journal homepage: www.fia.usv.ro/fiajournal
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XII, Issue 1 – 2013, pag. 18 - 23

THE EFFECT OF INTENSE LIGHT PULSED TREATMENT ON *ASPERGILLUS FLAVUS* (MI 148) SPORES

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Received 17 January 2013, accepted 25 February 2013

Abstract: *Aspergillus are highly aerobic, widely spread moulds species that can be found in almost all oxygen enriched environments, where they commonly grow on the surface of a substrate. Aspergillus species are common contaminants of cereals and grow on many plants. Not all Aspergillus strains are toxicogenic and cause illnesses. However, some highly toxicogenic strains can produce mycotoxins as secondary metabolites, with a strong negative impact on human and animal health.*
Intense light pulsed treatment (ILP) is one of the minimal technologies that has been proven effective for killing a wide variety of microorganisms. The objective of this study was to understand the influence of different ILP conditions on mould spores survival. The ILP treatment was applied for lamp tensions 1400, 1600 and 1800 V, for different durations (2 – 30) x 10-3 s. The effect of the experimental conditions was expressed by counting the colonies and the results were modeled with nonlinear regression logistic equation using SAS System for Windows 9 software.

Keywords: *pulsed light, moulds, inactivation, Aspergillus*

1. Introduction

Aspergillus flavus is responsible for the spoilage of foods and feed, and known to cause decay of the stored fruits damaged by insects, animals, early splits, and mechanical harvesting. Furthermore, *A. flavus* is able o produce aflatoxins in foods and feedstuffs [1]. Ingestion of aflatoxin from contaminated feed affects animal health, and is potentially dangerous to humans as the toxin metabolites are excreted in animal milk, meat and eggs [2].
New, non-thermal preservation techniques are being developed with the aim of inactivating both pathogenic and spoilage micro-organisms, while having a minimal effect on the quality and nutritional characteristics of the food itself. The intense light pulse system (ILP system) is one particularly promising minimal processing technologies. However, there is a need to determine whether a specific microorganism of concern is capable of surviving the treatment and/or can give rise to any food safety problems. These safety aspects are important when considering all the potential implications for the entire food supply chain, from farm to table.
The ILP method, which uses intense short duration pulses of white light, is capable of reducing the microbial load, for both

vegetative cells and spores, on the surfaces of food and food contact materials [3].
The mode of action of the pulsed light process is attributed to the effect of the high peak power and the UV component of the broad spectrum of the flash. Inactivation occurs by several mechanisms: electroporation, including chemical modification and cleavage of DNA, protein denaturation and other cellular materials alteration [4]. The lethal effect of the pulsed light on spores is stronger with the increase of light intensity or fluence.
Anderson et al. [5] reported the following IPL susceptibility, in decreasing order: Gram (-) bacteria, Gram (+), bacteria and fungal spores. The colour of the spores can plays a significant role in fungal spore susceptibility.

Aspergillus niger spores are more resistant than *Fusarium culmorum* spores, which could be because the pigment of the *A. niger* spores absorbs more in the UV-C region than that of *F. culmorum* spores, protecting the spore against UV [5]. In contrast, other researchers [6] did not observe any sensitivity pattern among different groups of microorganisms, after studying 27 bacterial, yeast and mould species.
More body of research is needed to clarify the kinetic of inactivation of different moulds.
The purpose of the present work was to study the survival of *Aspergillus flavus* (MI 148) spores at different ILP treatments.

2. Experimental

A spore suspension of *Aspergillus flavus* was used.

Preparation of the spore suspension of Aspergillus flavus
The aflatoxigenic strain *Aspergillus flavus* (MI 148) studied was obtained from the University of Agronomical Sciences and Veterinary Medicine Bucharest. The inoculum was obtained by growing the mold on a slant of stock cultures of Malt extract agar (MEA), which was kept at 5°C. The spore inoculum was prepared by growing *Aspergillus flavus* on MEA for 7 days at 30°C and spores were harvested aseptically using 10 mL of sterile 0.01% v/v Tween 80 solution. The spore suspension was filtered through three layers of sterile cheesecloth to remove hyphae, under aseptic condition. Finally, the number of spores in the filtrate was adjusted to about 10^9 spores x mL^{-1} by adding the necessary amount of physiological saline solution. The initial numbers of spores in the suspension was counted by direct microscopic counting method using a Thoma counting chamber. The spore suspension used in all experiments was freshly prepared every day and stored in refrigerator at 4°C during the experiments.

Samples inoculation
Initial suspension of *Aspergillus flavus* was spreaded proportionately on 16 pieces of cover glass (20×20 mm, thickness of 0.15 mm), of which 14 pieces were used for experiments and 2 pieces for control (untreated with intense light pulse), with each piece having 50 μL of *Aspergillus flavus* suspension in the shape of a liquid drop. The drops were dried in sterilized air at 20°C. The ILP experiments were operated in air, under atmospheric pressure, at room temperature, respectively 20°C.

Treatment with ILP
A draw of the experimental equipment is presented in Fig. 1. In the experimental an IFP-800 flashes lamp (5) with discharge through xenon and the following

characteristics: electromagnetic field, λ = 200–1000 nm, impulse regime, $\tau = 10^{-1}$–10^{-4} s, light intensity E = 1000–8000 J was used. We considered that the spectral distribution is similar to that of sunlight having peak emission between 200 and 500 nm [7].

