



Increased Resistance of *Salmonella enterica* Serovar Typhimurium and *Escherichia coli* O157:H7 to 222-Nanometer Krypton-Chlorine Excilamp Treatment by Acid Adaptation

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ABSTRACT In this study, we examined the change in resistance of *Salmonella enterica* serovar Typhimurium and *Escherichia coli* O157:H7 to 222-nm krypton-chlorine (KrCl) excilamp treatment as influenced by acid adaptation and identified a mechanism of resistance change. In addition, we measured changes in apple juice quality indicators, such as color, total phenols, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, during treatment. Non-acid-adapted and acid-adapted pathogens were induced by growing the cells in tryptic soy broth without dextrose (TSB w/o D) at pH 7.3 and in TSB w/o D at pH 5.0 (adjusted with HCl), respectively. For the KrCl excilamp treatment, acid-adapted pathogens exhibited significantly ($P < 0.05$) higher D_{5d} values, which indicate dosages required to achieve a 5-log reduction, than those for non-acid-adapted pathogens in both commercially clarified apple juice and phosphate-buffered saline (PBS), and the pathogens in the juice showed significantly ($P < 0.05$) higher D_{5d} values than those for pathogens in PBS because of the UV-absorbing characteristics of apple juice. Through mechanism identification, it was found that the generation of lipid peroxidation in the cell membrane, inducing cell membrane destruction, was significantly ($P < 0.05$) lower in acid-adapted cells than in non-acid-adapted cells for the same amount of reactive oxygen species (ROS) generated at the same dose because the ratio of unsaturated to saturated fatty acids (USFA/SFA) in the cell membrane was significantly ($P < 0.05$) decreased as a result of acid adaptation. Treated apple juice showed no significant ($P > 0.05$) difference in quality indicators compared to those of untreated controls during treatment at 1,773 mJ/cm².

IMPORTANCE There is a need for novel, mercury-free UV lamp technology to replace germicidal lamps containing harmful mercury, which are routinely utilized for UV pasteurization of apple juice. In addition, consideration of the changes in response to antimicrobial treatments that may occur when pathogens are adapted to the acid in an apple juice matrix is critical to the practical application of this technology. Based on this, an investigation using 222-nm KrCl excilamp technology, an attractive alternative to mercury lamps, was conducted. Our study demonstrated increased resistance to 222-nm KrCl excilamp treatment as pathogens adapted to acids, and this was due to changes in reactivity to ROS with changes in the fatty acid composition of the cell membrane. Despite increased resistance, the 222-nm KrCl excilamp achieved pathogen reductions of 5 log or more at laboratory scale without affecting apple juice quality. These results provide valuable baseline data for application of 222-nm KrCl excilamps in the apple juice industry.

KEYWORDS 222-nm KrCl excilamp, *Escherichia coli* O157:H7, ROS, *Salmonella* Typhimurium, acid adaptation, apple juice, cell membrane fatty acid, ultraviolet irradiation

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Fruit juices are widely popular and are consumed because of their characteristics of freshness, flavor, and health benefits, due to high vitamin, polyphenol, and antioxidant content (1–3). Apple juice is one of the most popular fruit juices, and its consumption has risen over the past 30 years because of its taste, flavor, and health benefits (4, 5). Increasing consumption of fruit juice has a positive impact on the economy, but it has a negative effect when problems occur, such as foodborne disease outbreaks or spoilage (6). To date, outbreaks associated with unpasteurized commercial apple juice consumption continue to occur, mainly due to *Escherichia coli* O157:H7 and *Salmonella* spp. (7). In fact, a large outbreak (70 cases) of *E. coli* O157:H7 infection occurred in 1996, during which 25 people were hospitalized, 14 experienced hemolytic-uremic syndrome, and 1 died (8, 9). In response to the increasing outbreaks associated with fruit juice, the U.S. Food and Drug Administration (USFDA) introduced regulations for all fruit juice processes that require manufacturers to perform processing steps that can achieve a minimum 5-log reduction of the pertinent pathogenic bacteria in the finished juice (10, 11).

The USFDA considers thermal processing to be an effective and proven way to ensure the safety of fruit juices from pathogenic microorganisms as well as prolonging the shelf life of refrigerated juices (11). Therefore, currently, thermal pasteurization is an industrial intervention that has been predominantly applied to ensure the microbial safety of juice (12–14). However, the adverse effects of high temperatures on the organoleptic and nutritional properties of juices and increased consumer demand for minimal processing and fresh-tasting products encourage researchers to investigate alternative, nonthermal food processing techniques (14–16). Among them, UV technology is considered one of the most promising alternatives to the conventional thermal pasteurization process due to its numerous advantages, such as no disinfectant residuals, an inactivation effect on a wide range of pathogens, ease of fit into existing processes, and low energy consumption (17, 18). In addition, the USFDA has approved UVC radiation to be applied to fruit juice processing to control human pathogens and other microorganisms (19). In the UV disinfection system, mercury vapor-filled lamps producing germicidal UV radiation are typically used, which are either low-pressure (LP) lamps, emitting a monochromatic light at 254.7 nm, or medium-pressure (MP) lamps, emitting polychromatic light across the electromagnetic spectrum (20, 21). To date, many studies have demonstrated that conventional mercury UV lamps, which replace thermal treatment, can effectively reduce pathogenic microorganisms in apple juice (22–25). However, the use of such UV radiation sources poses a potential risk of mercury release and resulting human health and environmental hazards due to their fragile quartz construction (21, 26). In addition, as the United Nations Environment Program (UNEP) has signed to the Minamata Convention on Mercury, which aims to gradually phase out the usage of mercury present in numerous products and processes by 2020, the application of a novel UV system to replace mercury UV lamps is essential (27, 28).

In that regard, UV-LEDs or dielectric barrier discharge (DBD)-driven excilamps (excimer or exciplex lamps) are a promising technology that may substitute for mercury lamps in the future. In particular, excilamps are considered an attractive UV source due to characteristics such as their wavelength selectivity, fast warm-up, long lifetime, and high radiant intensity, as well as the absence of mercury (29–31). Excilamps can emit nearly monochromatic radiation, with wavelengths ranging from 172 to 345 nm, depending on the type of rare gas and halogen used (31, 32). Recent studies, including ours, to evaluate the germicidal effects of various forms of excilamps (Xe₂, KrCl, and XeBr excilamps with maximum emissions at 172, 222, and 282 nm, respectively) and conventional mercury lamps emitting 254-nm radiation revealed that the KrCl excilamp, emitting at a 222-nm wavelength, resulted in the highest log reductions of pathogens among other excilamps as well as mercury lamps emitting at a 254-nm wavelength at the same incident dose, which means that the 222-nm KrCl excilamp has a greater inactivation effect than those of other excilamps or the mercury vapor lamp (29, 32–34). In particular, the Yin et al. study (35) comparing the inactivation effect at the same energy level that is imposed entirely on cells, excluding the matrix effect of the

medium, by using the actual dose imparted to apple juice, which was calculated by taking into account the absorption coefficient of the apple juice sample to be treated, reported that the 222-nm KrCl excilamp reduced *E. coli* O157:H7 in apple juice by almost 1 log more than the 282-nm XeBr excilamp or 254-nm mercury lamp. Meanwhile, according to Buonanno et al. (36), unlike the 254-nm wavelength of the conventional mercury lamp, the 222-nm wavelength of the KrCl excilamp did not show cytotoxicity on mammalian skin and rarely produced premutagenic DNA lesions associated with UV in a three-dimensional (3D) human skin model. This is due to the 222-nm wavelength being unable to penetrate either all of the tissues with a stratum corneum or the mammalian cell cytoplasm (37, 38). Thus, 222-nm KrCl excilamp technology can likely be utilized for juice processing without causing harmful effects to the operator by exposure to UV light. For these reasons, the 222-nm KrCl excilamp has great potential as an alternative to mercury lamps in the apple juice industry.

