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RESEARCH ARTICLE

Effect of environmental water activity on microbial inactivation by intense pulsed light (IPL)

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Abstract

In this study, the effect of environmental a_w on microbial inactivation by intense pulsed light (IPL) was investigated. Three different microorganisms (Gram-positive bacteria, Gram-negative bacteria, and yeast) were used as test organisms. The effect of environmental a_w was assessed by irradiating each microbial suspension in sodium chloride solutions with different environmental a_w levels (0.99–0.80). As the a_w decreased, the aggregation of intracellular material of cell interior was changed and the cell number was increased. However, there was no significant difference in microbial reduction according to the a_w after the 0.23–3.05 J/cm^2 of IPL treatment. It was confirmed that yeast had the highest resistance to IPL because of the differences in cell structure and cell wall components between yeast and bacteria. Additional research is needed to clearly understand the inactivation mechanism according to the type of microorganism by controlling a_w using various solutes.

Keywords Intense pulsed light · Water activity · bacteria · Yeast · Resistance

Introduction

Intense pulsed light (IPL) is a non-thermal processing in which food is irradiated with short and intense light pulses with a broad spectrum (180–1100 nm) to inactivate pathogens (Barbosa-Cánovas et al., 2000; Elmnasser et al., 2007). The IPL system consists of a treatment chamber, power supply, and xenon lamp. Electric energy is stored in a capacitor for a very short time and is then released to a xenon lamp. As the xenon ion becomes excited and returns to the ground state, it emits intense pulsed light. The light covers a spectrum ranging from UV to the near-IR region. (Elmnasser et al., 2007). This technology is known by several names, such as PL; high-intensity broad-spectrum PL; and pulsed white light (Farrell et al., 2010).

The UV region, which accounts for 25% of the spectrum, is a significant factor responsible for IPL inactivation (Takeshita et al., 2003). As the lethal effect of IPL is related to the UV region, its antimicrobial mechanism is also mainly attributed to the photochemical effect of UV, i.e., the formation of thymine dimer. A thymine dimer damages the replication and transcription of genetic information, resulting in cell death. However, the inactivation mechanism of IPL is considered to be different from that of a continuous UV system because IPL inactivation includes not only photochemical effects but also photophysical and photothermal effects. This was confirmed through the results of a study comparing the inactivation and cell damage of *Escherichia coli* O157:H7 and *Listeria monocytogenes* treated with UV-C and IPL (Cheigh et al., 2012). DNA damage, such as the accumulation of double-strand breaks (DSBs), single-strand breaks, and cyclobutane pyrimidine dimers, was noted in both UV- and IPL-treated cells, indicating that these treatments caused photochemical effects in cells. On the other hand, analysis of transmission electron microscope (TEM) images revealed that the cell structure was destroyed after IPL treatment but not after UV-C treatment, indicating that

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photothermal and photophysical effects appeared only in IPL-treated cells. Moreover, a study has suggested that IPL is about 4–6 times more effective in microbial inactivation than a continuous UV system (Fine and Gervais, 2004). This increase in efficiency is thought to be caused by the photophysical and photothermal mechanisms of IPL.

In the case of thermal processing, Syamaladevi et al. (2016) found that increase in microbial resistance at lower environmental a_w levels. Moreover, several studies have investigated the effect of environmental a_w during nonthermal processing. Hamanaka et al. (2006) investigated the inactivation tendency of *Bacillus subtilis* spores under nine different a_w conditions at three peak wavelengths in the IR region: 950, 1100, and 1150 nm. The inactivation tendency was found to differ depending on the peak wavelength and initial a_w conditions. In addition, when the wavelength decreased, a higher a_w level resulted in the maximum D-value. Also, Gayán et al. (2011 and 2014) assessed the inactivation effect of UV-C on *Staphylococcus aureus* and *Escherichia coli* in liquids with different physicochemical properties. They found that pH and a_w were not related to microbial resistance, while absorption coefficient and resistance were inversely correlated. In addition, more studies have been conducted on the effect of a_w on various nonthermal technique, such as PEF and ultrasonic treatment (Aronsson & Rönner, 2001; Alvarez et al., 2003). Chen et al. (2018) identified the sterilization efficiency of IPL according to the initial a_w (0.20–0.35) of fat-free milk powder and noted higher inactivation efficiency when the a_w level was relatively lower. It is important to understand the impact of environmental a_w on IPL inactivation. However, there is very little information about the effect of environmental a_w on IPL processing. Therefore, there is a need for a fundamental study that could be applied to more diverse conditions of a_w and microbial strains.

