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EVALUATION OF ULTRAVIOLET RADIATION TO CONTROL MICROORGANISMS ADHERING TO LOW-DENSITY POLYETHYLENE FILMS

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ABSTRACT

Efficiency of ultraviolet (UV) radiation in reducing the cell number of *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* K-12 adhered to low-density polyethylene (LDPE) films was evaluated. The microorganisms were let to adhere to the surface of LPDE bags for 12h at 18ºC, and then submitted to UV radiation at an intensity 196 µW.cm^{-2}, 254nm, for 2 seconds. *Staphylococcus aureus* was less resistant to UV radiation than *E. coli*, and the efficiency increased with the increase of the concentration of microbial suspension. After 1500 hours of use the UV radiation intensity of the lamp was reduced from 288 to 78 µW.cm^{-2}, and the higher decrease occurred in the first 100 hours of use. Also, the efficiency of the UV radiation decreased after 1500 hours of use. The number of mesophilic aerobes on the surface of LDPE films was reduced by 90% after irradiation with 137 µW.cm^{-2} for 2 seconds. Atomic force microscopy revealed cracks and crevices and protuberances on the LDPE surface, a topography that can protect the cells from UV radiation, reducing the efficiency of the process. The results showed that UV radiation can be a useful technique for reducing the microbiota adhered to LDPE films.

Key words: bacterial adhesion, ultraviolet radiation; low-density polyethylene, atomic force microscopy

INTRODUCTION

Surfaces, such as stainless steel, glass, rubber, polypropylene, polyethylene and cast iron, permit microbial adherence and biofilm formation. Such surfaces, widely used for food processing, should not contaminate or increase the incidence of spoilage and pathogenic microorganisms in food products. Biofilm formation is a complex process that can start with even one single adhered cell (3). Several factors including type of microorganism, hydrophobicity and surface's eletric charge and pH of the medium affect microbial adherence (3,10,12). Minimizing microbial contamination in plastic food packages increases the shelf life and maintains the quality of the food during storage and marketing (4). Microorganisms in plastic food packages can be controled by UV irradiation, which has some advantages i) does not leave residues on the surfaces, ii) does not require heat, iii) has low cost and is easy to use, and iv) is legal (2,11).

The microbial inactivation by UV is caused by absorption of 254-nm wavelength light by nucleic acids and their constituents (2,4,9). This has mutagenic effects and causes cell division retardation due to links between adjacent molecules of pyrimidine in DNA, forming dimmers. Bacterial sensitivity to UV radiation is affected by several factors such as medium pH and bacterial growth phase (1).

This study was done to evaluate the efficiency of UV radiation to reduce *Staphylococcus aureus* and *Escherichia coli* adhered to the low-density polyethylene (LDPE) films. Changes in efficiency of the process induced by the lamp use and by the microtopography of the LDPE film were also evaluated.

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MATERIALS AND METHODS

Microbial cultures

Cultures of *S. aureus* ATCC 25923 and *E. coli* K12 were maintained in semi-solid brain-heart infusion (BHI-BIOBRAS) at 6ºC. The bacteria were activated by three transfers in BHI broth at 35ºC for 24 h. The bacterial suspension for surface adherence was prepared by diluting the broth culture with sterile 0.31M phosphate buffer, pH 7.0 ± 0.1. The bacterial concentration in the final suspension was determined by plating in Plate Count Agar (PCA) (Merck).

Microbial adherence to LDPE (low density polyethylene) surface

LDPE fluid milk bags were obtained from the dairy plant at Food Science Department/Federal University of Viçosa, Minas Gerais, Brasil. The bags had 1000 ml capacity, 630 cm^2 internal surface area, and 70 mm thickness. They were decontaminated using 70% alcohol followed by 1 minute exposure to UV radiation. For bacterial adherence to the surface, the bags filled with 1000 ml of each bacterial suspension containing from 10^5 to 10^7cfu.mL^{-1}. After thermally sealed, they were incubated at 18ºC. After 12 h, the suspension was drained and the bags were filled with 1000 ml of sterile buffer and let stand for 1 minute to remove the plancktonic cells. The rinse solution was then drained and the packages washed manually with vigorous agitation with 100 ml sterile buffer for 90 seconds to remove the sessile cells from the film. The wash-buffer containing the cells was diluted and adequate aliquots were plated in PCA and incubated for 24 h at 35ºC. The results were expressed as log cfu.cm^{-2}.