Bacteria can be exposed to various stresses in all areas of the farm-to-table food chain, especially in fruit juice processing, where low pH is a major source of stress (39). Under such low-pH conditions, bacteria are induced to increase resistance to acidic environments, which has been termed the acid adaptation response, increasing their survival and consequently causing fruit juice to become contaminated more easily (40). Indeed, several studies have demonstrated that *E. coli* and *Salmonella enterica* serovar Typhimurium that are adapted to acidic conditions can survive longer in acidic environments (41–44). In addition, it has been reported that the acid adaptation response also leads to increased resistance of *E. coli* O157:H7 and *S. Typhimurium* in fruit juices to inactivation by other stresses in food processing, such as heat treatments (45–48), ozone (49), and ultrasound treatment (50). Another crucial concern is that acid-adapted pathogens may have lower infectious doses, leading to increased pathogenicity, because it is inferred that the greater the resistance to the acidity of the gastrointestinal environment, the more likely they are to overcome the human gastric barrier after ingestion (51, 52). Therefore, the acid adaptation response must be considered a real possibility which can have a great influence on the development of processing protocols by the fruit juice industry. Although a recent study reported that the 222-nm KrCl excilamp achieved a 2.81-log reduction of *E. coli* O157:H7 in apple juice following treatment at 75 mJ/cm² (35) and it yielded a D value of 28.48 mJ/cm² in another study (32) using a batch-type UV treatment system, to the best of our knowledge, no published research has been carried out to investigate the bactericidal effect of this intervention on acid-adapted pathogens.

Consequently, in our study, the changes in responses of *E. coli* O157:H7 and *S. Typhimurium*, the main organisms associated with apple juice outbreaks, to 222-nm KrCl excilamp treatment (Fig. 1) after acid adaptation were investigated, and the mechanism was elucidated. Also, apple juice qualities, such as color, total phenols, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, were observed under conditions in which pathogenic bacteria with higher resistance decreased 5 log or more in order to propose a practical pathogen inactivation protocol for apple juice without overestimating the sterilization effect of 222-nm KrCl excilamps.

RESULTS

Comparative resistance of acid-adapted and non-acid-adapted cells of *S. Typhimurium* and *E. coli* O157:H7 to 222-nm KrCl excilamp treatment. Figure 2 shows surviving populations of non-acid-adapted and acid-adapted *S. Typhimurium* and *E. coli* O157:H7 suspended in phosphate-buffered saline (PBS) (Fig. 2A and B) or apple juice (Fig. 2C and D) during treatment with the 222-nm KrCl excilamp. Incidentally, in order to ascertain whether the apple juice environment can affect pathogen reduction, non-acid-adapted or acid-adapted cells of *S. Typhimurium* and *E. coli* O157:H7 were inoculated into apple juice and maintained at room temperature (22°C ± 1°C) for 105.7 min (1,773 mJ/cm²), the maximum treatment time for this experiment, without subjecting them to 222-nm KrCl excilamp treatment, following which survival populations were enumerated. Results showed that a holding time of 105.7 min did not

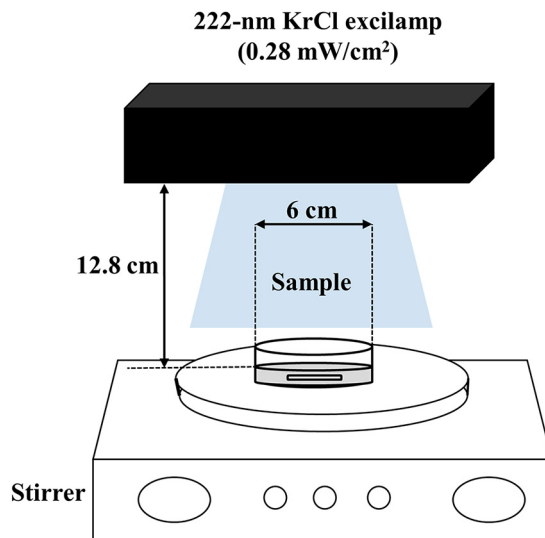


FIG 1 Schematic diagram of the batch-type 222-nm KrCl excilamp treatment system used in this study.

significantly ($P > 0.05$) change the survival population of the pathogens used in this study (data not shown). Thus, for this study, the reduction of pathogens that occurred during the UV treatment can be attributed solely to UV. In both PBS and apple juice, surviving populations of non-acid-adapted and acid-adapted *S. Typhimurium* and *E. coli* O157:H7 significantly ($P < 0.05$) decreased as the treatment dosage increased. To quantitatively compare the inactivation effects of the 222-nm KrCl excilamp based on the suspending matrix (PBS or apple juice) or cell conditions (non-acid adaptation or acid adaptation), survival curves were fitted to the suitable model equation to derive the D_{5d} value, which is the dosage required to achieve a 5-log reduction. The parameters of the models and the D_{5d} values derived from them are shown in Table 1. For both *S. Typhimurium* and *E. coli* O157:H7, when cells were adapted to acid, D_{5d} values were significantly ($P < 0.05$) increased compared to those for cells that were not acid adapted. This trend was the same when cells were treated in PBS or in apple juice. Specifically, non-acid-adapted cells of *S. Typhimurium* and *E. coli* O157:H7 treated in PBS yielded D_{5d} values of 6.32 and 2.42 mJ/cm², respectively, while cells of *S. Typhimurium* and *E. coli* adapted to acid and treated in PBS were 9.83 and 3.07 mJ/cm², respectively. In the case of pathogens treated in apple juice, cells of *S. Typhimurium* and *E. coli* O157:H7 that were not adapted to acid showed D_{5d} values of 927.71 and 1,023.72 mJ/cm², respectively, while acid-adapted cells of *S. Typhimurium* and *E. coli* had values of 1,887.09 and 1,807.29 mJ/cm², respectively. Also, this result revealed that regardless of cell condition (non-acid adaptation or acid adaptation), when cells were treated in apple juice, the D_{5d} value was significantly ($P < 0.05$) higher than that for cells treated in PBS.

When injured-cell recovery methods were compared to those for samples directly plated on selective media, there were no significant ($P > 0.05$) differences between either plating method at all treatment doses (197 to 1,773 mJ/cm²) (Table 2). That is, 222-nm KrCl excilamp treatment did not produce injured cells of either acid-adapted or non-acid-adapted *S. Typhimurium* and *E. coli* O157:H7.

Incidence of cell membrane damage and intracellular reactive oxygen species (ROS) generation during 222-nm KrCl excilamp treatment. Propidium iodide (PI) is a fluorescent dye that cannot pass through a structurally intact cell membrane because of its size and charge, but when pores form in the cell membrane, it can enter the cell and bind with nucleic acids (DNA or RNA) and emit fluorescence when exposed to an excitation wavelength of 493 nm (53, 54). Thus, the degree of pore formation in the cell membrane can be assessed by the increased fluorescence signal generated from PI binding with nucleic acids within the cell (55, 56). As shown in Fig. 3, PI uptake values

