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Decontamination Effect of the Spindle and 222-Nanometer Krypton-Chlorine Excimer Lamp Combination against Pathogens on Apples (*Malus domestica* Borkh.) and Bell Peppers (*Capsicum annuum* L.)

Jun-Won Kang,[a,b] Dong-Hyun Kang[a,b]

[a]Department of Agricultural Biotechnology, Center for Food and Bioconvergence, Research Institute for Agricultural and Life Sciences, Seoul National University, Seoul, Republic of Korea

[b]Institutes of Green Bio Science & Technology, Seoul National University, Pyeongchang-gun, Gangwon-do, Republic of Korea

ABSTRACT In this study, we developed a washing system capable of decontaminating fresh produce by combining the Spindle apparatus, which detaches microorganisms on sample surfaces, and a 222-nm krypton-chlorine excimer lamp (KrCl excilamp) (Sp-Ex) and investigated their decontamination effect against *Escherichia coli* O157:H7, *Salmonella enterica* serovar Typhimurium, and *Listeria monocytogenes* on apple (*Malus domestica* Borkh.) and bell pepper (*Capsicum annuum* L.) surfaces. Initial levels of the three pathogens were approximately 10^8 CFU/sample. Both *E. coli* O157:H7 and *S.* Typhimurium were reduced to below the detection limit (2.0 log CFU/sample) after 5 and 7 min of treatment on apple and bell pepper surfaces, respectively. The amounts of *L. monocytogenes* on apple and bell pepper surfaces were reduced by 4.26 and 5.48 logs, respectively, after 7 min of treatment. The decontamination effect of the Sp-Ex was influenced by the hydrophobicity of the sample surface as well as the microbial cell surface, and the decontamination effect decreased as the two hydrophobicity values increased. To improve the decontamination effect of the Sp-Ex, Tween 20, a surfactant that weakens the hydrophobic interaction between the sample surface and pathogenic bacteria, was incorporated into Sp-Ex processing. It was found that its decontamination effect was significantly ($P < 0.05$) increased by the addition of 0.1% Tween 20. Sp-Ex did not cause significant quality changes in apple or bell pepper surfaces during 7 days storage following treatment ($P > 0.05$). Our results suggest that Sp-Ex could be applied as a system to control pathogens in place of chemical sanitizer washing by the fresh-produce industry.

IMPORTANCE Although most fresh-produce processing currently controls pathogens by means of washing with sanitizers, there are still problems such as the generation of harmful substances and changes in product quality. A combination system composed of the Spindle and a 222-nm KrCl excilamp (Sp-Ex) developed in this study reduced pathogens on apple and bell pepper surfaces using sanitizer-free water without altering produce color and texture. This study demonstrates the potential of the Sp-Ex to replace conventional washing with sanitizers, and it can be used as baseline data for practical application by industry. In addition, implementation of the Sp-Ex developed in this study is expected not only to meet consumer preference for fresh, minimally processed produce but also to reduce human exposure to harmful chemicals while being beneficial to the environment.

KEYWORDS detachment, eco-friendly, foodborne pathogens, fresh produce, hydrophobicity, surfactant, the Spindle, ultraviolet

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Address correspondence to Dong-Hyun Kang, kang7820@snu.ac.kr.

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While the health benefits of fresh produce have led consumers to increase their intake of these foods for more than 20 years, a number of illness outbreaks that have been attributed to fresh produce have also occurred every year (1–3). In order to reduce the risk of foodborne illness caused by consumption of fresh produce, proper measures must be taken to decrease the initial microbial counts to prevent microorganisms from reaching undesirable levels in fresh produce (4). Preharvest strategies such as the application of good agricultural practices (GAPs) in cultivation and harvesting contribute to reducing the risk of contamination, but there is still heavy reliance on decontamination strategies applied during processing of produce (2). Thus, application of appropriate disinfection procedures during processing is an important step to ensure the safety of fresh produce (5).

The current utilization of sanitizers in fresh-produce processing is based predominantly on washing with water and sanitizers to detach soil and debris from fresh-produce surfaces and reduce microbes that have the potential to cause outbreaks (6). Currently, many different types of chemical sanitizers are available for the surface treatment of fresh produce (7). These consist primarily of chlorine compounds, peracetic acid, hydrogen peroxide, quaternary ammonium compounds (QACs), and aqueous ozone (8). However, some of these sanitizers leave chemical residues, some others lead to visible damage when not properly used, and chlorine compound-based materials react with organic matter to produce carcinogenic halogenated by-products (1, 8, 9). For this reason, the use of these chemicals has become more limited and is likely to be phased out in the near future (10).

In this regard, UV irradiation can be considered as an intervention which may supplant chemical treatment because of its characteristics of nonthermal treatment and advantages over conventional chemical treatment, such as no disinfectant residuals, negligible formation of disinfection by-products (DBPs), and ease of retrofitting into existing processes (11–14). Either low-pressure (LP) or medium-pressure (MP) mercury lamps are commonly used as a UV source in UV disinfection systems (15, 16). However, application of UV technology to fresh produce surface treatment has the following major limitations: first, because of the characteristic shallow penetration depth of UV, not only are surface microorganisms not directly facing the UV lamp not treated with UV irradiation, but the UV inactivation effect is further reduced due to the shadowing effect if microorganisms are located in pores or other natural irregularities of the food surface (17–19). Second, the commonly used mercury-containing UV lamps are constructed from fragile quartz, which implies the possibility of mercury release and, consequently, the risk of harming human health and the environment (20, 21). Therefore, there has been a need to overcome the limitations inherent in UV and chemical sanitizers.

Huang et al. developed a "water-assisted UV system" which is configured to process samples immersed in water with pulsed light (PL) while agitating them with a stirring bar (22, 23). This system detaches and disperses microorganisms on the sample surface into the water through agitation, so that the microorganisms can easily be inactivated by UV while the sample is moved randomly to expose all surfaces to UV irradiation, resulting in an increased UV inactivation effect. This system exhibited an excellent disinfectant effect on several kinds of fruit (blueberries, raspberries, and strawberries). Since this system uses mercury-free PL technology as the UV source and disinfects the sample with only water without using a chemical sanitizer, it has potential as a new fresh-produce disinfection technique that can overcome the limitations of conventional mercury-containing UV technology and chemical sanitizers. However, we considered that there is still a need for this novel decontamination intervention to be systematized by incorporating an effective agitating technology to facilitate practical application by the fresh-produce industry.

