Innovative Food Science and Emerging Technologies 39 (2017) 49–54

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Effect of sublethal temperatures on pulsed light inactivation of bacteria

S.T. Hilton, J.O. de Moraes, C.I. Moraru *

Department of Food Science, Cornell University, Ithaca, New York 14853, United States

ARTICLE INFO

Article history:
Received 10 October 2016
Received in revised form 1 November 2016
Accepted 4 November 2016
Available online 10 November 2016

Keywords:
Pulsed Light
Temperature
E. coli
L. innocua
P. fluorescens

ABSTRACT

This study investigated the effect of sublethal temperatures on the efficacy of pulsed light (PL) treatment for the inactivation of *Listeria innocua*, *Escherichia coli* ATCC 25922, and *Pseudomonas fluorescens*. A thin layer of clear, liquid phosphate buffer inoculated with one of the challenge organisms, at a concentration of about 10^8 CFU/mL, was equilibrated to a temperature ranging from 5 °C to 50 °C and then treated with PL, at doses between 1.02 and 12.29 J/cm^2. All treatments were performed in triplicate. In the temperature range of 5 °C to 40 °C, the average maximum reductions for *L. innocua*, *E. coli*, *P. fluorescens* were 6.27 ± 0.23 log CFU, 6.66 ± 0.36 log CFU, and 6.15 ± 0.19 log CFU, respectively. Temperature did not affect PL inactivation of *E. coli* or *P. fluorescens*, but a modest synergistic effect between PL and temperature was observed for *L. innocua* treated above 40 °C.

Industrial relevance: This study suggests that PL inactivation of bacteria is independent of temperature in the sublethal range 5 °C to 40 °C. The practical benefit of this finding is that PL treatments can be conducted in a wide range of environmental temperatures, without any change in the outcome of the treatment.

1. Introduction

Pulsed light (PL) technology is a non-thermal microbial inactivation method that can improve food safety and shelf life, with minimal effects on the food's original nutritional and sensory attributes. PL is approved by the U.S. Food and Drug Administration for the decontamination of food and food contact surfaces, at doses not exceeding 12 J/cm^2 (FDA, 1996). PL has been shown to be effective in the inactivation of foodborne pathogens and their surrogates on foods and food contact surfaces (Rajkovic et al., 2010; Proulx et al., 2015). Recent research also indicates a limited effectiveness of PL for the inactivation (Artíguez & de Marañón, 2015a) or germination (Artíguez & de Marañón, 2014) of bacterial spores.

The treatment consists of short duration pulses of high intensity broad-spectrum light ranging in wavelength from 200 to 1100 nm. It is generally accepted that PL treatment primarily inactivates microorganisms through the absorption of UV light by DNA (Takeshita et al., 2003; Wang, MacGregor, Anderson, & Woolsey, 2005; Woodling & Moraru, 2007; Uesugi, Hsu, Worobo, & Moraru, 2016). There are also reports that attribute some of the lethal effects of PL to photothermal effects, which lead to cytoplasmic membrane damage, cell content leakage, and ultimately cell death (Takeshita et al., 2003; Krishnamurthy, Tewari, Irudayaraj, & Demirci, 2010; Cheigh, Hwang, & Chung, 2012).

The efficiency of PL treatments is dependent on several factors, including fluence and spectral distribution of the light, the challenge microorganism, and the interaction of light with the substrate. Hsu and Moraru (2011) determined that fluence varies significantly within a PL chamber, making sample positioning relative to the lamp and lamp geometry crucial factors to consider when designing an efficient and uniform PL treatment. Surface properties of the substrate also affect the outcome of PL treatment. If the substrate contains UV absorbing components such as proteins, pigments, or phenolic compounds, PL effectiveness can be reduced, as less UV light is available to reach target microorganisms. For solid substrates, microorganisms may lodge in surface irregularities or penetrate into the solid beyond PL's penetration depth, limiting the microorganisms' exposure to PL (Woodling & Moraru, 2005). For instance, high UV reflectivity was found to reduce inactivation on Al packaging materials (Ringus & Moraru, 2013).