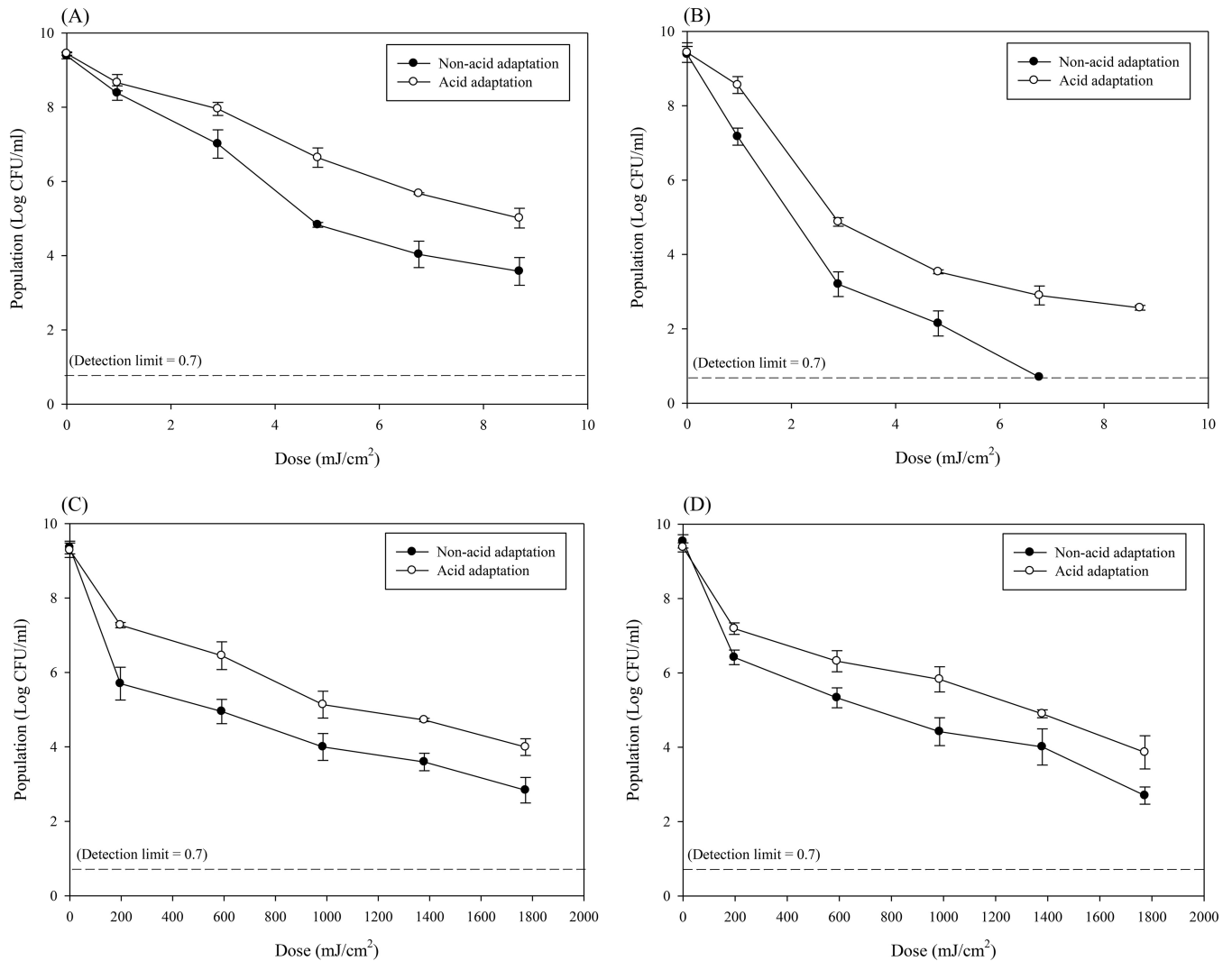


FIG 2 Surviving populations of non-acid-adapted or acid-adapted *Salmonella Typhimurium* suspended in PBS (A) or apple juice (C) or *Escherichia coli* O157:H7 suspended in PBS (B) or apple juice (D) and treated with a 222-nm KrCl excilamp. Each point and set of error bars represent the mean value for three replicates and the standard deviation, respectively.

significantly ($P < 0.05$) increased with increasing treatment doses for both pathogens. Moreover, for all treatments, PI uptake values for acid-adapted cells were significantly ($P < 0.05$) lower than those for non-acid-adapted cells. Therefore, treatment with the 222-nm KrCl excilamp caused membrane damage in the form of pore formation, and less membrane damage occurred to acid-adapted cells with the same UV treatment.

To measure the degree of lipid peroxidation of the cell membrane, we used the fluorescent dye diphenyl-1-pyrenylphosphine (DPPP) in this study. Nonfluorescent DPPP reacts with the lipid hydroperoxide in the cell membrane and is converted to fluorescent diphenyl-1-pyrenylphosphine oxide (DPPP=O) (57). Therefore, the generation of fluorescence resulting from formation of DPPP=O represents the degree of lipid peroxidation of the cell membrane. Figure 4 shows that lipid peroxide values for both non-acid-adapted and acid-adapted cells increased significantly ($P < 0.05$) as the treatment dose increased (0.97 to 8.69 mJ/cm²) for both pathogens. However, on comparing non-acid-adapted and acid-adapted cells, the lipid peroxidation value for non-acid-adapted cells was significantly ($P < 0.05$) higher for all treatments at the same treatment dose for both pathogens. Therefore, when *S. Typhimurium* and *E. coli* O157:H7 were adapted to acid, they exhibited a lower level of lipid peroxidation in the cell membrane in response to 222-nm KrCl excilamp treatment.

TABLE 1 Parameters of the Weibull or Weibull-with-tail model^a for inactivation of *Salmonella* Typhimurium and *Escherichia coli* O157:H7 treated with a 222-nm KrCl excilamp

Pathogen	Treatment condition	Cell condition	Parameter ^b					D _{5d} (mJ/cm ²) ^c
			δ	p	Log ₁₀ (N _{res})	RMSE	R ²	
<i>S. Typhimurium</i>	PBS	Non-acid adaptation	0.69 ± 0.20 A	0.72 ± 0.08 C		0.34	0.97	6.32 ± 0.54 A
		Acid adaptation	1.58 ± 0.26 B	0.89 ± 0.11 C		0.17	0.99	9.83 ± 0.72 B
	Apple juice	Non-acid adaptation	9.59 ± 9.49 ABC	0.33 ± 0.08 A		0.27	0.98	927.74 ± 184.27 C
		Acid adaptation	156.27 ± 40.97 D	0.64 ± 0.06 B		0.31	0.97	1,887.09 D
<i>E. coli</i> O157:H7	PBS	Non-acid adaptation	0.15 ± 0.03 A	0.58 ± 0.03 A		0.42	0.98	2.42 ± 0.15 A
		Acid adaptation	1.07 ± 0.26 B	1.55 ± 0.30 B	3.00 ± 0.10	0.29	0.99	3.07 ± 0.11 B
	Apple juice	Non-acid adaptation	34.11 ± 37.45 C	0.44 ± 0.12 A		0.35	0.97	1,023.72 ± 271.854 C
		Acid adaptation	95.94 ± 61.46 C	0.54 ± 0.13 A		0.31	0.97	1,807.29 ± 103.58 D

^aThe Weibull-with-tail model was applied only to the inactivation data for acid-adapted *E. coli* O157:H7 in PBS.

^bThe values are means ± standard deviations for three replications. Mean values with different uppercase letters within the same column for each pathogen are significantly different ($P < 0.05$). δ, time to first decimal reduction; p, shape parameter; log₁₀(N_{res}), residual population density; RMSE, root mean square error.

^cDosage necessary to achieve a 5-log reduction.

Intracellular ROS were measured using 5-(and -6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (CM-H₂DCFDA; Invitrogen), which is a cellular assay probe. This probe can readily penetrate the cell membrane and be hydrolyzed into the dichlorofluorescein (DCFH) carboxylate anion in the cell and then converted to 2',7'-dichlorofluorescein (DCF), which is highly fluorescent, upon oxidation by ROS (58–60). Therefore, the degree of fluorescence of DCF reflects the degree of generation of intracellular ROS. As shown in Fig. 5, ROS generation values significantly ($P < 0.05$) increased with increasing treatment doses for both non-acid-adapted and acid-adapted cells of both pathogens, while there were no significant differences ($P > 0.05$) in ROS generation values between non-acid-adapted and acid-adapted cells at each treatment dose for both pathogens. That is, 222-nm KrCl excilamp treatment generated the same amount of ROS whether or not *S. Typhimurium* and *E. coli* O157:H7 were adapted to acid.

Changes in fatty acid composition of the cell membrane. Changes in the membrane fatty acid composition, including the contents of individual major fatty acids, total minor fatty acid (MFA) content, and the ratio of unsaturated to saturated fatty acids (USFA/SFA), are shown in Table 3 for *S. Typhimurium* and *E. coli* O157:H7 relative to acid adaptation. Overall, for both pathogens, saturated fatty acid content increased and unsaturated fatty acid content decreased as a result of acid adaptation. Specifically, in the case of *S.*

TABLE 2 Reduction levels of non-acid-adapted and acid-adapted *Salmonella* Typhimurium and *Escherichia coli* O157:H7 in apple juice treated with a 222-nm KrCl excilamp at doses of 197 to 1,773 mJ/cm²

Organism and treatment dose (mJ/cm ²)	Log reduction ^a [log ₁₀ (N ₀ /N)] by treatment type and selection medium				
	Non-acid adaptation		Acid adaptation		
<i>Salmonella</i> Typhimurium	XLD	OV-XLD	XLD	OV-XLD	
	0	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa
	197	3.65 ± 0.39 Bb	3.65 ± 0.29 Bb	2.01 ± 0.19 Ba	2.12 ± 0.03 Ba
	591	4.40 ± 0.29 Cb	4.31 ± 0.39 Cb	2.83 ± 0.20 Ca	2.92 ± 0.33 Ca
	985	5.36 ± 0.19 Db	5.17 ± 0.52 Db	4.14 ± 0.20 Da	4.23 ± 0.17 Da
	1,379	5.76 ± 0.09 Db	5.40 ± 0.26 Db	4.56 ± 0.17 Ea	4.45 ± 0.06 Da
	1,773	6.65 ± 0.17 Eb	6.34 ± 0.14 Eb	5.29 ± 0.05 Fa	5.33 ± 0.43 Ea
<i>E. coli</i> O157:H7	SMAC	SPRAB	SMAC	SPRAB	
	0	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa
	197	3.11 ± 0.29 Bb	3.00 ± 0.02 Bb	2.20 ± 0.11 Ba	2.28 ± 0.10 Ba
	591	4.21 ± 0.34 Cb	4.19 ± 0.32 Cb	3.06 ± 0.24 Ca	3.22 ± 0.21 Ca
	985	5.12 ± 0.35 Db	4.84 ± 0.36 Db	3.55 ± 0.36 Ca	3.64 ± 0.19 Da
	1,379	5.53 ± 0.31 Db	5.43 ± 0.26 Eb	4.48 ± 0.22 Da	4.60 ± 0.17 Ea
	1,773	6.84 ± 0.10 Eb	6.69 ± 0.10 Fb	5.51 ± 0.33 Ea	5.55 ± 0.17 Fa

^aThe values are means ± standard deviations for three replications. Mean values with the same uppercase letter within the same column are not significantly different ($P > 0.05$). Mean values with the same lowercase letter within the same row are not significantly different ($P > 0.05$). XLD, xylose lysine desoxycholate agar; OV-XLD, overlay of XLD agar on TSA; SMAC, sorbitol MacConkey agar; SPRAB, phenol red agar base with 1% sorbitol; N₀, initial population (initial populations of *S. Typhimurium* and *E. coli* O157:H7 were approximately 9.0 and 9.5 log CFU/ml, respectively); N, population after treatment.

