UNIVERSIDAD DE LAS AMÉRICAS PUEBLA

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Inactivation of *Salmonella* Typhimurium on Sunflower Seeds by High Intensity Light Pulses and Lemongrass Essential Oil in Vapor Phase

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

Diana Paulina Sucar Escamilla

ID: 151076

***Licenciatura* in Chemical Engineering**

Advisor: Enrique Palou García, Ph.D.

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Signature sheet

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Advisor

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Dr. Enrique Palou García

President

_____

Dr. Aurelio López Malo Vigil

Secretary

_____

Dr. Fatima Reyes Jurado

Inactivation of *Salmonella* Typhimurium on Sunflower Seeds by High Intensity Light Pulses and Lemongrass Essential Oil in Vapor Phase

Diana Paulina Sucar Escamilla
Departamento de Ingeniería Química y Alimentos
Universidad de las Américas Puebla
San Andrés Cholula, Puebla 72810. México

ABSTRACT

The present study investigated the effect of a sequential treatment of high intensity light pulses (HILP) followed by lemongrass essential oil (EO) in vapor phase to inactivate *Salmonella* Typhimurium on sunflower seeds. First, the susceptibility of *S.* Typhimurium to HILP was evaluated *in-vitro*. Initial microbial load was inactivated with a treatment time of 8 s with a corresponding fluence of 10.80 J/cm^2 and a reduction of 10 log cycles. Then, the *in-vivo* effect of HILP on inoculated sunflower seeds was evaluated. A reduction of 3 log cycles was obtained with a treatment of 12 s; for larger treatments, the microbial load remained constant. The inactivation curves for both *in-vitro* and *in-vivo* tests were adequately fitted using the Weibull equation. Furthermore, surviving populations of *S.* Typhimurium on inoculated sunflower seeds were then exposed to an additional treatment with lemongrass EO in vapor phase. Studied microbial load was inactivated with a concentration of 294.12 mL EO/L_{air}, corresponding to a reduction of 3 log cycles. Thus, it was demonstrated that the HILP treatment in sequential association to vapor phase lemongrass EO increased the inactivation of *S.* Typhimurium on studied seeds. Further research is needed to evaluate the effect of each tested technology and its combination on sensory parameters of sunflower seeds.

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

INTRODUCTION

Sprouted seeds have become more popular in recent years due to their nutritional value including anti-cholesterol and anti-carcinogenic constituents (1). Raw seeds have gained attention as a ready-to-eat food and they can be added to salads, cereals, among other dishes. During their growth, the crops are susceptible to microbial contamination by different factors including soil, manure, irrigation water, and animals. Contamination is also likely to happen during storage, transport, and post-harvest processing of seeds (2). Pathogens in foods are responsible for the loss of quality and nutritional value (3). The rapid propagation of pathogens in seeds can occur in the presence of favorable conditions for microorganisms including pH, temperature, and aerobic conditions. High populations of 10^9 CFU/ml have been reported in sprouted seeds form retail stores (4).

Numerous seeds are potential vehicles for human pathogens including alfalfa, bean, sunflower and quinoa sprouts. Consumption of sprouted seeds has been associated with several outbreaks of foodborne illnesses in recent years. Approximately, there have been 279 outbreaks worldwide due to low-moisture foods (dry nuts and seeds) between the years 2004 and 2009. Out of those outbreaks, 85% were associated with *Salmonella* Typhimurium. The most common human pathogen found in sprouts is *S.* Typhimurium; however, there are also cases implicating *Escherichia coli* and *Listeria monocytogenes* (5). Thermal decontamination techniques such as microwave, radio-frequency, and food surface pasteurization, can cause changes in the physicochemical properties of foods. Therefore, alternative non-thermal seed decontamination methods have been developed including pulsed light and essential oils treatments.

Pulsed light is a non-thermal method used for food preservation by reducing microbial populations on the surface of foods and packaging materials. This technology uses short time pulses (1 μs to 0.1 s) of an intense broad-spectrum in a range of 200-1100 nm, including UV, visible and near-infrared light (6). The UV wavelength region (200-280 nm) of the spectrum provides a major contribution in order to achieve a lethal effect on microorganisms. The UV region is more efficient than other regions with longer wavelengths due to the fact that it processes higher energy levels. Additionally, the high peak power of pulsed light, combined with both the visible and near-infrared regions, can a cause a higher microbial death (7).

Different units including fluence, pulse width and pulse-repetition-rate characterize pulsed light treatment. Fluence is the energy received by the sample from the lamp per unit area (measured in J/cm^2). The pulse width measures the time interval during which energy is delivered. The pulse-repetition-rate (prr) expresses the quantity of pulses per unit of time (8). The effectiveness of the treatment depends on the quantity and intensity of the pulses delivered. The Food and Drug Administration of the United States of America has approved its practice as long as a Xenon flash lamp is used as a light source (6).

