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Effective non-thermal photosensitization-based decontamination of strawberries from microorganisms

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The methods recently applied for inactivation of food pathogens on the surface of fruits and vegetables are not always efficient, human and ecologically friendly. In this context photosensitization might serve as really promising antibacterial tool.

Decontamination of the surface of strawberry from inoculated pathogen *Listeria monocytogenes* ATCL3C 7644 by photosensitization diminishes the population of this pathogen by 98 %. It is important to note that even yeasts and microfungi are susceptible to photosensitization and their population was reduced by 86 %. High decontamination rate (97 %) was reached for naturally surface-distributed mesophylic bacteria. The following evaluation of shelf-life of treated strawberries in comparison with control ones indicate 40 % prolongation. The examination of nutritional quality of treated strawberries reveals that total antioxidant activity in treated strawberries increases by 33 %. No significant changes of colour in treated berries were detected. Certain photosensitization treatment algorithm is particularly interesting in the respect that it does not involve any thermal effects and does not produce toxic or volatile compounds.

Key words: inactivation photosensitization, strawberry, *Listeria monocytogenes*, yeasts, microfungi.

Introduction. Harmful and pathogenic microorganisms are one of the major food and food-related surface contamination problems, which reduce the self life of the product as well as can cause food-born diseases. The methods recently applied for inactivation of food pathogens (heat treatment, various chemical sanitizers, irradiation) are not always efficient, human and ecologically friendly. Besides, most of them often have associated disadvantages, for instance, unfavourable changes of organoleptic and nutritional characteristics. Moreover, bacterial resistance to most chemical and physical treatments recently is highly developed (Satin, 1996).

Strawberries (*Fragaria ananassa* Duch.) are one of the nourishment and popular fruits worldwide but also remarkably perishable, susceptible to mechanical injury and contamination during storage. Strawberry fruits have been reported to contain high phenolic and other antioxidant content (Kähkönen et al., 2001). Additionally they possess very short ripening and senescence periods, which handicap their selling. The loss of strawberries can reach 40 % during storage (Satin, 1996). The most widely known postharvest treatments to decrease microbial contamination degree and reduce water loss and respiration rate are low temperature and modified atmosphere packaging (Nielsen and Leufven, 2008). However, it has been reported that these procedures have impact on the quality of strawberries (Ayala-Zavala et al., 2007). Washing alone or conventional sanitizers have been shown as not enough effective to remove spoilage and pathogenic bacteria from the surface of product (Yuk et al., 2006)

Photosensitization as novel non-thermal and ecologically friendly treatment involves the administration of a photoactive compound (photosensitizer) and visible light. After spraying of photosensitizer, for instance, chlorophyll, on the surface of fruit or vegetable, most pathogens and harmful bacteria, distributed on the surface of the fruit are able to accumulate the photosensitizer. First data obtained on inactivation of food pathogens by photosensitization indicate that following illumination with visible spectrum of light induces a lot of photocytotoxic reactions and death inside microorganisms without any harmful effects on environment (Lukšienė et al., 2004; Lukšienė, 2005).

This study focuses on the possibility to decontaminate pathogens, yeasts, microfungi and naturally distributed mesophyls from strawberries, by photosensitization-based treatment under non-thermal conditions.

Object, methods and conditions. Sample preparation. 1. Pure culture of *Listeria monocytogenes*. *Listeria monocytogenes* ATCL3C 7644 was kindly provided by the National Veterinary Laboratory (Vilnius, Lithuania). The bacterial strain was cultured at 37 °C on Tryptone Soya Agar supplemented with 0.6 % Yeast Extract (TSYEA) (Liofilchem, Italy) for 24 h. For bacterial suspension preparation *L. monocytogenes* was grown overnight (~ 14 hours) at 37 °C in 20 ml of Tryptone Soya medium supplemented with 0.6 % Yeast Extract (TSYE) (Liofilchem, Italy), with aggitation of 120 rpm (Environmental Shaker-Incubator ES–20; Biosan, Latvia). The overnight bacterial culture grown in TSYE medium was diluted 20 times by the fresh medium (A = 0.164) and grown at 37 °C to mid-log phase (approx. 1.16×10^9 colony forming units/ml (cfu/ml), A = 0.9) in a shaker (120 rpm). Bacterial optical density was determined in a 10.01 mm glass cuvette at $\lambda = 540$ nm (Heλios Gamma & Delta spectrophotometers; ThermoSpectronic, Great Britain). Afterwards cells were harvested by centrifugation (20 min, 5 000 g) and resuspended to ~ 5.8×10^9 cfu/ml final concentration in 0.1 M phosphate buffer saline (PBS, pH = 7.2). This stock suspension was accordingly PBS-diluted to ~ 1 × 107 cfu/ml and used for the further photosensitization experiments.

