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# Relationship between optical properties of beverages and microbial inactivation by intense pulsed light



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#### ABSTRACT

This study determined the inactivation effects of the intense pulsed light (IPL) on *Pseudomonas aeruginosa* in various liquid foods and elucidated the relationship between microbial reduction and the optical properties of twelve liquid foods. In mineral water and isotonic beverage, 7.0 log reductions of *P. aeruginosa* were obtained with the IPL treatment at total fluence of 0.97 J/cm<sup>2</sup>. At 12.17–24.35 J/cm<sup>2</sup>, 7.0 log reductions were shown in five liquid samples (apple juice, carbonated beverages and plum juice), while only 0.5- to 2.0-log reductions were shown in the rest samples even after 29.21 J/cm<sup>2</sup> total fluence of the IPL treatment. High value of transmittance and low value of extinction coefficient have a decisive effect on the microbial inactivation because it allows the intensity of the IPL to be preserved as it penetrates into the sample. The best-fit regression kinetic which can explain the relationship between extinction coefficient and bactericidal effect was an exponential function. *Industrial relevance*: Intense pulsed light (IPL) is one of the nonthermal processing technologies for ensuring safe foods with satisfactory qualities. Through this study, transparent liquids showed a high microbial reduction level after IPL treatment in a short time. So it can be concluded that IPL has a potential as an excellent alternative or complement to conventional thermal processing of transparent liquids. Also, inactivation kinetic equation deducted from this study can be used to predict the microbial reduction level of specific liquid before IPL treatment by using its extinction coefficient.

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#### 1. Introduction

In the food industry, food safety and freshness are the most important factors for satisfying consumer demands. Prolonging the quality of foods requires treatment by appropriate methods like thermal processing. Heating is a very effective method of decontamination, but it can lead to undesirable changes such as the destruction of nutrients, changes in texture, and fragrance loss. As such, a new microbial inactivation method like nonthermal processing that provides both safety and high quality needs to be developed.

Intense pulsed light (IPL), one of the nonthermal processing technologies, involves applying intense, short-duration pulses of light to the food surface (Elmnasser et al., 2007; Wekhof, 2000). The IPL system consists of a power supply, a pulsed generator, a transformer, a light source and a treatment chamber. The electrical energy is produced in a power supply and accumulated in a high-power capacitor. The energy stored in capacitor is then delivered to a xenon lamp to produce pulsed light on a timescale of nanoseconds to milliseconds (Elmnasser et al., 2007; Oms-Oliu, Martín-Belloso, & Soliva-Fortuny, 2010).

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The inactivation mechanism of IPL is still inconclusive, but it is generally accepted that the main lethal action of IPL involves both photochemical and photothermal mechanisms. The photochemical mechanism involves dimers formed in a photochemical reaction inhibiting the formation of new DNA chains in the process of cell replication (mitosis) (Devi & Guttes, 1972). Many studies have shown that the germicidal effect of UV light on bacteria is primarily due to the formation of pyrimidine dimers (mainly thymine dimers), with the authors proposing that pyrimidine dimers can be quantified at the sequence level after an object has been irradiated by a UV light source (Giese & Darby, 2000; Kneuttinger et al., 2014; Poepping, Beck, Wright, & Linden, 2014). Many authors have also insisted that the UV spectral region is the most important aspect influencing the efficacy of IPL (Anderson, Rowan, MacGregor, Fouracre, & Farish, 2000; Rowan et al., 1999). Photothermal effects resulting from exposure to IPL have also been reported, such as a structural damage to cell membranes (Takeshita et al., 2003; Wekhof, 2000).

The disinfection performance of IPL in beverages has been demonstrated by several studies. However, the efficacy of IPL in disinfecting liquids varies widely due to the extent of light absorption differing markedly with the type of liquid. Palgan et al. (2011) achieved more than a 4.7-log reduction of *Escherichia coli* in apple juice after applying IPL at an energy dose of 1.17 J/cm<sup>2</sup>/pulse, while a smaller than 1.0-log reduction was produced orange juice and milk under the same treatment conditions. Moreover, Miller, Sauer, and Moraru (2012) observed a smaller than a 1-log reduction in E. coli when exposing concentrated milks containing 25% and 45% solids to 8.4 J/cm<sup>2</sup> IPL. It is difficult to sterilize beverages with a low translucency effectively due to the inability of IPL to penetrate them (Gómez-López et al., 2005; Sauer & Moraru, 2009). Artíguez, Arboleya, and de Marañón (2012) and Artíguez and de Marañón (2015) also reported that the degree of sample transmittance correlates with the light exposure of bacteria. Some studies have pretreated beverages in order to obtain a satisfactory microbial inactivation level. Pataro et al. (2011) diluted liquid samples with distilled water before applying IPL treatment at 4 J/cm<sup>2</sup> the total fluence and achieved 4.00- and 2.90-log reductions of E. coli in apple juice and orange juice, respectively. Sauer and Moraru (2009) observed a maximum 2.71-log reductions of E. coli O157:H7 in apple cider that was irradiated as a thin layer (1.3 mm).

