

**UNIVERSIDAD DE LAS AMÉRICAS PUEBLA**

**Engineering School**

**Chemical and Food Engineering Department**



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Lemongrass Essential Oil in Vapor Phase to Inactivate *Salmonella*  
Typhimurium on Quinoa Seeds**

Scientific Article that to complete the requirements of the Honors Program presents the  
student

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# Application of High Intensity Light Pulses in Combination with Lemongrass Essential Oil in Vapor Phase to Inactivate *Salmonella* Typhimurium on Quinoa Seeds

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## ABSTRACT

Contamination of seed products by *Salmonella* arises the concern for decontamination methods for raw or slightly cooked consumed seeds. In recent years, the demand for minimally processed food products has led to the development of alternative procedures to traditional techniques, such as thermal and chemical disinfection. This study is aimed to evaluate the efficiency of pulsed light technology in combination with vapor contact lemongrass essential oil treatment to inhibit *Salmonella* Typhimurium. The *in vitro* study showed high efficiency for the inhibition of *S. Typhimurium* with an applied fluence of 10.8 J/cm<sup>2</sup>. On the other hand, the maximum log-reduction achieved in the *in vivo* study was 2.91, with an applied fluence of 16.2 J/cm<sup>2</sup>. Because of the roughness and absorbance of ultraviolet (UV) light of the studied seed, the combination of pulsed light with an essential oil treatment in vapor phase was evaluated. The results then showed a further 2.39 log-reduction when the lemongrass essential oil was applied at the highest tested concentration (294.11 µl/l<sub>air</sub>).

**Keywords:** *Salmonella* Typhimurium; quinoa seed; high intensity light pulses; vapor phase essential oil; Weibull equation

## 1. Introduction

Quinoa seeds, a South American native cereal, are well-liked functional food products because of their high nutritional value. The quinoa seeds and sprouts have gained popularity due to their high content of amino acids, starch, minerals, vitamins, and lipids; as well as its antioxidant activity (Vega-Galvez et al., 2010). The structure of the food reserves of the quinoa seeds consists of three sites: i) large central perisperm, ii) peripheral embryo and iii) endosperm (Prego et al., 1998).

In the recent years, an increase in consumer demand for raw or slightly cooked seeds, as a result of the preservation of their nutritional properties, has led to diverse types of foodborne diseases (Kim et al., 2018). A possible contamination of seed products by *Salmonella* may arise at different stages of the preharvest and postharvest procedure. The main potential causes for seed contamination are related to the irrigation with contaminated water, due to sewage mismanagement, animal waste and improperly composed matter (Neetoo & Chen, 2015). Several recommendations from the U.S. Food and Drug Administration (FDA) for chemical disinfection of raw sprout seeds have been made to reduce pathogens that contribute to the contamination of seed coats (Gill et al., 2003). Thus, this study aims to analyze the effect of pulsed light (PL) technology in combination with an essential oil (EO) treatment to inactivate *Salmonella* Typhimurium on quinoa seeds.

A major global challenge in the food industry involves the development of alternative disinfection practices that can satisfy the demand for minimally-processed food concerning microbial safety and shelf life extension, taking into consideration the preservation of the nutrients and sensory properties (Bhavaya & Umesh Hebbar, 2017;

Kramer et al., 2017). Traditional food decontamination processes consist mainly on the microbial inactivation of food products by using thermal and chemical operations (Keklik et al., 2012). Although these methods represent effective decontamination techniques, they do not ensure the functionality of the products. Some of the adverse effects include the deterioration of the product quality, for instance, the alteration of proteins and polysaccharides structure, leading to changes in the texture, as well as the production of free radicals and changes in the physical appearance and functionality of the products (Bhavya & Umesh Hebbar, 2017; Luksiene et al., 2007).

High Intensity Light Pulses also known as PL technology consists of a non-thermal treatment to inactivate pathogenic and spoilage microorganisms on food products, as an alternative to the traditional surface decontamination techniques in the food industry (Bhavya & Umesh Hebbar, 2017; Oms-Oliu et al., 2008). The treatment consists of inactivating microbial cells through pulses of rich in UV-C light (200-280 nm) (Gómez-López et al., 2007).