2. Inoculation of *Listeria monocytogenes* onto surface of strawberries. Strawberries (*Fragaria ananassa* Dutch.) purchased in a local supermarket were stored at +6 °C and processed within one day. Prepared inoculum (described above) of *L. monocytogenes* was poured over strawberries and left for 30 min at room temperature for cells attachment.

Photosensitization treatment. After inoculum decant berries were soaked in 5×10^{-3} M Na-chl salt solution for 5 min. Dried strawberries were placed in the treatment chamber in a sterile Petri dish without cover and exposed to light intensity 20 mW/cm^2 at $\lambda = 400$ nm for 30 min. Light source necessary for photosensitization was constructed in the Institute of Applied Sciences of Vilnius University. LED-based prototype (light emitting diodes) emitted light $\lambda = 400$ nm with intensity 20 mW/cm^2 at the surface of samples. Light dose delivered to the surface of sample was calculated as light intensity multiplied on time.

Total aerobic microorganisms count. Strawberry samples were analyzed before and after photosensitization. Berries were weighted using sterile instruments under aseptic conditions. The samples (10–15 g) were soaked in a 1.5×10^{-3} M of Na-chl salt solution, the control ones – in 0.1 M PBS and kept in the dark for 5 min. Dried strawberries were placed in the treatment chamber in a sterile Petri dish without cover and exposed to light intensity 20 mW/cm^2 at $\lambda = 400$ nm for 20 min. (Control samples were not illuminated). Treated and not treated samples were separately mixed with appropriate volume of 0.1 M PBS (1g of sample – 10 ml buffer) and homogenized in a sterile BagPage bags using a BagMixer (model MiniMix 100 VP, Interscience, France). Total aerobic microorganisms count was obtained by means of serial dilutions (in 0.9 % NaCl) plated on TSYEA and incubated at 30 °C for 48 h. The surviving cell populations were enumerated and expressed by log10 (cfu/g) and afterwards recalculated as percents.

Shelf-life studies. For shelf-life studies one part of strawberries was soaked in 1.5×10^{-3} M Na-chl salt solution, the other one – in sterile distillated water. Samples, treated with Na-chl salt were illuminated for 20 min at 20 mW/cm^2 ($\lambda = 400$ nm), dried and stored in refrigerator (+6 °C). The control samples were not illuminated. Berries were observed until visually detectable spoilage occurred on the surface.

Temperature measurement. Precision Celsius temperature sensors (“Deltha Ohm”, Italy) were used for temperature measurements on the surface of strawberry.

Total antioxidant capacity. Total antioxidant capacity of strawberries was measured by FRAP (ferric reducing ability of plasma) method. Extracts for measurement were prepared by homogenization of 1 g of fruit with 50 ml 96 % alcohol (Minimix). FRAP working solution included 0.3 M acetate buffer (pH 3.6), 0.01 M 2, 4, 6-tripyridyl-s-triazine (TPTZ) in 0.04 M HCl and 0.02 M $FeCl_3 \times 6H_2O$ in distilled water. For measurement of antioxidant activity 1.5 ml FRAP reagent and 50 µl sample solution were mixed and reading was performed every 30 s up to 5 min at 593 nm, 1 cm light path. Fe (II) standard solution was tested in parallel.