The inactivation mechanism of pulsed light is based on the photochemical modification of microorganisms' DNA. UV wavelengths target nucleic acids by transforming pyrimidine bases into dimers. The bonds formed inhibit DNA unzipping for replication, which results in microorganisms being unable to form new DNA chains and reproduce. Furthermore, DNA transformation results in mutations, gene transcription, impaired replication, and the organism's death. This photochemical mechanism occurs in

several microorganisms including bacteria and viruses. Pulsed light treatment differs from conventional UV treatment regarding the degree of damage it causes. Conventional UV treatment operates in a continuous mode using medium-pressure lamps. Conventional UV treatment affects DNA through reversible mechanisms while pulsed light treatment causes permanent damage by inactivating the repair system. Pulsed light is able to suppress repair mechanisms because of its wider wavelength (7).

The microbial inactivation by pulsed light is also explained by a photo-thermal effect. The light pulses heat the surface of the product and the heat is spread into the interior of the food. Nevertheless, the quantity of heat is very small, being unable to raise the temperature of the whole sample. A fluence surpassing 0.5 J/cm^2 can cause bacteria to rupture during their momentous overheating. Pulsed light is considered a non-thermal process as long as short-duration pulses are applied (8). Consequently, pulsed light irradiation can represent a feasible solution for microbial inactivation in food and contact surfaces.

The efficacy of the treatment depends on several factors including shadow effect, degree of contamination, food composition, and heating. Decontamination is optimum in transparent and regular surfaces. Due to the fact that foods are commonly opaque with irregular surfaces, a greater shadow effect is generated. Therefore, the treatment is only effective for the first 2 mm of the surface. UV light penetrability is restricted by the sample thickness due to the overlapping of opaque layers. A contaminated sample with a high bacterial population produces a more significant shadow effect because of overlapping microorganisms. In that case, pulsed light can only target the upper layers. Moreover, food composition plays an important role in food decontamination. Microbial destruction is

reduced in high-protein and oily foods because proteins and oils can absorb part of the radiation dose available. Furthermore, it is important to consider the maximum procedure time and the distance between the sample and the lamp. As the distance from the sample to the lamp increases, the absorption diminishes (7).

Weibull distribution is a model used to describe microbial inactivation. Inactivation curves of pathogens are modeled using two Weibull parameters, *b* and *n*. Both parameters are dependent and are obtained by nonlinear regression. Weibull equation has a flexible structure capable of describing upward and downward concavity of the survival curve. A value of $n>1$ represents a monotonic downward concave survival curve while a value of $n<1$ represents an upward concave curve (16). Weibull model is shown in equation 1, where N_0 is the number of microorganisms before the treatment, N is the number of microorganisms at time t, b is the scale factor, n is the shape factor and t is time (17).

$$\log \frac{N}{N_0} = bt^n \qquad (1)$$

Essential oils (EOs) are aromatic extracts that have gained considerable attention for inactivating pathogens in foods (3). Essential oils are natural compounds found in plants that can be synthesized from flowers, stems, leaves, fruits or seeds. They are liquid, volatile, and characterized by a strong odor (9). Essential oils are used for food preservation because of their non-thermal mode of operation and their antimicrobial properties (10). The interaction of essential oils with the cell membrane of microorganisms leads to microbial inactivation in foods. The Food and Drug Administration of the United States has approved

the use of essential oils in foods (11). In consequence, this technique can be useful in the food industry to improve the quality of certain foods and expand their shelf life.

The effectiveness of essential oils depends on several factors including the quality and quantity of the active substance present. This is subject to the plant variety, the growing conditions, the time of harvest, and the way the essential oil is obtained (12). The degree of inactivation is linked to the volatile compounds present in essential oils and the sensitivity of the pathogens. It is reported in the literature that essential oils applied in the vapor phase have successfully inactivated microorganisms. An advantage of applying EOs in vapor phase rather than liquid phase is that smaller changes on organoleptic properties of foods can occur (13).

Lemongrass oil is an EO obtained from the herb *Cymbopogon citratus* by hydro-distillation. *C. citratus* is one of the 140 known species of the genus *Cymbopogon* and it is cultivated in many tropical countries in South America, Asia and Africa. Lemongrass oil has gained popularity due to its medicinal purposes, as well as its antibacterial and antifungal effects (14). Therefore, it represents an alternative technique for food preservation.

Essential oils have been combined with other preservation techniques to achieve a major effect in food decontamination. The treatment of essential oils in sequential association to other non-thermal technology such as pulsed light or hydrostatic pressure can increase the degree of inactivation reached. A synergistic treatment can be more effective than a technology acting alone, while still ensuring the preservation of the organoleptic properties of foods (15).

The objective of this study was to establish whether the application of pulsed light

treatment could completely inactivate *S.* Typhimurium colonies in sunflower seeds. If this was not possible, evaluate the microbial inactivation effect of a sequential non-thermal treatment of pulsed light followed by EOs.

