

## Inactivation of *Salmonella* Enteritidis on Almonds by Pulsed Light Treatment

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### ABSTRACT

The effect of pulsed light treatment on the inactivation of *Salmonella* Enteritidis PT 30 on almonds was determined based on voltage, distance between sample and lamp and treatment time. Almonds were spot-inoculated with 20 µL of *Salmonella* Enteritidis PT 30 culture and then dried for 24 h at an ambient temperature. Almonds were treated with pulsed light at 3000, 3400 and 3800V at distances of 14.1 or 19.1 cm for 20 or 60 s. Pulsed light treatments reduced *Salmonella* populations by 0.44 to 4.14 log CFU/almond. Almonds treated with 3800V at 14.1 cm distance for 60 s resulted in a reduction by 4.14 log CFU/almond in *Salmonella* counts on TSAYE and 4.09 log CFU/almond on XLD agar. After pulsed light exposure, almond surface temperature increased from 25 to 35-50°C depending on treatment conditions. Results indicated that pulsed light had a potential to be used in the microbial inactivation of *Salmonella* in almonds and other low moisture foods.

**Keywords:** *Salmonella*, Almonds, Microbial inactivation, Surface temperature, Pulsed light

### Atımlı Işık Uygulaması ile Bademde *Salmonella* Enteritidis İnaktivasyonu

#### ÖZ

Atımlı ışık uygulamasının badem yüzeyindeki *Salmonella* Enteritidis PT 30 inaktivasyonuna etkileri farklı voltaj, numune ile lamba mesafesi ve işlem süresi bakımından değerlendirilmiştir. Bademlerin yüzeyine 20 µL *Salmonella* Enteritidis PT 30 kültürü ekimi yapıp, sonra 24 saat oda sıcaklığında kurumaya bırakılmıştır. Ekim yapılmış bademlere, üç değişik voltajda (3000, 3400 ve 3800V); iki farklı numune ile lamba mesafesinde (14.1 ve 19.1 cm) 20 ya da 60 s süresince atımlı ışık uygulanmıştır. Badem yüzeyindeki *Salmonella* popülasyonu 0.44 ile 4.14 log CFU/badem oranında azalmıştır. Proses faktörlerinden 3800 V, 14.1 cm ve 60 s ile atımlı ışık uygulaması *Salmonella* miktarını TSAYE’de 4.14 log, XLD agarda ise 4.09 log CFU/badem azaltmıştır. Atımlı ışık yöntemi, uygulanan proses faktörlerine bağlı olarak, badem yüzeyindeki sıcaklığı 25’den 35-50°C’ye kadar yükseltmiştir. Bu çalışmaya göre atımlı ışık teknolojisi badem gibi nem oranı düşük olan gıdaların *Salmonella* mikrobiyal inaktivasyonda uygulanabileceği belirlenmiştir.

**Anahtar Kelimeler:** *Salmonella*, Badem, Mikrobiyal inaktivasyon, Yüzey sıcaklığı, Atımlı ışık

### INTRODUCTION

Low moisture foods are considered to be safe; however, researches indicate that pathogens, including *Salmonella*, can survive in low moisture environment for

long time [17, 20, 21]. Besides the low moisture environment conditions, *Salmonella* can also adapt to varied pH (3.8 and 9.5) and temperatures (2-54°C). Many seeds and nuts including sesame seeds, coconuts, macadamia nuts, pistachios, peanuts, and

almonds have been recalled in recent years due to contamination with pathogenic microorganisms [7]. Almonds, in particular, were associated with two recorded outbreaks of salmonellosis [2, 19], which led to the establishment of U.S. regulations that require treatment achieving a minimum 4-log reduction in *Salmonella* for California-grown almonds [10]. There are several treatments that can be used to meet the minimum reduction requirement including blanching [14], oil roasting [8], and propylene oxide fumigation [5, 6]. However, blanching and oil roasting technologies may lead to undesirable loss of nutritional and physical quality and propylene oxide fumigation was found less attractive treatment for pathogen reduction due to its toxicity.