Very limited data exists on the effect of temperature on PL efficacy. Artíguez and de Marañón (2015b) have shown a synergistic effect of PL and mild processing temperatures (60 °C) on the inactivation of *L. innocua* in whey, but a systematic evaluation of the effect of temperature on PL is not currently available. The effect of temperature is very relevant for practical applications of PL, since a temperature effect may on one hand lead to differences in inactivation if treatments are conducted at different temperatures, and on the other hand potential synergistic effects may allow the development of mild temperature – PL combination treatments. For instance, synergistic effects between mild heat and continuous UV were observed for *Escherichia coli* (Gayán, Monfort, Álvarez, & Condón, 2011), *Salmonella* (Gayán, Serrano, Raso, Álvarez, & Condón, 2012), *Staphylococcus aureus*

* Corresponding author.
E-mail address: cim24@cornell.edu (C.I. Moraru).

http://dx.doi.org/10.1016/j.ifset.2016.11.002
1466-8564/

(Gayán, García-Gonzalo, Álvarez, & Condón, 2014), and *Listeria monocytogenes* (Gayán, Serrano, Pagán, Álvarez, & Condón, 2015), when performing the UV treatments in the 50 °C–60 °C temperature range.

The objective of this study was to evaluate the effect of temperature on the PL inactivation of *Listeria innocua*, *E. coli* ATCC 25922, and *Pseudomonas fluorescens* in a liquid substrate. *L. innocua* and *E. coli* ATCC 25922 were determined to be non-pathogenic surrogates of *L. monocytogenes* and *E. coli* O157:H7 for PL treatments (Sauer & Moraru, 2009; Uesugi & Moraru, 2009). *Pseudomonas* spp. are commonly found spoilage bacteria in foods, particularly in dairy products (Ternström, Lindberg, & Molin, 1993). A sublethal temperature range from 5 °C to 50 °C was selected, to make sure that the challenge organisms were not inactivated by temperature alone.

2. Materials and methods

2.1. Challenge organisms

L. innocua FSL C2-008 and *P. fluorescens* D3-266 were obtained from frozen stocks maintained by the Food Microbiology and Safety Laboratory at Cornell University (Ithaca, NY). *E. coli* ATCC 25922 was obtained from frozen stocks maintained by the Worobo Laboratory at Cornell University. The cultures were streaked onto tryptic soy agar (TSA; Difco, Becto Dickinson, Franklin Lakes, NJ) plates and incubated for 24 ± 2 h at 37 ± 2 °C for *L. innocua* and *E. coli* and at 30 ± 2 °C for *P. fluorescens*.

2.2. Substrate preparation

A single colony of the challenge microorganism was isolated from TSA plates and transferred into tryptic soy broth (TSB; Difco, Becto Dickinson, Sparks, MD). The broth was incubated with shaking at 210 rpm for 24 ± 2 h at 37 ± 2 °C for *L. innocua* and *E. coli* and at 30 ± 2 °C for *P. fluorescens*. Incubation times were selected to yield cells in stationary growth state. The culture was diluted 10 fold in Butterfield's Phosphate Buffer (BPB; pH 7.2 ± 0.2 at 25 °C; Weber Scientific, Hamilton, NJ). The initial inoculum level was 10^8 CFU/mL for *L. innocua* and *E. coli* and 10^6 CFU/mL for *P. fluorescens*. One milliliter of the inoculated BPB was transferred into Nunc Lab-Tek II 1 well Chamber Slides (Thermo Fisher Scientific, Waltham, MA). The chamber slide and substrate were brought to the temperature of interest prior to PL exposure by placing the sample in an incubator for at least 15 min to ensure that the target temperature (±0.5 °C) had been reached. The temperatures studied were 5 °C, 20 °C, 30 °C, 40 °C, and 50 °C, which were in the sublethal range for the three challenge organisms were chosen. Some thermal inactivation of *E. coli* and *P. fluorescens* occurred at 50 °C, which will be discussed later. The temperature of each sample was measured immediately prior to PL treatment using a Type K temperature probe (ThermoWorks, Salt Lake City, UT).

