Biblid: 1450-5029 (2009) 13; 1; p.75-77
UDK: 614.48:591.133.1:655.222.6

Original scientific paper
Originalni naučni rad

DECONTAMINATION OF FOOD-RELATED SURFACES BY PHOTOSENSITIZATION
DEZINFEKCIJA POVRŠINA KOJE SU U KONTAKTU SA HRANOM METODOM FOTOSENZITIVNOSTI

Irina BUCHOVEC, MSc, Egle PASKEVICIUTE, MSc, Dr. Zivile LUKSIENE
Institute of Materials Science and Applied research, Vilnius University, Sauletekio 10, 10223, Lithuania, e-mail: Zivile.Luksiene@mtmi.vu.lt

SUMMARY

The use of effective decontamination technique on surface-attached microbes minimizes risks of foodborne diseases, enhances microbiological safety of product and expands its shelf-life. This study deals with the development of novel approach to decontaminate packaging from food pathogens by photosensitization.

For this purpose packaging samples with adhered pathogen were submerged Chlorophyll derivative solution (1.5 x 10^{-5} M) for 5 min. and afterwards illuminated with 20 mW/cm^2 (λ=400nm) light for 20 min up to the total dose 24 J/cm^2. Gram-positive Bacillus cereus as well as Listeria monocytogenes was inactivated by 3-4 log, depending on experimental conditions. Inactivation of Listeria biofilms by 1.7-3.1 log indicate that this treatment has potential to combat biofilms. Moreover, obtained data indicated that the bacillus spores are susceptible to this treatment as well. The spore population on the surface of packaging material was reduced by 3.8 log after photosensitization.

In conclusion, our data support the idea, that photosensitization is effective non-thermal and not-chemical antibacterial treatment, which inactivates food pathogens Bacillus cereus and Listeria monotytogenes as well as spores and biofilms on the surface of packaging material and has potential to be useful in the development of novel food safety technologies.

Key words: *Decontamination of packaging, photosensitization.*

INTRODUCTION

Contamination of food and food-related surfaces by various aerobic mesophils and food-borne pathogens is one of the main problems in food industry. Microbial contamination may begin in food post-processing, or in the packaging and distribution processes. Packaging material irradiation with γ rays is prevalent in U.S. (U.S. Food and Drug Administration, 2007). Some working groups are creating packaging materials with well known antimicrobial additives – nisin, lysozyme in combination with EDTA, bacteriocins (Limjaroen et al. 2003). Washing food-related surfaces with chemical sanitizers are commonly-used. Supposedly, it is one of the cheapest sanitation; do not require special equipment and skills and are conditionally effective against microbes. Disinfectants approved for use in the food industry are alcohols, chlorine-based compounds, quaternary ammonium compounds, oxidants (peracetic acid, hydrogen peroxide, ozone) persulphates, surfactants and iodophors (Wirtanen and Salo 2003). But, generally, microorganisms attached to the surfaces are concerned to build-up biofilms witch are more resistant to any treatment as compared with their planctonic counterparts (Pan et al. 2006). Moreover microbes have been found in disinfectant solutions, which is due to their ability to form resistant strains. This means, that microbial contamination can be spread on the surface to be cleaned instead of being cleansed (Wirtanen and Salo 2003)

One of the possible alternatives to chemical packaging decontamination treatments can be photosensitization. This treatment involves the administration of a photosensitizer that selectively accumulates in the target microorganism. After illumination of this microorganism with visible light, plethora of photochemical reactions induces selectively death of microorganism without any harmful effects on surrounding (Luksiene, 2005).

One of the most important advantages of photosensitization in comparison with other antibacterial tools is the absence of any bacterial resistance to this treatment (Nitzan and Ashkenazi 2001). It allows us to inactivate pathogens with minimal damage of the surrounding matrix (Jori 2006) and has no mutagenic or carcinogenic effects on living systems (Luksiene 2005; Luksiene et al. 2005).

