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Impact of High-Power Pulsed Light on Microbial Contamination, Health Promoting Components and Shelf Life of Strawberries

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Summary

The aim of this work is to evaluate the impact of high-power pulsed light (HPPL) on the microbial control and nutritional properties of strawberries. Berries were treated with HPPL and afterwards analyzed in terms of microbial contamination, shelf life extension, antioxidant capacity, firmness, total phenolic, total anthocyanin and ascorbic acid content, and colour. Results indicate that the decontamination of strawberries by HPPL was significant compared to control. Naturally distributed mesophilic bacteria on the surface of strawberries were inactivated by 2.2 log, and inoculated *Bacillus cereus* and *Listeria monocytogenes* were inactivated by 1.5 and 1.1 log, respectively. Yeasts/microfungi distributed on the surface of strawberries were inactivated by 1 log. The shelf life of treated strawberries was extended by 2 days. The increase of temperature on the surface of fruit never exceeded 42 °C. No significantly important differences were observed in total phenolic, total anthocyanin and ascorbic acid content, and antioxidant capacity of strawberry fruits before and after pulsed light treatment. Moreover, no impact on the strawberry colour or firmness was found after HPPL treatment. In conclusion, HPPL is fast, effective, non-thermal and environmentally friendly technique which can be applied for microbial control of strawberries.

Key words: non-thermal decontamination, food preservation, pulsed light, strawberries

Introduction

Consumption of fruits and vegetables is increasing all over the world. Strawberries have health-promoting properties and are at the fundament of the nutritional pyramid because they contain vitamins, minerals, dietary fibres, phenolic antioxidants and other bioactive compounds (*1*). However, fresh produce has been increasingly implicated as the vehicle of pathogen transmission and became the second leading cause of foodborne illnesses, which costs for instance the US economy $6.9 billion of loss in productivity and medical expenses (*2*). Worldwide, there are 1.5 billion cases of foodborne illnesses each year. More than 3 million deaths from foodborne illnesses are recorded per year. *Bacillus cereus* is one of the foodborne pathogens, often isolated from soil, foodstuffs, and fruit and vegetable production areas (*3*). Each year 63 400 people in the USA are infected with foodborne *B. cereus* (*4*). In 2010, 26 outbreaks, involving 561 cases of infection, caused by *Bacillus* toxins were reported in eight EU member states, with the distribution split evenly among them (*5*). *Listeria monocytogenes* is a highly pathogenic bacterium, which causes foodborne illness known as listeriosis. It is a primary cause of food-related mortality and morbidity (*6*). The Center for Disease Control and Prevention reported 568 cases of human listeriosis and 92 deaths in the USA in 2010 (*7*). Moreover, in 2010 a total of 1601 confirmed cases of listeriosis were

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reported by 26 EU member states (*5*). Many food products have been associated with *L. monocytogenes* contamination, including raw milk and cheese, raw fruits and vegetables, ready-to-eat meat, and raw and ready-to-eat seafood (*6*,*8–10*). Recently, *Listeria* has been isolated from strawberries (*11*).

Although intensive development of chemical treatments (fungicides, biocides, preservatives) and improved storage conditions during the last 40 years have greatly extended the shelf life of fruits after harvest, postharvest losses vary from 5 to 20 % and can be as high as 50 % in developing countries (*12*). However, the increasing health risks from chemical contamination of food have accelerated the need to reduce and even eliminate completely the chemical residues on strawberries (*13*).

Thus, during the past decade the emphasis in postharvest fruit protection has shifted from using chemicals to various alternative techniques including biological (*14*) and physical methods such as controlled atmosphere (*15*), high intensity pulsed electric field (*16*) or irradiation (*17*). However, biological control has met criticism due to high pathological and allergenic potential (*14*). Ionizing radiation is legal in 40 countries, but remains little used, mainly due to the suspicions of consumers (*18*). Obviously there is a need to develop a new preservation technology that will be more effective and will not diminish the organoleptic properties and nutritional value of the product.

High-power pulsed light (HPPL) is an emerging non-thermal food preservation technology that proposes decontamination of surfaces by intense and short duration (in μs) pulses of broad spectrum light (200–1000 nm) and was approved for food surface decontamination by the Food and Drug Administration in 1999 (*19*). This method offers effective inactivation of pathogens, has low energy costs, great flexibility and short exposure time. Other advantages of pulsed light are the lack of residual compounds and the absence of applied chemicals that can cause ecological problems or are potentially harmful to humans (*20*). Power emission from continuous UV light ranges from 100 to 1000 W, whereas pulsed UV light can produce a peak power of 35 MW. Moreover, pulsed light may reduce the temperature build-up compared to continuous UV light, due to short pulse duration and cooling period between pulses.