UV radiation

The internal surface of inoculated packages was exposed for approximately 2 seconds to UV radiation at an intensity of 196mW.cm^{-2} which corresponds to the conditions used in milk filling machine. The efficiency of UV radiation was evaluated by decimal reduction (DR) in the cell population, determined from the difference in counts (log cfu.cm^{-2}) of cells adhered to LPDE surface before and after UV treatment.

The intensity of UV radiation emitted by the lamp was determined at 50 h intervals during 1500h and was measured using a radiation meter (Optical Associate Inc., model 354). The bactericidal efficiency was determined after 70, 1000 and 1500 h of use, after 2 seconds radiation.

Bactericidal efficiency of UV radiation under user's conditions

In the experiment, a UV-lamp (15 W, 254nm, 2 seconds) recommended for fluid milk filling machines was used. As the UV radiation intensity emitted by lamp changes along the use, this parameter was measured before each experiment. Samples of LDPE packaging films similar to that used in the laboratory were withdrawn from three commercial rolls of films from the dairy plant and analyzed before and after UV irradiation. The cells from each LDPE surface were removed by vigorous shaking with 100 ml of phosphate buffer for 90 seconds. The number of cells in the rinse solution was determined using the most probable number (MPN) technique using 5 tubes for each aliquots of 10, 1, and 0.1 ml of sample (5). The number of cells adhered to the LPDE surfaces was expressed as log MPN.cm^{-2}. The efficiency of the UV radiation was determined as the difference between results obtained before and after treatment.

LDPE surface analysis

The microtopography of the LDPE surface was obtained using atomic force microscopy, with taping mode technique for surface photographing (8)

Data analysis

Three separate experiments were conducted. The first one evaluated the reduction of UV radiation intensity during 1500 hours of lamp use time and the bactericidal efficiency over *S. aureus* and *E. coli* adhered to LPDE surfaces after 70, 1000 and 1500 hours lamp use time. The second experiment evaluated the UV radiation efficiency on *S. aureus* and *E. coli* adhered to LPDE surfaces after exposure for 2 seconds at 196 µW.cm^{-2}. The third experiment evaluated the UV radiation efficiency in reducing the aerobic mesophilic bacteria adhered to three different samples of LPDE packaging films. Descriptive analysis was used to compare the results of the experiments.

RESULTS

UV radiation decreased the number of viable cells adhering to the LPDE surfaces (Table 1). The DR for *E. coli* varied from 0.52 to 1.37 and for *S. aureus* from 0.85 to 1.73. The UV radiation efficiency increased as the concentration of the microbial suspension used in the adhesion process increased (Table 1)

UV radiation intensity decreased gradually during the 1500 hours of use of the germicidal lamp, and 39.5% of the reduction occurred in the initial 100 hours (Fig. 1). Table 2 shows the DR values caused by 2 seconds of exposure to UV radiation after 70, 1000 and 1500 hours of use time. After 1500 hours, the lamp antibacterial efficiency was reduced by a factor of 2.6 and 1.8 for *E. coli* and *S. aureus*, respectively.

Under user's conditions, the initial average number of bacterial contaminants on the LDPE surface (0.13 MPN.cm^{-2}) was reduced to 0.014 after 2 seconds of radiation, giving a DR value of 0.97 (Table 3).

The microtopography of the LDPE surface showed two different areas: one relatively smooth and the other with refringent points. The smooth area (Fig. 2A) showed roughness of 5 nm indicating relatively plane surface and the other (Fig. 2B) presented 0.8 µm pick and 0.2 mm deep crack of 5 µm diameter.