Measurement of colour. Changes of strawberry colour after photosensitization with Na-chl salt was evaluated from absorption spectrum measuring optical density (OD) in visible region of spectrum. Samples weighting 10 g of fresh berry was blended in a food processor for 1minute with 75 ml of a mixture of methanol, acetic acid, and distilled water (M : A : W) at a ratio of 25 : 1 : 24. Afterwards the mixture was centrifuged at 12 000 rpm for 20 min and repeated 3 times. All three collected supernatants were used for following colour measurements. Optical density (310–650 nm) was measured using 1 cm path length quartz cuvettes. Each sample was extracted 3 times.

Statistics. The experiments were repeated at least three times. A standard error was estimated for every experimental point and marked in a figure as an error bar of mean. Sometimes the bars were too small to be visible. The data were analyzed with Origin 7.5 software (OriginLab Corporation, Northampton, MA 01060, USA).

Results. The data, depicted in Fig. 1 indicate that the incubation of strawberry with inoculated *Listeria monocytogenes* in 5×10^{-3} M Na-chl salt for 5 min and following illumination with visible light for 30 min reduces the population of *Listeria* by 98 %.

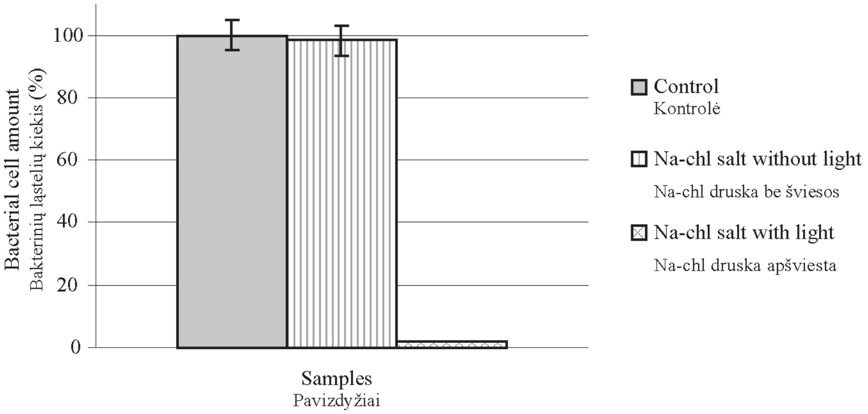

Fig. 1. Inactivation of *Listeria monocytogenes* ATCL3C 7644 on the surface of strawberries by photosensitization with 5 × 10^{-3} M Na-Chlorophyll salt (incubation time – 5 min, illumination time – 30 min)

1 pav. *Listeria monocytogenes* ATCL3C 7644 inaktyvacija braškių paviršiuje fotosensibilizacijos būdu: 5 × 10^{-3} M Na-chlorofilo druska (inkubacijos laikas – 5 min., švitinimo laikas – 30 min.)

Data presented in Fig. 2 indicate that total aerobic mesophyls naturally distributed on the surface of berries were effectively reduced by 97 % after photosensitization treatment. Moreover, so harmful microorganisms as yeasts and microfungi are susceptible to this treatment and can be reduced by 86 %.

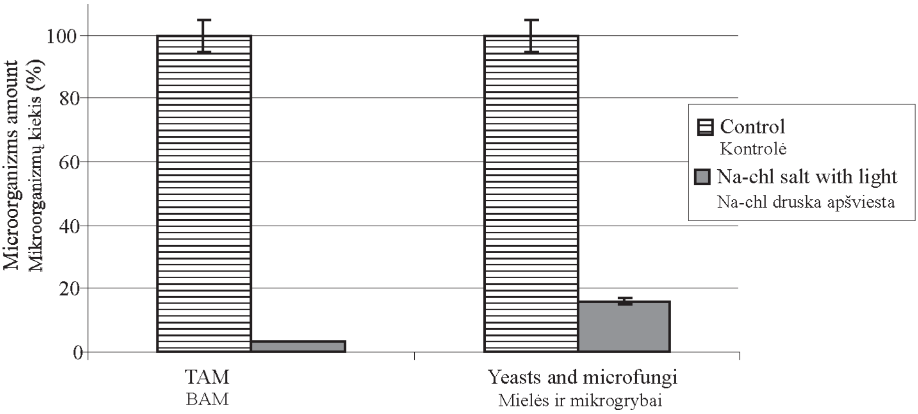

Fig. 2. Inactivation of total mesophylls, yeasts and fungi on the surface of strawberries by photosensitization with 1.5 × 10^{-3} M Na-Chlorophyll salt (incubation time – 5 min, illumination time – 20 min)

2 pav. Mezofilų, mielių ir pelėsinių grybų inaktyvacija braškių paviršiuje fotosensibilizacijos būdu: 1.5 x 10^{-3} M Na-chlorofilo druska (inkubacijos laikas – 5 min., švitinimo laikas – 20 min.)