Meanwhile a lot of work needs to be done on the optimization of this treatment as its efficiency strongly depends on critical process parameters (voltage, distance from the lamp and total light dose) (*21–23*). First attempts to find experimental parameters for effective *in vitro* inactivation of food pathogen *Salmonella enterica* were reported by Luksiene *et al.* (*24*).

The aim of this study is to evaluate the possibility of decontamination of strawberries from harmful and pathogenic microorganisms. For pathogen inoculation model, *B. cereus* and *L. monocytogenes* were used. In addition, examination of extention of shelf life and effects on nutritional properties of strawberries, as most perishable berries, after treatment would be interesting.

Materials and Methods

Bacterial strains and fruits

Bacillus cereus ATCC 12826 was kindly provided by the National Centre of Public Health (Vilnius, Lithuania). The bacterial culture was grown at 37 °C and maintained on Luria-Bertani agar (LBA; Liofilchem, Roseto degli Abruzzi, Italy). *Listeria monocytogenes* $ATC_{L3}C$ 7644 was kindly provided by the National Veterinary Laboratory (Vilnius, Lithuania). The bacterial culture was grown at 37 °C and maintained on tryptone soya agar supplemented with 0.6 % yeast extract (TSYEA; Liofilchem). Strawberries (*Fragaria* × *ananassa* Duch.) at partially ripe stage were purchased in a local supermarket, stored at 6 °C and processed within a day.

Preparation of pathogens for in vitro experiments

Bacillus cereus ATCC 12826 and *Listeria monocytogenes* $ATC_{L3}C$ 7644 were grown overnight (approx. 14 h) at 37 °C in 20 mL of Luria-Bertani medium (LB; Liofilchem) and tryptone soya medium supplemented with 0.6 % yeast extract (TSYE; Liofilchem), respectively, with aeration level of 120 rpm (Environmental Shaker-Incubator ES–20; Biosan, Latvia). The overnight bacterial cultures were then diluted 20 times with the fresh LB medium and bacterial absorbance was determined in a 1-cm glass cuvette at λ=540 nm (Heλios Gamma & Delta spectrophotometers; ThermoSpectronic, Cambridge, UK) as A= 0.164. Bacteria were grown at 37 °C to the mid-log phase (*B. cereus* approx. $6{\cdot}10^7$ CFU/mL, A=1; *L. monocytogenes* approx. $1.6{\cdot}10^9$ CFU/mL, A=0.9; bacterial count established by spread plate method) in a shaker (120 rpm). Afterwards, *B. cereus* and *L. monocytogenes* cells were harvested by centrifugation (3420×*g*, 10 min; Hettich Zentrifugen, Mikro-200, Tuttlingen, Germany) and resuspended to approx. $3.015{\cdot}10^8$ and $5.8{\cdot}10^9$ CFU/mL in 0.01 M phosphate buffer saline (PBS; pH=7.2), respectively. These stock suspensions were then diluted in PBS to approx. 10^7 CFU/mL and immediately used for the further experiments.

High-power pulsed light device

The device constructed in our laboratory consisted of a chamber, a reflector at a flash lamp fixed on the top of the chamber and a power supply unit. The chamber had three shelves at different distances from the flash lamp. Every shelf had a 16×16 cm^2 area, where illumination parameters were constant and uniform with 10 % fluctuation. The illumination spectrum of xenon lamp was broad (200–1000 nm) and had maximal emission at 260 nm (antibacterial UV light). Thus, in order to gain homogeneous light radiation, we placed the xenon flash lamp in the focus of the parabolic reflector. Moreover, the reflector was made from aluminium with raster (rough, patterned) surface. Such construction ensures that the light density variations are not greater than 10 % of total energy in all irradiation area (16×16 cm). Duration of light pulse was 112 μs, and frequency was 5 Hz. The power of each pulse ranged from 0.07 to 0.9 MW. In experiments, pulse power density of 133 W/cm^2 per 260 nm was used. UV light dose increments were obtained by increasing irradiation time (0–200 s).

In order to estimate the dose of total light energy delivered to the fruit, several mathematical calculations had to be performed. The pulse power density is a pulse energy (in mJ) delivered per cm^2 of the treated surface during pulse duration (112 μs), *i.e.* a ratio of the pulse energy to the pulse duration ($J/(cm^2 \cdot s)$). Traditionally, photobiological processes depend on the total light energy dose delivered to the object and are expressed in J/cm^2. In the case of pulsed light delivery, the total dose (D) is the energy of one pulse ($e_1/(J/cm^2)$), multiplied by a number of pulses during the whole treatment time (t/s):

$$D=e_1 \cdot t \cdot f \qquad /1/$$

where f is a pulse frequency (in Hz).

UV light power density measurements were performed at 260 nm with a 3sigma meter (Coherent Inc., Santa Clara, CA, USA) equipped with pyroelectrical detector J25LP04 (*24*).