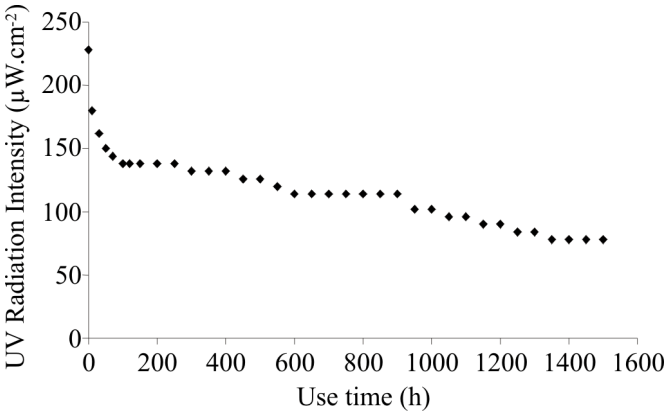

Figure 1. UV radiation intensity reduction as a function of the time of use of the UV lamp.

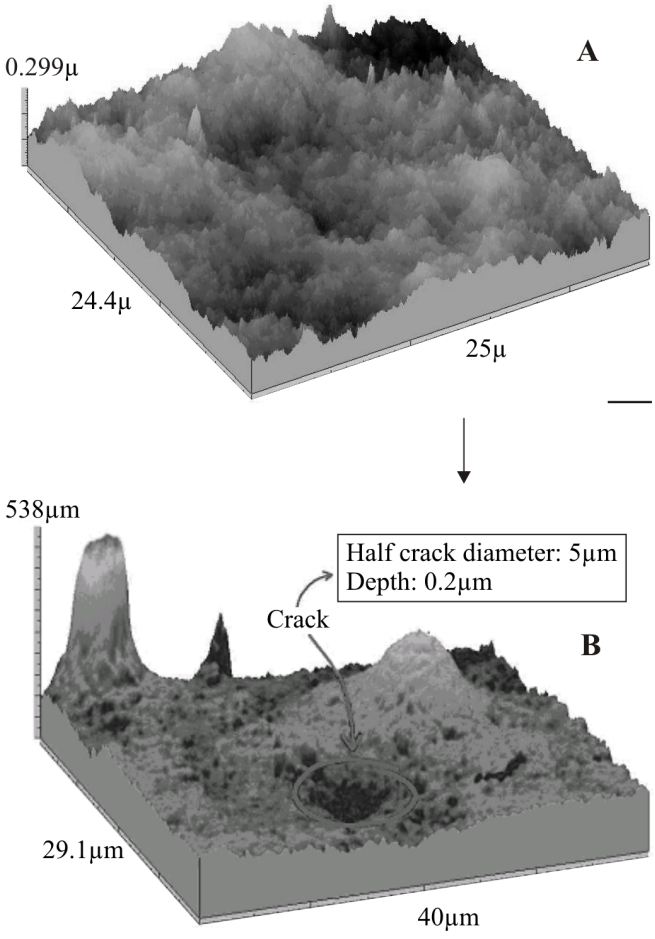

Figure 2. Low-density polyethylene surface microtopography obtained by atomic force microscopy: a) smooth areas and b) areas with cracks and crevices.

Table 1. Decimal reductions (DR), in seconds, for *Staphylococcus aureus* ATCC 25923 e *Escherichia coli* k12 cells adhered to low density polyethylene films after UV exposure for 2s, at 196 µW.cm^{-2}. Average of three repetitions.

Staphylococcus aureus		*Escherichia coli*	
Log IN	DR	Log IN	DR
4.6	0.85	5.0	0.52
5.7	1.23	6.1	0.73
6.8	1.73	7.5	1.37

IN = Number of cells in the adhesion suspension before UV exposure.

Table 2. Influence of the UV radiation intensity in the bactericidal efficiency on *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* K12 cells adhered to low density polyethylene films, after 2 seconds. Average of three repetitions.