Data presented in Fig. 3 reveal that some prolongation of strawberries shelf-life occurred when berries were treated by photosensitization. Thus, it is evident, that the reduction of all microbial contamination prolongs the shelf-life of berries by 40 %.

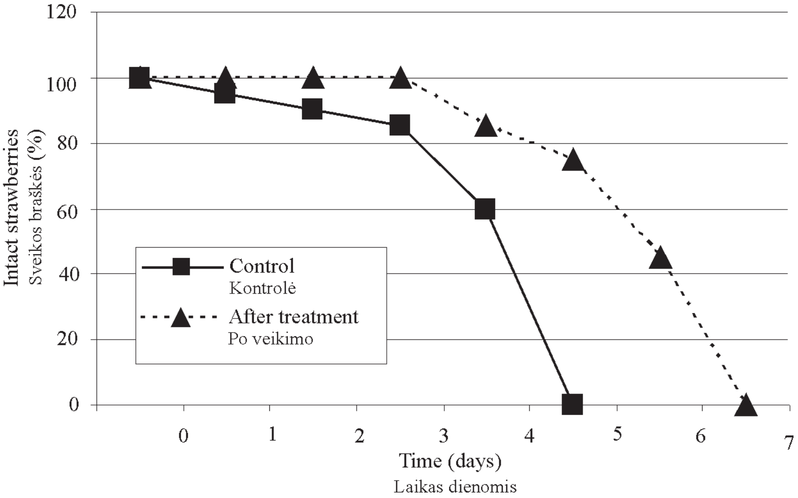

Fig. 3. Shelf life of strawberries after photosensitization with 1.5×10^{-4} M Na-Chlorophyll salt

3 pav. Braškių vartojimo trukmė kontroliniame variante ir taikant fotosensibilizaciją (1.5×10^{-4} M Na-chlorofilo druska)

Further investigations were focused on nutritional and organoleptic properties of strawberries after treatment. As depicted in Fig. 4, no some increase (33 %) of antioxidant activity was detected in the strawberries immediately after photosensitization in comparison with control.

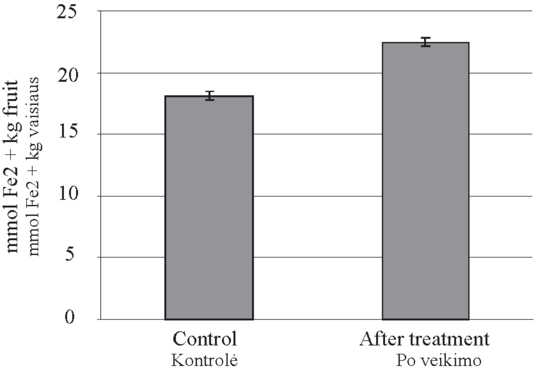

Fig. 4. Total antioxidant activity of strawberries after photosensitization

4 pav. Bendras antioksidantinis aktyvumas braškių uogose po fotosensibilizacijos

The other important characteristic, which can be influenced by photosensitization, is appearance of berry, especially colour. For this purpose absorption spectroscopy was used to analyze the spectrum of berry extract in visible region. As it is depicted in Fig. 5, no colour changes over all the visible spectrum region were detected in treated strawberries in comparison with control ones (Fig. 5).