Pulsed light treatment of the inoculated bacterial cells

The test culture (approx. 10^7 CFU/mL) was plated onto the LBA using spread plating method. Then the plates were placed on the middle shelf of the chamber and exposed to 0–500 pulses of high-power pulsed UV light (UV light dose of 0–1.95 J/cm^2). Pulse power density and input voltage were 133 W/cm^2 and 1200 V, respectively.

In order to check whether the destruction of microorganisms induced by pulsed light can be repaired by several photoenzymes, the treated pathogens were afterwards exposed to incoherent light source emitting at 365 nm with power density of 1 mW/cm^2 for 1–3 h (*22*). The control plates were wrapped in aluminium foil to prevent photoreactivation and incubated for 24 h at 37 °C. The total light dose was measured by a 3sigma meter (Coherent Inc.) equipped with pyroelectrical detector 25LP04 and was evaluated as 3.6–10.8 J/cm^2 on the surface of the sample.

Pulsed light treatment of inoculated strawberries

Strawberry samples of about 15 g were soaked in 50 mL of *B. cereus* ATCC 12826 or *Listeria monocytogenes* $ATC_{L3}C$ 7644 suspensions (approx. 10^7 CFU/mL). Then, the samples were kept in a sterile area for 30 min for further bacterial adhesion on the surface of the produce (*23*).

All strawberry samples inoculated with *B. cereus* or *L. monocytogenes* were placed sparsely (in order to minimize shadow effects) on a sterile Petri dish without cover on the middle shelf (10 cm from the lamp) in the treatment chamber and exposed to 0–1000 pulses of light (UV light dose of the 0–3.9 J/cm^2) from above and below (*24*). Input voltage was 1200 V. The control samples were not irradiated. For mesophiles, yeast and fungal counts, strawberry samples were treated under the same conditions.

Microbial enumeration

All samples (including control) were separately mixed with appropriate volume of 0.01 M PBS (1 g of sample in 10 mL of buffer) and homogenized for 60 s in sterile BagPage bags using a BagMixer (model MiniMix 100 VP, Interscience, Saint Nom La Bretèche, France) 4 h after the treatment. The homogenate was then serially diluted in 0.9 % NaCl and placed on the agar. For *B. cereus* and *L. monocytogenes*, Mossel's *Bacillus cereus* selective mannitol egg yolk polymixin (MYP) agar (Liofilchem) and PALCAM *Listeria*-selective agar (Liofilchem) were used, respectively. Plates were incubated at 37 °C for 24 h. Total aerobic bacterial, yeast and mould counts were determined by plating appropriately diluted samples onto TSYEA (Liofilchem) and dichloran glycerol (DG18) agar base, respectively, and incubated at 30 °C for 48 h. The surviving cell populations were enumerated and expressed as log CFU/g.

Evaluation of shelf life of strawberries

For the evaluation of the shelf life of the treated strawberries, the Kaplan-Meier survival curves were used. Part of berries was exposed to pulsed light from two sides. The treated samples were stored at (6±2) °C. The control samples were not irradiated and were stored under the identical conditions. Every experimental group consisted of 30 pieces. Strawberries were observed until visually detectable spoilage spots (visible fungi) occurred on the surface. The expansion of shelf life of the treated strawberries was evaluated according to Kittemann *et al.* (*25*). The level of infected berries was scored on a 1–6 scale. Results were expressed as a disease index between 0 and 100 (from 0=no infection to 100=all berries are infected).

The appearance of strawberries was examined according to the method described by Mahmoud *et al*. (*26*). The overall appearance of fruits was visually measured using the following scale: 8–9=excellent, 6–7=very good, 5=good, 1–4=not acceptable (visible mould grown).

Temperature measurements

The LM35 precision Celsius thermocouple (Delta Ohm, Padua, Italy) was used to measure temperature dynamics on the surface of the berries during treatment. Thus, the thermocouple with digital indication of temperature was fixed on the surface of strawberry which was placed in the chamber and treated with HPPL. In our previous work, it was found that the variation of pulse frequency from 1 to 5 Hz had no influence on the surface temperature (*24*). In order to shorten the exposition time, we used 5-Hz frequency for all experiments.

Determination of ascorbic acid, total phenolic and anthocyanin contents

All measurements of nutrition quality of the treated fruits were performed 4 h after the treatment. For ascorbic acid determination, metaphosphoric acid extracts of the strawberries were prepared and pH was adjusted to about 1.2. Ascorbic acid content was determined by titration with 2,6-dichlorphenolindophenol sodium chloride solution (*27*). In this oxidation-reduction reaction, ascorbic acid in the extract was oxidized to dehydroascorbic acid and 2,6-dichlorphenolindophenol dye was reduced to a colourless compound. End point of the titration was detected when excess of the unreduced dye gave a rose pink colour of acid solution.

