



Environmentally Friendly and  
Safe Technologies for Quality  
of Fruits and Vegetables

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Authors are responsible for content and accuracy of their papers.

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SECTION 5. NEW APPROACHES TO ENHANCE SAFETY  
AND QUALITY OF MINIMALLY PROCESSED FRUITS AND  
VEGETABLES

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# 33. PHOTSENSITIZATION AS NOVEL APPROACH TO DECONTAMINATE STRAWBERRY FRUIT SURFACES

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## Abstract

The methods recently applied for inactivation of food pathogens are not always efficient, safe for humans, or ecologically friendly. In this context, photosensitization might serve as a promising antibacterial tool. Data obtained in this study indicate that the important food pathogens *Listeria monocytogenes* and *Bacillus cereus* can be inactivated after photosensitization by 6 log *in vitro*. Moreover spores of *B. cereus* as well as biofilms of *L. monocytogenes* are susceptible to this treatment. Decontamination of strawberries from *L. monocytogenes* by photosensitization as well as from aerobic mezophylls reached 3 log. The shelf-life of treated berries in comparison with non-treated control strawberries increased by 40%. No significant changes of antioxidant activity in strawberries was detected.

**Keywords:** Food decontamination, Non-thermal, Photosensitization

## Introduction

Many procedures are required to be taken until food reaches consumers in an acceptable form. Consequently there are many possibilities for food to be lost between harvest and consumption. Food susceptibility to various losses demands novel technologies for its preservation after harvest and during storage. Microbial contamination is one of the major food and food-related surfaces contamination agent. Various microorganisms can cause human diseases under certain conditions. The methods recently applied for inactivation of food pathogens (heat treatment, various chemical sanitizers, irradiation) are not always efficient, safe for humans, or ecologically friendly. Current food treatment methods frequently have associated disadvantages, specifically resulting in unfavourable changes of organoleptic and nutritional characteristics. Moreover, under appropriate treatment conditions some pathogens resist destruction.

Food packaging cannot resolve all of these problems. Foodborne pathogens can easily contaminate packaging surfaces and food surfaces. According to the U.S. Food and Drug Administration, some disadvantages may be addressed using “smart” packaging when volatile compounds indicate packaged food freshness (US FDA 2002).

Photosensitization involves the administration of a photoactive compound that selectively accumulates in the target microorganism. After illumination with visible light plethora, photochemical reactions induce selectively death of microorganism without any harmful effects on surrounding (Luksiene *et al.* 2004; Luksiene 2005).

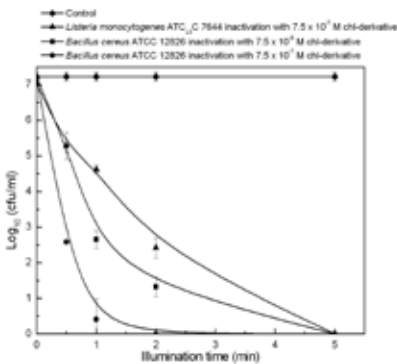
## Material & Methods

For photosensitization experiments *in vitro*, *Bacillus cereus* ATCC 12826 and *Listeria monocytogenes* ATC<sub>3</sub>C 7644 were grown at 37 °C in Luria-Bertani (LB) medium to the mid-log phase ( $\sim 6 \times 10^7$  colony forming units (cfu) mL<sup>-1</sup>, OD<sub>540</sub>=1) and were harvested by centrifugation (10 min, 5000 g). Cells were resuspended and diluted in PBS to give  $\sim 1 \times 10^7$  cfu mL<sup>-1</sup> final concentration. Aliquots (10 mL) of bacterial suspensions were incubated with a chlorophyll-derivative ( $7.5 \times 10^{-7}$  and  $7.5 \times 10^{-8}$  M) in darkness at 37 °C. Afterwards 150  $\mu$ L aliquots of bacterial suspension were withdrawn, placed into sterile flat bottom wells and exposed to light for different time (0-20 min). Light emitting diodes (LED) based light source for (constructed in the Institute of Applied Sciences of Vilnius university) emitted light  $\lambda=400$  nm with intensity 20 mW cm<sup>-2</sup> at the surface of samples. Light dose was calculated as light intensity multiplied on time.

