



Environmentally Friendly and
Safe Technologies for Quality
of Fruits and Vegetables

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The papers contained in this book report some of the peer reviewed Proceedings of the International Conference “Environmentally friendly and safe technologies for quality of fruit and vegetables”, but also other papers related with the subject were included. The manuscripts were reviewed by the Editor and Editorial Board, and only those papers judged suitable for publication were accepted. The Editor wish to thank to all the reviewers and authors for their contribution.

Authors are responsible for content and accuracy of their papers.

Proceedings of the International Conference “Environmentally friendly and safe technologies for quality of fruit and vegetables”, held in Universidade do Algarve, Faro, Portugal, on January 14-16, 2009. This Conference was a joint activity with COST Action 924.

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

35. NOVEL APPROACH TO DECONTAMINATE FRUITS AND VEGETABLES: COMBINED TREATMENT OF PULSED LIGHT AND PHOTOSENSITIZATION

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Abstract

Taking into account that in post - modern society the consumption of ready to eat meals is increasing all over the world, development of novel, more effective non-thermal technologies to increase food microbial control and reduce the risk of foodborne disease outbreaks becomes issue with global dimension. This study is concerned with the development of novel approach to decontaminate fruits and vegetables from pathogenic and harmful microorganisms. To decontaminate surface of vegetables from inoculated pathogen in non-thermal conditions 5.4 J cm⁻² broad spectrum UV (which UV light? A, B or C?) light dose was used and 1.3 log inactivation was achieved. For photosensitization experiments the vegetables inoculated with pathogen were submerged in chlorophyll salt solution (1.5×10⁻⁴ or 5×10⁻³ M) for 5 min and afterwards illuminated with 20 mW cm⁻² (λ=400 nm) light up to the total dose 36 J cm⁻². Inactivation of 2.4 log was achieved in this case. Combined treatment of photosensitization and pulsed light reduced the level of inoculated pathogen by more than 4 log, the natural contamination (mesophyls) - by 3.8 log. No effects on inactivation of antioxidant enzymes were detected. Moreover, the significant increase of shelf-life of treated vegetables was observed. In conclusion, fast and effective non-thermal technology might be developed for decontamination of fruits and vegetables for freshly cut or ready to eat meals.

Keywords: Food decontamination, non-thermal, photosensitization, pulsed light

Introduction

Despite the extensive efforts and scientific progress, food safety remains a worldwide problem. Frequently only one technology cannot insure efficient microorganisms decontamination and reduce others food losses. To obtain better result combination of two or more technologies (hurdle technologies) is used (Suparlan & Itoh 2003; Özdemir *et al.* 2005).

This study is focused on possibility to combine two light technologies: high power pulsed light and photosensitization for decontamination of freshly cut vegetables.

Material & Methods

For photosensitization and pulsed light experiments in vitro *Bacillus cereus* ATCC 12826 was grown at 37 °C in Luria-Bertani (LB) medium to the mid-log phase (~ 6×10⁷ colony forming units (cfu) mL⁻¹, OD₅₄₀=1) and collected by centrifugation (10 min, 5000 g), resuspended and accordingly PBS-diluted to ~1×10⁷ cfu mL⁻¹ final concentration. Aliquots (10 mL) of bacterial suspension with appropriate concentration of chlorophyll-derivative (7.5×10⁻⁸ cfu mL⁻¹) were incubated in the dark at 37 °C. Afterwards 150 μL aliquots of bacterial suspension were withdrawn, placed into sterile flat bottom wells and exposed to light for 60 s. Light emitting diodes (LED) based light source for (constructed in the Institute of Applied Sciences of Vilnius university) emitted light λ=400 nm with intensity 20 mW cm⁻² at the surface of samples. Light dose was calculated as light intensity multiplied on time. Then, 100 μL of appropriate dilutions (in 0.9% NaCl) of photosensitized bacterial suspension were placed on LB agar and illuminated with pulsed light (60 s, 1400 V, 0.162 J cm⁻²)

Cocktail tomatoes (*Solanum lycopersicum*) were obtained from a local supermarket and stored at

refrigerator temperature (+6 °C). For inoculation experiments, *B. cereus* ATCC 12826, grown at 37 °C in Luria-Bertani (LB) medium until mid-log phase was harvested by centrifugation (10 min, 5000 g), resuspended and diluted with phosphate-buffered saline (100 mM PBS, pH 7.2) to $\sim 1 \times 10^7$ cfu mL⁻¹ final concentration. Cell suspension was then mixed with chlorophyll derivative solution to obtain final 5×10^{-3} M photosensitizer's concentration and immediately used for further experiments. The control samples were soaked in PBS. All samples kept in the dark for 5 min and after inoculum decantation, dried in the thermostat at 37 °C for 20 min. Dried examples were placed in the treatment chamber in a sterile Petri dishes without cover and exposed to light intensity 20 mW cm⁻² at $\lambda=400$ nm for 30 min. After photosensitizing, samples were treated with pulsed light (1400V, 1000 pulses, 5.4 J cm⁻²). Control samples were not irradiated. After treatment, samples (also control samples) mixed with PBS buffer in sterile bags and homogenized with a BagMixer for 60 s. Then, 100 μ L of appropriate dilutions (in 0.9% NaCl) of homogenized tomatoes 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 (cfu g⁻¹).