Our previous data (Luksiene et al. 1989; 2005; 2007) indicate that yeasts as well as micromycetes might be inactivated by photosensitization *in vitro* as well as on the surface of food matrix. Moreover, food pathogens *Salmonella enterica, Bacillus cereus and Listeria monocytogenes* are susceptible to aminolevulinic-based photosensitization (Buchovec et al. 2009; Le Marc et al., 2009). It is important to note, that *Bacillus* spores being important target for food safety technologies can be inactivated by aminolevulinic acid-based photosensitization (Luksiene et al. 2009). Moreover, *Listeria* biofilms which spread on different food-contact surfaces and are resistant to hypochlorite can be destroyed by aminolevulinic acid-based photosensitization as well. It seems that plethora of harmful micromycetes and pathogenic bacteria might be inactivated by photosensitization, a method that is completely safe, reproducible, not-mutagenic, not-carcinogenic, environmentally and human friendly (Luksiene, 2005; Lithuanian patent, Nr 2008060).

However, there are no reports on the evaluation of applicability of chlorophyll sodium salt-based photosensitization to decontaminate packaging, as this food additive is much cheeper and more effective than aminolevulinic acid. This study was focused on the key issue - effectiveness of the chlorophyll sodium salt-based photosensitization for decontamination of packaging from several food pathogens (*Bacillus cereus, Listeria monocytogenes*). Moreover, it was important to evaluate the susceptibility of pathogenic spores and biofilms to this treatment.

MATERIALS AND METHOD

B. cereus ATCC 12826 and *Listeria monocytogenes* $ATC_{L3}C$ 7644 grown at 37 ^{0}C in Luria-Bertani (LB) medium to the mid-log phase (~ 6x10^7 colony forming units (cfu)/ml, OD_{540}=1) were harvested by centrifugation (10 min, 5000g), resuspended

and accordingly PBS-diluted to ~1 x 10^7 cfu/ml final concentration. For *B. cereus* ATCC 12826 spores preparation, culture was grown for 3 days at 37 ^{0}C in brain heart infusion (BHI) broth (Liofilchem) containing (per liter) 0.05 mg manganese until 80-90% sporulation was obtained. Spore suspension was prepared by washing with sterile distilled water, centrifuging (20 min, 6000g) and heating to 80 ^{0}C. *L. monocytogenes* biofilms were prepared according to the method of Pan et al. (2006). Packing yellow trays, cut into 4 cm × 8 cm pieces were soaked in 50 ml *B. cereus* ATCC 12826 and *L. monocytogenes* $ATC_{L3}C$ 7644 suspension separately for better cell adhesion. Afterwards the packaging samples were kept in laminar 30 min for further bacterial adhesion. Then appropriate packing samples were incubated in the dark with the 7.5 x 10^{-7} M and 1.5 x 10^{-4} M chlorophyll sodium salt for 5 min. The control samples were incubated with PBS (7.2 pH) buffer.

After incubation with chlorophyll sodium salt all packing samples (plastic packing yellow trays provided by LINPAC) were dried at room temperature for 20 min, placed in the treatment chamber and exposed to light for different time (2 min or 5 min) at λ= 400nm. The control sample was not illuminated. Then samples were mixed with 30 ml PBS and homogenized with a BagMixer separately. 100 µl of appropriate dilutions (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 (cfu/ml).

RESULTS AND DISCUSSION

In order to estimate the decontamination efficiency of photosensitization, food packaging material was submerged in *B. cereus* spore solution. Concentrations of Chl sodium salt solution used during these experiments was 7.5 x 10^{-7} M. Data, depicted in Fig. 1 describe the chlorophyll derivative-based photoinactivation of *B. cereus* on the surface of packaging. It is obvious, that photosensitizer alone without following illumination has no significant antibacterial effect. Moreover, even 20 min illumination of bacteria adhered on the surface has no effect on their viability. Just photosensitization treatment inactivated them to 0 log and cleaned the surface of packaging.