Pulsed light, a U.S. FDA (U.S. Food and Drug Administration) approved novel technology that rapidly inactivates bacteria, mold, yeasts, spores, and viruses on food surfaces, food contact surfaces, and packaging materials, may be a good alternative to conventional thermal or chemical decontamination processes [31]. It targets DNA of the microorganisms, inactivates DNA repair system thereby irreparable damage and then death occurs. This technology also utilizes intense, short-duration pulses of broad-spectrum light ranging from UV to infrared (100 to 1100 nm); UV light (100–400 nm), visible light (400–700 nm), and near-infrared light (700–1100 nm), which cause germicidal effects due to photochemical, photothermal and photophysical exposure [9, 4].

There have been several studies regarding with the microbial decontamination of foods by using pulsed light technology. It has been used in disinfection of fresh produce, meat and fish, beverages, and some other foods [24, 33]. In fresh produce, pulsed light applied to raspberries and strawberries [1], avocados [28], watermelon [29], mangoes [3], green onions [32], and apple [18]. Studies indicated that 1 to 3 log reduction is achievable in fresh-cut products in terms of microbial safety risks and quality. Maximum inactivation with pulsed light was about 1 to 2.5 log reduction in meat and fish products because intense treatments may lead to quality changes as in raw salmon [26], chicken frankfurter [22], and beef and tuna carpaccio [15]. Pulsed light was effective in microbial disinfection of highly transparent fluids compared to turbid fluids; however combined treatments such as ultrasound [11] and thermosonication [25] increased microbial inactivation.

The objective of this study was to investigate the effectiveness of pulsed light technology on inactivation of *Salmonella* Enteritidis PT 30 inoculated on the surfaces of almonds. Treatment parameters such as distance from the lamp, treatment time, and voltage were investigated. The almond surface temperature resulting from pulsed light treatments was also evaluated.

## MATERIALS AND METHODS

### Stock Culture Preparation

The almond outbreak isolate of *Salmonella* enterica Serovar Enteritidis phage type 30 (PT 30) was obtained from the culture collection of Dr. Linda Harris at the University of California, Davis and stored among the culture collection at the Institute for Food Safety and Health (Bedford Park, IL). A cryobead of the culture was removed from storage at  $-80^{\circ}\text{C}$ , quickly thawed, transferred to 10 mL tryptic soy broth (TSB) (Becton, Dickson and Co., Franklin Lakes, NJ), mixed thoroughly with a vortex (Fisher Scientific, Pittsburgh, PA), and then incubated at  $37^{\circ}\text{C}$  for 18 to 24 hr. After incubation, one sterile disposable loop-full of inoculum was plated on a tryptic soy agar supplemented with yeast extract (TSAYE, Becton, Dickson and Co.) and stored at  $4^{\circ}\text{C}$ . This pure culture of *Salmonella* Enteritidis PT 30 was used for the preparation of working cultures, which were utilized in subsequent experiments.

### Inoculum Preparation

An isolated colony was transferred from the previously described working culture to TSB and incubated at  $37^{\circ}\text{C}$  for 24 h. Then, 100  $\mu\text{L}$  of culture was spread evenly over the surface of TSAYE plates ( $n=3$ ) with an L-shaped spreader which were then incubated at  $37^{\circ}\text{C}$  for 24 h. Cells were harvested by adding 1 mL of phosphate buffered saline (PBS, 98.8 g in 1 L water) to the plate and then an L-shaped spreader was used to gently scrape the agar surface. The resultant suspension was removed with a sterile pipette and deposited in a sterile 50-mL conical tube (Fisher Scientific, Fair Lawn, NJ) along with cell suspensions from the other plates. The pooled culture was enumerated by serial dilution in buffered peptone water (BPW) followed by spreading on TSAYE and incubation at  $37^{\circ}\text{C}$  for 24 h.

### Almond Inoculation

Almonds (Sincerely Nuts, Brooklyn, NY) were stored at ambient temperature and humidity until treatment. Twenty microliters of pooled inoculum was spot-inoculated onto each of the almonds at the approximate geometric center of one side of the sample. A total of 50 almonds were inoculated per experiment, two of which were used as untreated controls. Spot-inoculated almonds were allowed to dry in a petri dish for 24 h at ambient temperature ( $23\pm 2^{\circ}\text{C}$ ).