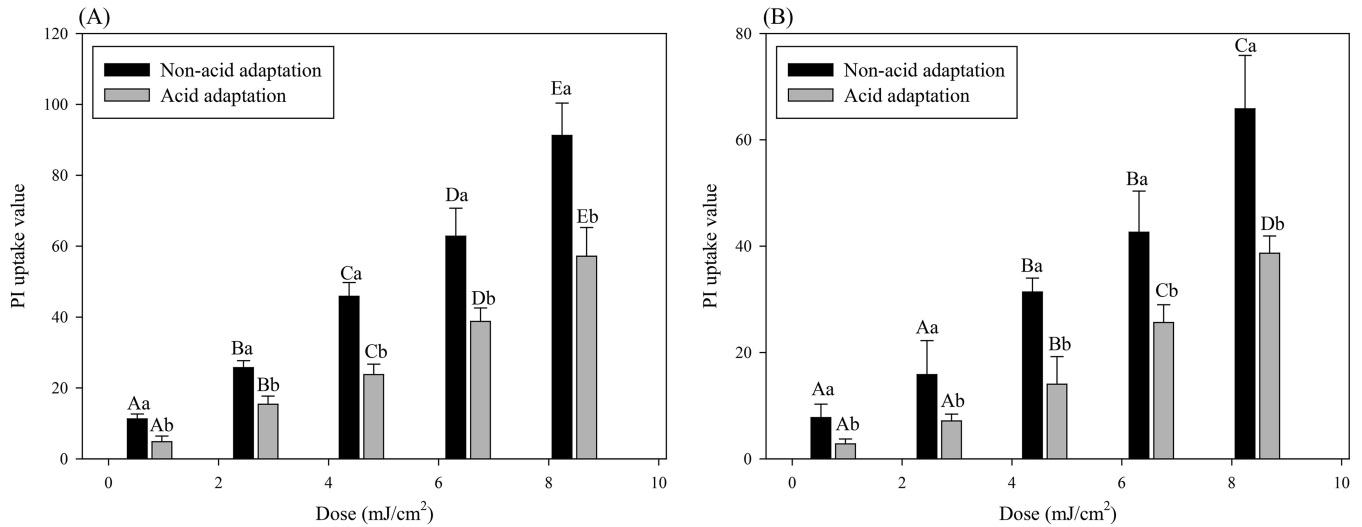


FIG 3 Destruction of cell membrane of non-acid-adapted or acid-adapted *Salmonella Typhimurium* (A) or *Escherichia coli* O157:H7 (B) as determined by the PI uptake assay. Data represent averages for three independent experiments, and error bars indicate standard deviations. The data were normalized by subtracting fluorescence values obtained for untreated cells and against the OD₆₀₀, as follows: (fluorescence value after treatment – fluorescence value for untreated control)/OD₆₀₀. Different uppercase letters for cells grown in the same matrix indicate significant differences ($P < 0.05$). Different lowercase letters for the same treatment dose indicate significant differences ($P < 0.05$).

Typhimurium, the content of C_{16:0} increased significantly ($P < 0.05$), from 53.51 to 66.78, while the content of C_{16:1} and C_{18:1} decreased significantly ($P < 0.05$), from 13.30 to 4.74 and from 6.64 to not detected (ND), respectively. Also, in the case of *E. coli* O157:H7, C_{14:0} and C_{15:0} contents increased significantly ($P < 0.05$), from 12.26 to 20.46 and from 5.18 to 9.16, respectively, while the contents of C_{16:1} and C_{18:1} decreased significantly ($P < 0.05$), from 10.70 to 0.77 and from 1.78 to ND, respectively. Furthermore, we derived the value of USFA divided by SFA because the calculated USFA/SFA ratio can be used as an indirect indicator of membrane fluidity (61). Accordingly, significantly ($P < 0.05$) decreased USFA/SFA ratio values were observed following acid adaptation in both *S. Typhimurium* and *E. coli* O157:H7. In other words, *S. Typhimurium* and *E. coli* O157:H7 showed reduced fluidity of the cell membrane under acidic conditions.

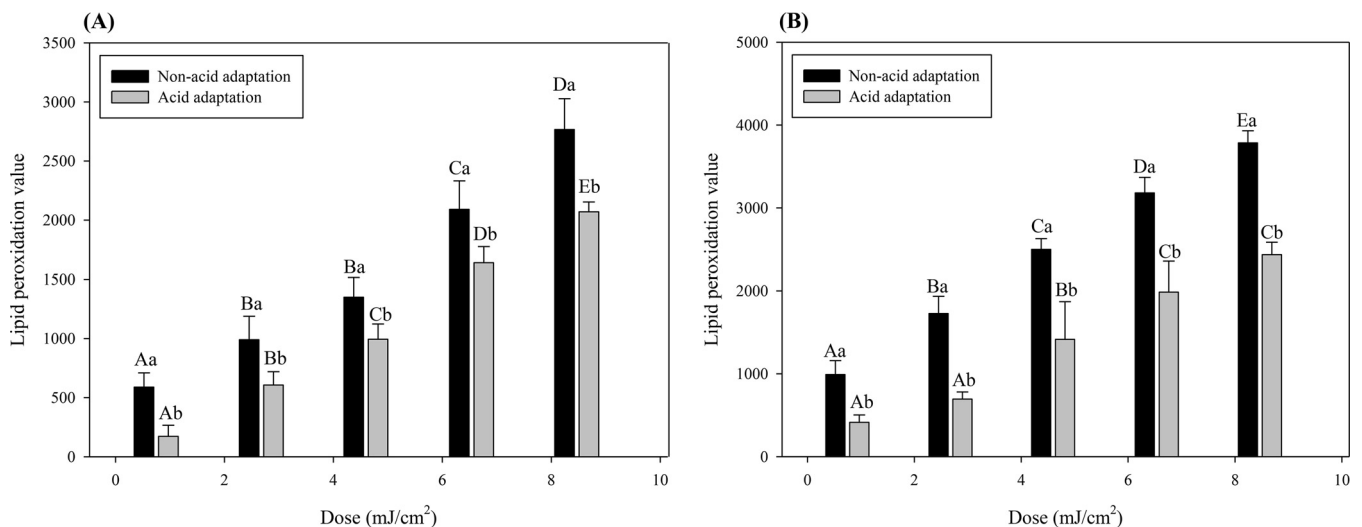


FIG 4 Lipid peroxidation of cell membrane of non-acid-adapted or acid-adapted *Salmonella Typhimurium* (A) or *Escherichia coli* O157:H7 (B) as determined by the DPPH assay. Data represent averages for three independent experiments, and error bars indicate standard deviations. The data were normalized by subtracting fluorescence values obtained for untreated cells and against the OD₆₀₀, as follows: (fluorescence value after treatment – fluorescence value for untreated control)/OD₆₀₀. Different uppercase letters for cells grown in the same matrix indicate significant differences ($P < 0.05$). Different lowercase letters for the same treatment dose indicate significant differences ($P < 0.05$).