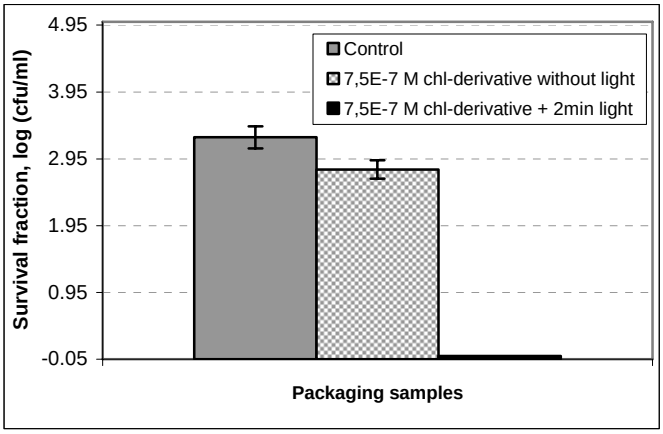


Fig. 1. Inactivation of Bacillus cereus ATCC 12826 by 7.5E-7 M chl-derivative based photosensitization onto packaging samples.
Sl. 1. Aktivacija Bacillus cereus ATCC 12826 by 7.5E-7 M chl-derivatom zasnovan fotosenzitivnošću na upakovan uzorak

The data, depicted in Fig. 2, clearly indicate that the inactivation of *Listeria* cells attached to the surface of packaging after photosensitization treatment reached 4.5 log from initially attached 4.5 log. Very low (7.5 x10^{-7} M) photosensitizer concentration was enough effective and cleaned the surface to 0 log.

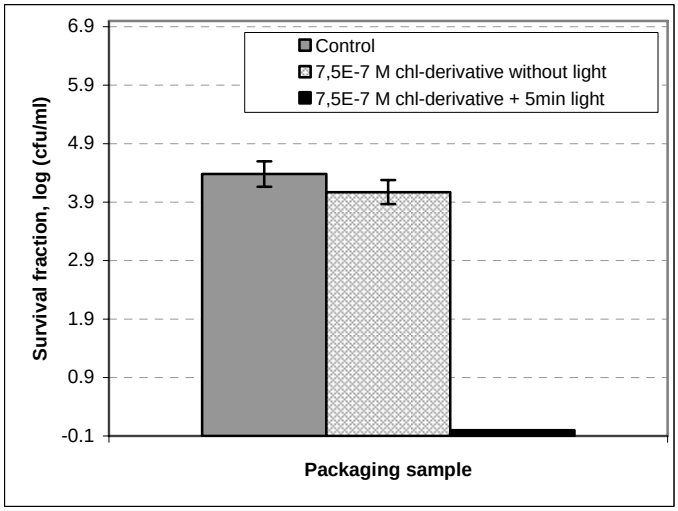


Fig. 2. Inactivation of Listeria monocytogenes $ATC_{L3}C$ 7644 by 7.5E-7 M chl-derivative based photosensitization onto packaging samples.
Sl. 2. Aktivacija Listeria monocytogenes $ATC_{L3}C$ 7644 by 7.5E-7 M chl derivatom zasnovan fotosenzitivnošću na upakovan uzorak

Data, shown in Fig. 3 indicate that *B. cereus* spores are able to attach on plastic food-related packaging material and can be inactivated by chlorophyll sodium salt-based photosensitization (3.8 log).