### Treatment of Almonds

Almonds were treated with Steripulse XL-3000 pulsed light system (Xenon Corporation, Wilmington, MA). The system operated at 3 pulses/s, produced polychromatic radiation in the wavelength range of 100 to 1100 nm and generated 1.27  $\text{J}/\text{cm}^2$  per pulse at 3800 V based on the manufacturer's specifications. One almond per trial was placed in a sample holder located at the axial and longitudinal center of lamp and affixed to a movable shelf. Almonds were treated at three different voltages

(3000, 3400 and 3800 V) and two different distances (14.1 and 19.1 cm) as measured from sample to quartz window for two different treatment times (20 and 60 s) to determine inactivation of *Salmonella* Enteritidis PT 30. Each treatment condition was replicated 5 times.

In separate trials aimed at measuring almond heating during pulsed light treatment, a K-type thermocouple (Model HH306, Omega Engineering Inc., Stamford, CT) was inserted into a 0.41 mm diameter drilled hole from the underside of the almond until it was 0.5 mm below the top surface. Thermocouple equipped almonds were treated at 3 different voltages (3000, 3400 and 3800 V) and distances (14.1, 16.6 and 19.1 cm) for 6 different treatments times (0, 20, 30, 40, 50 and 60 s). Each treatment was replicated 3 times.

### Microbial Enumeration

After pulsed light treatment, each treated almond was placed in sterile 50-mL conical tube to which 10 mL of BPW was added and then the tube was vortexed for 1 min. The resultant suspension was serially diluted in BPW and spread-plated in duplicate on TSAYE and xylose lysine deoxycholate (XLD, Becton, Dickson and Co.) agar plates which were then incubated at 37°C for 24 h prior to enumeration.

### Statistical Analysis

The experimental design was a completely randomized, 3-way factorial ( $3 \times 2 \times 2$ ) design with five replications. Microbial data were analyzed by analysis of variance (ANOVA) using JMP software (JMP version 9.0; Cary, NC, USA). Tukey's multiple comparison at 95% level was performed to determine significant differences between treatments.

## RESULTS and DISCUSSION

### Effect of Pulsed Light Treatment on Microbial Inactivation

Initial populations of spot-inoculated almonds were  $6.96 \pm 0.37$  log CFU/almond and  $6.66 \pm 0.49$  log CFU/almond as enumerated on TSAYE and XLD agars, respectively. As shown in Table 1, pulsed light treatments reduced *Salmonella* populations on almonds by  $0.44 \pm 0.44$  to  $4.14 \pm 1.45$  log CFU/almond when plated on TSAYE. Results were comparable using XLD agar indicating that cells exposed to pulsed light were inactivated rather than injured. Thus, reductions reported hereafter refer to those determined with TSAYE. As expected, treatment at the highest voltage (3800 V), closest distance (14.1 cm) and longest time (60 s) resulted in the greatest reductions of *Salmonella* ( $4.14 \pm 1.45$  log CFU/almond). However, treatment time had a significant effect ( $p < 0.05$ ) on inactivation levels achieved as a  $2.79 \pm 0.75$  log CFU/almond reduction was observed with 20 s treatment at the same voltage (3800 V) and distance (14.1 cm). Greater variability in log reduction was seen as treatment time increased. These results are consistent with the findings of other studies.

After pulsed light treatment of inoculated raspberries at distance of 5 cm to quartz window, 1.1 and 4.3 log *Salmonella* reduction was determined at 5 and 60 s of treatment times, respectively [1]. In addition, similar results were obtained in inactivation of *Salmonella* and *E. coli* on blueberry surface by using pulsed light technology [35]. Researchers found that higher frequency (100Hz) did not affect the microbial inactivation when compared to lower frequencies (1.8 or 3 Hz) [34]. However, reducing voltage from 3800 V to 3400 and 3000 V resulted in significantly less microbial inactivation ( $p < 0.05$ ). At a lamp distance of 14.1 cm and treatment time of 20 s, voltages of 3800, 3400, and 3000 V reduced *Salmonella* by 2.79, 1.29, and 0.89 log CFU/almond, respectively. Statistical analysis confirmed that voltage, distance and treatment time were factors significantly affecting *Salmonella* inactivation, and that there were interaction effects between these treatment parameters.