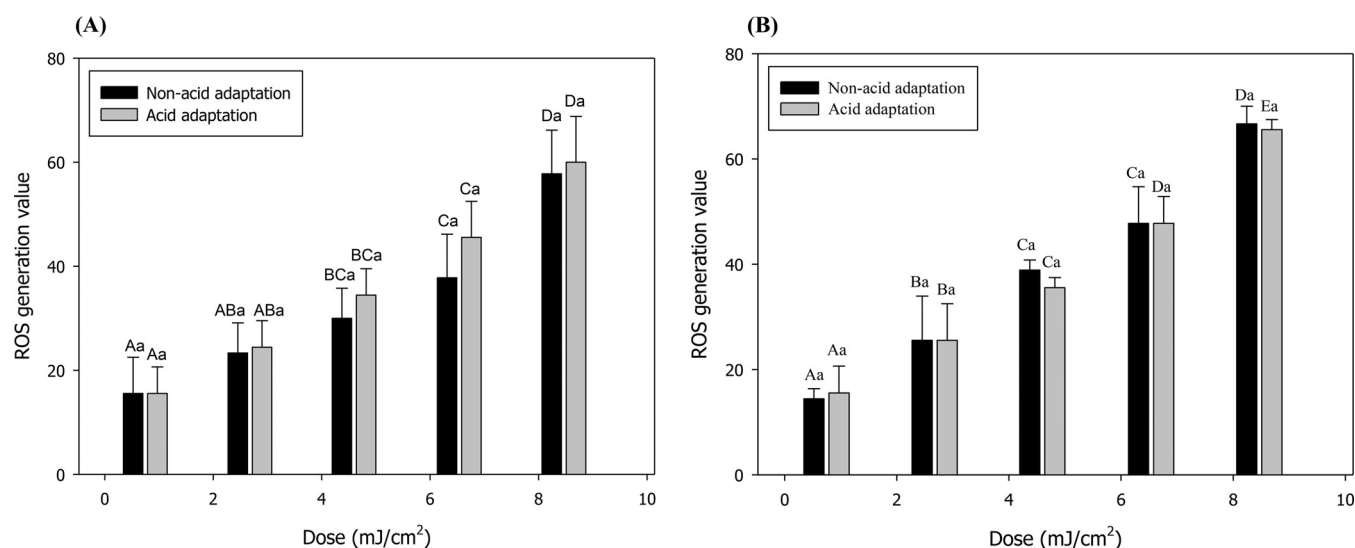


FIG 5 Intracellular ROS generation values for non-acid-adapted or acid-adapted *Salmonella Typhimurium* (A) or *Escherichia coli* O157:H7 (B), obtained by the ROS detection assay using CM-H₂DCFDA. Data represent averages for three independent experiments, and error bars indicate standard deviations. The data were normalized by subtracting fluorescence values obtained for untreated cells and against the OD₆₀₀ as follows: (fluorescence value after treatment – fluorescence value for untreated control)/OD₆₀₀. Different uppercase letters for cells grown in the same matrix indicate significant differences ($P < 0.05$). Different lowercase letters for the same treatment dose indicate significant differences ($P < 0.05$).

Observation of apple juice quality during 222-nm KrCl excilamp treatment.

Color (L^* , a^* , and b^* , indicating color lightness, redness, and yellowness, respectively), total phenols, and DPPH free radical scavenging activity values were chosen as quality indicators because these values are commonly investigated for comparisons of quality changes in studies involving apple juice disinfection (62–66), and accordingly, these values were measured after 222-nm KrCl excilamp treatment. During treatment at 197 to 1,773 mJ/cm², there were no significantly ($P > 0.05$) different values compared to those for untreated control samples for all three values (Table 4). This means that 222-nm KrCl excilamps can reduce the number of cells of *S. Typhimurium* and *E. coli* O157:H7 that are not acid adapted, as well as those of *S. Typhimurium* and *E. coli* O157:H7 that are adapted to acid, more than 5 log without inducing quality deterioration of apple juice.

DISCUSSION

Our results showed that in both PBS and apple juice, both acid-adapted *S. Typhimurium* and *E. coli* O157 exhibited increased resistance to the 222-nm KrCl excilamp

TABLE 3 Membrane fatty acid compositions of non-acid-adapted and acid-adapted *Salmonella Typhimurium* and *Escherichia coli* O157:H7

Fatty acid	Content (%) ^a			
	<i>S. Typhimurium</i>		<i>E. coli</i> O157:H7	
	Non-acid adaptation	Acid adaptation	Non-acid adaptation	Acid adaptation
C _{12:0}	10.23 ± 3.80 A	9.20 ± 1.53 A	12.26 ± 2.19 A	20.46 ± 2.39 B
C _{14:0}	13.85 ± 1.06 A	12.80 ± 1.28 A	14.26 ± 1.73 A	16.01 ± 1.52 A
C _{15:0}	2.13 ± 2.18 A	4.20 ± 0.73 A	5.18 ± 1.82 A	9.16 ± 0.95 B
C _{16:0}	53.51 ± 6.68 A	66.78 ± 3.64 B	52.58 ± 3.25 A	52.44 ± 2.84 B
C _{16:1}	13.30 ± 1.38 A	4.74 ± 4.13 B	10.70 ± 2.63 A	0.77 ± 1.33 B
C _{18:1}	6.64 ± 6.51	ND	1.78 ± 1.61	ND
MFA	0.15 ± 0.27 A	2.28 ± 4.74 A	3.24 ± 4.08 A	1.15 ± 0.25 A
USFA/SFA	0.25 ± 0.09 A	0.05 ± 0.04 B	0.15 ± 0.05 A	0.01 ± 0.01 B

^aThe values are means ± standard deviations for three replications. MFA, total minor fatty acids; USFA, total unsaturated fatty acids; SFA, total saturated fatty acids; ND, not detected (absent or below the detection limit). Mean values with the same uppercase letter within the same row for each pathogen are not significantly different ($P > 0.05$).

TABLE 4 Changes in color, DPPH free radical scavenging activity, and total phenols in apple juice during 222-nm KrCl excilamp treatment

Treatment dose (mJ/cm ²)	Quality value ^a				
	Color			DPPH free radical scavenging activity (%)	Total phenols (mg GAE/liter)
	L*	a*	b*		
0	24.52 ± 0.63	0.41 ± 0.05	4.76 ± 0.10	84.12 ± 0.52	42.39 ± 0.63
197	24.44 ± 0.61	0.43 ± 0.01	4.75 ± 0.08	84.44 ± 0.16	42.20 ± 0.36
591	24.69 ± 0.85	0.42 ± 0.03	4.78 ± 0.10	84.48 ± 0.44	42.58 ± 0.51
985	24.35 ± 0.67	0.43 ± 0.06	4.79 ± 0.23	84.76 ± 0.50	42.44 ± 0.60
1,379	24.43 ± 0.43	0.42 ± 0.06	4.82 ± 0.13	84.26 ± 0.60	42.29 ± 0.55
1,773	24.00 ± 0.92	0.43 ± 0.08	4.86 ± 0.09	84.58 ± 0.18	42.63 ± 0.36

^aData are means ± standard deviations for three replications. All quality values showed no significant ($P > 0.05$) difference from untreated controls during treatment at 197 to 1,773 mJ/cm².

compared to that of cells not adapted to acid (Fig. 2 and Table 1). Acid-exposed cells exhibit cross protection against many other environmental challenges, such as heat, oxidative stress, high osmolarity, and DNA damage, which may be related to the activation of various global regulators due to changes in internal and external pH (67). This means that stress from the acidic environment led to increased resistance of *S. Typhimurium* and *E. coli* O157:H7 to 222-nm KrCl excilamp treatment by inducing the acid adaptation response. Indeed, this increase in pathogen resistance due to acid adaptation is consistent with other studies showing increased resistance when acid-adapted cells are subjected to fruit juice processing interventions, such as heating, ultrasound, and ozone treatment (45–50). Therefore, it is important to consider the acid adaptation response, because there is a possibility of overestimating the bactericidal effect of 222-nm KrCl excilamp treatment on pathogenic bacteria in apple juice if these bacteria are adapted to acid and exhibit increased resistance.