MATERIALS AND METHODS

Sunflower seeds characterization. Sunflower seeds (brand OKKO Superfoods) were purchased from a local supermarket and stored at 20 ºC until use. The dimensions of the seeds (length, width and thickness) were measured using a Vernier caliper to an accuracy of 0.001 mm. A total of 100 sunflower seeds were randomly selected and the average length, width and thickness were obtained. Measurements were repeated twice (18).

Microbiological analysis of sunflower seeds. A microbiological analysis was carried out in sunflower seeds of brand OKKO Superfoods, which assures 100% sanitation. Enumeration of coliform colonies was realized by using violet red bile agar medium. Furthermore, aerobic mesophilic bacteria colonies were identified by using standard methods agar medium (19). 1 gram of sunflower seeds was added to autoclaved glass tubes containing 9 ml of peptone water and the solutions were stirred. Next, 1 ml of the solution was poured into plastic Petri dishes followed by the addition of either RBA or STM, and homogenized. Tests for each agar medium were performed in duplicates. All plates where incubated at a temperature of 37 ºC for 24 h. After incubation, the mean number of colonies was determined.

Preparation of bacterial cultures. *S. enterica* subsp. *enterica* serovar Typhimurium (ATCC 14028) was obtained from the frozen culture collection of the Food Microbiology Laboratory Collection at the Universidad de las Américas Puebla, Mexico. The organism was kept in a Trypticase soy agar slant at a temperature of 4 ºC. The bacteria was activated at 37 ºC and cultured in Trypticase soy broth for 24 h.

For in-vitro experiments, 100 μl of bacterial culture were added to sterile glass tubes containing 9 ml of peptone water and subjected to serial dilutions. The solutions were mixed using a vortex (20). Depositions of 50 μl of dilution were plated on Typticase soy agar Petri dishes and grown overnight at 37 ºC. Plating was carried out using a spiral plater (Autoplate 4000 from Spiral Biotech, Norwood, MA, USA) (5). These samples were then exposed to pulsed light treatment for different exposure times.

For in-vivo experiments, *S.* Typhimurium was inoculated in sunflower seeds. For feed inoculation, 40 grams of sunflower seeds were incorporated to a solution containing 400 ml of sterilized TSB and 4 ml of adjusted inoculum. The jar was left in a water bath incubator (BT25 from Yamato Scientific Co., Japan) for two hours at 35 ºC with shaking of 120 rpm. Next, the seeds were drained and dried at 35 ºC in a laminar flow hood, with the help of filter paper sheets. Dried seeds were spread on plastic Petri dishes (21).

Pulsed light treatment. Pulsed light treatment was executed in the pilot plant of the Chemical and Food Engineering Department at Universidad de las Américas Puebla. The experiments were carried out using a Xenon PL applicator (Model LH840 from Xenon Corporation, Wilmington, MA, USA). The equipment consists of a controller unit and a

treatment chamber with a Xenon flash lamp mounted on the top. There are 11 stainless steel trays located inside the chamber (length 16 in, width 6.2 in, and height 0.35 in). The system generates a broadband spectrum of 200-1100 nm, including infrared light, visible light, and a maximum emission in the ultraviolet range. The pulse power dose supplied is 505 joules per pulse electrical and the pulse rate is 3 pulses per second (6).

For *in-vitro* tests, TSA Petri dishes with *S.* Typhimurium inoculum were exposed to increasing duration times of 1-13 seconds. For *in-vivo* tests, the seeds were treated by PL for duration times of 3, 6, 9, 12, and 15 s. The inoculated samples were placed on the 8th steel tray in the PL chamber, located 4.26 in below the light source (chamber window). Pauses of 1 min after each treatment were made to prevent overheating of the chamber. Temperature was measured before and after the treatment using an infrared thermometer (Fluke 59 MAX, Everwood, WA, USA). All PL experiments were performed by duplicate.

Microbiological determination. For *in-vivo* tests, plating was carried out after the pulse light treatment. 1 g of each exposed sample was transferred to 9 mL of sterile peptone water. The solution was stirred and 50 μl were plated on Trypticase soy agar Petri dishes. Plating was carried out using a spiral platter.

Both *in-vitro* and *in-vivo* test plates were incubated at 37 ºC for 24 hours. The surviving colonies were counted on Q-Counter (Spiral Biotech, Norwood, MA, USA) and expressed as CFU/ml per plate. Plate counting analyses were done in duplicates. Weibull distribution model was used to describe microbial inactivation kinetics. Weibull parameters were obtained by equation 1 using Microsoft Excel.

Lemongrass essential oil chemical analysis. Lemongrass essential oil was analyzed by a gas chromatography–mass spectrometry method. The technique was carried out using a 6850 Series Network GC System gas chromatograph (Agilent Technologies, Santa Clara, CA) paired to a 5975C VL mass selective detector with triple-axis detector (Agilent Technologies) and a split-splitless injector (1:10 split ratio). A fused silica HP-5MS (5% phenyl–95% polydimethylsiloxane) capillary column (with dimensions of 30 m by 0.250 mm and a film thickness of 0.25 um) was employed. Helium was the carrier gas used and it was supplied at a flow rate of 1.1 ml/min. The analyzed samples were prepared by diluting the essential oil at 5:100 (v/v) in ethanol, with an injection volume of 1 μl. The temperature of the column oven was programmed from 60 ºC (4 min) to 240 ºC (10 min) at 4 ºC/min. The injector temperature was set at 250 ºC while the detector temperature at 280 ºC. Retention times were obtained by means of a homologous series of n-alkanes C8.0 to C18.0 (Sigma, St. Louis, MO). Eluted compounds were identified by comparing their retention times with the literature. Moreover, the mass profile of the same compounds available from the U.S. National Institute of Standard Technology library was consulted (22).