The process is accomplished by accumulating electromagnetic energy in the capacitor of the PL system and subsequently releasing the high voltage short-duration electric pulses through an inert xenon gas flash lamp, exposing the food product to the intense flashes of white light with a broad spectrum ranging from 200 to 1100 nm, including the ultraviolet, visible and near the infrared regions of the spectrum (Abida et al., 2014; Gómez-López et al., 2007; Kramer et al., 2017).

Research on PL technology has been conducted with the aim of disinfecting diverse types of food, such as meat and fish, fresh produce, beverages, and other food products; additionally, it has been applied to sterilize surfaces of equipment and packaging materials

(Kramer et al., 2017; Levy et al., 2012). The studies performed for the evaluation of the PL treatment efficiency have had different targeted microorganisms, i.e., bacteria, molds, yeasts, spores, and others (Levy et al., 2012).

The fundamental factors influencing the efficiency of the microbial inactivation caused by the PL treatment are: i) energy received by the sample during the treatment, ii) distance between the target product and the xenon lamp, iii) spectral range of the light bursts, iv) sensitivity of the microorganisms, v) product matrix, and vi) absorbance of UV light by the product (Aron-Maftei et al., 2014; Kramer et al., 2017).

The germicidal effect of the PL treatment is based on two primary mechanisms: the photochemical and photo-thermal effect of the UV radiations (Abida et al., 2014; Levy et al., 2012). First, the photochemical inactivation mechanism is achieved when the UV light is absorbed by the carbon-carbon double bonds of proteins and nucleic acids, leading to DNA and RNA structure alterations. The formation of pyrimidine dimers, principally thymine dimers is the leading cause of the lethal effect of UV light. On the other hand, the photo-thermal effect is presented when the fluence exceeds  $0.5 \text{ J/cm}^2$ , caused by the rupture of bacteria due to the temporary overheating caused by the UV light absorption (Abida et al., 2014; Gómez-López et al., 2007). Although it has been shown that the PL treatment is more efficient than continuous UV irradiation treatments, the microbial inactivation of the PL treatment is attributed to the UV-C spectral range, particularly at 250-260 nm (Abida et al., 2014).

In order to maximize the microbial inactivation, the PL treatment can be applied in combination with other decontamination operations. Compatible treatments with possible synergic results include sub-lethal stress conditions, thermal treatment, modified

atmosphere packaging, high hydrostatic pressure, and antimicrobial essential oils, among others (Heinrich et al., 2015).

The antimicrobial and antifungal activity of essential oils (EOs) has led to increased interest from researchers to evaluate their application in the food industry. EOs consist of lipid-soluble mixtures of volatile compounds that can be obtained by the distillation or extraction of diverse types of plants. The chemical composition of the EOs is highly dependent of the plant and even of the biological species (Boukhatem et al., 2014). The antimicrobial activity is attributed to their low molecular weight organic constituents, such as terpenes, terpenoids, phenylpropenes and others (Hyldgaard et al., 2012; Valgimigli, 2012).

The mode of action of EOs is related with the cell membrane damage, caused as a result of the hydrophobicity of their constituents, leading to their accumulation in the cell membranes structures (Goñi et al., 2009). The volatile nature of EOs increases the efficiency for inhibiting microbial spoilage in the vapor phase (Kloucek et al., 2012).

Several studies have demonstrated the high antimicrobial activity of thyme, oregano, rosemary, basil and lemongrass essential oils against the bacterial spoilage on food products (Boukhatem et al., 2014; Klein et al., 2013; Valgimigli, 2012). Among 14 different EOs, cinnamon bark, thyme and lemongrass (*Cymbopogon citratus*) essential oil (LGEO) showed high efficiency against microbial activity by gaseous contact, inactivating *H. influenzae*, *S. pyogenes*, *S. pneumoniae*, *S. aureus* and *E. coli* (Inouye et al., 2001).