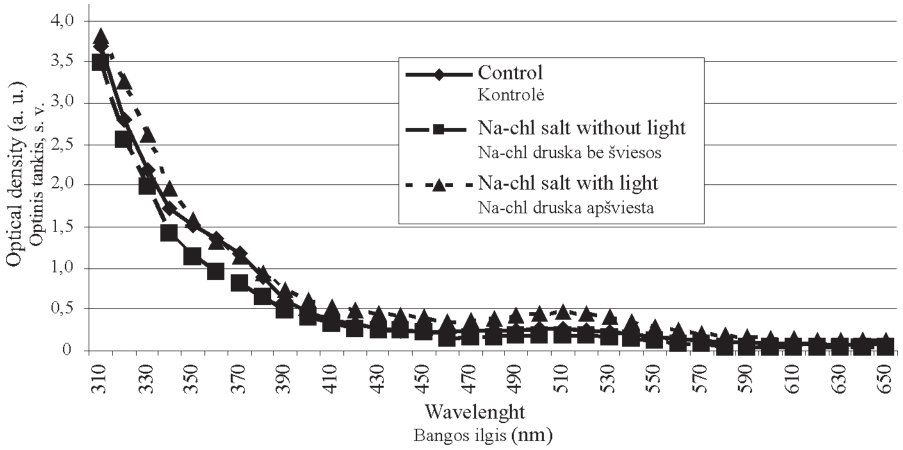

Fig. 5. Absorption spectrum in visible region of control, chlorophyll treated and photosensitization treated strawberry extract sample

5 pav. Braškės ekstrakto sugerties spektras matomoje dalyje po fotosensibilizacijos

As it was mentioned before, distinguishing feature of photosensitization is its non-thermal action. Thus, we evaluated the increase of temperature on the surface of berry during treatment. Data presented in Fig. 6 clearly indicate that photosensitization is non-thermal treatment, as during all illumination time the temperature increase was very slow and never exceeded 27 °C.

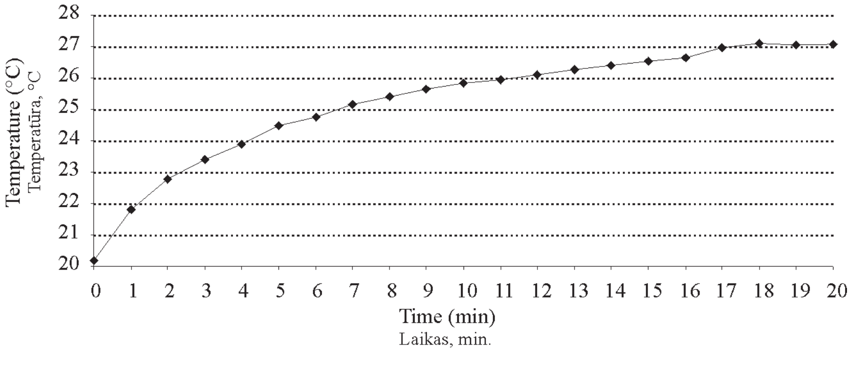

Fig. 6. The increase of temperature on the surface of strawberries placed in light emitting diode-based light prototype during 20 min of illumination. Thermometer (Delta Ohm, Italy) was used for temperature measurements on the surface of berry

6 pav. Temperatūros kitimas braškės paviršiuje per 20 min. fotosensibilizacinio poveikio metu. Temperatūra matuota „Delta Ohm“ termometru.

Discussion. Data obtained in our previous work reveal that photosensitization-based treatment can inactivate food pathogens *Listeria monocytogenes* ATC$_{L3}$C 7644 and *Bacillus cereus* ATCC 12826 by 6 log *in vitro* (Buchovec et al., 2009; Luksiene et al., 2009). Moreover, spores of *Bacillus cereus* as well as biofilms of *Listeria monocytogenes* are susceptible to this treatment as well (Buchovec et al., 2009; Lukšienė et al., 2009). Thus, photosensitization was patented as method to decontaminate food surface as well as food-related surfaces (Lukšienė and Buchovec, 2009).

These results prompted us to investigate further the susceptibility of pathogen *Listeria*, yeasts, microfungi and mesophyls to this treatment when they are distributed on the surface of strawberry.

According to the data obtained, photosensitization-based treatment can decontaminate surface of strawberries from inoculated *Listeria* by 98 %, from naturally distributed mesophyls by 97 %, from harmful yeasts and microfungi by 86 %. It is worldwide accepted, that photosensitization efficiency depends on illumination time and light intensity. Taking it into account the antibacterial efficiency of photosensitization obtained in Fig. 1 is not ultimate and final result and can be enhanced by the usage of more powerful light sources (LED) or by increase of illumination time.