Essential oil treatment. Inoculated seed samples were set in plastic Petri dishes and exposed to a PL treatment of 12 s. To expose the treated seeds to the vapor of lemongrass oil, 6 grams of sunflower seeds were placed on the upper part of a plastic container with a hermetic seal. A plastic grid was set on a second level using small plastic cups. Over the grid, sterile gauzes were placed and the seeds were set over them. The lemongrass oil was

poured into small Petri dishes in concentrations of 58.82, 117.65, 176.47, 235.29, and 294.12 μl of EO per L of air. A control experiment without EO was also assessed. The six tested concentrations were located under the grid of the plastic container. The hermetic containers were incubated for 24 h at 37 °C. After incubation, plating was carried out using the methodology previously explained. The remaining *S.* Typhimurium colonies were counted using Q-Counter and expressed as CFU/ml per plate after the plates were incubated for 24 h at 37 °C (13).

RESULTS AND DISCUSSION

Sunflower seeds characterization. The seeds have a length ranging from 0.56 to 1.098 cm, a width ranging from 0.412 to 0.490 cm, and a thickness ranking from 0.198 to 0.262 cm. The average length, width, and thickness of sunflower seeds obtained were 1.035 cm, 0.446, and 0.235 cm, respectively.

Microbiological analysis of sunflower seeds. No colonies of coliforms were present in the studied sunflower seeds. Moreover, a mean value of 3 colonies of aerobic mesophilic bacteria was determined. The sanitary quality of the tested seeds was determined to be adequate for the following *S.* Typhimurium inoculation. Therefore, inoculation and treatment results will not be influenced by seed contamination (23).

High intensity light pulses *in-vitro* tests. Preliminary experiments consisted of in-vitro inoculated *S.* Typhimurium exposed to different duration times of pulsed light treatment.

The behavior of *S.* Typhimurium populations in TSA is shown in Table 1. It can be seen that the microbial colonies decrease with the increase of the number of pulses emitted. The microbial load was inactivated with a treatment time of 8 seconds, corresponding to a fluence of 10.80 J/cm^2. Moreover, for an 8-second treatment a microbial reduction of 10 log cycles was detected. The obtained results reveal that pulsed technology can completely inhibit *S.* Typhimurium grown in TSA after a short duration treatment of 8 seconds.

TABLE 1. *In-vitro* inactivation of *Salmonella* Typhimurium by pulsed light treatments

Treatment times (s)	Number of pulses	Total fluence (J/cm^2)	*S.* Typhimurium counts (log CFU/mL)
0	0	0.00	10.28
1	3	1.35	9.56
2	6	2.70	9.23
3	9	4.05	8.21
4	12	5.40	7.73
5	15	6.75	6.00
6	18	8.10	3.93
7	21	9.45	1.27
8	24	10.80	< 10
9	27	12.15	< 10
10	30	13.50	< 10
11	33	14.85	< 10
12	36	16.20	< 10
13	39	17.55	< 10

High intensity light pulses *in-vivo* tests. The experiments were performed on inoculated sunflower seeds with an initial microbial concentration of 10^6 CFU/ml. The log cycles reduction due to pulsed light treatment was evaluated. The results are shown on Table 2. The efficacy of pulsed light treatment is directly proportional to the energy dose absorbed by the target microorganism. *S.* Typhimurium colonies decreased significantly as a function of HILP fluence and temperature. *S.* Typhimurium reduction results were 2 log cycles for duration times of 3, 6, and 9 seconds, and 3 log cycles for duration times of 12 and 15 seconds. A greater reduction was achieved until reaching 12 s. After that, the population remained constant. Particularly, a significant reduction of 3 log cycles was obtained with a duration treatment of 12 s. Results demonstrate colony numbers decreased with the increase of pulsed light exposure.

TABLE 2. *In-vivo* inactivation of *Salmonella* Typhimurium by pulsed light treatments

Treatment times (s)	Number of pulses	Total fluence (J/cm^2)	*S.* Typhimurium counts (log CFU/mL)
0	0	0.00	6.81
3	9	4.05	4.49
6	18	8.10	4.17
9	27	12.15	4.09
12	36	16.20	3.59
15	45	20.25	3.59

The most significant limiting factor of pulsed light treatment is heating caused by the absorption of light. Long applications surpassing one minute result in physical and

nutritional changes in foods. Therefore, temperature was a liming factor during this study to avoid overheating of the samples. The final temperature of the samples increases with the number of PL emissions. The microbial inactivation observed at low temperatures is mainly caused by the photochemical effect of pulsed light treatment. At higher temperatures, inactivation of toxins occurs because of both photochemical and photothermal effects (24). The results show that the temperature of untreated sunflower seeds increased from 31.5 ºC ± 1 ºC to 36.8 ºC, 37.6 ºC, 47.2 ºC, 49.6 ºC, and 56.4 ºC for 3, 6, 9, 12, and 15 s treatment times, respectively. Temperature results are shown in Figure 1.