## **2. Materials and methods**

### **Seed characterization**

The experiments were performed using quinoa seeds purchased (OKKO Super Foods) from a local supermarket in Puebla, Mexico. The seeds were labeled with a 100% sanitization guarantee. The mean values of the diameter and thickness of the seeds were measured with a Vernier Caliper; for this calculation ten quinoa seeds were measured.

### **Seed microbiological analysis**

In order to confirm that seeds were sanitized, a microbiological analysis was carried out. For the enumeration of coliform organisms and mesophilic aerobic bacteria, 1 g of the seed samples was aseptically transferred to a 9 ml sterile peptone water test tube and homogenized for 1 minute, using a Vortex mixer (M63215, Barnstead Thermolyne Corp., USA). Afterward, one ml of the dilution was poured on a petri dish, and subsequently, the standard methods agar (SMA; Difco, BD, NJ, USA) was added. The petri dish was manually stirred. The same process was carried out for the violet red bile glucose agar (VRBGA; Difco, BD, NJ, USA).

### **Essential oil chemical analysis**

The LGEO was purchased from Grupo TECCNAL (Jalisco, Mexico) and analyzed employing gas chromatography-mass spectrometry, using a gas chromatograph system (6850 Series Network GC, Agilent Technologies, Santa Clara, CA) combined with a mass selective detector with triple axis (5975C VL, Agilent Technologies, Santa Clara, CA) and

a split-splitless injector (split ratio=1:10). A fused silica HP-5MS (5% phenyl–95% polydimethylsiloxane) capillary column (30 m by 0.250 mm; film thickness, 0.25  $\mu\text{m}$ ) was used. The carrier gas was helium at a 1.1 ml/min flow rate. The samples were prepared by dilution of the EO at 5:100 (v/v) in ethanol, and the injection volume was one  $\mu\text{L}$ . The column oven temperature was programmed from 60 °C (4 min) to 240 °C (10 min) at 4 °C/min. The injector and detector temperatures were set at 250 and 280 °C, respectively (Gomez-Sanchez, Palou, & Lopez-Malo, 2011). Retention indices were calculated using a homologous series of *n*-alkanes C<sub>8,0</sub> to C<sub>18,0</sub> (Sigma, St. Louis, MO). Eluted compounds were identified by comparing their retention indices from the literature and the mass profile of the same compounds available from the U.S. National Institute of Standard Technology library (Avila-Sosa et al., 2012).

### ***In vitro* experiments**

Sample preparation for *in vitro* tests: 50  $\mu\text{L}$  of the *S. Typhimurium* (ATCC 14028) inoculum were plated on tryptic soy agar in duplicate, employing a microprocessor controlled spiral plater (AP4000, Spiral Biotech, Advanced Instruments Inc., USA). After the PL treatment, the cultivated agar plates were incubated at 37 °C for 24 h.

*Salmonella* Typhimurium enumeration for *in vitro* analysis: in order to develop the microbiological analysis, the colonies were counted using an automatic colony counter QCount (M530, Spiral Biotech, Advanced Instruments Inc., USA). The results were expressed in CFU/g.

### ***In vivo* experiments**

Seed inoculation for *in vivo* tests: 40 g of the quinoa seeds were aseptically transferred to a 400 ml sterile tryptic soy broth (TSB; Difco, BD, NJ, USA) flask. Subsequently, the solution was mixed with 44 ml sterile TSB containing 4 ml of *S. Typhimurium* inoculum. The solution was placed in a water bath incubator (BT25, Yamato Scientific Co., Japan) with shaking (120 rpm), at 35 °C. After an hour, the seeds were aseptically drained in a laminar flow hood using What-man filter paper sheets and then left for drying in the same environment for 1.5 hours. Subsequently, the samples were treated using PL and EO techniques.