The Spindle apparatus was developed in our laboratory to effectively detach microorganisms while keeping food samples intact in order to analyze levels of microorganisms on the food surface. This apparatus operates on the principle of the container holding the sample and solution being subjected to whirlpool movement generated by

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TABLE 1 Surviving populations of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on sample surfaces after 222-nm KrCl excilamp treatment

Treatment sample	Treatment time (min)	Surviving population[a] (log CFU/sample)		
		E. coli O157:H7	*S.* Typhimurium	*L. monocytogenes*
Apples	0	8.33 ± 0.03 Aa	8.40 ± 0.19 Aa	8.33 ± 0.12 Aa
	0.5	7.83 ± 0.20 Ba	7.84 ± 0.12 Ba	7.65 ± 0.05 Ba
	1	7.82 ± 0.22 BCa	7.77 ± 0.15 BCa	7.52 ± 0.05 Ba
	2	7.61 ± 0.03 BCDa	7.62 ± 0.12 BCDab	7.47 ± 0.03 Bb
	3	7.58 ± 0.06 CDa	7.47 ± 0.07 CDa	7.52 ± 0.08 Ba
	5	7.46 ± 0.17 DEa	7.34 ± 0.20 DEa	7.42 ± 0.26 BCa
	7	7.23 ± 0.06 Ea	7.08 ± 0.27 Ea	7.16 ± 0.27 Ca
Bell peppers	0	8.21 ± 0.11 Aa	8.29 ± 0.04 Aa	8.38 ± 0.37 Aa
	0.5	7.75 ± 0.15 Ba	7.72 ± 0.03 a	7.84 ± 0.14 Ba
	1	7.70 ± 0.15 BCa	7.62 ± 0.07 BCa	7.84 ± 0.14 Ba
	2	7.45 ± 0.06 Da	7.47 ± 0.13 CDa	7.41 ± 0.08 Ca
	3	7.48 ± 0.13 CDa	7.30 ± 0.17 DEa	7.31 ± 0.05 Ca
	5	7.05 ± 0.21 Ea	7.27 ± 0.06 Ea	7.35 ± 0.09 Ca
	7	6.86 ± 0.07 Fa	6.93 ± 0.11 Fab	7.14 ± 0.16 Cb

[a]Surviving populations are expressed as means ± standard deviations from three replications. Mean values with different uppercase letters within the same column for each sample are significantly different ($P < 0.05$). Mean values with different lowercase letters within the same row are significantly different ($P < 0.05$).

the motor, thereby effectively detaching microorganisms on the sample surface, and its detachment ability has been verified (24). Consequently, we have devised a systemized fresh-produce decontamination technique through combining the detachment technique (the Spindle) with UV technology. In this case, a relatively new 222-nm krypton chloride excimer lamp (KrCl excilamp), which could replace mercury lamps, was used as a UV source due to its long lifetime, nearly instantaneous warm-up, high radiation intensity, and absence of mercury (25–27).

The aim of this study was to evaluate the decontamination effect of a newly designed combination system consisting of the Spindle and 222-nm KrCl excilamp (Sp-Ex) against pathogens on fresh-produce (apple [*Malus domestica* Borkh.] and bell pepper [*Capsicum annuum* L.]) surfaces. In this case, *Escherichia coli* O157:H7, *Salmonella enterica* serovar Typhimurium, and *Listeria monocytogenes* were used as target pathogens because they are a major concern in fresh produce (28).

RESULTS AND DISCUSSION

Effect of individual 222-nm KrCl excilamp and Spindle treatment on pathogen inactivation on sample surfaces. Table 1 shows the inactivation effect of the 222-nm KrCl excilamp against *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on apple and bell pepper surfaces. Treatment with the 222-nm KrCl excilamp for 7 min resulted in 1.10-, 1.32-, and 1.17-log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on apple surfaces, respectively. In the case of bell pepper surfaces, 1.35-, 1.36-, and 1.24-log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively, occurred after the same treatment time. However, beyond 7 min of treatment (up to 30 min), there were no significant further reductions for any pathogen on both apple and bell pepper surfaces ($P > 0.05$) (data not shown), and thus in this study, individual 222-nm KrCl excilamp treatment was performed for up to 7 min. These results, therefore, indicate that individual 222-nm KrCl excilamp treatment is insufficient to meet the recommended 5-log reduction guidelines proposed by the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) when applied to the disinfection of apple and bell pepper surfaces (29). Unlike these results, our previous study (30) revealed that 222-nm KrCl excilamps resulted in a 5-log reduction of pathogenic bacteria under ideal conditions (phosphate-buffered saline [PBS] buffer) with doses of 1.6 to 3.8 mJ/cm^2 (in the present study, 7 min of treatment produced a dose of 180.6 mJ/cm^2). Woodling and Moraru (31) indicated that the ineffectiveness of UV treatment for decontaminating pathogens on surfaces is attributable to several factors, such as roughness, hydrophobicity, and reflectivity. Specifi-

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TABLE 2 Surviving populations of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on sample surfaces and in tap water after Spindle treatment