The temperature of the samples surpassed 50 ºC after treatment durations greater than 12 s. A temperature of 49.6 ºC was reached for a duration time of 12 s. A temperature below 50 ºC is not expected to cause any physicochemical change in the treated seeds. However, sensory analyses need to be carried out to verify this hypothesis.

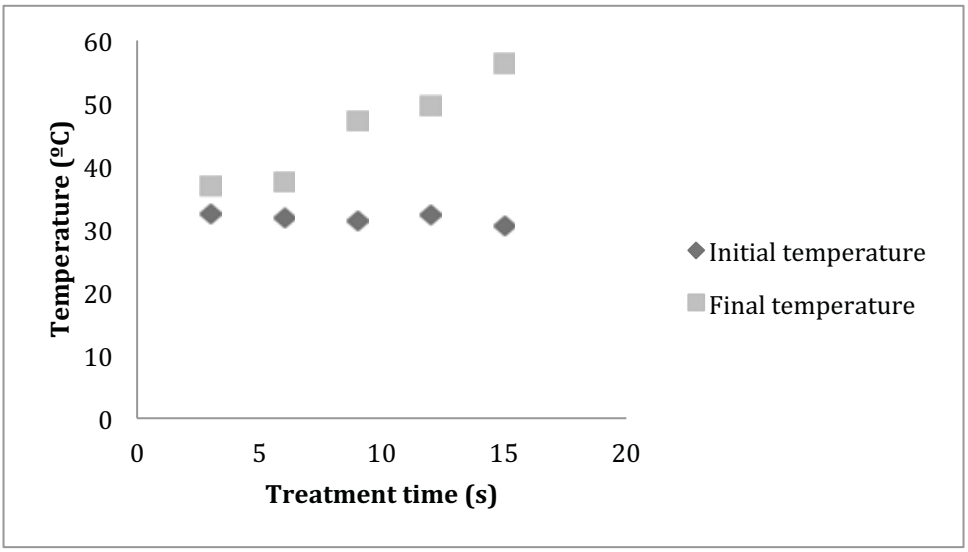

FIGURE 1. Initial and final temperature of inoculated sunflower seeds samples exposed to different times of pulsed light treatments

Weibull model fitting results. Weibull equation (see equation 1) was used to model the nonlinear distribution of the PL curve. The equation was fitted to the inactivation curve of *S.* Typhimurium for both in-vitro and in-vivo tests. The logarithm log (N/N_0) was plotted against time, and the Weibull parameters *b* and *n* were obtained with the help of Excel Solver tool. Experimental and calculated inactivation data can be observed in figures 2 and 3 for in-vitro and in-vivo tests, respectively. It can be seen that the Weibull model was a good fit to model microbial inactivation in sunflower seeds by pulsed light treatment. The obtained Weibull parameters for the pulsed light inactivation of *S.* Typhimurium *in-vitro* and *in-vivo* are shown in Table 3.

For *in-vitro* tests, the *n* parameter value obtained was >1, indicating a downward concavity. Downward concavity means the microorganisms are perceptive to the pulsed light treatment. This curve shape also indicates a gradual weakening of the survivors. That is to say, as pulsed light emissions increase, the microorganisms become more sensitive to the treatment (17). It can be observed that inactivation is directly proportional to the treatment time.

On the other hand, an *n* parameter value <1 was obtained for *in-vivo* tests, indicating an upward concavity. An upward concavity means a rapid inhibition of the more susceptible microorganisms, leaving behind the survivors of higher resistance (17). It can be seen that after 12 seconds, the microbial population remains constant. This indicates that the resistant survival microorganisms will not be affected by a longer treatment. Therefore, a treatment surpassing 12 seconds is not feasible.