For statistical analysis bacterial populations cfu mL⁻¹ were transformed into log₁₀ mL⁻¹. Analysis of variance (Anova) was performed ($P < 0.05$). In addition, Bonferroni tests were performed between means. Each experimental point is an average of 3-5 experiments. A standard error was estimated for every experimental point and marked in a figure as an error bar. The data were analyzed with Origin 7.5 software (OriginLab Corporation, Northampton, MA, USA).

Results

Our previous data indicate that food pathogens *B. cereus*, *Listeria monocytogenes* as well as *Salmonella typhimurium* are susceptible to photosensitization as well as to pulsed light and can be inactivated by 6-7 log. These results prompted us to investigate the efficiency of combination of these two antibacterial treatments in different sequences. Data presented in Fig 1 clearly indicate that combined treatment of pulsed light and photosensitization exhibits synergy in inactivation of food pathogen *B. cereus*. It is important to note that sequence of treatments plays crucial role. For effective decontamination algorithm photosensitization must be used before pulsed light. Otherwise, photosensitizer bleached from pulsed light and was useless for photosensitization.

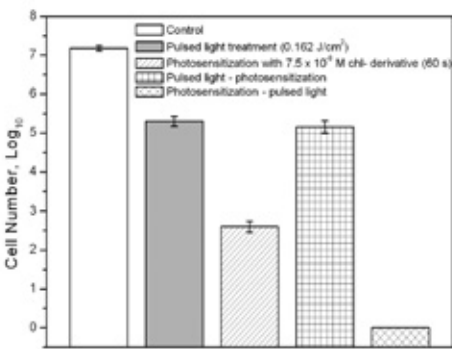


Fig 1. Inactivation of *B. cereus* in vitro by photosensitization, pulsed light and combined treatment.

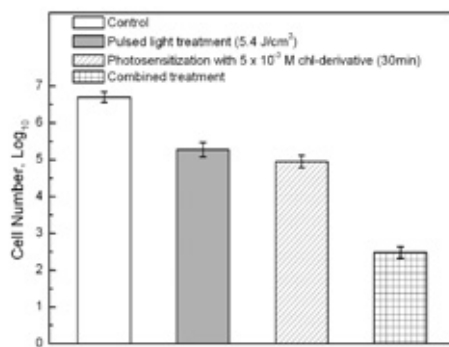


Fig 2. Inactivation of *B. cereus* inoculated on cocktail tomatoes by photosensitization, pulsed light and combined treatment.

Data depicted in Fig 2 show the decontamination of cocktail tomatoes from aerobic mesophyls by photosensitization. It is clear that more than 4 log inactivation is possible to reach, when photosensitization is combined with pulsed light technique. In the next step *B. cereus* was inoculated on the surface of cocktail tomatoes and treated by this combination.

Data obtained indicate that more than 4 log inactivation of *B. cereus* is possible to achieve after combined treatment of photosensitization and pulsed light (Fig 3).

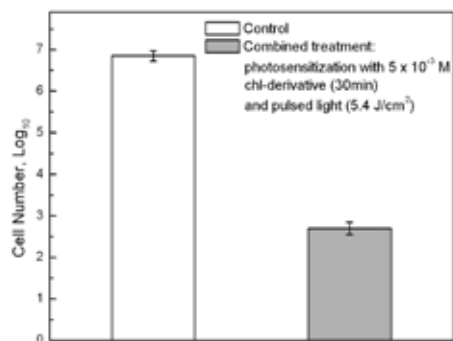


Fig 3. Decontamination of natural contaminants (mesophyls) by combined treatment of photosensitization and pulsed light.

Discussion

The lack of proper sanitation procedures can cost a lot of money for food processing companies. Chemical sanitizers like hypochlorite, iodine, ozone and chloramines are largely used in food industry but these hazardous materials did not ensure its safety and change food chemical parameters. Also, various microorganisms are able to form very resistant forms like biofilms and spores whose are more resistant to chemical treatment than vegetative cells. However, combined treatments of chemical sanitizers and modified atmosphere packaging demonstrate sufficient efficacy on food matrix (Lee & Baek 2008).

Our data in vitro reveals that *B. cereus* decontamination with combined treatment of photosensitization and pulsed light is more effective than individually. Combined treatment reduced the level of inoculated tomatoes by more than 4 log, the natural contamination (mesophyls) - by 3.8 log.

In conclusion, fast and effective non-thermal technology might be developed for decontamination of fruits and vegetables for freshly cut or ready to eat meals.

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