*Pseudomonas aeruginosa* can grow in a wide range of habitats. Several studies have isolated *P. aeruginosa* from liquid foodstuffs (Hardalo & Edberg, 1997; Olorunjuwon, Temitope, Muibat, & Afolabi, 2014). This psychrophilic bacterium is also the most frequent pathogen causing pneumonia (Sawa et al., 1997), and some regulators suggest that it should be eliminated from food and water due to a risk of serious infections (Hardalo & Edberg, 1997).

The purpose of the present study was to determine the effectiveness of IPL in the microbial inactivation of various liquid samples contaminated by *P. aeruginosa*. This was achieved by inoculating various liquid samples with *P. aeruginosa*, then treating them with IPL and subsequently measuring the microbial reduction. We also aimed to derive the mathematical relationship between certain properties of the liquid samples and the bactericidal effect. Several types of liquid samples with different properties were treated with IPL at the same dose, and the relationship between the liquid properties and bactericidal effect was analyzed to derive an equation for predicting the bactericidal effect of any liquid prior to applying IPL treatment.

#### 2. Materials and methods

#### 2.1. Tested samples

In this study, various commercial beverages with different degrees of transparency and color were selected: mineral water (W), isotonic beverage (I), two types of apple juice (A1 and A2), orange juice (O), grape juice (G), plum juice (P), three types of carbonated drink that have different color and transmittance each (C1, C2, C3), milk (M), and coffee beverage without milk (C). Samples were purchased from a market and stored at 4  $^{\circ}$ C in a refrigerator prior to inoculation.

#### 2.2. Inoculation of microorganisms

In order to inoculate the liquid samples with microorganisms, *P. aeruginosa* ATCC 10145 were cultured. This culture was grown in tryptic soy agar (TSA) (Difco<sup>TM</sup>, Sparks, MD, USA) and stored at 4 °C refrigeration. One or two colonies from an agar plate were transferred to 5 ml of tryptic soy broth (TSB) (Bacto<sup>TM</sup>, Sparks, MD, USA), and then precultured at 37 °C for 24 h. Then, 1 ml of precultured fluid was transferred to 100 ml TSB and incubated at 37 °C for 5 h in a shaking incubator to obtain the cells in the early stationary phase. The cells were harvested by a centrifuge (Gyrozen, Seoul, South Korea) for 10 min at 8000g. The supernatant was discarded and the cell pellet was stored in 4 °C refrigeration after washing in sterile 0.85% NaCl solution.

To inoculate the microorganisms in the liquid samples, the cells were re-suspended in 0.85% NaCl solution until approximate population reached  $10^8$  CFU/ml. Then, 1 ml of this suspension was inoculated into 10 ml of liquid samples. Before the inoculation, the temperature of liquid samples was 24 °C.

#### 2.3. Device of IPL and treatment condition

A power system to generate pulsed light and a treatment chamber were designed and manufactured in our laboratory (Fig. 1). The input power of a 220 AC supply source at 25 A is rectified and transformed to a maximum permissible voltage (30 kV) and supplied to a capacitor. When sufficient electrical energy is stored in the capacitor and momentary discharge occurs, the xenon lamp generates the IPL. The xenon lamp used in this study was the Heraeus Noblelight XAP Series (Type NL4006, Cambridge, UK). The lamp was 145 mm long with a 7.14 mm diameter and it was equipped where the distance between the lamp and the base of the sample is 3.5 cm. At this point, the distribution and the intensity of the IPL were determined using a spectroradiometer (ILT-900, International Light Technologies, Peabody, MA, USA). As shown in Fig. 2, spectroradiometer measured the absolute irradiance of xenon lamp, which produced a broad spectrum of light between 200 and 1100 nm of wavelengths. The integrated area of the instantaneous light spectrum indicates the dose of IPL per second.

Before the IPL treatment, 10 ml of the samples were spread equally on 90 mm diameter Petri-dishes. The temperature of samples before



Fig. 1. Schematic diagram of intense pulsed light treatment system.

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