Sample position and orientation to the lamp and the distance between the sample and the lamp are important parameters for microbial inactivation by pulsed light. Others have shown that the greater the distance between the sample and the lamp, the lower the lethality [16, 30]. Results obtained in this study are consistent with these findings as *Salmonella* was reduced by 4.14 log CFU/almond at 14.1 cm and 3.01 log CFU/almond at 19.1 cm for treatments at 3800 V for 60 s. Similar results were obtained in treatments at 3400 and 3000 V and 60 s treatment time. Since only one almond was treated at a time, additional consideration will have to be given in order to scale-up the technology as efficacy is expected to be lower due to lamp position and orientation if a cluster of almonds were to be placed very close to the lamp [13]. There were no visually observable changes in almond quality for samples treated for up to 60 s.

### Effect of Pulsed Light Treatment on Surface Temperature

In pulsed light treatments, when the light intensity is high or treatment duration is long, the product surface temperature may increase and ultimately lead to product burning. There have been several studies that indicate surface temperature of food product increases when exposed to pulsed light treatment [16, 12, 26]. As seen in Figures 1,2 and 3 after pulsed light exposure, temperature at 1 mm below the almond surface increased from 25 up to 50°C depending on the treatment conditions of voltage, distance and time. At 14.1 cm, the highest temperatures (48 to 50°C) after 60 s treatment were obtained in samples regardless of system input voltage. At each distance, temperature increased significantly with treatment time. Similar results obtained in decontamination of *Listeria monocytogenes* on inoculated unpackaged and packaged chicken frankfurters by using pulsed light system. The temperature difference between the treated and untreated (initial) unpackaged samples varied between 2.4 and 46.5°C depending on treatment conditions [22]. At 3800 and 3400 V, little difference in almond temperatures were observed at 16.6 and 19.1 cm; however, at lower voltage (3000 V) there existed

greater differentiation between temperature with respect to distance. None of the temperatures reached just below the almond surface with up to 60 s treatment were high enough to contribute thermal inactivation of *Salmonella* [27, 23]. Nevertheless, in other study, temperature increased to higher levels on skin sides of

salmon fillets within 60 s of pulsed light treatment; 100, 86, 76°C at 3, 5, and 8 cm distances to quartz window, respectively, indicating that surface temperature may vary based on food type as well [26].

Table 1. Log reductions of *Salmonella* Enteritidis PT 30 on almonds after pulsed light treatment.

Pulse Light Treatment Conditions <sup>b</sup>			<i>Salmonella</i> Reduction log (CFU/almond) <sup>a</sup>	
Voltage (V)	Distance (cm)	Time (s)	TSAYE	XLD
3800	14.1	20	2.79±0.75 <sup>B</sup>	3.01±0.84 <sup>AB</sup>
		60	4.14±1.45 <sup>A</sup>	4.09±1.48 <sup>A</sup>
	19.1	20	0.84±0.75 <sup>CD</sup>	0.93±0.93 <sup>D</sup>
		60	3.01±1.19 <sup>B</sup>	3.02±1.56 <sup>AB</sup>
3400	14.1	20	1.29±0.46 <sup>CD</sup>	1.60±1.05 <sup>BCD</sup>
		60	3.54±1.18 <sup>AB</sup>	3.87±1.48 <sup>A</sup>
	19.1	20	0.44±0.44 <sup>D</sup>	0.44±0.17 <sup>D</sup>
		60	1.52±0.74 <sup>C</sup>	1.75±0.59 <sup>BCD</sup>
3000	14.1	20	0.89±0.47 <sup>CD</sup>	0.83±0.34 <sup>D</sup>
		60	2.86±0.67 <sup>B</sup>	2.66±1.38 <sup>ABC</sup>
	19.1	20	0.50±0.39 <sup>D</sup>	0.38±0.32 <sup>D</sup>
		60	1.46±0.65 <sup>C</sup>	1.32±0.43 <sup>D</sup>

<sup>a</sup> Values in the same column followed by different letters are significantly different (p<0.05). Experiment was replicated five times. <sup>b</sup> Initial populations of spot-inoculated almonds are 6.96±0.37 log CFU/g and 6.66±0.49 log CFU/g on TSAYE and XLD agars, respectively.