Regardless of cell condition, more UV energy was needed to inactivate pathogens in apple juice than those in PBS at the sample matrix level. This tendency is consistent with the results of Gabriel and Nakano (12), who showed that pathogens in apple juice exhibited greater D_{5d} values on exposure to UV radiation treatment than those for pathogens in PBS. Ngadi et al. (68) reported that the pH of the suspending food matrix did not affect UV inactivation of *E. coli* O157:H7, as pathogens in apple juice and liquid egg white (pH 3.5 and 9.1, respectively) showed similar inactivation rates for UV radiation treatment. Therefore, it can be considered that PBS and apple juice exhibit different D_{5d} values that are not due to the pH of the medium. Instead, this phenomenon can be explained by the transmission characteristics of UV radiation in the suspending medium. Because of the presence of organic solutes, colored compounds, or suspended matter, fresh juice products allow only relatively little UV light transmission, and this low UV light transmission in fresh juice affects the effectiveness of the UV pasteurization process (69). In fact, it is widely reported that the inactivation effect of UV light decreases as the absorbance or amount of suspended particles in the suspension medium increases (70, 71). Specifically, Murakami et al. (71) showed that the turbidity and absorption coefficient of apple juice reduce the UV inactivation capacity as their levels increase. They interpreted this to mean that as the turbidity of apple juice increased, the UV light shielding effect of the particles increased, which would have a negative impact on the UV inactivation effect. Also, they explained the effect of the absorption coefficient of apple juice on the UV inactivation effect by using Beer-Lambert-Bouguer's law (72), by which the amount of light transmitted through the solution decreases as the absorption coefficient of the solution increases. Since the apple juice used in this study had a turbidity of 0 nephelometric turbidity units (NTU) and an absorption coefficient of 20.8 cm⁻¹, the results of this study indicate that pathogens in apple juice show larger D_{5d} values than those for pathogens in PBS, which can be explained by the absorption coefficient of apple juice. That is, unlike the case in PBS, in which 222-nm UV light is transmitted completely (data not shown), in apple juice, with an absorption coefficient of 20.8 cm⁻¹ for the 222-nm wavelength, 222-nm UV light

penetrates as its light intensity decreases in proportion to the absorption coefficient value of apple juice according to Beer-Lambert-Bouguer's law, so the actual UV dose imparted in apple juice is lower than the UV dose imparted in PBS, which ultimately results in a lower UV inactivation effect on the pathogens in apple juice than on those in PBS. Expanding on this, it can be expected that juice samples with different turbidities or absorption coefficients for the same UV treatment can produce different inactivation results. Moreover, the decreased capability of UV radiation to penetrate a food medium with high turbidity or absorption coefficient characteristics can create limitations in the practical application of UV light by the juice industry. In response, various strategies have been proposed to optimize the efficacy of UV radiation. In one of them, new UV reactors have been devised to guarantee turbulent flow patterns to ensure that all portions of the liquid sample are exposed to UV (69, 73, 74). Another strategy is to incorporate UV light and other novel processing techniques or milder conventional conservation methods, called hurdle technology, which can achieve acceptable inactivation of pathogens (75). In particular, a study by Yin et al. (35) reported that the absorption coefficient increased from 16.15 to 30.25 cm^{-1} as the wavelength decreased from 282 to 222 nm, indicating that the shorter the UV wavelength, the smaller the penetration depth of UV photons becomes. Therefore, for practical application of 222-nm KrCl excilamps by the apple juice industry, it is necessary to consider the transmittance of the UV light and to establish an appropriate strategy for optimizing treatment.

Since elucidation of the mechanism of increased resistance can be provided as important baseline data for future related research or industrial applications, we sought to determine the mechanism by which resistance of pathogens to 222-nm KrCl excilamp inactivation increases as they become adapted to acid. Our previous studies that identified the inactivation mechanism of the 222-nm KrCl excilamp suggested that it mainly induces lipid peroxidation of the cell membrane, resulting in physical damage with increased permeability and eventually leading to cell death (34, 76). Thus, we hypothesized that the cause of differential resistance to 222-nm KrCl excilamp treatment between non-acid-adapted and acid-adapted cells is related to the occurrence of cell membrane damage. From the results shown in Fig. 3, it can be seen that when pathogens are adapted to acid, less physical membrane damage, such as pore formation, occurs than that in pathogens not adapted to acid when exposed to the same UV treatment dose. This physical damage in the cell membrane induces events such as increased permeability of the cell membrane, changes in the membrane potential and the internal pH, and a decrease in the concentration of the cellular ATP, which can make it difficult for cells to maintain homeostasis, eventually leading to cell death (56, 77, 78). Thus, the results for cell membrane damage can be linked to results which show that more acid-adapted cells tend to survive against 222-nm KrCl excilamp treatment than is the case for non-acid-adapted cells. In other words, for 222-nm KrCl excilamp treatment, acid-adapted cells show greater resistance because less membrane damage occurs than that for cells that are not acid adapted. For a specific mechanism analysis, it is necessary to identify the cause of membrane damage by investigating what form of changes are caused by 222-nm KrCl excilamp treatment and result in generation of physical damage, such as pore formation. In particular, this cause of cell membrane damage must exhibit a difference in the degree of occurrence between acid-adapted and non-acid-adapted cells. As mentioned above, it is known that 222-nm KrCl excilamps induce lipid peroxidation, resulting in cell membrane damage. Thus, we measured the extent of lipid peroxidation of the cell membrane during 222-nm KrCl excilamp treatment through DPPH assay. As shown in Fig. 4, it was confirmed that 222-nm KrCl excilamp treatment induced lipid peroxidation of the cell membrane for both pathogens, and at the same 222-nm KrCl excilamp treatment dose, the generation of lipid peroxidation of the cell membrane was lower in acid-adapted than in non-acid-adapted cells. If lipid peroxidation occurs in the cell membrane, it will cause damages, such as loss of fluidity, decreasing cell membrane potential, and increasing permeability, leading to cell death (79). Therefore, 222-nm KrCl excilamp treatment induces lipid peroxidation in the cell membrane, which results in physical damages

leading to cell death, such as pore formation, and when cells become acid adapted, less lipid peroxidation occurs in the cell membrane than that in cells that are not acid adapted, resulting in increased resistance to 222-nm KrCl excilamp treatment.

In order to fully analyze the mechanism, there was still the question of how different amounts of lipid peroxidation occurred with the same treatment dose. In general, it is known that when intracellular constituents, such as chromophoric amino acids, absorb UV light, excited state species or radicals are generated as a result of photoionization through the photosensitization process (80–82). Based on this principle, it is widely reported that UV-induced ROS cause oxidative damage to cells (82–85). In particular, based on several studies, it is well demonstrated that ROS are one of the major causes that induce lipid peroxidation in cell membranes, resulting in cell death (86–89). Indeed, our previous study (34) found that the 222-nm KrCl excilamp produced lipid peroxidation in the cell membrane as a result of ROS generation. Therefore, we assumed that different amounts of ROS were produced with the same treatment dose, resulting in a difference in the occurrence of lipid peroxidation, and thus we measured the intracellular ROS of cells that were adapted as well as not adapted to acid and subjected to treatment. However, unlike our hypothesis, during 222-nm KrCl excilamp treatment, the same amounts of ROS were generated in both non-acid-adapted and acid-adapted cells at the same dose for both pathogens. In other words, different levels of lipid peroxidation of cell membranes occur between non-acid-adapted and acid-adapted cells for the same amount of intracellular ROS. Additionally, it is known that an acid stress environment causes various physiological changes to bacteria, including a decrease in cytoplasmic pH (90), an increase in activity and production of the F_1F_0 -ATPase that excretes protons out of the cell (91), and an alteration in the fatty acid composition of the cell membrane (92). Based on these facts, along with the results of this study showing that levels of lipid peroxidation in fatty acids of the cell membrane between non-acid-adapted and acid-adapted cells were different, we hypothesized that the different levels of lipid peroxidation might be related to a change in fatty acid composition of the cell membrane among several physiological changes induced by the acid adaptation response, and thus we examined the change of the fatty acid composition of the cell membrane after acid adaptation. We found that the content of saturated fatty acids increased and that of unsaturated fatty acids decreased, and thus the value of the USFA/SFA ratio, which can indicate fluidity of the cell membrane, decreased remarkably, when cells were adapted to acid (Table 2). Other studies investigating the fatty acid composition of bacteria adapted to acidic conditions have also reported a decrease in cell membrane fluidity (increased USFA and decreased SFA) as a result of acid adaptation (92–95). Therefore, our results are consistent with the results of several other studies. A study by Jordan et al. (96) demonstrated that resistance of *E. coli* O157:H7 to acids increased due to reduced proton penetration through cell membranes in acidic environments when cells were adapted to acid. Thus, if bacteria become adapted to acid, they improve their ability to maintain intracellular pH, thereby increasing their survival under acidic conditions. Linking this to our results, the increase in USFA and the decrease in SFA in the cell membrane as a result of acid adaptation are interpreted to mean that pathogens reduce their membrane fluidity by altering the fatty acid composition as a way of decreasing their permeability to protons in order to maintain their internal pH, consequently increasing their resistance to acids under acidic conditions. Meanwhile, it is well known that the presence of the double bond in fatty acids weakens the C-H bonds adjacent to the double bond to facilitate the removal of the hydrogen ion, so the unsaturated fatty acid is particularly sensitive to the induction of peroxidation and thus the unsaturated fatty acid in the cell membrane is extremely vulnerable to damage caused by ROS, in contrast to saturated fatty acids (79, 97). Based on this principle, our findings can be interpreted as follows: fluidity of the cell membrane is reduced by decreasing USFA and increasing SFA levels, which increases resistance to acidic conditions as a result of acid adaptation, and thus damage to the cell membrane caused by ROS is reduced, resulting in an increase in the resistance of pathogens to 222-nm KrCl excilamp treatment.