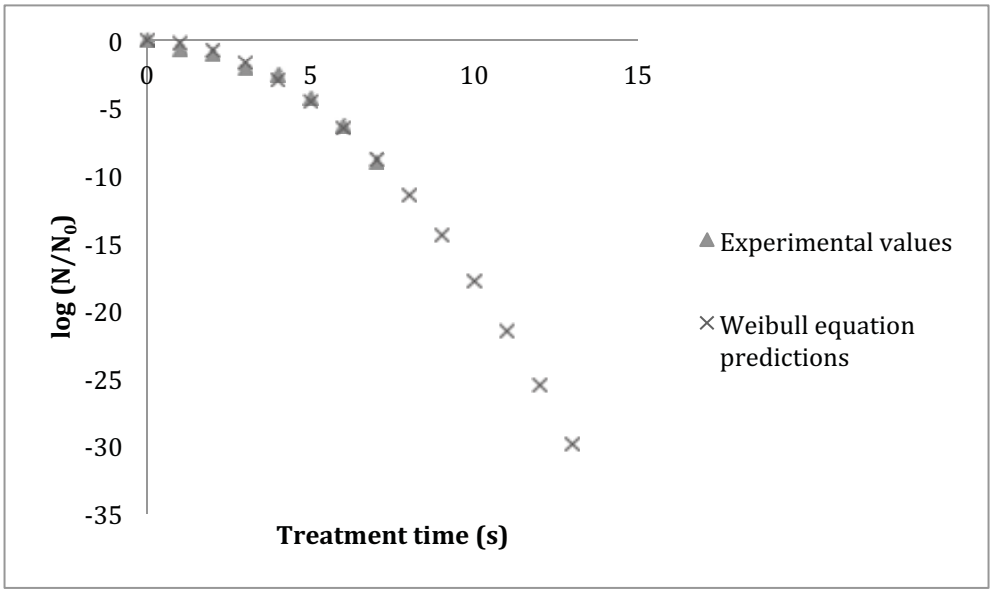

FIGURE 2. *In-vitro* inactivation of *Salmonella* Typhimurium by pulsed light treatments

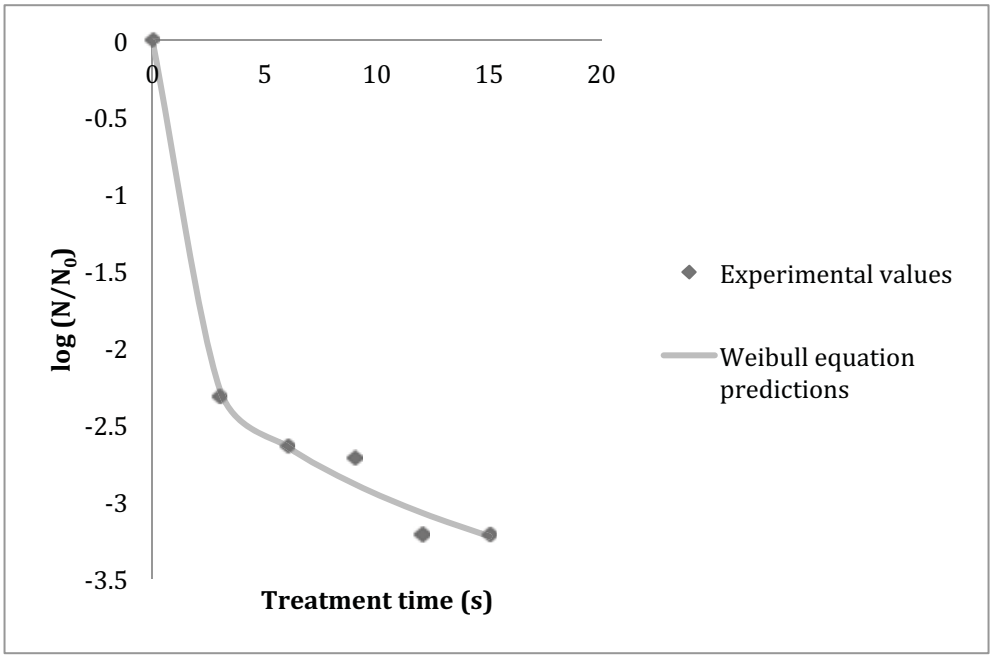

FIGURE 3. *In-vivo* inactivation of *Salmonella* Typhimurium by pulsed light treatments

TABLE 3. Weibull model parameters (*b* and *n*) for *in-vitro* and *in-vivo* tests

	b	*n*
In-vitro	0.1883	1.9752
In-vivo	1.7872	0.2181

Lemongrass essential oil chemical analysis. The main compounds found in lemongrass essential oil are reported in Table 4, as well as their CAS number, average retention time, and percentage area. A total of 42 chemical constituents were found in lemongrass EO, accounting for 98.85% of the total composition. The major components identified in lemongrass essential oil are the following: neral (β-citral) (39.21%), ϒ-muurolene (8.20%), farnesol (5.79%), and camphene (5.54%). Other compounds that contributed significantly to lemongrass EO composition include limonene (4.87%), isopulegol (4.16%), (+) camphene (4.07%), isogeraniol (3.38%), geranial (α-Citral) (3.41%), and (3Z)-β-ocimene (2.19%).