Total phenolic content (TPC) was determined as described in the work by Bobinaitė *et al.* (*28*). The extracts for the TPC assay were obtained by extracting 5 g of homogenised strawberries (using Polytron PT 1200E homogenizer, Thomas Scientific, Swedesboro, NT, USA) with 50 mL of methanol at ambient temperature for 1 h with constant shaking. The solution was filtered and the residue was repeatedly extracted with 50 mL of methanol for 1 h. Finally, the extracts were combined and diluted to 500 mL with methanol. The TPC in strawberry extracts was determined using Folin–Ciocalteu reagent according to the method of Slinkard and Singleton (*29*) using gallic acid as a reference. The reagent was prepared by diluting a stock solution (Sigma-Aldrich Chemie, Steinheim, Germany) with distilled water (1/10, by volume). The samples (1.0 mL, three replicates) were introduced into the test cuvettes and mixed with 5.0 mL of Folin-Ciocalteu reagent and 4.0 mL of Na_2CO_3 (7.5 %). The absorbance was measured at 765 nm in a Genesys-10 UV-VIS spectrophotometer (Thermo Spectronic, Rochester, NY, USA) after incubation at ambient temperature for 1 h. The TPC was determined from the calibration curve and expressed in mg of gallic acid equivalents (GAE) per 100 g of fresh mass (fm) of berries.

Total anthocyanins were extracted from 5 g of strawberries with methanol acidified with 0.2 M HCl. The strawberries were ground with quartz sand and the extraction was performed with 20-mL portions of solvent until the sample became colourless. The extract was diluted with acidified methanol, and the absorption was measured using a Cintra 202 UV-VIS spectrophotometer (GBC Scientific Equipment, Dandenong, Australia) at 510 nm. Total anthocyanin content was estimated as predominantly pelargonidin 3-glucoside at 510 nm, using a molar absorptivity coefficient of 36 000 and expressed as mg per 100 g of fm (*30*).

Measurements of antioxidant capacity

The antioxidant capacity of berry extracts was evaluated using a stable 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) radical (Sigma-Aldrich) by a slightly modified spectrophotometric method of Brand-Williams *et al.* (*31*) as described in the paper by Viskelis *et al.* (*32*) and expressed as Trolox equivalents (TE) (*33*).

Evaluation of firmness changes

Firmness of strawberries was neasured with a texture analyzer (TA.XT Plus, Stable Micro Systems, Godalming, UK). A P/5 probe (cylinder with a 5-mm diameter and flat end) was used for piercing the flesh of berries. The start of penetration test was the contact of the probe with the strawberry surface, and the finish when the probe penetrated the tissue to the depth of 8 mm. The probe speed was 1 mm/s. Ten fruits from each test group were used for analysis. The results of the tests were processed with the program Texture Exponent (Stable Micro Systems, Ltd).

Evaluation of colour changes of pulsed light-treated strawberries

Measurements of colour coordinates (L^*, a^*, b^*) were made with a portable spectrophotometer MiniScan XE Plus (Hunter Associates Laboratory, Inc., Reston, VA, USA). The colorimeter was set to measure total reflectance with illuminant C and a 10° observation angle. The parameters L^*, a^* and b^* (lightness, red value and yellow value, respectively, on the CIELAB scale) were measured and converted into hue angle (h^*) and chroma (C^*) (*34*). The values of L^* were measured as a percentage; a^* and b^* were measured in National Bureau of Standards (NBS) units; h^* was expressed in degrees from 0 up to 360°. The spectrophotometer was calibrated on a standard white tile (X=81.3, Y=86.2, Z=92.7) before each series of measurements. Colour coordinates were processed with the program Universal software v. 4.10 (Universal Software Corporation, Nashua, NH, USA).

Statistical analysis

The experiments were carried out in triplicate for each set of exposure, using different batches of strawberries. The data were analyzed with Origin v. 7.5 software (OriginLab Corporation, Northampton, MA, USA). A standard error was estimated for every experimental point and marked as an error bar. Sometimes the bars were too small to be visible. To estimate the significance of *in vitro* inactivation on the surface of fruits, analysis of variance (ANOVA) with Bonferroni test was used. To estimate the significance of shelf life of fruits and the effect of pulsed light on the amount of total antioxidants, Student's *t* test was used.

Results

In vitro inactivation of bacteria by high-power pulsed light

According to the results presented in Fig. 1a, *Bacillus* and *Listeria* are susceptible to pulsed light treatment and can be inactivated *in vitro* by 7 log (detection level of 10 CFU/mL). Data presented in Fig. 1b indicate that neither low level destruction of microorganisms (obtained at pulsed light dose of 0.078 J/cm^2) nor higher (obtained at pulsed light dose of 0.195 J/cm^2) were repaired by pathogen photoreactivation mechanism. No statistically significant difference ($p<0.05$) between the survival ratios of control and photoreactivation-treated samples was found.