Materials and methods

Strains and microbial cultures

Gram-positive bacteria *Staphylococcus aureus* ATCC 12,692, Gram-negative bacteria *Salmonella* Enteritidis ATCC 13,076, and yeast *Saccharomyces cerevisiae* IFO 1950 were used in the experiments. All these microorganisms were acquired from the Korean Culture Center of Microorganisms (Seoul, Korea). They were cultured on nutrient agar (NA; Difco™, BD, Sparks, MD, USA) or tryptic soy agar (TSB; BBL™, BD, Sparks, MD, USA, Agar; SAMCHUN, Pyeongtaek, Korea) and stored at 4 °C. A colony from each agar plate was inoculated into 10 mL of tryptic soy broth (TSB; BBL™, BD, Sparks, MD, USA) for precultivation of *S. aureus* and *S.* Enteritidis. In case of *S. cerevisiae*, the yeast malt broth (YMB; Difco™, BD, Sparks, MD, USA) was used. They were precultured at 30 °C for 48 h for *S. cerevisiae* or at 37 °C for 24 h for other organisms in a shaking incubator. Then, 3 mL of the precultured medium was taken and inoculated into 300 mL of TSB for main culture of *S. aureus* and *S.* Enteritidis, and YMB for *S. cerevisiae*. Each inoculated broth was cultured under the same conditions as those used for the preculture step in order to obtain final microbial cultures with an initial cell density of 10^8 CFU/mL.

Adjustment of environmental water activity

To assess the effect of a_w of the treatment environment on IPL treatment, the a_w condition of the sample was adjusted using NaCl referring to the study of Mazas et al. (1999). Water activities according to each aqueous NaCl solution of a specific concentration were measured using a water activity meter (AquaLab LITE, Decagon Devices, USA) (Fig. 1). Accordingly, samples were prepared using aqueous NaCl solutions adjusted to a_w levels of 0.99, 0.95, 0.90, 0.85, and 0.80, which the concentrations are corresponded to 0.15 M, 1 M, 2 M, 3 M and 4 M, respectively. Both the preparation of solution and the measurement of a_w were conducted at room temperature.

Sample preparation

In total, 10 mL of each microbial culture (initial density of about 10^8 CFU/mL) was taken, added to a conical tube,

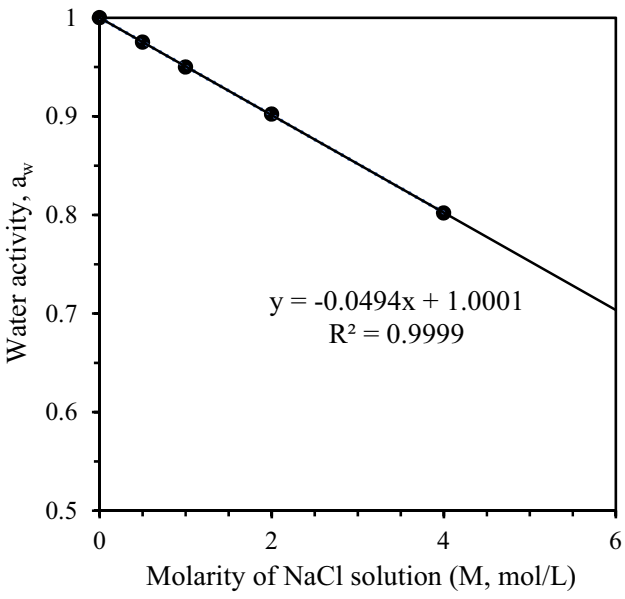

Fig. 1 Water activity level according to the concentration of NaCl solution at 20 °C

and centrifuged at 4000×*g* for 15 min at room temperature. The supernatant was removed to obtain the cell pellet. The pellet was washed with 0.85% NaCl solution. The same process was repeated for 3 times to leave only the cells. Then, the pellets were resuspended in NaCl solutions with five different a_w levels to obtain the microbial population of approximately 10^7–10^8 CFU/mL. Each suspension was mixed thoroughly and left at room temperature for 1 h. As cell death may occur due to low a_w, an adaptation time of 1 h was given to confirm the same and to prevent the occurrence of experimental errors. The long-term effect of high NaCl concentration on microbial cells was not considered, since the present study investigated the effect of environmental a_w of treatment media where the light is irradiated. After an hour, 4 mL of each suspension was poured into 50 × 15 mm Petri dish for treatment.