*Salmonella Typhimurium* enumeration for *in vivo* analysis: in order to develop the microbiological analysis for each of the treated-seed samples, 1 g of the seeds was aseptically transferred to a 9 ml sterile peptone water test tube and homogenized for 1 minute, using a Vortex mixer (M63215, Barnstead Thermolyne Corp., USA). The plating, incubation and colonies counting methods were the same as the one for the *in vitro* experiment

### **Pulsed light treatments**

A PL sterilization system (Z1000, XENON Co., USA) was employed for the treatment. The emitted spectrum of light ranged from 200 to 1100 nm. The light pulses were produced by one linear inert xenon gas flash-lamp (40.64 cm arc length) situated 10.8 cm above the sample, which was placed on a removable tray made of metal grade stainless steel. Each

pulse had a width of 360  $\mu\text{s}$  and a fluence of 0.45  $\text{J}/\text{cm}^2$ , while the pulse rate and electrical energy were set at three pulses/s and 505 J/pulse, respectively.

Two sets of experiments were carried out for analyzing the effects of the PL exposure time as a function of the *Salmonella* decontamination in the i) agar cultivated plates and the ii) inoculated seeds, the *in vitro* and *in vivo* conditions, respectively. In the first set of experiments, the agar plates were exposed to the treatment for 0 to 9 s, with an interval of 1 s. For the second set, the seed samples were exposed to the PL treatment for 3, 6, 9, 12, 15, 18 and 21 s. The input and output temperatures were registered using an infrared thermometer (59 MAX, Fluke Co., USA).

### **Vapor phase essential oil treatment for *in vivo* experiments**

Taking into consideration the *in vivo* results of the *S. Typhimurium* log-reduction after the PL treatment, it was decided to additionally treat the quinoa seeds with a LGEO vapor phase technique. According to the results, after 12 s of exposition, the log-reduction did not experience an increase. Thus, the inoculated-seed sample was exposed to a PL treatment for 12 s. Subsequently, the seeds were spread above a sterile gauze and then left on a grid inside a plastic-recipient (1.7 L) with hermetic sealing. A petri dish with LGEO was placed below the grid. The experiment was carried out using six different EO concentrations: 0, 117.64, 176.47, 235.29 and 294.11  $\mu\text{l}/\text{l}_{\text{air}}$ . The recipients were left 24 h in incubation at 37 °C.

### **Modeling of the microbial reductions**

In order to fit the microbial log-reduction curves of *S. Typhimurium* for the *in-vitro* and *in-vivo* experiments, the Weibull model was chosen (Peleg & Cole, 1998). The equation for the cumulative form of the Weibull distribution is given by Eq. 1:

$$\log S(t) = \log \left( \frac{N}{N_0} \right) = -b \cdot t^n \quad \text{Eq. 1}$$

Where S is the survival ratio, N is the number of surviving organisms (CFU/g) after the treatment time, t (s).  $N_0$  represents the initial organism count (at  $t=0$ ), whereas b and n are parameters. The parameters b and n were estimated by fitting the experimental data in Eq. 1, using the least squares regression method with the Solver tool in Microsoft Excel 2018 (Microsoft Corp., Redmond, WA).

## **3. Results**

### **Seed characterization**

The values of the mean diameter and thickness of the studied quinoa seeds were 2.065 mm and 1.17 mm, respectively.

### **Seed microbiological analysis**

It was confirmed that the seeds were effectively sanitized. The results of the microbiological analysis are presented in Table 1.

**Table 1. Enumeration of coliform organisms and mesophilic aerobic bacteria of studied quinoa seeds**

	Mean
Coliform organisms (CFU/g)	<10
Mesophilic aerobic bacteria (CFU/g)	25.5

### Chemical composition of lemongrass essential oil

The composition of the tested LGEO is shown in Table 2.

