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 join activity with COST Action 924.

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

37. DECONTAMINATION OF PACKAGING BY ALA-BASED PHOTOSENSITIZATION

Zivile Luksiene, Egle Paskeviciute*
Institute of Applied Research, Vilnius University, Sauletekio 10, 10223 Vilnius, Lithuania
*E-mail: Zivile.Luksiene@mtmi.vu.lt

Abstract

This study deals with the development of a novel approach to decontaminate packaging from food pathogens by photosensitization. For this purpose, packaging samples with adhered pathogen were submerged in aminolevulinic acid (ALA solution (3-7.5 mM) for 10 min. Samples were then illuminated with 20 mW cm^{-2} (λ=400 nm) for 5-20 min up to the total exposure of 24 J cm^{-2}. Gram-positive *Bacillus cereus* and Gram-negative *Salmonella enterica* were inactivated, with population reductions of 4.2 and 2.5 log, respectively. Inactivation of Gram-positive *Listeria monocytogenes* biofilms ranged from 1.7-3.1 log. Moreover, our data indicated that the *B. cereus* spores were susceptible to this treatment, with as much as a 3.1 log reduction in spore population observed after ALA-based photosensitization *in vitro* and 2.7 log on the surface of packaging material.

Introduction

Interest in non-thermal processing of food and food-related packaging among scientists, consumers and producers is increasing. This interest is based on the fact that these technologies have minimal impact on the nutritional and sensory properties of foods (Pirttijarvi *et al.* 1996; Wainwright 1998). The aim of this study was to investigate the susceptibility of foodborne bacterial pathogens to novel emerging non-thermal treatment- photosensitization.

Materials & Methods

Bacillus cereus ATCC 12826 and *Listeria monocytogenes* $ATC_{L3}C$ 7644 were grown at 37 °C in Luria-Bertani (LB) medium to the mid-log phase (~6×10^7 colony forming units (cfu) mL^{-1}, OD_{540}=1). Cells were harvested by centrifugation (10 min, 5000 g), resuspended and diluted in phosphate buffer solution (PBS) to give a final concentration of ~1×10^7 cfu mL^{-1}. For *B. cereus* ATCC 12826, spores were prepared by growing the strain for 3 d at 37 °C in brain heart infusion (BHI) broth (Liofilchem) containing (per liter) 0.05 mg manganese until 80-90% sporulation was obtained. Spore suspensions were prepared by washing with sterile distilled water, centrifuging (20 min, 6000 g) and heating to 80 °C. *L. monocytogenes* biofilms were prepared according to the method of Pan *et al. (2006)*. Yellow packing trays cut into 4 cm×8 cm pieces were soaked in 50 mL of suspensions of *B. cereus* ATCC 12826 and *L. monocytogenes* ATCL3C 7644 to ensure pathogen cell adhesion to the packaging surfaces. After inoculation with the pathogens, the packaging samples were dried in a laminar flow hood for 30 min. Samples were incubated in darkness with a 3-10 mM concentration of ALA for different periods (5, 10, 20 min). Control samples were treated with PBS not containing pathogens and incubated under the same conditions.

After incubation with ALA, all packing samples were dried at room temperature for 20 min, placed in the treatment chamber and exposed to light for different times ranging from 5 to 20 min at λ=400nm. The control samples were not illuminated. Then cells, spores or biofilms were washed by mixing with 30 ml PBS separately. Appropriate dilutions of 100 μl (in 0.9 % NaCl) of suspension were placed on LBA plates. The colonies were counted after 24 h incubation at 37 °C. The surviving cell populations were enumerated and expressed as $\log_{10}$ (cfu mL^{-1}) and N/N_0 where N_0 is the number of cfu mL-1 in the untreated culture and N is the number of cfu mL^{-1} in the treated culture. Precision Celsius temperature sensors (Deltha Ohm Italy) were used for temperature measurements.

Results

Data describing ALA-based photoinactivation of *B. cereus* as function of illumination time are presented in Fig 1. Clearly, 20 min illumination was sufficient to inactivate *B. cereus* (4 log population decrease), when with ALA incubation time was 10 min. In order to estimate the decontamination efficiency of ALA-based photosensitization, food packaging material was submerged in *B. cereus* spore solution. Different concentrations of ALA solution (3-7.5 mM) were exploited for experiments. Data shown in Fig 2 show that *B. cereus* spores attached to plastic food-related packaging material and were inactivated by ALA-based photosensitization (2.7 log).

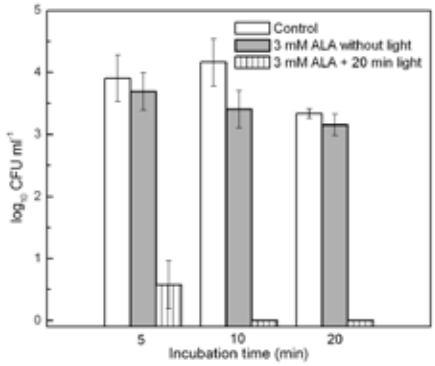

Fig 1. Inactivation of *Bacillus cereus* by 3 mM ALA-based photosensitization onto packaging samples as function of incubation time. Illumination time was 20 min.