Decontamination of strawberries by high-power pulsed light treatment

To evaluate the antibacterial efficiency of HPPL, strawberries were illuminated in the chamber with pulsed light (total UV light dose of of 3.9 J/cm^2) and then examinated for microbial counts. Data presented in Table 1 indicate that inactivation of the surface-inoculated *Bacillus* and *Listeria* (these pathogens were selected for inoculation-based experiments as they had the highest surface attachment level) by HPPL technique is significant and practically important. The amount of microbial count in untreated strawberries reached 5.0–5.9 log. After HPPL, this amount was reduced by 1–1.5 log. Data presented in Table 1 indicate that decontamination of strawberries from the naturally distributed mesophiles on fruit surface by pulsed light was effective as well. More than 99 % decontamination of strawberries was

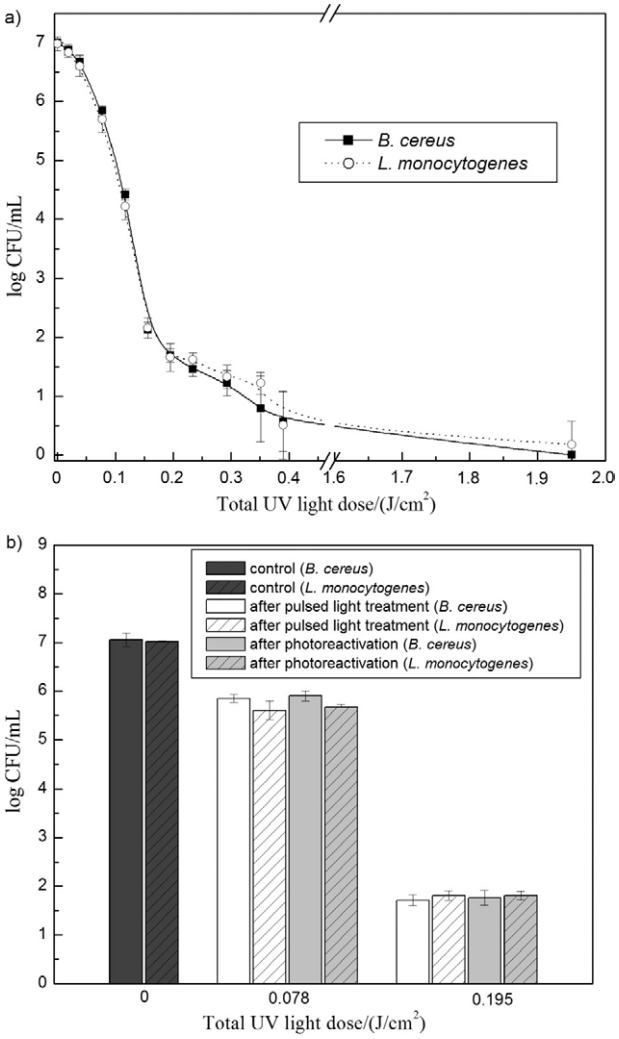

Fig. 1. High-power pulsed light-based inactivation (a) and light--based photoreactivation at 365 nm (b) of *B. cereus* and *L. monocytogenes in vitro* in dependence of total light dose

obtained (2.2 log) ($p<0.05$). It is evident that mesophilic bacteria are more susceptible to pulsed light treatment than *Bacillus* and *Listeria*.

Table 1. Decontamination of strawberries by high-power pulsed light treatment (UV light dose of 3.9 J/cm^2)

Microorganism	Control sample log CFU/g	Treated sample log CFU/g
Bacillus cereus	5.9±0.2	4.4±0.2
Listeria monocytogenes	5.0±0.3	3.9±0.5
mesophiles	6.0±0.1	3.8±0.3
microfungi/yeasts	5.2±0.2	4.2±0.2

Results are expressed as mean values of three replicate measurements±standard deviation

Generally, the reduction of bacterial viability on the surface of fruit is a function of light dose, when the distance from the light source, pulse repetition rate and voltage are constant. In order to increase the light dose, the exposition time was increased. It was important to identify whether naturally distributed microfungi/yeasts on most perishable fruits, *i.e.* strawberries, were susceptible to pulsed light. Data presented in Table 1 indicate that after 4 h of pulsed light treatment, microfungi/yeasts on the surface of strawberries were reduced from 5.2 to 4.2 log.

Shelf life of strawberries after pulsed light treatment

It is obvious that one of the advantages of any antimicrobial technology is the ability to extend the shelf life of the treated strawberries. Shelf life of strawberries was evaluated visually by observing surface colour changes (visible fungi) using Maier-Kaplan curves. As depicted in Fig. 2, the half-time of disease-free period (time necessary for 50 % of berries to survive) of the treated strawberries was prolonged in all cases. For instance, the shelf life of strawberries treated with pulsed light increased for 2 days, *i.e.* from 2.5 to 4.5 days during which the number of healthy strawberries remained at 50 %.

