $\pm$ 1.16 ^A
500	4.16 $\pm$ 0.01 ^A	31.36 $\pm$ 0.50 ^A	16.74 $\pm$ 0.46 ^A	24.52 $\pm$ 0.62 ^A	14.60 $\pm$ 0.41 ^{AB}	106.43 $\pm$ 1.61 ^A
1,000	4.15 $\pm$ 0.01 ^A	31.43 $\pm$ 0.57 ^A	17.19 $\pm$ 0.45 ^A	24.61 $\pm$ 0.14 ^A	15.04 $\pm$ 0.80 ^{AB}	106.93 $\pm$ 1.32 ^A
10,000	4.15 $\pm$ 0.00 ^A	31.46 $\pm$ 0.14 ^A	16.84 $\pm$ 0.07 ^A	24.71 $\pm$ 0.55 ^A	14.98 $\pm$ 1.41 ^{AB}	106.79 $\pm$ 0.64 ^A
20,000	4.15 $\pm$ 0.01 ^A	31.98 $\pm$ 0.33 ^A	17.23 $\pm$ 0.37 ^A	25.29 $\pm$ 0.17 ^A	15.43 $\pm$ 1.85 ^{AB}	107.51 $\pm$ 2.27 ^A

^a Values in the same column that are followed by the same superscript uppercase letter are not significantly different ($P > 0.05$).

^b Color values are L* (lightness), a* (redness), and b* (yellowness).

sulting from higher applied frequencies. In contrast to our results, some studies reported that heating rate increased with decreasing frequency due to the resulting reduction of impedance (12–14). However, such a reduction of impedance, which acts as the resistance of several factors in the alternating current circuit, can result in more rapid heat generation by increasing the electrical conductivity shown in the as equation 1. Therefore, ohmic heating at high frequencies is very effective for rapid heating of food products. The waveform of alternating current also has an influence on the heat generation of salsa during ohmic heating. We observed that the heating rate of salsa for a 60-Hz square wave was significantly ($P < 0.05$) lower than for 60-Hz sine and sawtooth waves. This result was in agreement with other research studies which showed that square wave was less efficient than the sine wave at 60 Hz (14, 15). These investigators mentioned that changing of the waveform affected electrical conductivity, and this effect increased as the frequency decreased (14). For this reason, no significant ($P > 0.05$) difference in the ohmic heating rate between waveforms was observed as frequency increased above 500 Hz. Also, ohmic heating at high frequencies above 1 kHz is desirable in order to prevent electrode corrosion. Therefore, the change of waveforms in the low frequency range (below 500 Hz) is not an important factor in ohmic heating.

Although numerous research studies of ohmic heating inactivation of microorganisms have been reported (28–31), to our knowledge there is no information concerning the inactivation of *E. coli* O157:H7, and *S. Typhimurium* in solid-liquid food mixtures using high-frequency ohmic heating. The treatment time required to reduce *E. coli* O157:H7 and *S. Typhimurium* to below the detection limit (1 log CFU/g) decreased as the frequency increased. At a frequency of 60 Hz, 82 s was required to reduce the levels of *E. coli* O157:H7 and *S. Typhimurium* in salsa to undetectable levels, but only 50 s was required at 1 kHz. In addition, there were no significant ($P > 0.05$) differences in pH, color, and nutritional content between salsa treated at frequencies above 300 Hz and that of nontreated salsa. Other studies also reported that ohmic heating treatment resulted in food products of superior quality. Leizeron and Shimoni (32) found that the ohmic heated orange juice maintained higher amounts of the five representative flavor compounds than did heat-pasteurized juice. Vikram et al. (33) and Lee et al. (30) reported that the ohmic heating method produced a higher vitamin C retention in orange juice compared

to conventional heating. In the present study, the significant differences in ascorbic acid content observed in salsa treated at low frequencies (60 and 100 Hz) might be related to severe electrode corrosion as a result of electrochemical reactions. Based on our results, there was no significant ($P > 0.05$) difference in treatment time required to inactivate both pathogens in salsa, and no significant quality differences were observed between untreated salsa and salsa treated at frequencies ranging from 1 to 20 kHz. Therefore, frequencies >1 kHz offer the most effective treatment condition for inactivating pathogens in salsa.

Our results indicate that application of high-frequency (above 1 kHz) ohmic heating leads to effective inactivation of *E. coli* O157:H7 and *S. Typhimurium* in salsa, as well as preventing undesirable electrode corrosion and quality deterioration. The effect of inactivation depends on applied frequency, electrical conductivity, and treatment time rather than waveform. Therefore, ohmic heating is a very promising alternative technology to control of food-borne pathogens in salsa, allowing the processor to obtain products with superior microbiological and organoleptic quality.

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