Transmission electron microscopy (TEM)

The changes in the internal structure and morphology of cells according to the environmental a_w conditions were analyzed using TEM (JEM-1011, JEOL, Tokyo, Japan) at an acceleration voltage of 80 kV equipped with a Megaview III CCD camera (Soft imaging system-Germany). The analysis was performed in collaboration with Yonsei Biomedical Research Institute. To assess the changes in cells according to the environmental a_w levels, each microorganism was suspended for 1 h in NaCl solutions with a_w levels of 0.99 and 0.80, before the fixation procedure.

IPL treatment

Each microbial suspension in aqueous NaCl solutions under different a_w conditions (0.99–0.80) was treated using a laboratory-scale IPL device, as shown in Fig. 2. The device consists of treatment chamber, power supply, and cooling device. At the top and side of the treatment chamber, there were cooling fans to prevent overheating of treatment chamber. There was a plate below the lamp to place the sample. By moving the position of this plate, the distance between the sample and the lamp could be adjusted. In this experiment, the distance between the sample and the lamp was 9 cm, the frequency of the IPL was 5 Hz, and the charging voltages of IPL were 800, 1000, and 1200 V. The total fluences of IPL applied to the microorganisms were 0.23–3.05 J/cm^2 (Fig. 3). The energy fluence was measured using a spectroradiometer (ILT-900, International Light Technologies, Peabody, MA, USA) immediately before all the experiments so that the energy fluence received by the sample was kept constant for each repetitive experiment. The probe of spectroradiometer was at the same distance as the sample, and the sensor side of the probe faced toward the xenon lamp. After treatment, the treated suspension samples were immediately diluted serially using 0.85% NaCl solution and spread on yeast malt agar (YMA) in the case of *S. cerevisiae* or on TSA in the case of other bacterial samples, *S. aureus* and *S.* Enteritidis. The agar plates were then incubated at 30 °C for 48 h for *S. cerevisiae* or at 37 °C for 24 h for other organisms. After incubation, the number of colonies was determined. The microbial reduction value was calculated as $\log_{10}(N/N_0)$, where N_0 is the initial population