Use time (h)	Intensity (mW.cm^{-2})	*Staphylococcus aureus* DR	*Escherichia coli* DR
70	144	1.04	0.94
1000	102	0.72	0.58
1500	78	0.58	0.36

Table 3. MPN of mesophilic aerobes (log.cm^{-2}) in the low-density polyethylene surface before and after UV radiation exposure at 137 µW.cm^{-2} for 2 seconds. Average of three repetitions.

Polyethylene films rolls	Log_{10} of the MPN in the non irradiated packages	Log_{10} of the MPN in the irradiated packages	Decimal Reduction
1	1.47	0.67	0.80
2	2.38	1.20	1.18
3	1.57	0.64	0.93
Average	1.80	0.83	0.97

DISCUSSION

The number of cells adhered to LDPE surface increased as the cell concentration in the suspension increased in the adhesion process. The efficiency of UV radiation was affected by the cell concentration. It appears that low cell numbers in the adhesion suspension lead to a better cell distribution over the surface and the cells can be protected from UV radiation if deposited in the cracks and crevices of the surface. Since the LDPE surface has limited capacity for bacterial protection, the radiation efficiency is higher when the surface is contaminated with high number of adhered cells.

The UV radiation was able to kill *S. aureus* and *E. coli* cells adhered to the LDPE surface, but its efficiency was not enough to cause the 3 DR required for physical and chemical sanitizers (7). The number of mesophillic aerobes on the user's LDPE surface decreased to 0. 014MPN.cm^{-2} after radiation, which is lower than 0.1MPN.cm^{-2}, as recommended for this kind of surface (4). The radiation intensity reduction occurring in the initial 100 hours of the germicidal lamp use was also reported by Fluckiger (4).

Food processing surfaces, such as polyethylene, have microtopography marked with cracks and crevices whose diameter and depth are large enough to protect microorganisms from UV radiation. The roughness of the LDPE surface can decrease UV radiation efficiency, because the bactericidal effect occurs only in the direction of the radiation beam (6). In this study, the LDPE microtopography surface analysis (Fig. 2A, B) showed irregularities. As the cell dimensions range from 0.5 to 1.5 µm for *S. aureus* and 1.5 to 2.0 x 6.0 for *E. coli*, it is possible that surface cracks and crevices of 5 µm diameter and 0.2 µm deep present on LDPE surface could protect these microorganisms from direct contact with UV radiation.

The results of this study showed that UV radiation is a good technique for bacterial decontamination of LDPE surfaces used for fluid food packaging. In addition, this technique is easily applicable, has low operational cost and does not leave residues on the radiated surface.

RESUMO

Avaliação da radiação ultravioleta no controle de microrganismos aderidos em filmes de polietileno de baixa densidade

A eficiência da radiação ultravioleta (UV) na redução do número células de *Staphylococcus aureus* ATCC 25923 e *Escherichia coli* k12 aderidas a filmes de polietileno de baixa densidade (PEBD) foi avaliada. Os microrganismos em suspensões foram aderidos à superfície de sacos de PEBD a 18ºC durante 12 horas, e, em seguida, submetidos à exposição de raios UV na intensidade de 196 µW.cm^{-2} a 254 nm, durante 2 segundos. *Staphylococcus aureus* foi menos resistente do que *E. coli* à ação da radiação UV. Após 1500 horas de uso, a intensidade da radiação UV reduziu-se de 288 para 78 µW.cm^{-2}, com declínio acentuado nas 100 horas iniciais. A eficiência da radiação UV na inativação dos microrganismos decresceu após 1500h de uso da lâmpada germicida. O numero de mesófilos aeróbios nas superfícies dos filmes de polietileno de baixa densidade (PEBD) foi reduzido em 90% após a irradiação com 137 µW.cm^{-2}. Fendas e elevações, observadas na superfície do PEBD através de microscopia de força atômica, podem proteger as células do contato com a radiação UV, reduzindo sua eficiência. Os resultados mostram que a radiação UV é uma técnica útil na redução da microbiota aderida à superfície de filmes de PEBD.

Palavras-chave: adesão bacteriana, radiação ultravioleta, polietileno de baixa de densidade e microscopia de força atômica

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