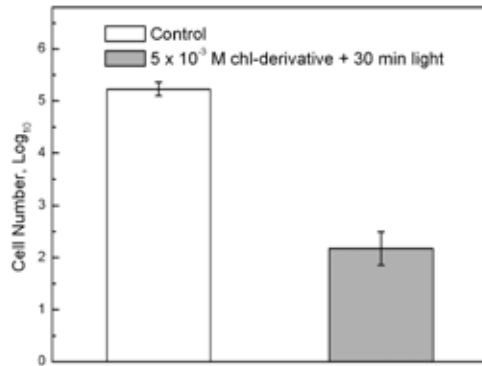
Strawberries (*Fragaria ananassa* Dutch.) purchased in a local supermarket were stored at +6 °C and processed within one day. The samples (strawberries of about 15 g) were soaked in  $1.5 \times 10^{-4}$  M concentration of chlorophyll derivative solution, the control samples – in phosphate-buffered saline (100 mM PBS, pH 7.2). All samples kept in the dark for 5 min and after inoculum decantation, dried in the thermostat at 37 °C. Dried strawberries were placed in the treatment chamber in a sterile Petri dish without cover and exposed to light intensity  $20 \text{ mW cm}^{-2}$  at  $\lambda=400 \text{ nm}$  for 30 min. Control samples were not irradiated. After treatment, each sample (also control samples) mixed with 135 mL 100 mM sterile PBS buffer in a sterile 100 BagPage and homogenized 60 s with a BagMixer. Then,  $100 \mu\text{l}$  of appropriate dilutions (0.9 % NaCl) of homogenized strawberries placed on LB agar. All plates were kept in the thermostat for 48 h at 37 °C. The surviving cell populations enumerated and expressed by  $\log_{10}$  (cfu/g). For shelf-life studies one part of samples were soaked in  $1.5 \times 10^{-4}$  M chl-derivative solution, the other one – in sterile distilled water. Samples, treated with chl-derivative were illuminated for 30 min at  $20 \text{ mW/cm}^2$  ( $\lambda=400 \text{ nm}$ ) and stored with refrigeration at 6 °C. The control samples were not illuminated. Total antioxidant capacity was measured by FRAP (ferric reducing ability of plasma) method. Precision Celsius temperature sensors (Deltha Ohm, Italy) were used for temperature measurements.

## Results

The data depicted in Fig 1 indicate that main pathogens *L. monocytogenes*, *B. cereus* were susceptible to chlorophyll-based photosensitization and can be inactivated by 7 log *in vitro*. The decontamination of berries inoculated with pathogens by photosensitization seems promising, as about 3 log decrease in pathogen population was observed. Moreover, total aerobic mesophylls on the surface of berries were reduced by 3 log as well (Fig 2).

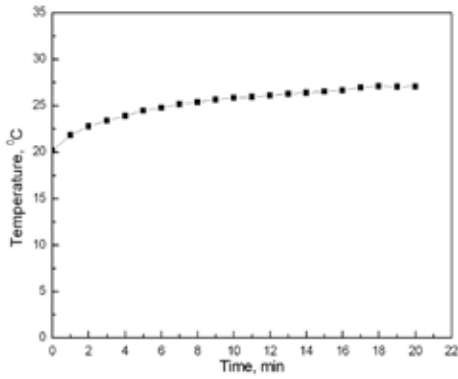


**Fig 1.** Inactivation of *Listeria monocytogenes* ATC<sub>L3</sub>C 7644 and *Bacillus cereus* ATCC 12826 by chlorophyll-derivative based photosensitization, when different concentration of chlorophyll-derivative was used.

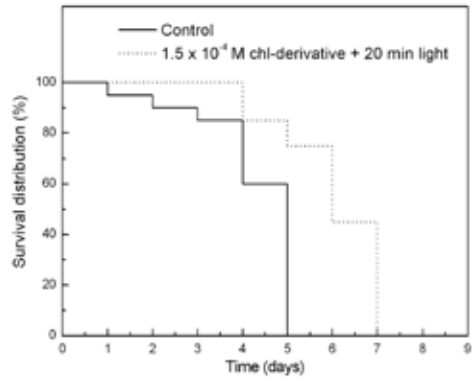


**Fig 2.** Inactivation of total mesophylls from strawberries by photosensitization with  $5 \times 10^{-3}$  M chlorophyll-derivative.

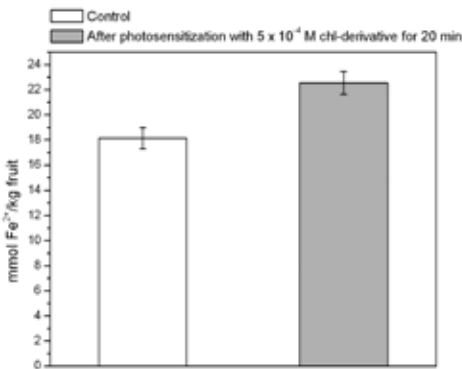
Data presented in Fig 3 clearly indicate, that photosensitization is non-thermal treatment, as during all illumination time the temperature increase was very slow and never exceeded 27 °C. Afterwards shelf-life of treated berries was evaluated. As depicted in Fig 4, the shelf-life of treated strawberries prolonged about 40% in comparison with control. Data indicate, that no inactivation of antioxidant enzymes was detected under certain experimental conditions (Fig 5).



**Fig 3.** The increase of temperature on strawberries placed in LED-based light source during 20 min of illumination. Thermometer (Delta Ohm, Italy) was used for temperature measurements.



**Fig 4.** Shelf-life of strawberries after photosensitization with  $1.5 \times 10^{-4}$  M chlorophyll-derivative.



**Fig 5.** Total antioxidant activity (FRAP) of strawberries after photosensitization.

## Discussion

Strawberries are an important, nourishing, and popular fruit worldwide. Unfortunately they are also particularly perishable, and are highly susceptible to mechanical injury and contamination during storage. Strawberries 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 that aggravate 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 & Leufven 2008) However, it has been reported that these procedures can also impinge on strawberries quality (Ayala-Zavala *et al.* 2007). According to the data obtained, photosensitization-based treatment can decontaminate surface of strawberries by 3 log, prolong their shelf-life 40%, without any inactivation of antioxidant enzymes.

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