It is obvious that most important advantage of any antimicrobial technology is its ability to prolong the self-life of treated berries. Thus, as depicted in Fig. 3, the shelf-life of treated strawberries after photosensitization treatment prolonged about 40 % in comparison with control.

Therefore, it might be possible that this treatment modality can affect and make some negative impact on the nutritional properties of strawberries. Thus, it was necessary to investigate whether some changes of antioxidant activity take place after photosensitization in strawberries. According to the obtained results, depicted in Fig. 4, slight increase of total antioxidant activity was observed in the strawberries after photosensitization (33 %) in comparison with control not treated ones. Measurements of berry colour indicate that no remarkable changes in all visible light spectrum were detected – this means that effects on berry colour are undetectable.

One of the most important characteristics of this treatment is its non-thermal action. Results presented in Fig. 5 reveal that decontamination of *Listeria*, mesophyls, yeasts and microfungi from strawberry can be reached without thermal effects on strawberry.

Microbial decontamination of strawberries was studied by other authors as well. For instance, Marquenie et al. (2003) studied combined effect of three physical methods – high power pulsed light, heat, and UV-C illumination on *Botrytis cinerea* decontamination fromstrawberry surface. Their results reveal that pulsed light alone was ineffective against selected fungus, 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, increasing the shelf-life by 1–2 days and this is comparative with our results.

Allende and others (2007) determined the effect of UV-C light, gaseous O_3, superatmospheric O_2 and CO_2-enriched atmospheres applied individually and in combination on the shelf-life of strawberries. Individual treatments did not affect the microbial contamination, whereas combination of them reduced the growth of yeast and microfungi by approximately 1 log for up to 5 days and prolong the storage period, respectively. The advantage of photosensitization technology is that it can reduce population of fungi and yeasts on the surface of strawberry by 86 % without any combination with other treatments in non-thermal and not-chemical way.

Conclusion. Our data obtained in this study clearly indicate that photosensitization being first time in the history applied for food safety might be useful technology to decontaminate pathogen *Listeria*, yeasts, microfungi and mesophyls distributed on the surface of strawberry without any harmful effect on antioxidant activity or colour. Moreover, photosensitization prolonged significantly strawberry shelf-life (40 %). This addresses to the understanding that in some special cases (for instance, to increase the safety of ready to eat fruits, confectionary, pastry products, etc) photosensitization might be useful non-thermal and not-chemical antimicrobial tool.

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Neterminis mikroorganizmų sunaikinimas braškių paviršiuje – fotosensibilizacija

E. Paškevičiūtė, Ž. Lukšienė

Santrauka

Šiuolaikiniai ligų sukėlėjų sunaikinimo metodai, taikomi vaisių ir daržovių mikrobiologinėje kontrolėje, ne visada efektyvūs ir nekenksmingi žmogui ir aplinkai. Šiuo požiūriu fotosensibilizacija gali būti tikrai veiksminga ir perspektyvi antimikrobinė priemonė. *Listeria monocytogenes* ATCL3C7644, inokuliuotos braškės paviršiuje, populiacija po fotosensibilizacijos poveikio sumažėjo 98 %. Svarbu pažymėti, kad net mielės ir pelėsiniai grybai yra jautrūs šiam metodui ir jų gali būti sunaikintą 86 %, o paviršiuje natūraliai egzistuojančių mezofilų – net 97 %. Mikroorganizmų sunaikinimas fotosensibilizacijos būdu pailgino braškių vartojimo trukmę 40 % ir padidino bendrą antioksidantinį aktyvumą 33 %, o braškių spalva visiškai nepakito. Ši technologija ypač vertinga tuo, kad nesukelia terminio efekto vaisiaus matricoje ir nepalieka cheminių teršalų aplinkoje.

Reikšminiai žodžiai: braškės, mikroorganizmų sunaikinimas, fotosensibilizacija, *Listeria monocytogenes*, mielės, grybai.