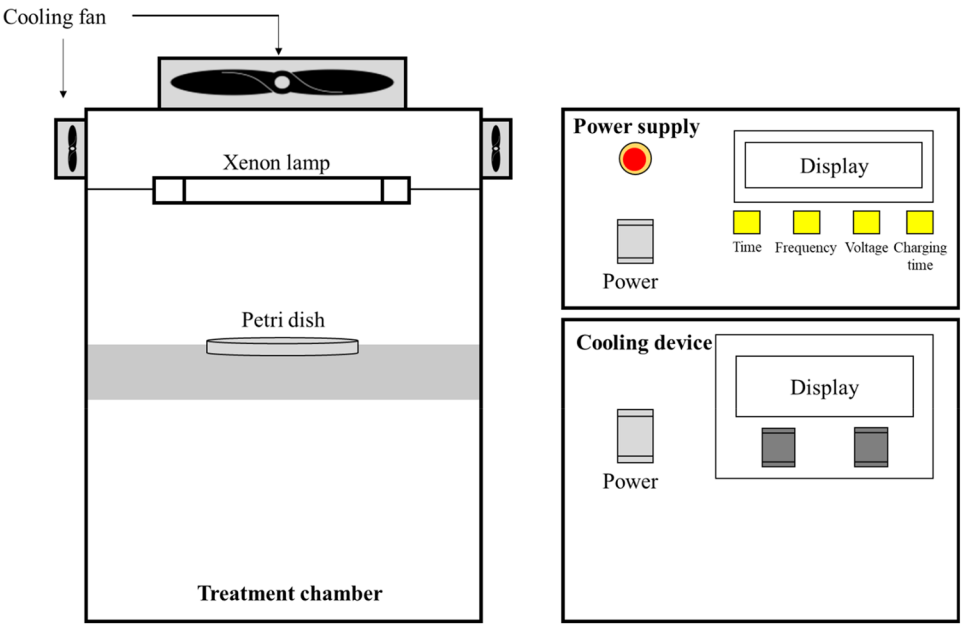

Fig. 2 Diagram of a laboratory-scale IPL device

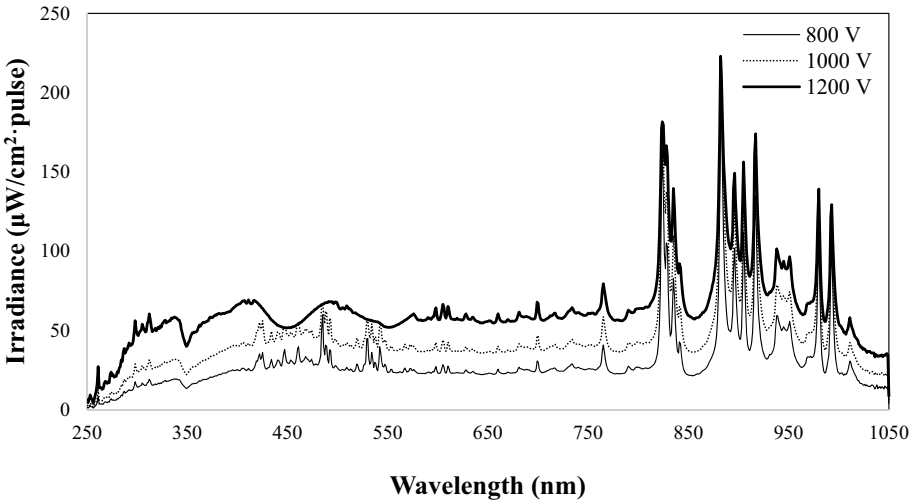

Fig. 3 Light energy distribution of IPL irradiated at different voltages

of the pretreatment sample and *N* is the number of surviving populations.

Statistical analysis

One-way ANOVA and Scheffe test with $p < 0.05$ were performed using the statistical package SPSS Statistics (IBM, Chicago, IL, USA) to assess if there were significant differences between each treatment condition.

Results and discussion

Changes in microbial cells according to the condition of a_w

In this study, the a_w was adjusted by varying the NaCl concentration in the microbial suspension before the IPL treatment. Figure 4 showed the TEM images of cross-sections of untreated cells that were suspended for 1 h in each aqueous NaCl solution with high environmental a_w (0.99) or low