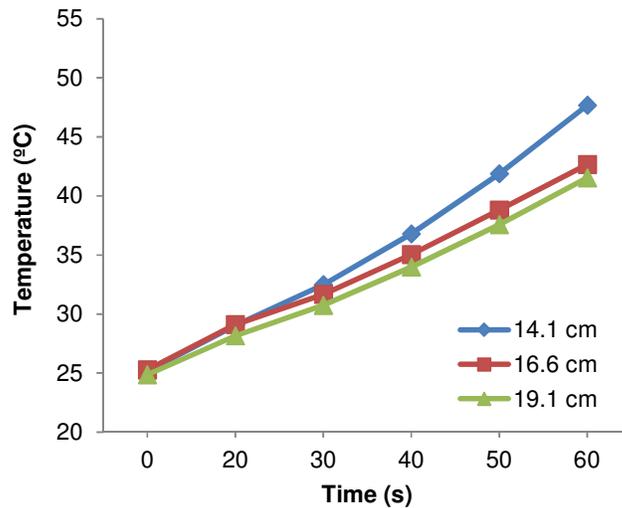


Figure 1. Change in surface temperature of almonds during pulsed light treatment with 3800 V.

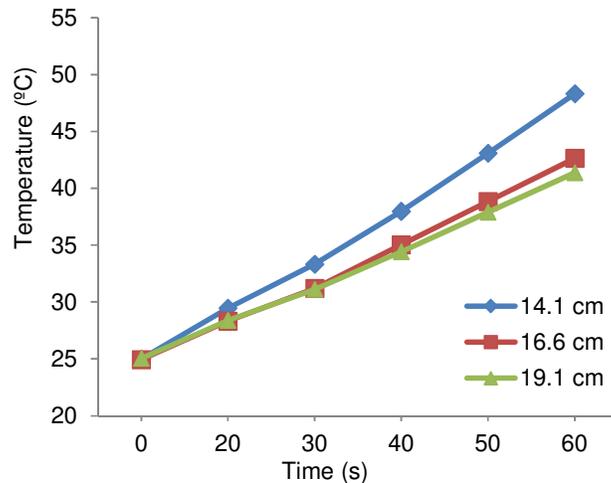


Figure 2. Change in surface temperature of almonds during pulsed light treatment with 3400 V

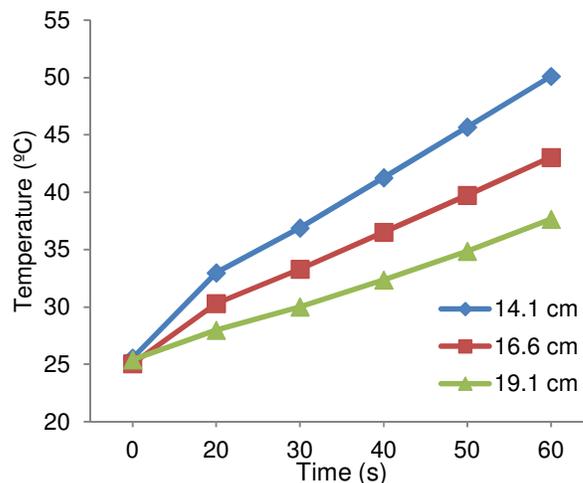


Figure 3. Change in surface temperature of almonds during pulsed light treatment with 3000 V.

## CONCLUSION

The highest voltage, the shortest distance and the longest treatment time increased the inactivation of *Salmonella* Enteritidis PT 30 on the surface of almonds. The maximum microbial inactivation was observed in almond treated with 3800 V, 14.1 cm distance, 60 s and the surface temperature reached to 48°C. There were no visually observable changes in almonds for samples treated for up to 60 s. Results of this study indicate that pulsed light technology is a promising technology for microbial inactivation of *Salmonella* Enteritidis PT 30 on almonds. However, further studies are needed to determine the effectiveness of pulsed light on other low moisture food products including, nuts, peanuts, and sesame seeds. In addition, scaling-up pulsed light system with a proper design of lamp position and an orientation should be evaluated; thereby cluster of food pieces might be treated homogeneously by using this

technology instead of treating one spot on the surface of the food product.

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