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# Reduction of microbial contamination of fruits and vegetables by hypericin-based photosensitization: Comparison with other emerging antimicrobial treatments

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ABSTRACT

The aim of this study is focused on the evaluation of possibility to control microbes on the surface of fruits and vegetables (FV) by hypericin (Hyp)-based photosensitization. The effect of Hyp-based photosensitization on survival of *Bacillus cereus in vitro* and on the surface of FV was examined using different Hyp concentrations ($1.5 \cdot 10^{-5}$–$1 \cdot 10^{-8}$ M) and illumination (0–9.2 J/cm$^2$; $\lambda$ = 585 nm; intensity – 3.84 mW/cm$^2$). Results indicate that Hyp-based photosensitization effectively (4.4 log CFU/mL) reduces the population of *Bacillus in vitro*. Inactivation of mesophilic bacteria on the surface of FV reached 0.6–0.72 log CFU/g and was comparable with that of high power pulsed light (HPPL) treatment. No significant increase of temperature was detected on the surface of treated FV. Data reveal that this treatment has no significant impact on antioxidant activity and color of treated FV and was comparable with the effects of HPPL. Hyp-based photosensitization as nonthermal, environment-friendly and cost effective antimicrobial treatment seems promising for development of innovative preservation of fruits and vegetables.


## 1. Introduction

Foodborne diseases are a serious and global problem. World Health Organization estimates that worldwide foodborne and waterborne diarrheal diseases taken together kill about 2.2 million people annually (WHO, 2010). Fresh produce has been increasingly implicated as the vehicle of transmission and became 2nd leading cause of foodborne illnesses, which cost for instance the U.S. economy $6.9 billion of loss in productivity and medical expenses (ERS, 2005). *Bacillus cereus* spores and vegetative forms are frequently found on fresh vegetables, berries, cereals and fruits (Haque and Russell, 2005).

Traditionally, in order to decrease microbial contamination of fruits and vegetables (FV), sodium hypochlorite and acids as disinfection agents are commonly used. Yet, the usage of chemical sanitizers in 21st century is suspected to be environmentally unsound as it is associated with occupational and operational hazards. Moreover it is potentially harmful for humans (Ölmez and Kretzschmar, 2009). Consequently, the need of novel technologies which would be more effective and would meet high nowadays standards is obvious.

One of the novel antimicrobial approaches may be photosensitization. This treatment is based on the interaction of photoactive compound (photosensitizer) and visible light. After spraying of the photosensitizer on the surface of fruit or vegetable most pathogens and harmful bacteria distributed on the surface of the fruit are able to bind the photosensitizer. The following illumination of fruits with visible light induces various photocytotoxic reactions and selective death of surface-attached microorganisms without any harmful effects on fruit matrix or environment (Luksiene and Brovko, 2013). This treatment has been successfully applied for decontamination of strawberries. Such microorganisms as naturally surface-distributed yeasts, microfungi, and mesophilic microorganisms have been reduced on the strawberries by 1.7 log (97%) (Luksiene and Paskeviciute, 2011a). Food pathogens, inoculated on the surface of berries were inactivated by photosensitization as well. It is important to note, that no negative impact on antioxidant activity, amount of phenols, anthocyanins or color was found in treated strawberries (Luksiene and Paskeviciute, 2011a).

Hypericin (Hyp) is a naturally occurring plant pigment, found in *Hipericum perforatum* (also called Saint John's Wort) and well-known for its antidepressant (Wang et al., 2010), antiviral (Waser

*Abbreviations:* Hyp, hypericin; FV, fruits and vegetables; Chl, chlorophyll sodium salt; HPPL, high power pulsed light.

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and Falk, 2011) and antitumor effects (Darbinian-Sarkissian et al., 2006). The *H. perforatum*-derived products are available as teas, tinctures, juices, and oily macerates (Bhatia et al., 2011) and are often used both for therapeutic purposes and as a flavoring in the preparation of foods and alcoholic beverages (Mašković et al., 2011). Hyp is polycyclic phenanthroperylenedione exhibiting high lipophilicity what results in its high aggregation and preferential binding to the cellular membrane (Kairyte et al., 2012). Just few studies are published on the inactivation of yeasts and clinically isolated pathogens by Hyp-based photosensitization (López-Chicón et al., 2012; Rezusta et al., 2012). Our previous results indicate, that foodborne pathogens *Listeria monocytogenes* and *Salmonella enterica* were susceptible to photoactivated hypericin *in vitro* and combined treatment with high power pulsed light can diminish bacterial population by 7 log (Kairyte et al., 2012).

Thus, the aim of this study is to extend the potential application of photoactivated hypericin as antimicrobial treatment. Hence, it is focused on the evaluation of possibility to control pathogenic and harmful microorganisms on the surface of FV, comparing its efficiency with other emerging antimicrobial treatments.

## 2. Materials and methods

### 2.1. Chemicals

Hypericin was synthesized from anthraquinone derivative emodin and gifted by Dr. P. Adomėnas (Institute of Applied Sciences, Vilnius University). A stock solution of $1 \cdot 10^{-2}$ M Hyp was prepared in dimethyl sulfoxide (DMSO) (5 mg/mL) and stored at −20 °C in the dark. Three different concentrations of Hyp ($1.5 \cdot 10^{-5}$, $1 \cdot 10^{-7}$ and $1 \cdot 10^{-8}$ M) were used in experiments. Appropriate final concentrations of Hyp were prepared by further dilution of a stock solution in 0.01 M phosphate-buffered saline (PBS, pH 7.2). Not copperized chlorophyll sodium salt (Chl) was purchased from ROTH (Karlsruhe, Germany) and dissolved in water ($1.5 \cdot 10^{-5}$ M).