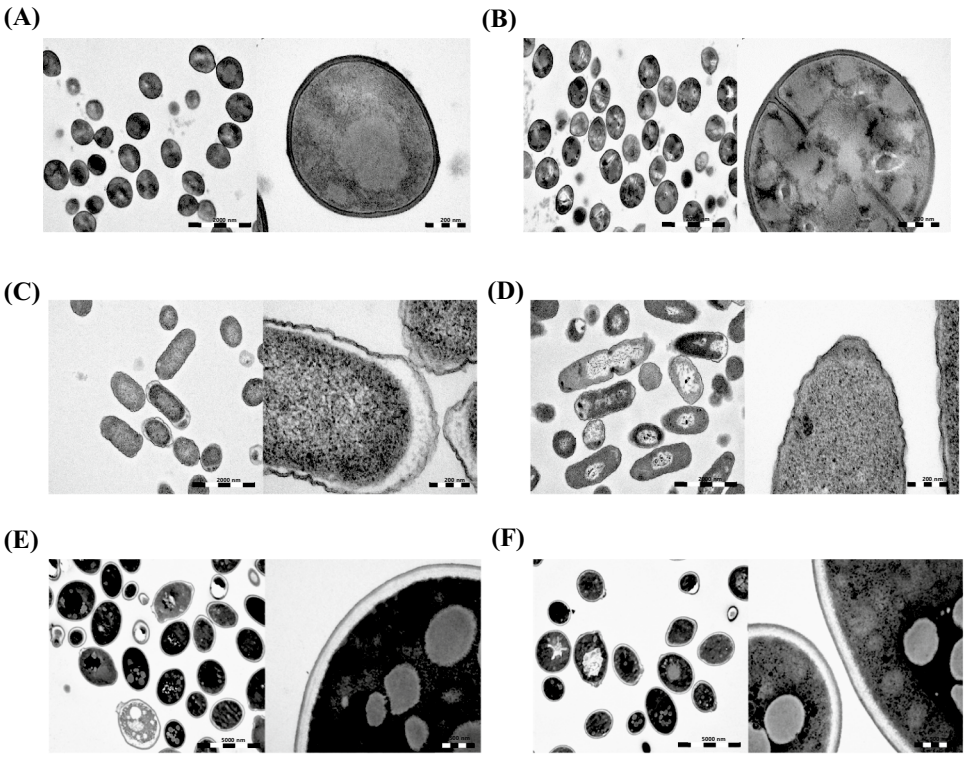

Fig. 4 TEM images of microorganisms at high or low environmental a_w **A** *S. aureus* at a_w of 0.99 (scale bar 2000 nm), **B** *S. aureus* at a_w of 0.80 (scale bar 200 nm), **C** *S.* Enteritidis at a_w of 0.99 (scale bar 2000 nm), **D** *S.* Enteritidis at a_w of 0.80 (scale bar 200 nm), **E** *S. cerevisiae* at a_w of 0.99 (scale bar 5000 nm), **F** *S. cerevisiae* at a_w of 0.80 (scale bar 500 nm)

environmental a_w (0.80). Contrary to expectations, there was no significant change in the cell surface or thickening of the cell wall according to the environmental a_w conditions. Instead, changes inside the cell were noticeable, particularly in *S. aureus* and *S.* Enteritidis cells. It was observed that the water present inside the microorganisms escaped to the outside. Also, structural changes in the internal components of the microorganisms were seen and this seemed the general mechanism to prevent water flux. Aronson and Rönner (2001) mentioned that when microorganisms are exposed to an environment of low aw, microoragnisms synthesize or increase the number of intracellular molecules to try to prevent water loss. In our study, the aggregation of intracellular material and increase in cell number were clearly observed in *S. aureus* and *S.* Enteritidis. However, there was no significant change in *S. cerevisiae*. This was because the range of a_w required for survive was different depending on the type of microorganism. According to Tapia et al. (2020) and Barbosa-Cánovas et al. (2020), the minimum a_w required for most *Saccharomyces* species to grow was 0.87–0.80, which was relatively lower than that of *S. aureus* and *Salmonella* spp., which was 0.87–0.95.

Microbial inactivation by IPL treatment

The microbial reduction curves of *S. aureus*, *S.* Enteritidis, and *S. cerevisiae* treated with IPL at a total fluence of 0.23–3.05 J/cm^2 according to the a_w were shown in Fig. 5. Judging from the overall flow of microbial reduction, the yeast *S. cerevisiae* showed higher resistance to IPL than the other two microorganisms, regardless of the a_w level. Several studies have shown that when IPL is applied to microorganisms, the microbial reduction curve takes the form of a double-Weibull model (Harguindeguy and Gómez-Camacho, 2021; Hwang et al., 2023). The form of the inactivation curve can be simply divided into the shoulder section which shows the resistance to IPL in the beginning, the linear section where microorganisms are reduced in earnest, and the tail section which is difficult to reduce because microorganisms are not exposed to IPL (Hwang et al., 2023). In our study, however, the shoulder, linear, and tail parts were not observed clearly in the shape of the reduction curve of three microorganisms. So, a higher fluence of IPL was required to observe the entire parts. Anderson et al. (2000) explained that the resistance of the fungi, *Aspergillus niger*, to pulsed light was higher than bacteria, due to the presence of protective pigments in the wall layer surrounding the spore forms. Although yeast was used in our study, it was assumed that a cell structure different from that of general bacteria caused the difference in resistance to IPL.