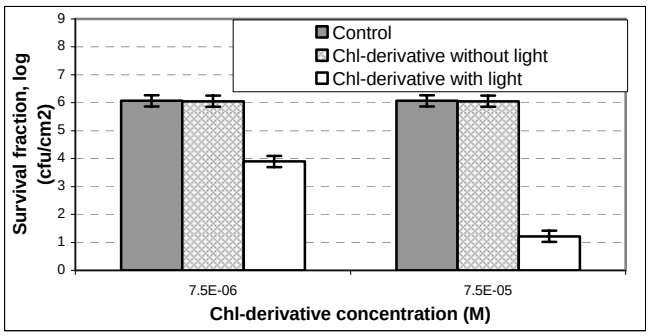


Fig. 3. Inactivation of Bacillus cereus ATCC 12826 spores by 7.5E-6 M and 7.5E-5 M chl-derivative based photosensitization onto packaging samples.
Sl. 3. Aktivacija Bacillus cereus ATCC 12826 spore 7.5E-7 M chl- derivatom zasnovan fotosenzitivnošću na upakovan uzorak

Our task was to evaluate susceptibility of *Listeria* biofilms to chlorophyll derivative-based photosensitization treatment. For this purpose, bacterial biofilms were adhered on the surface of packaging material. The treatment of biofilm-associated cells by 1.5 x 10^{-5} - 1.5 x 10^{-4} M chlorophyll sodium salt destructed them by 4.5 log from 4.5 log of attached cells (Fig. 4).

Nowadays, several technologies are practicing to reduce the risk of surface contamination. Meanwhile all of them, as described in Introduction, have specific disadvantages. First results obtained on packaging decontamination by chlorophyll sodium salt-based photosensitization seems promising, as antibacterial efficiency of this method seems high in comparison with chemical disinfectants. Wirtanen et al. (2001) evaluated efficacy of common commercial disinfectants (based on hypochlorite, hydrogen peroxide, alcohol and peracetic acid) against some foodborne pathogens and spoilage microbes in biofilm-constructs. It came clear, that Gram-positive bacteria tested in poloxamer hydrogels was reduced from ~0.1- to ~2-log and Gram-negative

bacteria were even more resistant (Wirtanen et al. 2001). Furthermore, survived cells are able to grow and recontaminate surfaces. Also harmful chemical compounds commonly are unstable, corrosive and toxic (Wirtanen and Salo, 2003).

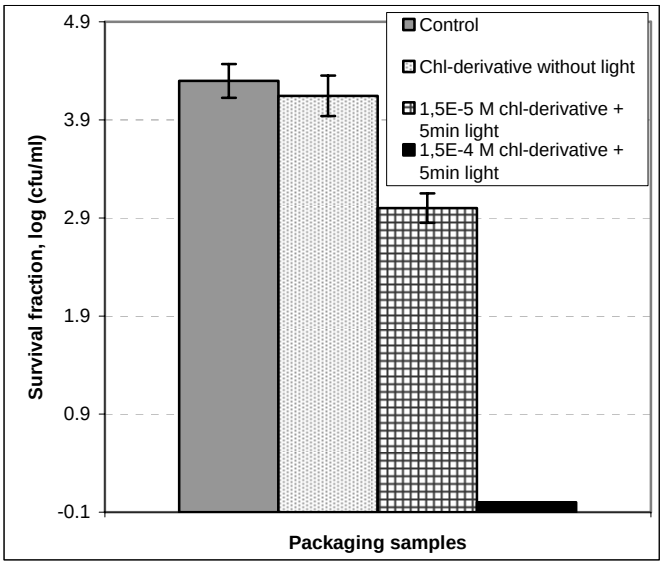


Fig. 4. Inactivation of Listeria monocytogenes $ATC_{L3}C$ 7644 biofilms by 1.5E-5 M and 1.5E-4 M chl-derivative based photosensitization onto packaging samples.
Sl. 4. Aktivacija Listeria monocytogenes $ATC_{L3}C$ 7644 biofilmovi pomoću 1.5E-5 M and 1.5E-4 M chl-- derivatom zasnovan fotosenzitivnošću na upakovan uzorak

Destruction of biofilms can be a problem not even in food packaging, but in food processing as well. Due to insufficient efficiency of cleaning the remaining cells start to grow and contaminate the product (Hood and Zottola 1995). According to our data, chlorophyll sodium salt-based photosensitization can inactivate biofilms on the surface of packaging by 4.5 log from 4.5 log of attached cells.