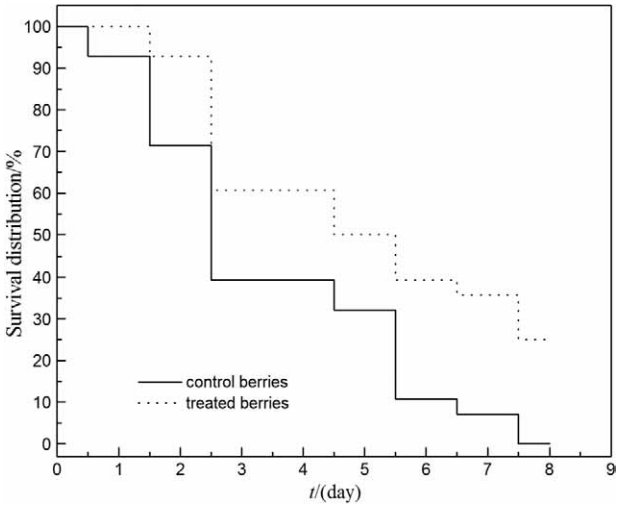

Fig. 2. Shelf life of strawberries before (control) and after high--power pulsed light treatment

In addition, the extension of the shelf life of treated strawberries was evaluated according to Kittemann *et al.* (*25*) using a 1–6 scale. The obtained data indicate that after 7.5 days of storage at 6 °C all control strawberries (100 %) were infected (disease index 6). Only 75 % (disease index 4.75) of treated strawberries were damaged during the same storage period when kept under identical conditions. The appearance of strawberries was examined according to the method described by Mahmoud *et al.* (*26*). The overall appearance of fruits was visually measured using the following scale: 8–9=excellent, 6–7=very good, 5=good, 1–4=not acceptable (visible mould grown). Data presented in Table 2 show the effect of HPPL on the overall appearance of treated strawberries during storage at (22±2) °C for 8 days. There were no significant differences between the control and treated samples on day 0 and day 2 of storage. The visual overall quality of strawberries gradually decreased over storage time. The score decreased from 9 to 3.9 after 4 days and from 9 to 3.5 after 5 days in control and in UV light--treated samples.

Table 2. Changes in the visual overall quality of strawberries treated with high-power pulsed light during storage at (22±2) °C

Storage time/day	Control sample	Treated sample
0	9.0±0.0	9.0±0.0
1	9.0±0.0	9.0±0.0
2	8.8±0.5	8.9±0.4
4	3.9±1.6	5.4±1.9
5	2.4±1.7	3.5±2.1
6	1.9±1.4	2.1±1.6
7	1.1±0.4	1.8±1.5
8	1.0±0.0	1.4±0.7

Results are expressed as mean values of three replicate measurements±standard deviation; the overall appearance of fruits was visually measured using the following scale: 8–9=excellent, 6–7=very good, 5=good, 1–4=not acceptable (visible mould grown)

Temperature values on the surface of the produce during pulsed light treatment

Data presented in Fig. 3 clearly indicate that fruit surface temperature during HPPL treatment tends to increase. It is obvious that the rise of temperature depends on the delivered light dose and distance from the lamp.

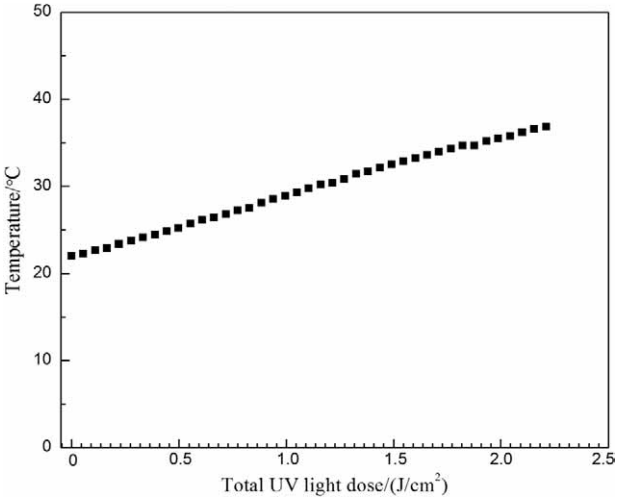

Fig. 3. The increase of temperature on the surface of strawberry fruits as a function of total UV light dose in comparison with the control (untreated fruits with a constant temperature on the surface of 22 °C)

Thus, in order to avoid thermal effects, the main experiments were performed on the middle shelf (10 cm) using such distance and UV light density (5.4 J/cm^2) that the temperature on the surface of fruit never exceeded 42 °C. Control (untreated) samples were kept at 22 °C surface temperature.