TABLE 4. Main compounds in studied lemongrass essential oil

Compound	CAS	RT	Area %
2-methyl-3-buten-2-ol	115-18-4	1.892	0.10
2,4-dimethyl; 2,4-dimethylcyclopentan-1-ol	89794-28-5	2.159	0.02
2-Heptanol, 5-methyl-; 5-methylheptan-2-ol	54630-50-1	3.195	0.01
Dodec-1-yne	765-03-7	3.750	0.01
(E)-non-3-en-1-ol	10339-61-4	4.539	0.02
(1R,4R,8S)-rel-9-Oxabicyclo[6.1.0]nonan-4-ol	69853-85-6	4.762	0.01
2,2'-(1,12-dodecanediyl)bis-Oxirane	800375-27-3	12.049	0.88
1,7,7-trimethyl-Tricyclo[2.2.1.02,6]heptane	508-32-7	6.239	0.21
2,6,6-trimethyl- (1*R*,5*R*)-Bicyclo[3.1.1]hept-2-ene	7785-70-8	6.845	0.71
2,2-dimethyl-3-methylene-	79-92-5	8.477	5.54

Bicyclo[2.2.1]heptane			
6-methyl-5-Hepten-2-one	110-93-0	11.039	1.02
1-methyl-4-(1-methylethenyl)-Cyclohexene	138-86-3	13.042	4.87
2,2-dimethyl-3-methylene-, (1*R*,4*S*)-Bicyclo[2.2.1]heptane	5794-03-6.	14.229	4.07
(3*Z*)-3,7-dimethyl	3338-55-4	15.649	2.19
3,7-dimethyl-1,6-Octadien-3-ol	78-70-6	17.315	1.87
3-Ethoxy-3,7-dimethyl-1,6-octadiene	72845-33-1	18.072	1.9
(3Z)-3,7-dimethylocta-3,6-dien-1-ol	5944-20-7	19.632	3.38
(1R,2S,5R)-5-methyl-2-prop-1-en-2-ylcyclohexan-1-ol	89-79-2	20.467	4.16
(2Z)-3,7-dimethylocta-2,6-dienal	106-26-3	24.144	39.21
(2E)-3,7-dimethylocta-2,6-dienal	141-27-5	26.820	3.41
(2E)-1-ethenoxy-3,7-dimethylocta-2,6-diene	17957-93-6	27.284	1.01
(2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-ol	106-28-5	27.461	5.79
[(2E)-3,7-dimethylocta-2,6-dienyl] acetate	16409-44-2	28.039	1.81
2-methoxy-4-[(E)-prop-1-enyl]phenol	5932-68-3	29.075	2.07
(1S,4aS,8aR)-7-methyl-4-methylidene-1-propan-2-yl-2,3,4a,5,6,8a-hexahydro-1H-naphthalene	24268-39-1	29.821	8.2
3as,3br,4s,7r,7ar)-7-methyl-3-methylidene-4-(propan-2-yl)octahydro-1h-cyclopenta[1,3]cyclopropa[1,2]benzene	13744-15-5	29.527	1.46
2-[(1R,3S,4S)-4-ethenyl-4-methyl-3-prop-1-en-2-ylcyclohexyl]propan-2-ol	639-99-6	30.757	0.64
(1R,4R,6R,10S)-9-Methylene-4,12,12-trimethyl-5-oxatricyclo[8.2.0.04,6]dodecane	1139-30-6	31.618	0.83
(1S,4R,4aR,8aR)-4,7-dimethyl-1-propan-2-yl-2,3,4,5,6,8a-hexahydro-1H-naphthalen-4a-ol	21284-22-0	32.125	0.37
(4aS,9aR)-3,5,5-trimethyl-9-methylidene-2,4a,6,7,8,9a-hexahydro-1H-benzo[7]annulene	3853-83-6	32.677	0.14
(1aR,4S,4aR,7R,7aS,7bS)-1,1,7-trimethylspiro[2,3,4a,5,6,7,7a,7b-octahydro-1aH-cyclopropa[e]azulene-4,2'-oxirane]	85710-39-0	33.258	0.07
1,3b,6,6-Tetramethyldecahydro-1H-cyclopropa[7,8]azuleno[4,5-b]oxirene	159366*	33.461	0.12
11. (2E,6E,10E)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-ol	24034-73-9	34.193	0.06

Aromadendrene oxide-(2)	85710-39-0	34.723	0.02
cis-Z-alfa-Bisabolene epoxide	1000131-71-2	35.140	0.02
1,1,7-trimetilspiro [2,3,4a, 5,6,7,7a, 7b-octahidro-1aH-ciclopropa [e] azuleno-4,2'-oxirano]	94020-95-8	35.655	0.02
(Z,E)-2,13-octadecadienol; (Z)-2-(E)-13-Octadecadien-1-ol	123551-47-3	35.873	0.02
3,7,11,15-tetramethylhexadec-2-en-1-ol	102608-53-7	36.022	0.03
5α-Cholestan-3β-ol, 2-methylene-	22599-96-8	36.956	0.04
1,5,9-trimetil-12-propan-2-ilciclotetradeca-4,8,13-trieno-1,3-diol	7220-78-2	42.083	1.96
(6E,10E)-3,7,11,15-tetramethylhexadeca-1,6,10,14-tetraen-3-ol	1113-21-9	41.640	0.53
Triciclo [20.8.0.0 (7,16)] triacontano, 1 (22), 7 (16) -diepoxi-	155907*	46.029	0.05