When a_w was 0.99, 0.95, 0.90, 0.85, and 0.80, the highest reduction effect of *S. cerevisiae* through IPL treatment was 3.96, 3.65, 4.15, 4.30, and 4.30 log, respectively. It seemed that the reduction effect of microorganisms increased as the water activity decreased, and this tendency was also shown in *S. aureus* and *S.* Enteritidis. In the case of *S. aureus*, the maximum reduction level was 5.04, 5.43, 5.47, 5.95, and 6.18 log when a_w was 0.99, 0.95, 0.90, 0.85, and 0.80, respectively. And *S.* Enteritidis showed 4.70, 4.70, 5.20, 4.53, and 5.91 log reduction in the same sequence of a_w. However, in the overall IPL fluence, the microbial reduction effect according to a_w did not show a clear trend. The value of the microbial reduction effect at the maximum fluence mentioned above was reduced, but it was analyzed that there was no significant difference. Research data on the influence of nonthermal technologies on microorganisms suspended in different a_w solutions are rare. Aronsson and Rönner (2001) showed that when *E. coli* and *S. cerevisiae* with a_w adjusted to 1.00–0.94 were treated with a PEF of 25–30 kV/cm, the reduction effect decreased as the a_w value decreased. They

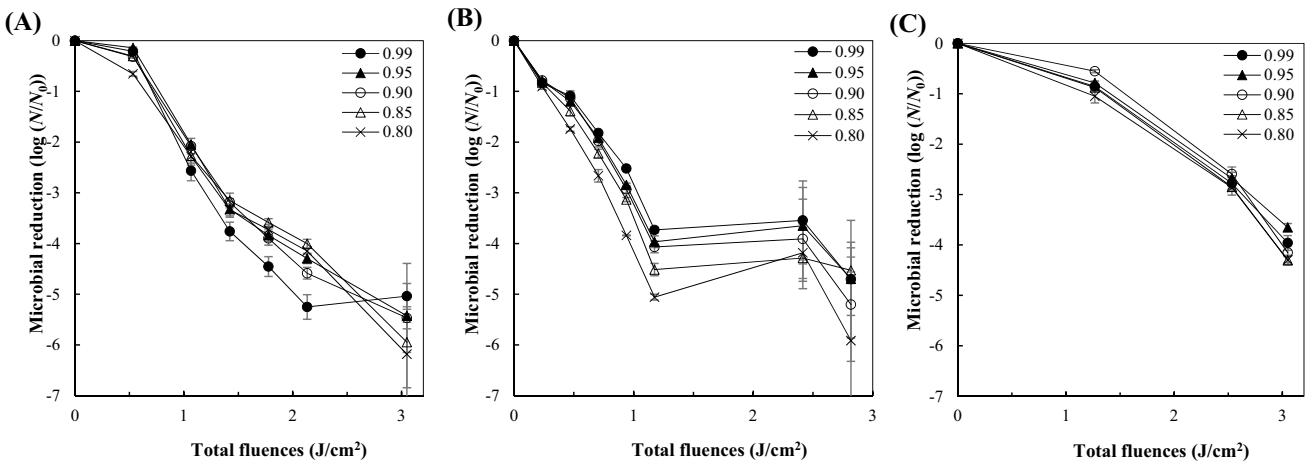

Fig. 5 Reduction of microorganisms after IPL treatment at different a_w levels. **A** *S. aureus*, **B** *S.* Enteritidis, **C** *S. cerevisiae*

explained that reduced a_w values caused cell shrinkage, which made them resistant to PEF because it thickened the cell wall and reduced membrane permeability and fluidity. Our study also expected similar research results, but no significant data could be obtained. Meanwhile, the effect of a_w on IPL inactivation depends on various factors, such as not only the concentration of the solution but also the type of solute, the type of bacteria, and the temperature of the solution (Mazas et al., 1999; Sevenich et al., 2015). However, in the present study, only NaCl was used as the only solute to control the aw of the sample solution. Therefore, follow-up studies using solutes other than NaCl, such as glucose, glycerol, or sucrose, are needed to understand the effect of only a_w on the inactivation efficiency of IPL. In addition, as food is a complex system composed of various solutions, the inactivation efficiency of IPL according to the a_w of actual food should be studied.

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Declarations

Conflict of interest There is no conflict of interest between the authors of this manuscript.

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