2.3. PL treatments

PL treatments were performed using a Xenon RS-3000C SteriPulse System (Xenon Corp., Wilmington, MA). A xenon flashlamp within the unit emits light in the wavelength range 200 to 1100 nm, with a pulse width of 360 μs and a pulse frequency of 3 pulses per second. Chamber slides were centrally placed on an adjustable stainless steel shelf located 5.8 cm below the flashlamp housing. Samples were exposed to 1, 3, 6, 9, or 12 pulses of light, corresponding to fluence (PL dose) values of 1.02, 3.07, 6.14, 9.22, and 12.29 J/cm^2, respectively. A waiting period of at least 60 s between treatments was allowed, to prevent overheating of the flashlamp.

For the verification of the developed PL inactivation models, PL inactivation tests were also conducted for each of the three challenge microorganisms at fluence values of 2.18, 5.28 and 10.2 J/cm^2. These PL experiments were conducted at 45 °C for *L. innocua*, and 35 °C for *E. coli* and *P. fluorescens*.

2.4. Fluence measurements

A pyroelectric detector head (PE25BB-DIF) connected to a Nova II energy meter (Ophir Optronics, North Andover, MA) was used to quantify the fluence received by the samples. The detector was placed inside the PL unit treatment chamber with the diffusor opening of the detector centrally located 5.8 cm below the flashlamp housing. The detector cable and base were covered in aluminum foil, leaving only the diffuser opening exposed. Fluence measurements were conducted for 1, 3, 6, 9, and 12 pulses. Measurements were performed at least 60 s apart to prevent overheating of the flashlamp and pyroelectric detector. All measurements were performed in triplicate.

2.5. Recovery and enumeration of survivors

Following PL treatment, the 1 mL sample was transferred from the chamber slide to a test tube containing 7 mL of TSB. The chamber slide was rinsed twice with 1 mL of TSB to yield a final volume of 10 mL. Serial dilutions in BPB were performed and the appropriate dilutions were plated in duplicate on TSA plates. Plates were incubated for 24 ± 2 h at 37 ± 2 °C for *L. innocua* and *E. coli* and at 30 ± 2 °C for *P. fluorescens*. Survivors were enumerated using standard plate counting. When counts below 25 CFU/plate were expected the three-tube most probable number technique was used (Downes, Ito,, & APHA, 2001). Microbial reductions were calculated as $\log[N/N_0]$, where N is the number of survivors after PL treatment and N_0 is the initial inoculum level as determined by positive controls. As positive controls, samples prepared in the same manner as the test samples and exposed to temperature conditions, but not PL treatment, were used.

2.6. Modeling of inactivation kinetics

The PL inactivation kinetics for *L. innocua*, *E. coli*, and *P. fluorescens* across the temperature range of 5 °C to 50 °C was described using the Weibull model. The Weibull model (Eq. (1)) has been shown before to accurately predict PL inactivation of *L. innocua* in clear liquid substrates at room temperature (Uesugi, Woodling, & Moraru, 2007):

$$\mathrm{Log}(N/N_0) = -b \times F^n \quad (1)$$

where N is the number of survivors after the PL treatment, N_0 is the initial inoculum level, b is the scale parameter, n is the shape parameter, and F is the fluence (J/cm^2). The shape parameter indicates the shape of the survivor curve, with $n > 1$ indicating concave down survival curves, $n < 1$ concave up survival curves, and $n = 1$ indicates linear survival curves.

2.7. Replication and statistical analysis

Both biological and technical triplicates were conducted for all experimental conditions. ANOVA and Tukey's HSD test were used to determine if differences in inactivation between treatments at different temperatures were significant ($p < 0.05$), using the statistical program RStudio (R Core Team, 2014).

3. Results and discussion

3.1. Effect of temperature on PL inactivation of the challenge organisms

PL inactivation curves for *L. innocua*, *E. coli* ATCC 25922 and *P. fluorescens* at different sample temperatures are shown in Figs. 1-3. It should be noted that in all cases inactivation levels ($\log N/N_0$) were calculated using as control a sample that was handled and equilibrated at