Treatment sample	Treatment time (min)	Surviving population[a] (log CFU/sample or log CFU/250 ml)					
		E. coli O157:H7		*S.* Typhimurium		*L. monocytogenes*	
		Sample[b]	Tap water[c]	Sample	Tap water	Sample	Tap water
Apples	0	8.16 ± 0.08 Aa		8.24 ± 0.18 Aa		8.20 ± 0.14 Aa	
	0.5	6.57 ± 0.20 Ba	7.88 ± 0.14 Aa	6.60 ± 0.15 Ba	7.91 ± 0.17 Aa	6.49 ± 0.25 Ba	7.99 ± 0.13 Aa
	1	6.60 ± 0.18 Ba	7.90 ± 0.16 Aa	6.51 ± 0.11 Ba	7.95 ± 0.17 Aa	6.61 ± 0.12 Ba	7.93 ± 0.19 Aa
	2	6.57 ± 0.17 Ba	7.91 ± 0.21 Aa	6.50 ± 0.16 Ba	7.95 ± 0.13 Aa	6.50 ± 0.12 Ba	8.03 ± 0.13 Aa
	3	6.49 ± 0.14 Ba	7.96 ± 0.14 Aa	6.44 ± 0.16 Ba	8.10 ± 0.15 Aa	6.44 ± 0.13 Ba	8.06 ± 0.13 Aa
	5	6.45 ± 0.16 Ba	7.99 ± 0.14 Aa	6.41 ± 0.17 Ba	8.10 ± 0.15 Aa	6.44 ± 0.15 Ba	8.15 ± 0.07 Aa
	7	6.48 ± 0.15 Ba	8.04 ± 0.19 Aa	6.45 ± 0.12 Ba	8.12 ± 0.15 Aa	6.37 ± 0.26 Ba	8.14 ± 0.12 Aa
Bell peppers	0	8.12 ± 0.11 Aa		8.32 ± 0.09 Aa		8.24 ± 0.16 Aa	
	0.5	6.44 ± 0.13 Ba	7.95 ± 0.15 Aa	6.49 ± 0.15 Ba	7.98 ± 0.21 Aa	6.55 ± 0.15 Ba	8.04 ± 0.13 Aa
	1	6.41 ± 0.19 Ba	7.87 ± 0.41 Aa	6.32 ± 0.20 Ba	7.97 ± 0.20 Aa	6.48 ± 0.15 Ba	8.04 ± 0.26 Aa
	2	6.42 ± 0.09 Ba	7.97 ± 0.29 Aa	6.43 ± 0.07 Ba	8.00 ± 0.22 Aa	6.48 ± 0.16 Ba	8.01 ± 0.14 Aa
	3	6.44 ± 0.17 Ba	7.93 ± 0.40 Aa	6.33 ± 0.12 Ba	8.06 ± 0.19 Aa	6.44 ± 0.17 Ba	8.09 ± 0.15 Aa
	5	6.42 ± 0.18 Ba	7.96 ± 0.29 Aa	6.42 ± 0.10 Ba	8.04 ± 0.16 Aa	6.41 ± 0.10 Ba	8.01 ± 0.15 Aa
	7	6.41 ± 0.26 Ba	8.04 ± 0.26 Aa	6.38 ± 0.13 Ba	8.08 ± 0.19 Aa	6.51 ± 0.12 Ba	8.05 ± 0.20 Aa

[a]Surviving populations are expressed as means ± standard deviations from three replications. Mean values with different uppercase letters within the same column for each sample are significantly different ($P < 0.05$). Mean values with different lowercase letters within the same row for sample or tap water are significantly different ($P < 0.05$).
[b]Log surviving population of pathogen on sample surface (log CFU/sample).
[c]Log surviving population of pathogen in tap water (log CFU/250 ml).

cally, a high degree of surface roughness hides microorganisms inside surface crevices and prevents them from being exposed to UV light. Also, on surfaces with high hydrophobicity, cells accumulate into thick aggregations which reduce the direct effect of UV light. In addition, surfaces with high reflectivity decrease the absorption of UV light by microorganisms, thereby reducing the effectiveness of UV light. Thus, such features make it difficult for UV to inactivate pathogens on surfaces.

This study assumes that Spindle technology provides solely a detachment effect and that pathogens can be inactivated by 222-nm KrCl excilamp treatment only after being released into tap water from sample surfaces by means of Spindle technology. However, if the Spindle also exhibits an intrinsic inactivation effect on pathogens, the actual decontamination principle of the combined Spindle and 222-nm KrCl excilamp (Sp-Ex) system should be considered different from what is assumed in this study. Therefore, in order to identify the actual decontamination principle of Sp-Ex, the decontamination effect of the Spindle was examined using apples and bell peppers as model foods. As shown in Table 2, Spindle treatment for 0.5 min resulted in 1.59-, 1.64-, and 1.71-log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on apple surfaces, respectively. In the case of bell peppers, Spindle treatment for 0.5 min resulted in 1.68-, 1.83-, and 1.69-log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. For both model foods, however, Spindle treatment did not achieve significant further pathogen reductions ($P > 0.05$) despite treatment exceeding 0.5 min. For both apples and bell peppers, levels of pathogens released into tap water during Spindle treatment were not significantly different from initial sample pathogen levels ($P > 0.05$). From this result that pathogens in tap water were not inactivated, it can be inferred that recontamination of the samples occurred due to a high concentration of dispersed pathogens in tap water following spindle treatment. This caused pathogen populations on sample surfaces not to decrease even with increasing treatment time. That is, it can be considered that Sp-Ex operates according to the principle assumed in our study, because the Spindle has been shown to be a technology for detaching pathogens on surfaces without exhibiting any inactivation effect. Failure to effectively reduce pathogens on samples is a typical limitation when washing with water only, and pathogens surviving in tap water can cause more serious problems throughout fresh-produce processing lines by means of cross-contamination (32). Therefore, effective

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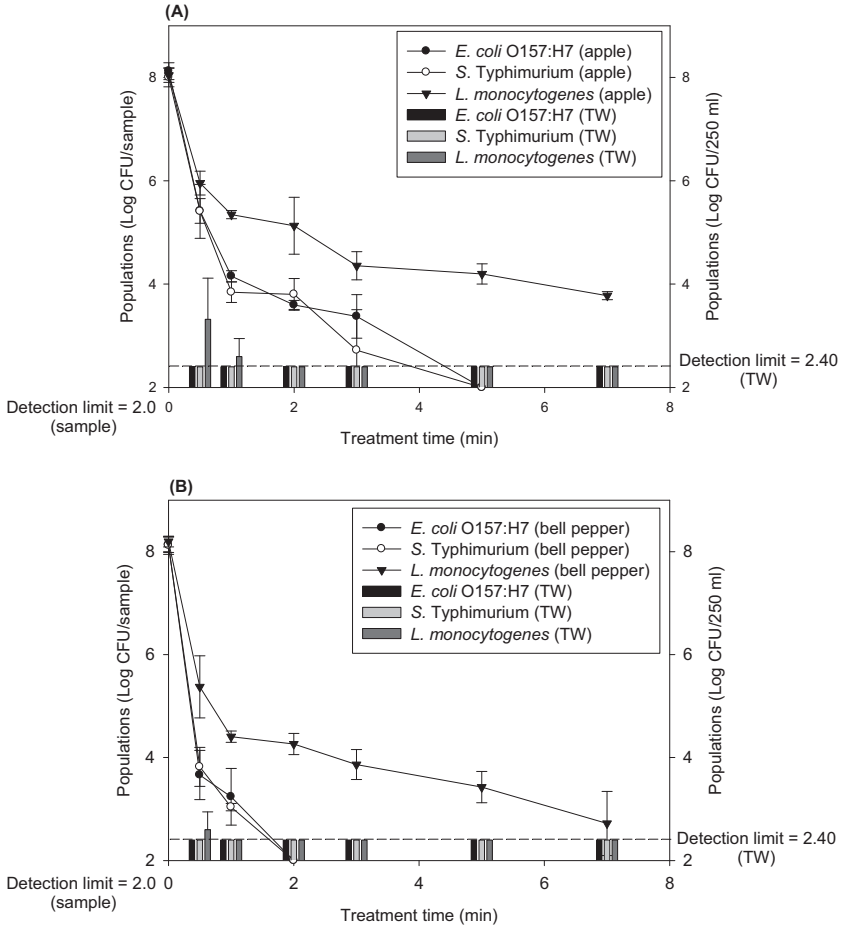