**Table 2. Chemical composition of tested lemongrass essential oil**

Compound	Area (%)	Compound	Area (%)
Neral ( $\beta$ -citral)	39.21	Elemol	0.64
$\gamma$ -Muuroolene	8.2	Geranyllinalool	0.53
Farnesol	5.79	Cubenol	0.37
Camphene	5.54	Triciclina	0.21
Limonene	4.87	$\alpha$ -Himachalene	0.14
Isopulegol	4.16	Isoaromadendrene epoxide	0.12
(+) Camphene	4.07	Dimethylvinylcarbinol	0.10
Geranial ( $\alpha$ -Citral)	3.41	Aromadendrene oxide 2	0.07
Isogeraniol	3.38	Geranylgeraniol	0.06
(3Z)- $\beta$ -Ocimene	2.19	AC1LBWRG	0.05
Isoeugenol	2.07	2-Methylenecholestan-3-ol	0.04
$\alpha$ -cembrenediol	1.96	Phytol	0.03
Ethyl Linalyl Ether	1.9	2,4-dimethyl-Cyclopentanol	0.02
Linalol	1.87	(3E)-3-Nonen-1-ol	0.02
Geraniol acetate	1.81	Aromadendrene epoxide	0.02
$\beta$ -Cubebene	1.46	cis-Z-alfa-Bisabolene epoxide	0.02
Methyl Heptenone	1.02	Z,E-2,13-Octadecadien-1-ol	0.02
Geranylvinylether	1.01	Aromadendrene oxide-(1)	0.02
1,2-15,16-Diepoxyhexadecano	0.88	5-methyl-2-Heptanol	0.01
$\beta$ -Caryophyllene oxide	0.83	1-Dodecyne	0.01
$\alpha$ -Pinene	0.71	(1R,4R,8S)-rel-9-Oxabicyclo[6.1.0]nonan-4-ol	0.01

### Inactivation of *Salmonella* Typhimurium by pulsed light treatments

The inactivation effect of the PL treatment on *S. Typhimurium* spread on the TSA plate surface is shown in Table 3. The maximum inactivation level, with a non-existent population of *S. Typhimurium*, was reached when the level of pulsed light energy was 10.8 J/cm<sup>2</sup>.

**Table 3. *In vitro* inactivation of *Salmonella* Typhimurium as a function of the applied fluence of pulsed light treatments**

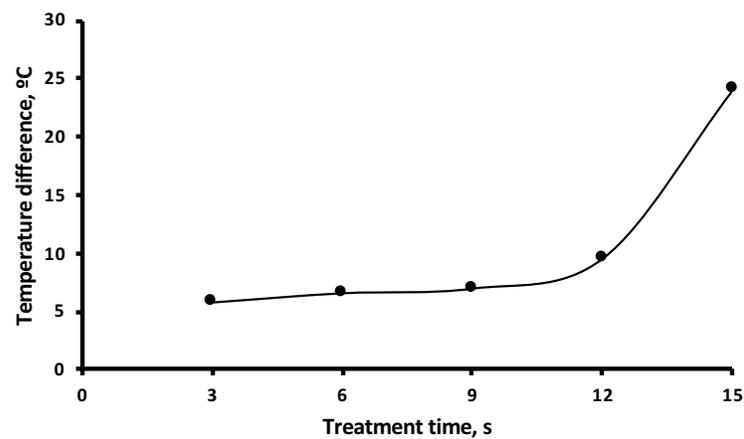
Treatment time, s	Number of pulses	Fluence, J/cm <sup>2</sup>	Population, CFU/ml
0	0	0	1.92E10
1	3	1.35	3.66E+09
2	6	2.70	1.70E+09
3	9	4.05	1.60E+08
4	12	5.40	5.35E+07
5	15	6.75	9.99E+05
6	18	8.10	8.57E+03
7	21	9.45	1.85E+01
8	24	10.80	0.00E+00

Furthermore, the inactivation effect for the *in vivo* conditions is presented in Table 4. The maximum *Salmonella* inactivation was achieved when the total fluence was 16.2 J/cm<sup>2</sup>, reaching a 2.91 log CFU/g.