In conclusion, *S. Typhimurium* and *E. coli* O157:H7, which are the predominant pathogens implicated in apple juice-related outbreaks, were resistant to 222-nm KrCl excilamp treatment when adapted to acid. Therefore, acid adaptation of pathogens must be considered in order to avoid overestimation of the bactericidal effect in application of 222-nm KrCl excilamps in the apple juice pasteurization process. Conventional 254-nm mercury lamp treatment has been reported to effectively reduce the microbial load in fruit juice while minimizing quality changes (23, 98–100). In the present study, the 222-nm KrCl excilamp reduced levels of acid-adapted pathogens more than 5 log without adversely affecting apple juice quality indicators, such as color, total phenols, and DPPH free radical scavenging activity. This result supports the possibility that the 222-nm KrCl excilamp is a promising technology for apple juice disinfection processing by replacing existing mercury UV lamps. However, this study shows only the laboratory-level disinfection effect of 222-nm KrCl excilamps and the limited quality analysis results. For practical application of 222-nm KrCl excilamps, further studies on commercial-scale application, changes in nutritional or organoleptic properties, sensory evaluation by use of panels, consumer acceptance, and cytotoxicity after treatment are necessary. Nevertheless, since this study, which confirmed the disinfection effect of the 222-nm KrCl excilamp in apple juice and identified the increased resistance of pathogens to the 222-nm KrCl excilamp as a result of acid adaptation, provides valuable baseline data for practical industry application, it can be expected to be utilized in further studies to help facilitate 222-nm KrCl excilamp treatment by the juice industry in the near future.

MATERIALS AND METHODS

Bacterial strains. Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, and ATCC 43890) and *S. Typhimurium* (ATCC 19585, ATCC 43971, and DT104), obtained from the bacterial culture collection of Seoul National University (Seoul, South Korea), were used in this study. Stock cultures were prepared by incubating strains in 5 ml of tryptic soy broth (TSB; Difco, BD) at 37°C for 24 h, mixing 0.7 ml with 0.3 ml of sterile 50% glycerol, and then storing cultures at –80°C. To prepare working cultures, bacterial strains were streaked onto tryptic soy agar (TSA; Difco, BD), incubated at 37°C for 24 h, and then stored at 4°C and used within 1 week.

Preparation of non-acid-adapted and acid-adapted bacterial cultures and inoculums. All strains of *E. coli* O157:H7 and *S. Typhimurium* were separately cultured in 5 ml of TSB overnight at 37°C, and then 200- μ l aliquots of overnight cultures were transferred to 20 ml of TSB without dextrose (TSB w/o D; pH 7.3) (Difco, BD) and TSB w/o D at pH 5.0 (adjusted with 6 N HCl) to develop the non-acid adaptation and acid adaptation responses, respectively (39, 68, 101), and incubated for 18 h at 37°C. The incubation time of 18 h was the time required for the cells to grow to early stationary phase in TSB w/o D, pH 7.3 or 5.0, which was determined from growth curves plotting the mean optical density at 600 nm (OD_{600}) versus the incubation time of each strain (data not shown). This incubation time was applied because it is commonly known that bacteria in stationary phase exhibit increased UV resistance (102, 103). Furthermore, in this study, TSB w/o D instead of the usual TSB formulation (containing 0.25% dextrose) was used to prevent the acid adaptation response of the pathogens from being induced in non-acid-adapted cells by acids produced as a result of dextrose fermentation (101, 104). After incubation, all three strains of each pathogen were combined, and the cell pellet was harvested by centrifugation at 4,000 \times g for 20 min at 4°C and washed twice with phosphate-buffered saline (PBS; 0.1 M). To prepare the inoculum, the final pelleted cells were resuspended in PBS at a concentration of approximately 10^{10} to 10^{11} CFU/ml.

UV treatment. In this study, a DBD excilamp (29 \times 9 \times 8 cm; Unilam, Ulsan, South Korea) with 20 W of output power and filled with a KrCl gas mixture was used as a UV radiation source; detailed specifications of this apparatus were described in our previous study (34). A batch-type processing system was used in this study and was constructed as follows: a 222-nm KrCl excilamp was placed vertically at a 12.8-cm distance from the center of the sample surface to allow the sample to vertically face the UV radiation (Fig. 1). In this experimental setup, a light intensity of 0.29 mW/cm² at the center of the sample surface (incidence intensity) was measured using a UV fiber optic spectrometer (AvaSpec-ULS2048; Avantes, Eerbeek, Netherlands). However, since the UV light of the excilamp used in this study is emitted without being collimated, the intensity decreases as the distance from the center of the sample surface contained in the petri dish increases, resulting in no uniform intensity throughout the sample surface. Therefore, the petri factor, defined as the ratio of the average incident irradiance over the petri dish area to the irradiance at the center of the petri dish, was used to correct the intensity at the petri dish center to more accurately reflect the incident fluence average over the surface (105). The petri factor was calculated by scanning the probe over every 5 mm of the surface area of the sample, and the value was 0.97. Therefore, the incident intensity (0.29 mW/cm²) measured at the center of the sample surface was corrected to 0.28 mW/cm² by multiplying by the petri factor of 0.97, and this value was used in this study. Commercially clarified apple juice (Woongjin Foods Co., Seoul, South Korea) purchased from a local grocery market and PBS were used as samples. Samples were kept in a refrigerator at 4°C until use. Before inoculation, samples were taken out of the refrigerator for more than 1 h to equilibrate to room temperature (22°C \pm 1°C). Five milliliters of each sample was dispensed into a petri dish (60 \times 15

mm²), inoculated with 0.1 ml of inoculum, and then gently mixed by hand for approximately 5 s to allow for an even distribution. At this time, the sample depth was 2 mm. Immediately after inoculation and mixing, apple juice and PBS samples were treated with a 222-nm KrCl excilamp at dosages of 0.97 to 8.69 mJ/cm² and 197 to 1,773 mJ/cm², respectively, while being mixed with a magnetic stirrer (1.5 cm × 0.1 cm; TM-17R; Jeio Tech, South Korea) at 300 rpm to allow for even irradiation at room temperature (22°C ± 1°C) in a dark chamber. UV doses were calculated by multiplying the incident intensity of UV light by the irradiation times. The value of the UV dose in our study implies the amount of energy delivered to the sample surface, with the change in energy due to the sample matrix not taken into consideration, because it is expressed as the energy value for the intensity of the UV light reaching the sample surface. In order to make the results of this study comparable to those of other relevant studies, the characteristics of apple juice, such as pH, sugar concentration (degrees Brix [°Bx]), turbidity (NTU), and UV absorption coefficient (α), were investigated. The pH, sugar concentration, and turbidity of apple juice samples were measured with a pH meter (Mettler Toledo, Switzerland), a digital refractometer (Atago Co., Ltd., Japan), and a turbidity meter (Lutron Electronic Enterprise Co., Ltd., Taiwan), respectively, and their values were pH 2.95, 12.6 °Bx, and 0 NTU, respectively. To obtain the absorption coefficient (α) of apple juice samples at 222 nm, absorbance values for various dilutions (1:10, 1:25, 1:50, 1:100, 1:250, and 1:500 [vol/vol]) of apple juice sample were measured at 222 nm by use of a quartz cuvette with a 1-cm path length in a spectrophotometer (Spectramax M2e; Molecular Devices, Sunnyvale, CA, USA), and then the absorption coefficient was estimated from the slope of the absorbance value-versus-sample concentration plot; the absorption coefficient of the apple juice sample was 20.8 cm⁻¹.

For investigation of destruction and lipid peroxidation of the cell membrane and ROS generation, non-acid-adapted or acid-adapted *E. coli* O157:H7 and *S. Typhimurium* were adjusted to an OD₆₀₀ of approximately 0.3 in PBS and then treated with a 222-nm KrCl excilamp at 8.69 mJ/cm².

Bacterial enumeration. For microbial enumeration after treatment, 1-ml aliquots of treated samples were 10-fold serially diluted in 9 ml of sterile 0.2% buffered peptone water, and 0.1-ml aliquots of samples or diluents were spread plated onto each selective medium. If small populations of surviving cells were anticipated, 1-ml aliquots of the undiluted original samples were equally divided between four plates of each medium and spread plated. Xylose lysine desoxycholate agar (XLD; Difco) and sorbitol MacConkey agar (SMAC; Difco) were used as selective media for the enumeration of *S. Typhimurium* and *E. coli* O157:H7, respectively. All plates were incubated at 37°C for 24 h, and typical colonies were enumerated and calculated as log₁₀ CFU per milliliter (detection limit, 0.70 log CFU/ml).