FIG 1 Surviving populations (log CFU/sample or log CFU/250 ml) of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on the sample surface or in washing solution (tap water [TW]) treated with the Spindle in combination with a 222-nm KrCl excilamp. The target samples were apples (A) and bell peppers (B). The error bars indicate standard deviations calculated from triplicates.

control of pathogens released into the washing solution during washing of fresh produce is of great importance in preventing cross-contamination as well as reducing pathogens on the sample surface (33).

Decontamination effect of Sp-Ex treatment. To address the aforementioned issues, despite inadequate pathogen inactivation of apple and bell pepper surfaces using single 222-nm KrCl excilamp or Spindle treatment, we attempted to control the pathogens on the model foods by combining the 222-nm KrCl excilamp, which effectively inactivated pathogens in solution, and the Spindle, which is a technology to detach microorganisms from sample surfaces. Figure 1 depicts surviving populations of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on sample surfaces or in the washing solution during treatment with the Sp-Ex. Initial populations of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on samples were approximately 10^8 CFU/sample, there were no significant differences in initial population regardless of pathogen species or samples ($P > 0.05$), and the limit of detection was 2.0 log CFU/sample. The Sp-Ex treatment showed a decontamination effect against pathogens on both apple and bell pepper surfaces, and cell levels were significantly ($P < 0.05$) decreased with increasing treatment time. In the case of apples, specifically, counts of *E. coli* O157:H7 and *S.* Typhimurium were reduced to below the detection limit, and those of *L. monocytogenes* were reduced by 4.20 log CFU/sample, at 5 min of Sp-Ex treatment. In the case of bell peppers, counts of *E. coli* O157:H7 and *S.* Typhimurium were reduced to below the detection limit and those of *L. monocytogenes* by 4.26 log CFU/sample at 2 min of treatment.

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Individual Spindle treatment detached and dispersed high levels of pathogenic cells into the washing solution, which were substantially reduced by the Sp-Ex treatment component. This is because pathogens released into the washing solution by the Spindle were inactivated by UV treatment. In the case of apples, specifically, surviving cells of *E. coli* O157:H7 and *S.* Typhimurium in the washing solution were not detected (detection limit = 2.40 log CFU/250 ml) at 0.5 min of Sp-Ex treatment, and those of *L. monocytogenes* were not detected after 2 min. In the case of bell peppers, surviving cells of *E. coli* O157:H7 and *S.* Typhimurium in the washing solution were not detected at 0.5 min of Sp-Ex treatment and those of *L. monocytogenes* were not detected after 1 min. Therefore, the Sp-Ex showed the decontamination effect by the principle that Spindle treatment detaches pathogens from sample surfaces and disperses them into the washing solution, where they are inactivated by simultaneous 222-nm KrCl excilamp treatment.

Factors affecting the decontamination effect of Sp-Ex. From our results of investigating the decontamination effect of the Sp-Ex, it was found that its efficacy depended on certain factors. First, the decontamination effect of the Sp-Ex varied depending on the type of pathogen. As shown in Fig. 1, for both apple and bell pepper surfaces, there was no significant difference in the numbers of surviving cells between *E. coli* O157:H7 and *S.* Typhimurium ($P > 0.05$), but *L. monocytogenes* survivors were significantly more numerous ($P < 0.05$) than the others at the same treatment time. In other words, *L. monocytogenes* showed higher resistance than *E. coli* O157:H7 and *S.* Typhimurium, and *E. coli* O157:H7 and *S.* Typhimurium showed similar resistance to the Sp-Ex. We hypothesized that differences in the adhesion force of microbes on fresh-produce surfaces depending on the species of pathogen caused differences in resistance to Sp-Ex treatment, because this system operates on the principle that pathogens can be inactivated mainly when they are detached from the sample surface, and thus detaching ability is directly related to the decontamination effect. Attachment characteristics of microorganisms to surfaces are affected by various factors, such as flagella and fimbriae, extracellular polysaccharides, and bacterial hydrophobicity and surface charge (34–36). Especially, several studies have reported that cell surface hydrophobicity has a major effect on microbial attachment between solid and liquid (35, 37). Therefore, we postulated that the degree of microorganism attachment to food surfaces during water immersion under the treatment conditions of this system would be affected mainly by cell hydrophobicity, because the Sp-Ex has the ability to detach microorganisms from surfaces of samples suspended in tap water. Generally, hydrophobic cells exhibit stronger surface adhesion properties than hydrophilic cells due to hydrophobic interaction (38–40). Okuku and Fett (36) demonstrated that bacterial cell hydrophobicity was highly correlated with the ability of bacteria to resist being washed off cantaloupe surfaces with water. The cell surface hydrophobicities of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* were 7.39 ± 1.17, 6.15 ± 0.73, and 21.14 ± 4.87% (means ± standard deviations from three replications), respectively. The hydrophobicity level of *L. monocytogenes* was significantly ($P < 0.05$) the highest, and those of *E. coli* O157:H7 and *S.* Typhimurium were not significantly different ($P > 0.05$). This result is consistent with the tendency of pathogen reduction using the Sp-Ex. In other words, the higher the cell surface hydrophobicity of the pathogen, the greater the resistance to Sp-Ex detachment. Therefore, it can be interpreted that pathogens which exhibit higher cell surface hydrophobicity show greater resistance to Sp-Ex treatment because they are more strongly attached to fresh-produce surfaces and thus less detached by spindle treatment, resulting in fewer cells undergoing UV treatment in tap water.