**Table 4. *In vivo* inactivation of *Salmonella* Typhimurium as a function of the applied fluence of pulsed light treatments**

Treatment time, s	Number of pulses	Total fluence, J/cm <sup>2</sup>	Population, CFU/g
0	0	0	2.02E+06
3	9	4.05	2.49E+04
6	18	8.10	1.82E+04
9	27	12.15	1.73E+04
12	36	16.20	2.48E+03

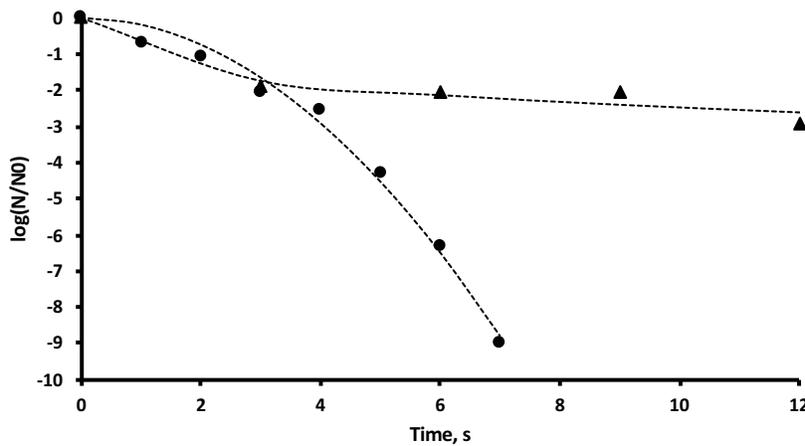
In order to analyze the temperature change of the seed samples, the input and output temperatures were registered. The results are presented in a plot shown in Fig. 1. Until a treatment duration of 12 s, the temperature difference between the input and output temperature was lower than 10 °C. When the sample was treated for 15 s, the temperature difference reached 24.1 °C, that is, the temperature difference increased approximately 2.5 times from the one of 12 s. Hence, no treatments with longer duration were applied to the samples.



**Figure 1. Temperature difference of the treated seeds as a function of treatment time**

A comparison of both, *in vitro* and *in vivo*, inactivation effects by PL was made employing the Weibull model to fit the log-reduction curves. The results are shown in Fig. 2, where the calculated  $b$  and  $n$  parameters for the *in vitro* conditions were 0.0038 and 4.123 respectively. For the *in vivo* experiment, the same calculated parameters had a value of 1.2723 and 0.2908.

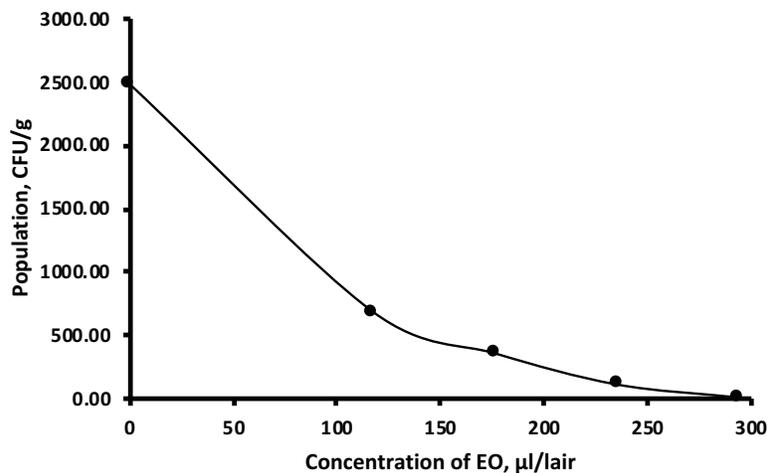
Although the highest reduction was observed on the TSA cultivated plates, the log-reduction of both experiments when the treatment was applied for 3 s was similar, for longer durations, the *in vivo* results did not achieve more significant reductions.



**Figure 2. Experimental data of the *in vitro* (●) and *in vivo* (▲) experiments and fitting of log-reduction curves (---) employing the Weibull model for each of the conditions, for the *in vitro* tests  $b=0.0038$  and  $n=4.123$ , while  $b=1.2723$  and  $n=0.2908$  were obtained for the *in vivo* tests.**

### Inactivation of *Salmonella* Typhimurium by essential oil treatments

According to the results obtained by the PL treatment of the inoculated seed samples, the inactivation of *S. Typhimurium* was not total. Thus, an EO treatment was additionally applied to the PL-treated seeds (12 s of treatment duration) to analyze the EO effectiveness as a microbial inactivation method. Fig. 4 presents the results of the EO treatment as a function of the LGEO dose. A 2.39 log-reduction was the most significant reduction, achieved when the EO treatment was applied at the highest concentration (294.11  $\mu\text{l}/\text{l}_{\text{air}}$ ). The final population under this condition was 10.2 CFU/g.