CONCLUSION

In conclusion, obtained results reveal, that chlorophyll sodium salt–based photosensitization treatment is effective antibacterial tool against such food pathogens as *Bacillus cereus and Listeria monocytogenes* and can totally decontaminate packaging from these bacteria adhered on the surface of packaging. It is important to note, that chlorophyll sodium salt–based photosensitization treatment can combat resistant *Listeri*a biofilms and *Bacillus* spores on the surface of packaging.

REFERENCES

[1] U.S. Food and Drug Administration, CFSAN/Office of Food Additive Safety, 2007, available at: http://www.cfsan.fda.gov/~dms/irrapack.html

[2] Limjaroen, P., Ryser, E., Lockhart, H. and Harte, B: Development of a food packaging coating material with antimicrobial properties, Journal of Plastic Film and Sheeting, 19(2003), p. 95-109.

[3] Wirtanen, G. and Salo, S: Disinfection in food processing – efficacy testing of disinfectants, Reviews in Environmental Science and Bio/Technology, 2(2003), p. 293-306.

[4] Pan, Y. Breidt, F. Jr. and Kathariou, S: Resistance of *Listeria monocytogenes* biofilms to sanitizing agents in a simulated food processing environment, Applied and Environmental Microbiology, 72(2006), p. 7711–7717.

[5] Luksiene, Z: New approach to inactivate harmful and pathogenic microorganisms: photosensitization. Food Technology and Biotechnology, 43(2005), p.1-8.

[6] Nitzan, Y. and Ashkenazi, H: Photoinactivation of *Acinetobacter baumannii* and *Escherichia coli B* by a cationic hydrophilic porphyrin at various light wavelengths. Current Microbiology, 42(2001), p. 408-414.

[7] Jori, G: Photodynamic therapy of microbial infections: state of the art and perspectives. Journal of Environmental Pathology, Toxicology and Oncology, 25(2006), p. 505-519.

[8] Luksiene, Z., Peciulyte, D., Jurkoniene, S. and Puras, R: Inactivation of possible fungal food contaminants by photosensitization, Food Technology and Biotechnology, 43(2005), p. 1-7.

[9] Luksiene, Z., Bagdoniene, I., Smilgevicius, V., Piskarskas, A. and Juodka B: Action of sensitizers and light on biomolecules and cells: inactivation of S. cerevissiae growth after laser and porphyrin treatment. Biology, 27(1989), p. 161-169.

[10] Luksiene, Z., Danilcenko, H., Taraseviciene, Z., Anusevicius, Z., Maroziene, A. and Nivinskas, H: New approach to the decontamination of germinated wheat from microfungi: effects of aminolevulinic acid, International Journal of Food Microbiology, 116(2007), p. 153-158.

[11] Buchovec, I., Vaitonis, Z. and Luksiene, Z: Novel approach to control *Salmonella enterica* by modern biophotonic technology: photosensitization. Journal of Applied Microbiology, 2009, in press.

[12] Le Marc,Y., Buchovec, I., Baranyi, J. and Luksiene Z: Modeling the photosensitization-based inactivation of *Bacillus cereus*, Journal of Applied Microbiology, 2009, in press.

[13] Luksiene, Z., Buchovec, I. and Paskeviciute, E: Inactivation of food pathogen *Bacillus cereus* by photosensitization *in vitro* and on the surface of packaging material. Journal of Applied Microbiology, 2009, in press.

[14] Wirtanen, G., Aalto, M., Harkonen, P., Gilbert, P. and Mattila-Sandholm, T: Efficacy testing of commercial disinfectants against foodborne pathogenic and spoilage microbes in biofilm-constructs, European Food Research and Technology, 213(2001), p. 409-411.

[15] Hood, S. K. and Zottola, E.A: Biofilms in food processing. Food control, 6(1995), p. 9-18.

Primljeno: 10.02.2009. Prihvaćeno: 12.03.2009.