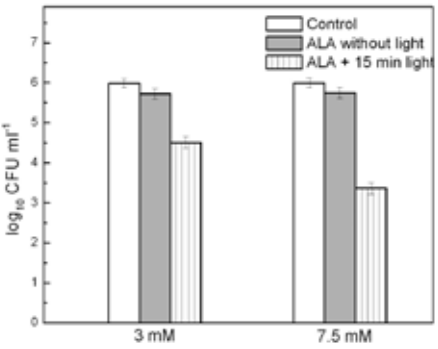

Fig 2. Decontamination of food-related packaging from *B. cereus* spores.

The data, depicted in Fig 3, clearly indicate that the inactivation of *Listeria* cells after photosensitization treatment decreased from 2.3 up to 3.7 log, depending on the ALA concentration used. Our task was to evaluate susceptibility of *Listeria* biofilms to ALA-based photosensitization treatment. For this purpose, bacterial biofilms were adhered on the surface of packaging material. The treatment of biofilm-associated cells by 7.5-10 mM ALA and subsequent illumination reduced significantly the formation of biofilms. Depending on the used ALA concentration (3-7.5-10 mM) inactivation of biofilm-associated cells decreased from 1.7 log to 3.0 log, respectively.

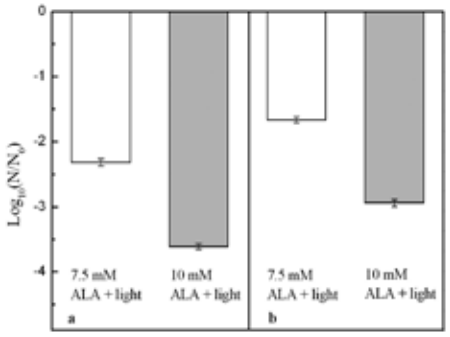

Fig 3. Susceptibility of *Listeria monocytogenes* $ATC_{L3}C$ 7644 to ALA - based photosensitization: cells (a) and biofilms (b) adhered to the surface of packaging material. ALA concentration 7.5-10 mM, illumination time – 15 min, total light dose 18 J cm^{-2}. Control, not treated sample = 0 log (N/N_0).

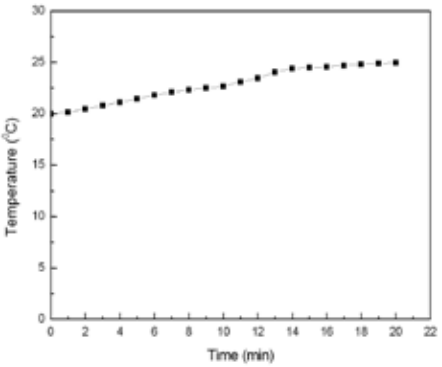

Fig 4. The increase of temperature in the chamber of LED-based light source during 20 min of illumination.

One of our tasks in this study was inactivation by photosensitization in a non-thermal way. For this purpose, precise thermophora were used. Dynamics of temperature inside the chamber was monitered every minute. Data presented in Fig.4 clearly indicate, that the temperature in the chamber of LED-based light source slowly increased up to 24 °C. Some saturation of temperature started from 14 min into the treatment and continued up to 20 min. Even after 20 min, of illumination temperature in the chamber did not exceed 25 °C.

Discussion

Due to very high resistance of bacterial spores to UV (Nicholson 2000), germicidal lamps are insufficient to decontaminate packaging materials. Decontamination of packaging material from *B. cereus* adhered to the surface by this treatment seems promising. More than 4 log inactivation was achieved after ALA-based photosensitization. Moreover, obtained data indicated that the *B. cereus* spores are susceptible to this treatment as well. As much as a 3.1 log reduction in spore population was observed after ALA-based photosensitization *in vitro* and 2.7 log inactivation was observed when spores were placed on the surface of packaging material (Fig 1).

The data presented in Fig 2 demonstrate that inactivation of cells after photosensitization can reach up to 2.3-3.7 log reduction. Inactivation of biofilms by 1.7-3.1 log indicate that this treatment has potential to combat biofilms.

Conclusions

The decontamination of packaging material from adhered *B. cereus* after ALA-based photosensitization reached 4 log. Of importance to note, that spores of *B. cereus* are susceptible to this treatment and can be inactivated by 3.1 log *in vitro* or 2.7 log on the surface of packaging material. Efficient photoinactivation of food pathogens onto packaging materials looks promising and, may be, could serve as a background for the development of a novel non-thermal or hurdle technology for decontamination of foods or food-related surfaces.

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