Second, the decontamination effect of the Sp-Ex varies depends on the type of food as well as pathogen. Overall, the Sp-Ex showed a significantly ($P < 0.05$) greater reduction effect on cells attached to bell pepper surfaces than on those on apple surfaces for the same type of pathogen and treatment time. Surface attachment of microorganisms is affected not only by microorganism properties but also by the physicochemical characteristics of the sample surface (41). Among them, surface hy-

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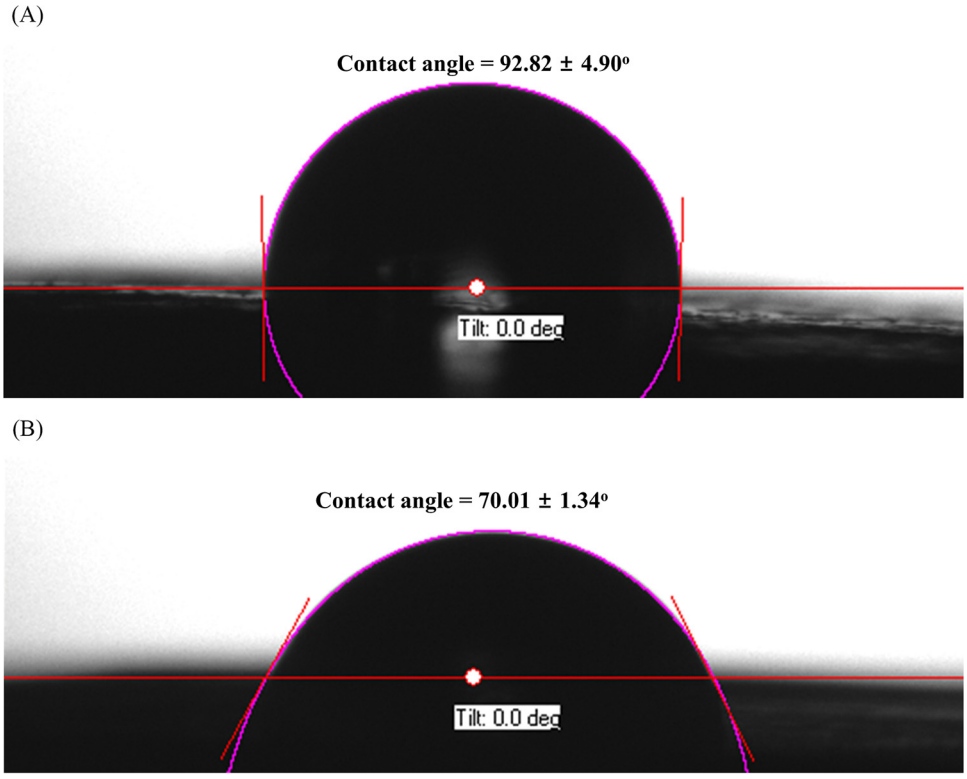

FIG 2 Photographs of a water droplet and its contact angle on apple (A) and bell pepper (B) surfaces. Contact angle values are means ± standard deviations from three replications. There was a significant difference ($P < 0.05$) in contact angle values between apple and bell pepper surfaces.

drophobicity and surface roughness are the main factors mediating microbial adhesion (42). Wang et al. (43) found that the surface roughness and attachment rate of *E. coli* O157:H7 showed a positive linear correlation when washing *E. coli* O157:H7 on four types of fruit surfaces exhibiting different roughnesses. In addition, several studies have reported that bacterial adhesion increases with increasing surface hydrophobicity (44–46). Thus, based on these factors, we quantified and compared the surface roughness and surface hydrophobicity of apples and bell peppers. The two-dimensional parameter surface roughness is quantified as an R_a value, which is the arithmetical mean deviation of the height profile. Surface hydrophobicity was also quantified by the contact angle formed between a water droplet and the surface of the sample. When comparing surface roughness, there was no significant difference in the R_a values of apples and bell peppers ($P > 0.05$) (data not shown). This means that surface roughness does not cause a difference in the decontamination effect of the Sp-Ex, because these two samples have similar surface roughness characteristics. Meanwhile, when the hydrophobicity of each surface was quantified according to the contact angle, apple and bell pepper surfaces had values of 92.82° and 70.01°, respectively, and the contact angle value of the apple surface was significantly ($P < 0.05$) larger than that of the bell pepper surface (Fig. 2). The contact angle is positively proportional to the hydrophobicity of the surface. Therefore, it can be interpreted that the decontamination effect of the Sp-Ex for apple surfaces is lower than that for bell pepper surfaces because the attachment strength of pathogens on apple surfaces is greater due to the higher hydrophobic interaction of pathogens with apple surfaces. From these findings, we expected that a strategy to reduce the attachment strength of pathogens could enhance the decontamination effect of this combination system. In particular, establishing this strategy is crucial because it can eventually reduce processing time and ensure greater food safety.