Total antioxidant capacity and ascorbic acid, phenolic and anthocyanin contents in strawberries before and after pulsed light treatment

In order to estimate specific changes of the nutritional quality of strawberries, total antioxidant capacity and total phenolic and anthocyanin content in the treated and control strawberries were evaluated. According to the results presented in Table 3, total antioxidant activity in control strawberries was (9.8±2.1) μM of TE per g of fm, whereas in the HPPL-treated it was insignificantly ($p<0.05$) reduced to (8.8±1.8) μM of TE per g of fm. Concerning the amount of total soluble phenolics, it was found that immediately after HPPL treatment no significantly important reduction (from (168.7±11.2) mg per 100 g of fm in control to (160.3±12.4) mg per 100 g of fm in treated strawberries) of total phenolics was found. In addition, the level of total anthocyanins in strawberries after the treatment was the same as in control group ((27.74±2.30) mg per 100 g of fm in control and (28.87± 2.10) mg per 100 g of fm in the treated ones). Eventually, vitamin C content was evaluated in the control and in the HPPL-treated strawberries. Data presented in Table 3 reveal that no significantly important changes in vitamin C content were found between the treated and the control groups: in control samples (60.0±1.8) mg of vitamin C per 100 g of fm was detected, whereas in the treated samples the of amount vitamin C was slightly reduced to (58.0±2.1) mg per 100 g of fm ($p<0.05$).

Firmness of strawberries before and after pulsed light treatment

The firmness of strawberries was measured directly after HPPL treatment and compared with that of control. Data presented in Table 3 indicate that the average firmness of 10 berries treated by pulsed light was (17.6±4.2) N/cm^2. It must be noticed that the firmness of strawberries in control group was (19.1±3.2) N/cm^2, which means that high-power pulsed light had no impact on strawberry firmness.

Table 3. Ascorbic acid, total phenolic and total anthocyanin content, and antioxidant capacity per 100 g of fresh mass, and firmness of strawberries before and after high-power pulsed light treatment

Variant	w(ascorbic acid)/mg/100 g	w(TPC as GAE)/mg/100 g	w(TA)/mg/100 g	Antioxidant capacity $\frac{c(\text{TE})}{m(\text{fresh fruit})}$/(μM/g)	Firmness/N/cm^2
control samples	60.0±1.8	168.7±11.2	27.74±2.30	9.8±2.1	19.1±3.2
treated samples	58.0±2.1	160.3±12.4	28.87±2.10	8.8±1.8	17.6±4.2

Results are expressed as mean values of three replicate measurements±standard deviation; TPC=total phenolic content, GAE=gallic acid equivalent, TA=total anthocyanins, TE=Trolox equivalent

Table 4. Strawberry fruit colour coordinates before and after high-power pulsed light treatment

Variant	L^*	a^*	b^*	C^*	$h^*/°$
control samples	35.8±3.8	34.7±3.3	20.9±6.1	40.6±5.7	30.4±5.4
treated samples	37.2±3.6	36.4±3.9	23.4±4.6	43.4±5.5	32.4±3.3

Results are expressed as mean values of three replicate measurements±standard deviation

Impact of pulsed light treatment on the strawberry colour

To determine whether the treatment had any negative effects on the colour of the strawberries, samples were analyzed 4 h after the treatment. For this purpose the parameters L^*, a^* and b^* were measured in reflection mode, and colour chroma C^* and colour tone were calculated. The results in Table 4 show that no significant changes of colour parameters were detected, which means that pulsed light has no impact on strawberry colour parameters.

Discussion

The most widely known postharvest treatments to reduce microbial growth are low temperature and modified atmosphere packaging (*35*). However, it has been reported that these technologies are not enough effective, they induce microbial resistance, and include high costs and the use of chemical contaminants (*36*). For instance, it has been reported that a low storage temperature decreased the antioxidant capacity and flavonoid content in strawberries, and the carbon dioxide treatment reduced the anthocyanin content and changed the internal fruit colour (*37,38*). Hot-water rinsing and brushing significantly reduce the microbial population on fruit surface and stop decay development. However, the loss of fruit mass and heat-induced damage of the fruit surface were observed (*39*). Washing with tap water reduced mesophiles on the fresh-cut asparagus by 1.4 log, washing with chlorinated water by 2 log and with ozonated water by 1.9 log (*40*). Conventional sanitizers have been shown to have limited efficacy in removing spoilage and pathogenic bacteria from the surface of a product (*41*). One of the ways to control the loss and quality of strawberries is based on the application of physical treatment such as pulsed light.