**Figure 3. Inhibitory effect on *Salmonella* Typhimurium of the vapor phase lemongrass essential oil (EO)**

#### 4. Discussion

The data supported the stated hypothesis. PL technology can be employed as an alternative to traditional thermal treatments to inactivate spoilage microorganisms. Thus, it is essential to take into consideration the temperature difference experienced by the food product after the PL treatment. According to the results of temperature difference as a function of the treatment time, under the same operating conditions, the treatment should not be of longer duration than 12 s, since it would be no longer possible to attribute the inactivation effect to the PL treatment.

The PL treatment was efficient when carrying out the *in vitro* experiment after the application of a  $10.8 \text{ J/cm}^2$  energy dose, the inactivation of *Salmonella* was fully accomplished. Nevertheless, the total fluence applied to the cultivated TSA plate was higher than the one applied by Luksiene et al. (2007) for achieving corresponding log-reductions. Still, the log-reduction was higher than the obtained by Gomez-Lopez et al. (2005) for the *S. Typhimurium in vitro* inactivation, when 50 pulses (pulse duration of 30  $\mu\text{s}$  and pulse intensity of 7 J) were applied to the sample.

Although the results for the *in vitro* experiment were efficient, the treatment only achieved a 2.91 log-reduction for the *in vivo* conditions with a 12 s duration treatment time; at the following treatment times, the *Salmonella* population remained constant. It is possible that the absorbance of UV light by the seed, due to the shading effect, was an essential factor for the microbial inactivation (Aron-Maftei et al., 2014; Kramer et al., 2017). The efficiency of the treatment for the inactivation of *S. Typhimurium* was dependent on the surface morphology and roughness, as specified in Kim et al. (2018).

Comparing the *in vivo* results with the literature, a log-reduction of 2.8 was achieved when applying the PL treatment with a fluence of 16.9 J/cm<sup>2</sup> to fresh blueberries with a similar initial *Salmonella* population (Bialka & Demirci, 2007).

Due to the opaque and uneven surface of the seeds, a combination of the PL treatment with another minimal processing technology was recommended (Aron-Maftei et al., 2014). The addition of the LGEO treatment to the PL technology demonstrated to be efficient to inactivate the *Salmonella* on the inoculated seeds. A total log-reduction of 5.29 was accomplished when both treatments were applied to the seeds.

The antimicrobial activity of the LGEO in the vapor phase achieved a 2.39 log-reduction when the concentration was 294.11 µl/l<sub>air</sub>. The obtained results differ from the ones of Kloucek et al. (2012), where the LGEO for the *Salmonella enteritidis in vitro* experiment did not exhibit total microbial inhibition when applied in the vapor phase with a concentration of 500 µl/l<sub>air</sub>.

## 5. Conclusion

This work allowed us to evaluate and compare the effectiveness of the pulsed light treatments against *Salmonella* Typhimurium for *in vitro* and *in vivo* conditions. However, the microbial reduction difference for both conditions is still a limiting factor for employing this technology as an individual disinfection technique on rough surfaces that can experience a shading effect. The combination of this treatment with the vapor phase lemongrass essential oil showed that microbial contamination of food products could be inhibited by using the combined treatment. Future experiments should focus on evaluating

the treatment effect on other seed products, due to the increasing demand of raw-seeds consumption.

## 6. Acknowledgements

This work was supported by the National Council of Science and Technology (CONACyT) of Mexico [grant number CB-2016-01-283636] and Universidad de las Américas Puebla (UDLAP) [grant number 3555]. Author M. Etchegaray-Bello acknowledges financial support for her BSc studies in Chemical Engineering from UDLAP.

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