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Enhanced decontamination effect of the Sp-Ex by addition of surfactant. Since surfactants have both a hydrophobic and a hydrophilic group, altering the surface properties of liquids at the liquid-solid interface, surfactants can reduce the interfacial tension between a liquid and a solid (47, 48). Thus, water containing surfactants has a lower interfacial tension and is spread more easily to solid surfaces (49). In particular, Paul and Jeffrey (50, 51) reported that surfactants led to decreasing hydrophobic interaction associated with bacterial attachment based on properties of surfactants. In many studies, based on this principle, surfactants have been applied with various decontamination techniques, such as chlorine (49, 52–54), ozone (55), organic acid (56–58), hydrogen peroxide (56), and ultrasound (59) washing, to enhance the decontamination effect by promoting the detachment of pathogens on fresh-produce surfaces. In this study, we found that the degree of attachment through the hydrophobic interaction between pathogens and the sample surface is a primary factor affecting the decontamination effect of the Sp-Ex. Thus, we expected that the application of a surfactant to the Sp-Ex combination treatment will improve the detachment effect on pathogens on sample surfaces so that the decontamination effect will be enhanced by increasing the number of suspended pathogenic cells to be treated with UV in tap water. Accordingly, we tried to confirm this hypothesis. Polysorbates such as Tween 20, 40, 60, 80, and 85, which are nonionic surfactants and generally recognized as safe (GRAS) (49), are in common use, and among those, 0.1% Tween 20 was selected and used in this study because it facilitated the greatest pathogen reductions when combined with ultrasound in the study by Sagong et al. (59). As shown in Fig. 3, Sp-Ex treatment including 0.1% Tween 20 significantly ($P < 0.05$) reduced pathogens remaining on apple and bell pepper surfaces compared with Sp-Ex treatment without incorporation of Tween 20 (Fig. 1). Specifically, Sp-Ex treatment including Tween 20 reduced *E. coli* O157:H7 and *S.* Typhimurium on apple surfaces to below the detection limit with 2 min of treatment and reduced *L. monocytogenes* to below the detection limit with 7 min of treatment, whereas at the same treatment times (2 min for *E. coli* O157:H7 and *S.* Typhimurium and 7 min for *L monocytogenes*), Sp-Ex treatment without Tween 20 left 3.60, 3.80, and 3.78 logs of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively, remaining on apple surfaces. In the case of bell peppers, Sp-Ex treatment including Tween 20 reduced *E. coli* O157:H7 and *S.* Typhimurium to below the detection limit after 1 min of treatment and reduced *L. monocytogenes* to below the detection limit following 7 min of treatment, whereas at the same treatment times (1 min for *E. coli* O157:H7 and *S.* Typhimurium and 7 min for *L. monocytogenes*), Sp-Ex treatment without Tween 20 left 3.24, 3.04, and 2.72 logs of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively, remaining. In accord with our expectation, therefore, application of a surfactant to Sp-Ex treatment can lead to an improvement in its decontamination effect based on the principle that surfactants weaken adhesion between pathogens and fresh-produce surfaces through hydrophobic interaction, thus increasing the detachment effect of the Spindle and resulting in an enhanced decontamination effect due to increased numbers of pathogen cells in aqueous suspension subject to UV inactivation in tap water.

Effect of the Sp-Ex on apple and bell pepper quality. It is necessary to assess changes in quality during storage before commercial application of the Sp-Ex can occur. UV irradiation from a 222-nm KrCl excilamp applied to the sample surface potentially could cause visible damage, and the Spindle could possibly affect sample texture because it acts mechanically on the sample to detach pathogens from the sample surface. Thus, we measured color and texture changes of sample surfaces to examine the effect of treatment with the combination system on apple and bell pepper quality. For color and texture change analysis of the sample, Sp-Ex treatment with or without 0.1% Tween 20 was applied for the time interval in which *L. monocytogenes*, the pathogen with greatest resistance, was reduced to below the detection limit, and then Hunter's color values (L^*, a^*, and b^*) and the maximum load value (g), which represents the hardness of the sample, were compared between untreated controls and samples

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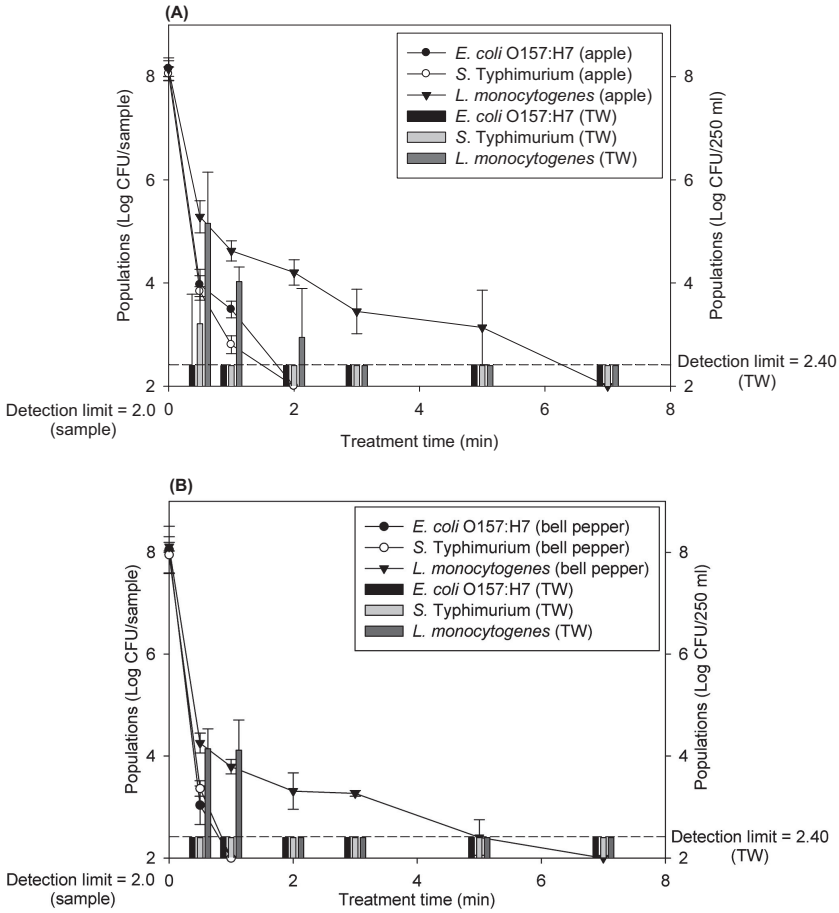

FIG 3 Surviving populations (log CFU/sample or log CFU/250 ml) of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on sample surfaces or in washing solution (tap water [TW] plus 0.1% Tween 20) treated with the Spindle in combination with a 222-nm KrCl excilamp. Target samples were apples (A) and bell peppers (B). The error bars indicate standard deviations calculated from triplicates.

treated with the Sp-Ex during storage at 4°C for 7 days. The results demonstrated that there were no significant differences in either color or maximum load values between the controls and treated samples within those 7 days ($P > 0.05$) (data not shown). Therefore, these results indicate that the Sp-Ex effectively reduces pathogens without generating visible color or texture damage to apples and bell peppers.

Conclusion. The findings obtained from this study suggest that use of the combination system consisting of the Spindle and 222-nm KrCl excilamp is a method that effectively reduces pathogens on apple and bell pepper surfaces by the principle that pathogens on sample surfaces are detached by the Spindle and simultaneously inactivated by the 222-nm KrCl excilamp. Therefore, this result supports the possibility that the Sp-Ex can be applied to apple or bell pepper processing to effectively control pathogens instead of using conventional washing with sanitizers. Furthermore, since addition of a surfactant to weaken the hydrophobic interaction between pathogens and sample surfaces helps to improve the decontamination effect of the Sp-Ex, if a surfactant is incorporated into Sp-Ex processing, it is expected that not only an improvement in productivity by reducing the processing time but also an enhancement in safety by increasing the decontamination effect of the Sp-Ex can be achieved.