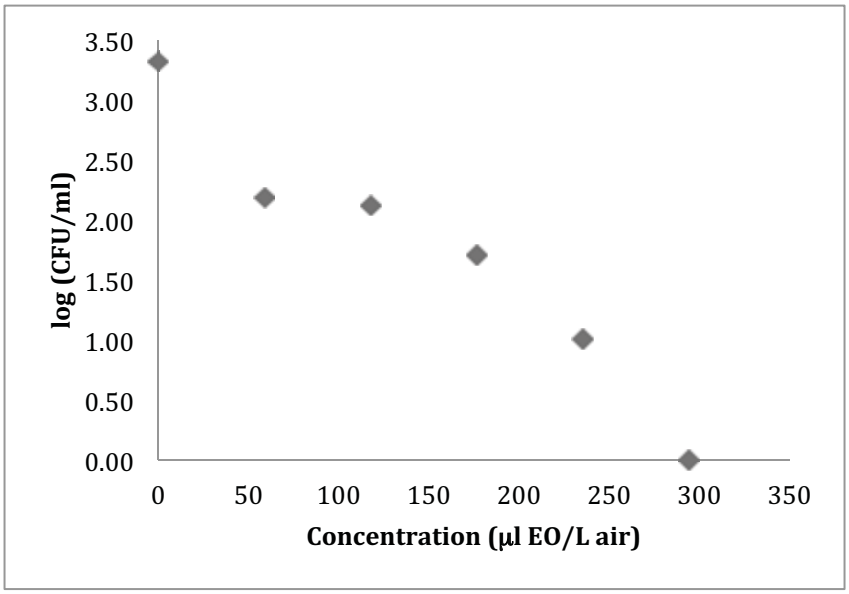

FIGURE 4. Inactivation of *Salmonella* Typhimurium inoculated on sunflower seeds exposed to different concentrations of lemongrass essential oil in vapor phase after being exposed to pulsed light treatments

Treatments with lemongrass essential oil in vapor phase. The surviving populations of *S.* Typhimurium in inoculated sunflower seeds were exposed to an additional treatment of EO. Lemongrass EO was applied in vapor phase in sequence to pulsed light treatment. The experiments were performed on inoculated sunflower seeds exposed to 12 seconds of pulsed light treatment, with an initial microbial concentration of 10^3 CFU/ml. Results are shown in Figure 4. It is shown that the microbial colonies decrease with the increase of EO concentration. The microbial load of *S.* Typhimurium was completely inactivated with an amount of 294.12 μl of EO per L of air, corresponding to a microbial reduction of 3 log cycles.

CONCLUSIONS

The present study investigated the effect of a sequential treatment of pulsed light followed by essential oils on sunflower seeds. A preliminary in-vitro test consisted of inoculated *S.* Typhimurium exposed to different duration times of high intensity light pulses. The microbial load was inactivated with a treatment time of 8 seconds, corresponding to a fluence of 10.80 J/cm^2 and a reduction of 10 log cycles. Weibull equation was used to model the inactivation curve. The *n* parameter value obtained was >1, indicating a downward concavity. Downward concavity means the microbial populations are perceptive to the pulsed light treatment, resulting in gradual weakening of the microorganisms.

In case of the inoculated sunflower seeds, the samples were treated by PL for duration times of 3, 6, 9, 12, and 15 s. It was observed that the efficacy of pulsed light treatment was directly proportional to the energy dose absorbed by the target

microorganism. *S.* Typhimurium reduction results were 2 log cycles for duration times of 3, 6, and 9 seconds, and 3 log cycles for duration times of 12 and 15 seconds. A greater reduction was achieved until reaching 12 s. This behavior was fitted by Weibull equation. An *n* parameter value <1 was obtained, indicating an upward concavity. An upward concavity means a rapid inhibition of the more susceptible microorganisms, leaving behind the resistant survivors. It was observed that after 12 seconds, the microbial load remained constant. The resistant survival microorganisms will not be affected by a longer treatment, so a sequential nont-hermal technique was proposed.

The surviving populations of *S.* Typhimurium on inoculated sunflower seeds were exposed to an additional treatment of EO. Lemongrass EO was applied in vapor phase in sequence to pulsed light treatment in different concentrations of 58.82, 117.65, 176.47, 235.29, and 294.12 μl of EO per L of air. The microbial colonies decreased with the increase of EO volume. The microbial load of *S.* Typhimurium was completely inactivated with an amount of 294.12 μl EO/L air, corresponding to a microbial reduction of 3 log cycles.

In conclusion, both high intensity light pulses and essential oils treatments represent emerging non-thermal food preservation technologies. It was demonstrated that the treatment of essential oils in sequential association to pulsed light emissions increases the degree of inactivation reached. Therefore, these promising techniques are capable of inhibiting pathogens in sunflower seeds, without compromising the food quality. A future investigation regarding the unique application of EOs treatment needs to be assessed.

Furthermore, investigations need to be executed to evaluate the effect of each technology on sensory parameters.

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