Our previous work indicates that the main food pathogen *Salmonella enterica* was susceptible to HPPL treatment *in vitro* (*24*). Data obtained in this study reveal that *B. cereus* and *L. monocytogenes* are also sensitive to HPPL and can be inactivated to an undetectable level *in vitro* (Fig. 1a). The obtained results are in accordance with prior reports (*38–40*), where susceptibility of *B. cereus* and *L. monocytogenes* to HPPL was detected. Meanwhile, some authors reported that repetitive use of pulsed light treatment induced an increased resistance of surviving bacterial cells (*42*). For instance, Rajkovic *et al.* (*43*) found approx. 1 log CFU/mL increase of the resistance of a mixture of *L. monocytogenes* strains. It is important to note that no photoreactivation of HPPL-induced bacterial damage was observed (Fig. 1b). These promising results prompted us to evaluate decontamination efficiency of this treatment on real food systems. Data presented in Table 1 indicate that decontamination of strawberries from naturally distributed mesophiles was rather effective (2.2 log). It is interesting to note that *B. cereus* and *L. monocytogenes* inoculated on the surface of strawberries at similar levels as mesophiles (5.0–5.9 log) were inactivated by 1.5 and 1.1 log, respectively. It looks like these food pathogens are less susceptible to pulsed light than mesophilic bacteria. These data are in line with Gómez-López *et al.* (*19*), who found 0.56–2.04 log reduction of bacteria when treating minimally processed vegetables.

It is important to note that harmful yeasts and fungi naturally distributed on the surface of the most perishable fruit, *i.e.* strawberry, exhibited slightly lower susceptibility to pulsed light and were inactivated by 1 log (Table 1). These data are in line with the results of other authors (*44*). Hence, overall reduction of the microbial contamination prolongs the half-time of disease-free period of strawberries by 2 days (Fig. 2). Marquenie *et al.* (*45*) studied the combined effect of three physical methods: HPPL, heat, and UV-C illumination on the removal of *Botrytis cinerea* from strawberries. Their results revealed that pulsed light alone used at rather low dose was ineffective, although combined treatment of all three techniques reduced visually *B. cinerea* mycelia and did not affect fruit firmness. This technique also prolonged disease-free period and increased the shelf life by 1–2 days.

However, there is a concern about heat generation during pulsed light treatment. Data on the temperature dynamics on the surface of produce obtained during treatment indicate that it is possible to find operational parameters at which temperature increase on the surface of fruit never exceeds 42 °C (Fig. 3). During 20 min of treatment the temperature on the surface of the fruit changed from 22 to 37 °C. Bialka and Demirci (*46*) found higher *Salmonella* and *E. coli* inactivation level (3.4 log) in strawberries after HPPL treatment, but the temperature on the surface of the strawberry increased to unacceptable 80 °C after 60 s of illumination.

Colour is one of the important quality indicators of fresh strawberry appearance. The bright red colour of strawberry fruit is due to the presence of anthocyanin pigments in the fruit epidermis and cortex (*47*). To find out whether pulsed light has any effect on the colour of strawberry, the fruits were analyzed immediately after the treatment. According to the obtained data (Table 4), the colour of strawberry was not markedly affected by the pulsed light. These data are in line with the results of Bialka and Demirci (*46*), who used HPPL to decontaminate strawberries and did not find any changes in fruit skin colour.

The main beneficial properties of strawberries have been partially attributed to the high content of antioxi-

dant compounds (*1*). It was observed that after exposure to pulsed light, the nutritional quality of strawberries did not diminish significantly (Table 3). Gil *et al.* (*48*) studied the effect of carbon dioxide treatment on the amount of anthocyanins and other polyphenols in strawberries. Their results revealed that anthocyanin content in CO_2--treated fruits was reduced as compared to those of stored in air. Fan *et al.* (*49*) studied the effect of yeast antagonist (*Cryptococcus laurentii*) entrapped in sodium alginate film on strawberry fruits and found no significant effects on external colour parameters and anthocyanin content of berries during storage. Odriozola-Serrano *et al.* (*16*) found that high-intensity pulsed electric field (HIPEF) affected strawberry nutritional qualities too. The loss of phenolic content after HIPEF and thermal treatment of strawberry juices was in the range of 21.5–24.1 mg per 100 mL after 56 days of storage at 4 °C. The firmness of strawberries is an important parameter which partly determines their shelf life. Our results show that high-power pulsed light treatment does not affect strawberry fruit firmness as no changes were observed in comparison with control (Table 3).

Conclusions

The obtained data indicate that high-power pulsed light is fast and effective non-thermal and non-chemical tool to control microbial contamination of most perishable fruits, *i.e.* strawberries. It is important to note that high-power pulsed light significantly prolonged the shelf life of strawberries without negative impact on their nutritional quality (total phenolic, total anthocyanin and ascorbic acid content, and antioxidant capacity) and overall appearance (firmness, colour coordinates L^*, a^*, b^*, C^* and h^*).

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