As shown in Fig. 4, the Sp-Ex used in this study was a small experimental device, and its effect was verified at the laboratory scale for samples with an area of 10 cm^2. However, for practical application, a scaled-up or pilot-scale system should be developed, and the decontamination effect on samples, such as whole fresh produce,

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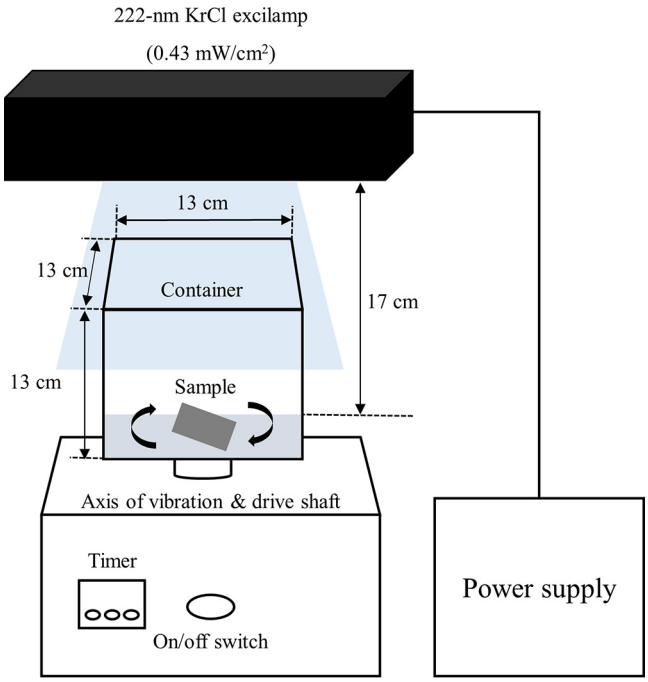

FIG 4 Schematic diagram of the decontamination system composed of the Spindle and 222-nm KrCl excilamp used in this study.

processed under commercial conditions should be investigated. In this case, since the system capacity, the volume of the solution, the power of the motor, and the number and size of the samples may affect the decontamination effect, for effective application, optimization studies considering these factors should be performed. Meanwhile, in this study, apples and bell peppers, which are vulnerable to contamination by pathogenic bacteria and thus are implicated in numerous foodborne disease outbreaks (60–65), were selected as representative target foods, and the decontamination effects on these samples were verified. In order for the Sp-Ex to be widely applied to fresh-produce processing, however, it is also necessary to identify its applicability with various other kinds of fresh produce besides apples and bell peppers, such as sprouts or leafy vegetables, which are frequently involved in foodborne disease outbreaks (66, 67). Consequently, in order for the Sp-Ex system developed in this study to be effectively applied by industry, it is essential to develop a scaled-up system that can be applied in practice and to optimize this system by studying the deconmtaination effect considering various factors and sample types through further research.

MATERIALS AND METHODS

Bacterial strains and inoculum preparation. Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, and ATCC 43890), *S.* Typhimurium (ATCC 19585, ATCC 43971, and DT 104), and *L. monocytogenes* (ATCC 19111, ATCC 19114, ATCC 19115) obtained from the bacterial culture collection of Seoul National University (Seoul, South Korea) were used. Stock and working cultures were prepared according to a previously described method (30). A single colony of each strain of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* cultured from stocks on tryptic soy agar (TSA) (Difco, Becton, Dickinson, Sparks, MD), were inoculated individually into 15 ml tryptic soy broth (TSB) (Difco), incubated at 37°C for 24 h, and then collected by centrifugation at 4,000 × *g* for 20 min at 4°C. After three washes with 0.2% peptone water (PW) (Bacto, Becton, Dickinson, Sparks, MD), suspended pellets of the three pathogens were combined to constitute a mixed-pathogen-species culture cocktail corresponding to approximately 10^8 to 10^9 CFU/ml.

Sample preparation and inoculation. Bell peppers and unwaxed apples were purchased from a local market (Seoul, South Korea), stored under refrigeration (4 ± 2°C), and used within a week. Since the reduction effect of each treatment should be compared using the same sample surface area to examine the decontamination effect on pathogens on sample surfaces, intact portions of sample surfaces were cut into 2- by 5-cm (10-cm^2) pieces. A spot inoculation method was used to inoculate pathogens onto the prepared samples (68). Briefly, sample pieces were placed on sterile aluminum foil in a laminar flow

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hood and inoculated with 0.1 ml of the above-described culture cocktail by depositing small droplets with a micropipette at 10 to 15 locations. The inoculated samples were dried for 1 h in the laminar flow biosafety hood at room temperature (22 ± 2°C) to allow sufficient attachment of bacteria and then were subjected to treatment.

Experimental setup. The Spindle apparatus used in this experiment consisted of an electric motor, a time controller, and a container to contain samples and solutions. The drive motor vibrated the container connected to the motor very quickly and vigorously. The body (20 by 21 by 16 cm^3) consisted of the motor and container (13 by 13 by 13 cm^3) connected vertically. A dielectric barrier discharge (DBD)-driven excilamp (20 by 10 by 10 cm^3; Unilam, Ulsan, South Korea) filled with a KrCl gas mixture was used for UV irradiation treatment in this study. The cylindrical geometry excilamp connected to the power supply (110 W) was covered by a metal case having a UV exit window with an area of 60 cm^2 (10 by 6 cm^2). The Spindle and excilamp combination decontamination system was constructed by vertically and directly placing a 222-nm KrCl excilamp above the Spindle using a stainless steel holder. The distance between the 222-nm KrCl excilamp and the surface of 250 ml of sterile tap water inside the container attached to the Spindle was 17 cm, and the radiation intensity at the surface of the sterile tap water was 0.43 mW/cm^2 as measured with a UV fiber optic spectrometer (AvaSpec-ULS2048; Avantes, Eerbeek, Netherlands) calibrated to a range of 200 to 400 nm within the UV-C spectrum. A schematic of this experimental setup is depicted in Fig. 4. Meanwhile, for single 222-nm KrCl excilamp treatment, the excilamp was placed vertically at a position 17 cm away from the sample surface using a stainless steel holder.