### 2.2. Bacterial cultures and growth conditions

A food pathogen *Bacillus cereus* ATCC 12826 was provided by the National Centre of Public Health (Vilnius, Lithuania). *Bacillus* cultures were maintained at 37 °C for 24 h onto Luria Bertani Agar (LBA; Liofilchem, Roseto degli Abruzzi, Italy). Later bacteria were grown overnight (~16 h) at 37 °C in 20 mL of Luria–Bertani medium (LB; Liofilchem, Roseto degli Abruzzi, Italy) with agitation of 120 rev/min (Environmental Shaker-Incubator ES-20; Biosan, Latvia). Afterwards bacterial culture was 20 times diluted by the fresh LB medium (absorbance ($A$) = 0.164), and grown at 37 °C in a shaker (120 rev/min) to the mid-log phase (~$6 \cdot 10^7$ CFU/mL, $A$ = 1). Bacterial optical density was determined in a 10.01 mm cuvette at $\lambda$ = 540 nm (Heλios Gamma & Delta spectrophotometers; ThermoSpectronic, Waltham, MA, USA). Cells were then harvested by centrifugation (10 min, 3420 × $g$) (Hettich zentrifugen; Mikro 200, Germany) and resuspended in a 0.3 mL of 0.01 M PBS (pH 7.2) to $3 \cdot 10^8$ CFU/mL concentration of *Bacillus* cells. This stock suspension was accordingly PBS diluted to ~$1 \cdot 10^{-7}$ CFU/mL and used for the further experiments.

### 2.3. LED-based light sources for inactivation of bacteria

Light source with emission maximum at 585 nm was constructed for the inactivation of microorganisms by Hyp-based photosensitization. BL-3000 light emitting diode (LED) array (“Lamina”) was used for light prototype. Light intensity at surface of samples (at 3.5 cm from the light source) reached 3.84 mW/cm$^2$.

Light source with emission maximum at 405 nm was constructed for the inactivation of microorganisms by Chl-based photosensitization. An InGaN light emitting diodes array (LED Engine, Inc. LZ1-00UA00) was used for construction of light source. The light intensity at surface of samples (at 6 cm from the light source) reached 9.6 mW/cm$^2$.

As presented in Fig.1 on general light prototype consisted of illumination chamber and power supply unit. Cooling system was integrated in the light prototype to dissipate heat from the source and minimize any heat transfer to the sample.

Light dose was calculated as light intensity multiplied by irradiation time. Light intensity was measured by *3 Sigma* power and energy meter “Coherent” equipped with a piro-electrical detector J25LP04. The sample exposure time was adjusted according to the equation:

$$E = Pt, \tag{1}$$

where $E$ is the energy density (dose) in J/cm$^2$, $P$ is the irradiance (power density) in W/cm$^2$, and $t$ is the time in seconds.

### 2.4. Hyp-based photosensitization treatment in vitro

Aliquots (20 mL) of bacterial suspension (~$1 \cdot 10^7$ CFU/mL in 0.01 M PBS (pH 7.2)) with appropriate concentration of Hyp ($1 \cdot 10^{-7}$ and $1 \cdot 10^{-8}$ M) were incubated in the dark at 37 °C. For the following experiments, the cells were incubated in the shaker (130 rev/min) for different periods (2 and 60 min). Afterwards, 150 μL aliquots of bacterial suspension were withdrawn, placed into sterile flat bottom wells and exposed to light for different time (0–40 min). The depth of bacterial suspensions was 0.5 cm. Light dose was calculated as light intensity multiplied on irradiation time.

The antibacterial effect of photosensitization treatment on *Bacillus* cells was evaluated by the spread plate method. Thus, 100 μL of appropriate dilutions (in 0.9% NaCl) of bacterial test culture after treatment *in vitro* were surface inoculated on the LBA plates. Afterwards the bacteria were incubated in the thermostat for 24 h at 37 °C. The surviving cell populations were enumerated and expressed by $\log_{10}$ (CFU/mL).

### 2.5. Decontamination of FV from inoculated B. cereus by Hyp-based photosensitization

Apricots (*Prunus armeniaca*), plumes (*Prunus domestica*), and cauliflowers (*Brassica oleracea*) in partially ripe stage were purchased in a local supermarket, stored at 6 °C and processed within a day. For the inoculation with the pathogen, samples of about 15 g were soaked in 50 mL *Bacillus* suspension (~$1 \cdot 10^7$ CFU/mL) and

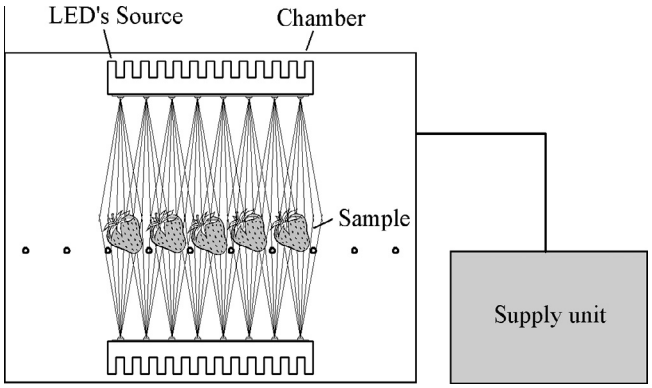


**Fig. 1.** Schematic presentation of LED-based light source prototype.