Enumeration of sublethally injured cells. To identify the occurrences of sublethally injured cells of *S. Typhimurium* in apple juice after treatment, the overlay (OV) method was used (106). A nonselective medium, tryptic soy agar (TSA; Difco), was used to resuscitate injured cells. Aliquots of appropriate dilutions or undiluted original samples were duplicate spread plated onto TSA, and the plates were incubated at 37°C for 2 h to facilitate injured-cell recovery. Following incubation, the plates were overlaid with 7 ml of the selective medium XLD. After solidification, the plates were incubated for an additional 22 h at 37°C, and then typical black colonies were counted.

In the case of enumeration of injured *E. coli* O157:H7 cells, phenol red agar base with 1% sorbitol (SPRAB; Difco) was used (29). After incubation at 37°C for 24 h, typical white colonies characteristic of *E. coli* O157:H7 were enumerated, and simultaneously, serological confirmation (RIM *E. coli* O157:H7 latex agglutination test; Remel, Lenexa, KS, USA) was performed on randomly selected presumptive *E. coli* O157:H7 colonies because SPRAB is not generally used as a selective medium for enumerating *E. coli* O157:H7.

Modeling of survival curves and calculation of D_{5d}. In order to quantitatively compare our inactivation results, the D_{5d} value, which indicates the UV dosage necessary to achieve a 5-log reduction by 222-nm KrCl excilamp treatment, was calculated using predictive modeling equations. To identify the appropriate model to apply to our results among the various models, the goodness of fit of each model for each data set was evaluated using R² and root mean square error (RMSE) values, which meant that the closer the value was to 1 and the lower the value, respectively, the better the model fit the data. When survival curves were fitted with several model equations by use of GlnaFIT (107), it was shown that the Weibull model and the Weibull-with-tail model were well fitted to all inactivation data except those for acid-adapted *E. coli* O157:H7 in PBS and to the inactivation data only for acid-adapted *E. coli* O157:H7 in PBS, respectively, because these models represented high (≥0.97) R² and low (<0.50) RMSE values. Thus, these two models were applied in this study. The related model equations are as follows:

The Weibull model equation (108) is described as follows:

$$\log(N) = \log(N_0) - \left(\frac{d}{\delta}\right)^p$$

where N (CFU/ml) is the surviving population of microorganisms, N_0 is the initial population, d (mJ/cm²) is the treatment dosage, δ (mJ/cm²) is the dosage for the first decimal reduction, and p is the parameter related to the shape of the line, such as a concave upward curve when $p < 1$ and a concave downward survival curve when $p > 1$.

The Weibull-with-tail equation (109) is a modification of the Weibull model and is described as follows:

$$\log(N) = \log\left[(N_0 - N_{res}) \times 10^{-\left(\frac{d}{\delta}\right)^p} + N_{res}\right]$$

where N (CFU/ml) is the surviving population of microorganisms, N_0 is the initial population, d (mJ/cm²) is the treatment dosage, δ (mJ/cm²) is the dosage for the first decimal reduction, N_{res} is the residual population density, and p is the parameter describing the shape of the line. There is no point of inflection when $0 < p < 1$, but the line has an inflection point when $p > 1$.

After the model parameters for each model (Weibull or Weibull with tail) were obtained by fitting survival curves to model equations by use of GlnaFIT, D_{5d} was derived using Microsoft Excel 2010: describing the appropriate equations for independent variables followed by calculating the dose value for a particular log reduction was performed using the "goal seek" function.

Measurement of incidence of cell membrane damage and generation of intracellular ROS.

Treated samples were incubated with PI (Sigma-Aldrich), DPPP (Sigma-Aldrich), or CM-H₂DCFDA (Molecular Probes, Thermo Fisher Scientific) at a final concentration of 2.9, 50, or 5 μ M for 10, 20, or 15 min at 37°C to assess pore formation in the cell membrane, incidence of lipid peroxidation of the cell membrane, or generation of intracellular ROS, respectively. After incubation, cells were collected by centrifugation at $10,000 \times g$ for 10 min and washed twice with PBS, and fluorescence was measured with a spectrofluorophotometer (Spectramax M2e; Molecular Devices, Sunnyvale, CA, USA) at excitation/emission wavelengths of 493/630 nm for PI uptake, 351/380 nm for DPPP assay, and 495/520 nm for CM-H₂DCFDA assay. For quantitative comparison of the values, the obtained fluorescence signal was divided by the OD_{600} . Because this study was intended to quantitatively compare only the extents of damage to the cell membrane and generation of ROS, quantitative comparisons were performed using average values at the population level rather than comparing values at the individual cell level by using a tool such as flow cytometry.

Analysis of fatty acid composition of the cell membrane. Cells grown under nonacidic or acidic conditions were collected by centrifugation at $4,000 \times g$ for 20 min at 4°C, and the obtained pellets were subjected to fatty acid extraction following protocols described in MIDI technical note 101 (110). The fatty acid methyl esters (FAMES), extracted with mixtures of hexane and methyl *tert*-butyl ether, were analyzed with an Agilent gas chromatograph (model 7890 A; Agilent Technologies, Santa Clara, CA, USA) equipped with a split-capillary injector and a flame ionization detector (111). A DB-23 column (60 mm \times 0.25-mm internal diameter \times 0.25 μ m; Agilent Technologies) was used to separate fatty acids. The injection and detector temperatures were set at 250 and 280°C, respectively. The initial column oven was maintained at 50°C for 1 min, and then the temperature was increased to 130°C at a rate of 15°C/min, 170°C at a rate of 8°C/min, and 215°C at a rate of 2°C/min and then held for 10 min. The carrier gases were hydrogen, air, and helium at flow rates of 35, 350, and 35 ml/min, respectively. The fatty acid compositions were analyzed using a Supelco 37 component FAME mix (Supelco, Inc., PA, USA). The relative contents of fatty acids were expressed as percentages by peak area normalization.

Quality measurement. To analyze changes of apple juice quality after treatment, color, total phenols, and DPPH free radical scavenging activity of apple juice were investigated. Apple juice was treated with the 222-nm KrCl excilamp at doses of 197 to 1,773 mJ/cm², and untreated apple juice was used as a control. Color values for apple juice were measured with a Minolta colorimeter (CR400; Minolta Co., Osaka, Japan) and expressed as L*, a*, and b* values, indicating color lightness, redness, and yellowness, respectively (112).

Total phenols were measured by the Folin-Ciocalteu assay (113, 114). Briefly, 1 ml of apple juice sample diluted 10-fold in distilled water was incubated with 5 ml of 0.2 N Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO) for 3 min at room temperature (22°C \pm 1°C). This mixture was then mixed with 4 ml of 7.5% sodium carbonate (Samchun Pure Chemicals Co., Ltd., Pyeongtaek, South Korea) solution. After maintenance at room temperature (22°C \pm 1°C) for 2 h in the dark with shaking, the absorbance at 765 nm was measured against that of a blank sample by use of a spectrophotometer. The blank sample was prepared using distilled water instead of apple juice. Gallic acid (Sigma-Aldrich) was used as a standard solution to construct the calibration curve, and the results were expressed as milligrams of gallic acid equivalents (GAE) per liter.

DPPH free radical scavenging activity was measured according to the method described by Abid et al. (62), with some modification. Two milliliters of 0.2 mM DPPH solution dissolved in methanol was added to 2 ml of apple juice sample. This mixture was incubated for 30 min at room temperature (22°C \pm 1°C) in the dark. After the reaction, the absorbance at 517 nm was measured using a spectrophotometer (Spectramax M2e; Molecular Devices, Sunnyvale, CA). A control was prepared with methanol instead of apple juice, and the absorbance of the control was measured with the same procedure. DPPH free radical scavenging activity was calculated as follows: DPPH free radical scavenging activity (%) = $(1 - \text{absorbance of sample/absorbance of control}) \times 100$.

Statistical analysis. All experiments were duplicate plated and replicated three times. All data were analyzed by the analysis of variance (ANOVA) procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC, USA) and the least significant difference (LSD) *t* test to determine whether there were significant differences in mean values for log reduction, fluorescence signal normalized to OD_{600} , fatty acid composition, color, DPPH free radical scavenging activity, and total phenols at a probability level (*P*) of <0.05.

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