Treatment. For treatment with the Sp-Ex, inoculated samples were immersed in 250 ml of sterile tap water in the container, and the Spindle and 222-nm KrCl excilamp were operated simultaneously at room temperature (22 ± 2°C) for 0.5, 1, 2, 3, 5, or 7 min. For convenience, the container was lined with polyethylene (PE) film before the sample and solution were added to the container and the lining changed with every treatment run, because if not, the container would have to be disinfected before every treatment. The speed of the Spindle's motor was 7,000 × *g*. Moreover, the single Spindle or Sp-Ex treatment with surfactant was performed based on the procedure described above, either with or without operation of the 222-nm KrCl excilamp or use of Tween 20 (Sigma, St. Louis, MO, USA) diluted to 0.1% in sterile tap water. For single UV treatment, the inoculated samples were placed on sterile aluminum foil and treated with the 222-nm KrCl excilamp at room temperature (22 ± 2°C) for 0.5, 1, 2, 3, 5, or 7 min.

Bacterial enumeration. Following treatment, samples were immediately transferred into sterile stomacher bags (Labplas Inc., Sainte-Julie, QC, Canada) containing 100 ml of PW and homogenized with a stomacher (Easy Mix; AES Chemunex, Rennes, France) for 2 min. Simultaneously, aliquots of the tap water in which samples were immersed were sampled to enumerate surviving bacteria suspended in the tap water after treatment. After homogenization or sampling, 1-ml aliquots of stomacher-treated samples or tap water suspensions were 10-fold serially diluted in 9 ml of PW, and 0.1-ml aliquots of samples or diluents were spread-plated onto selective media. Sorbitol-MacConkey agar (SMAC) (Difco), xylose-lysine-desoxycholate agar (XLD) (Difco), and Oxford agar base with Bacto Oxford antimicrobial supplement (MOX) (Difco) were used as selective media for the enumeration of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. Where low bacterial numbers were anticipated, 250 μl of undiluted sample was plated onto 4 plates of each respective medium. After incubation at 37°C for 24 h, colonies were counted and calculated as $\log_{10}$ CFU/sample or $\log_{10}$ CFU/250 ml for treated sample or tap water, respectively. In this case, the detection limits for treated sample or tap water were 2.00 $\log_{10}$ CFU/sample or 2.40 $\log_{10}$ CFU/250 ml, respectively, because the sample was homogenized in 100 ml of PW and then 1 ml of homogenized PW was collected to count the surviving population, and in the case of tap water, 1 ml of 250 ml tap water was collected to confirm the surviving population.

If a homogenized solution of apples or bell peppers has an antimicrobial effect because of antimicrobial substances that may be present, this may cause an overestimated result in this study. To examine this effect, culture cocktail was inoculated into homogenized solutions of uninoculated apples or bell peppers, and then the inoculated solutions were spread-plated onto each selective medium after 1 h of incubation time to enumerate surviving bacterial populations. In this case, the control was the surviving population of culture cocktail inoculated into tap water. The results showed that there was no significant difference between the bacterial populations in tap water and in the homogenized sample solutions ($P > 0.05$) (data not shown). Since this suggests that the above-described method is an appropriate procedure that did not adversely affect the results, no further steps to neutralize the reactivity of homogenized solution that could affect bacterial survival were performed in this study.

Cell surface hydrophobicity of pathogens. Cell surface hydrophobicity was measured by microbial adhesion to *n*-hexadecane (MATH assay) as described previously (69). Briefly, cocktails composed of the three strains of a single pathogen species were suspended in phosphate-buffered saline (PBS) (0.1 M) to obtain an optical density at 600 nm (OD_{600}) of ~0.8. This bacterial suspension was mixed with an equal volume of *n*-hexadecane by vortexing for 1 min and then left for 20 min to allow for complete phase separation. After phase separation, the hydrophobicity of the pathogen was quantified to the extent that the cells migrated to nonpolar solvent (*n*-hexadecane) and calculated as follows: hydrophobicity (%) = $[1 - (A_1/A_0)] \times 100$, where A_0 is the initial aqueous phase absorbance and A_1 is the aqueous phase absorbance mixed with *n*-hexadecane after 20 min of incubation.

Analysis of surface characteristics. The contact angle and roughness of the surface were measured to compare the surface characteristics of apples and bell peppers. The static contact angles of water droplets on sample surfaces were measured with a contact angle goniometer (Theta Lite, Attension, Finland) at room temperature (22 ± 2°C). A sample specimen was fixed onto a glass slide using adhesive

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tape, and 4-μl drops of deionized water were placed on five different points of the sample surface. Immediately after applying a drop, the contact angle was measured within 1 s, and the average of five different measurements obtained at the five different points was used for the analysis.

White light scanning interferometry (WLSI) was used to quantify roughness of the sample contact surface. A sample specimen was placed on the stage of a noncontact three-dimensional (3D) surface profiler (NanoView-E1000; NanoSystem, Daejeon, South Korea) with a 50× lens objective. The surface roughness values were measured using a software package (NanoMap version 2.5.17.0; Nanosystem, Daejeon, South Korea), and the average of five different measurements was acquired from five randomly selected scan areas (125 by 95 μm) on the sample surface.

Color and texture measurement. Samples were treated with the Sp-Ex with or without surfactant. After treatment, treated and untreated (control) samples were stored at 4°C for 7 days to investigate color and texture changes of samples during storage. Color values of sample surfaces were measured using a Minolta colorimeter (model CR400; Minolta Co., Osaka, Japan) at 3 different locations on the same surface and expressed as L*, a*, and b* values. The L*, a*, and b* values represent color lightness, redness, and yellowness of the sample, respectively (70). The averaged values measured from 3 different locations were used for the analysis. Sample texture was evaluated with a texture analyzer (TA-CT3; Brookfield Engineering Laboratory, Inc., Middleboro, MA, USA) with a cylinder set probe with a 35-mm diameter. A sample was placed onto the press holder, and a cylinder probe was moved to the sample at 2 mm/s (path length, 10 mm). Hardness was used as an indicator of texture change because apples and bell peppers are solid samples, and it was quantified by measuring the maximum force value (*g*) of a deformation curve recorded using Texturepro CT software (Brookfield Engineering Laboratory, Inc.). The averaged values measured from three independently prepared samples per treatment were used for the analysis.

Statistical analysis. All experiments were repeated three times. All data were analyzed by analysis of variance (ANOVA) using the Statistical Analysis System (SAS Institute, Cary, NC, USA), and mean values were separated using the least significant difference (LSD) *t* test. A probability level of $P < 0.05$ was used to determine significant differences between values.

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