Cover glasses spread with *A. flavus* suspension were placed at the center of the wire sieve at a distance of 7 cm from the flash lamp and treated with ILP, excepting the control sample

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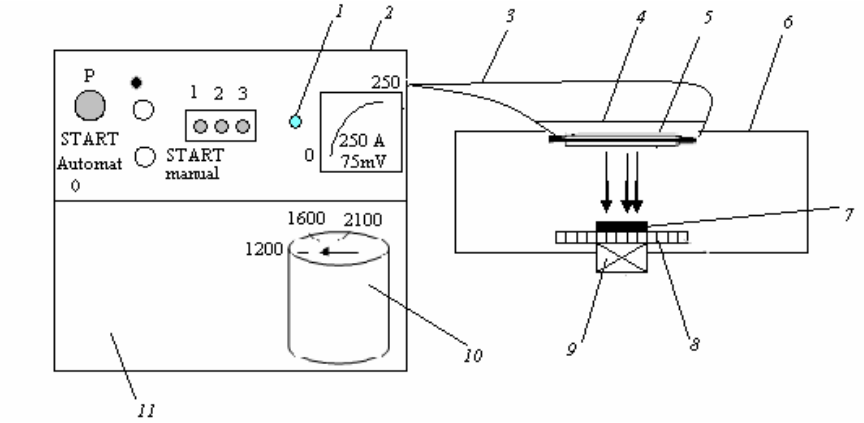

Figure 1. Pulsed light instalation 1 – led; 2 - pulse generator; 3 – control cable; 4– lid; 5- IFP-800 flash lamp; 6 – treatment space; 7 – glass pieces spreaded with mould spores; 8 – plate of wire sieve; 9 – support system; 10– energy autotransformer; 11 – system with five capacitors.

The intense flash pulsed lamp (5) IFP-800 is fixed above a plate of wire sieve (8) on which the sample (7) has to be treated. A pulse generator (2) feeds the lamp. The electrical energy is accumulated in an energy storage capacitor being multiplied by several times, rather than being released in a very short time in the IFP lamp as a very short and intense light pulse (flash).

The effect of the energetic density on the mould survival was determined with 1.364 – 2.256 J/cm^2 for the same time of exposure. The number of pulses was varied for the same energetic density: 2, 5, 10, 15, 20, 25, 30 pulses, respectively 2 x10^{-3} s, 5 x10^{-3} s, 10 x10^{-3} s, 15 x10^{-3} s, 20 x10^{-3} s, 25 x10^{-3} s, 30 x10^{-3} s.

Viability studies

The viable spores` number of *Aspergillus flavus* was determined after incubation of mould suspension and cfu/mL values were calculated. After the intense light pulse treatment each cover glass was introduced in 5 mL physiological saline solution, shacked on a rotary shaker (Lab. Companion SI-300R) for 300 s to completely remove the spores of *Aspergillus flavus* from the cover glass into the physiological saline solution.

The surviving colonies were then counted by inoculation into the Petri dishes with Malt extract culture medium. Afterwards the Petri dishes were incubated for 5 days at 25°C. The suspension was diluted until the cell number on the Petri dishes could be counted with the naked eye (with the number range of 15-300). All the treatments were replicated two times.

Statistical analysis

To model the results a logistic equation was used and non-linear regression was applied for the data set. SAS System for Windows 9, software was used and the fitness of data was evaluated.

$$\lg(N) = \lg(N_0) + \frac{a}{1+\exp(b-ct)} \qquad (1)$$

where: N=count x ml^{-1}; N_0 =initial count x ml^{-1} when t=0; t=time (milisec.), a, b and c are model parameters.

3. Results and Discussion

Intense light pulse treatment was applied at different energetic densities to demonstrate the viability of the microorganisms.
The decrease of viable spore number of *A. flavus* after 30x10^{-3}s at different energetic densities is shown in Fig. 2.
From the picture we can notice the obvious inactivation of *A. flavus* spores produced by IPL treatment. The inactivation of *Aspergillus flavus* spores at different energetic densities (1.364 J/cm^2, 1.782 J/cm^2 and 2.256 J/cm^2) for 2x10^{-3} s and 30 x 10^{-3} s of ILP treatment is presented in Fig. 3, respectively Fig. 4.
From the Fig. 3 and 4 it could be observed that there was a reduction of the microbial population with an increase of the energetic density, for the same duration of the ILP treatment.

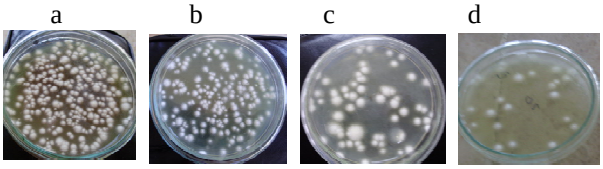

Figure 2. Photos of the Petri dishes with colonies of *A. flavus* control after before and after the IPL treatment at 30x10^{-3}s a) initial; b) 1.364 J/cm^2 ; c) 1.782 J/cm^2 ; d) 2.256 J/cm^2

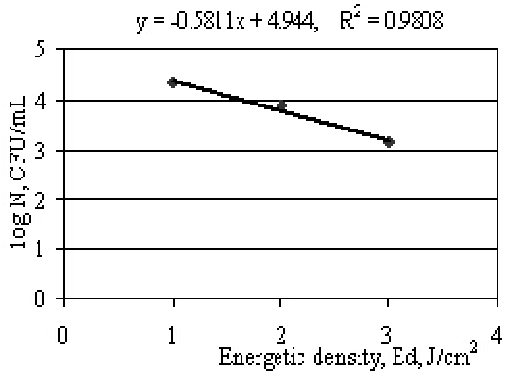

Figure 3. Viability variation of *A. flavus* spores against energetic densities for 30x10^{-3}s of ILP treatment

A one log reduction of the *A. flavus* spores was obtained with the increase of energetic density from 1 to 3 J/cm^2 when the treatment was applied for 30 x10 $^{-3}$ s and 0.5 log reduction for the same increase in energetic density, at a shorter time (2 x 10^{-3}s).

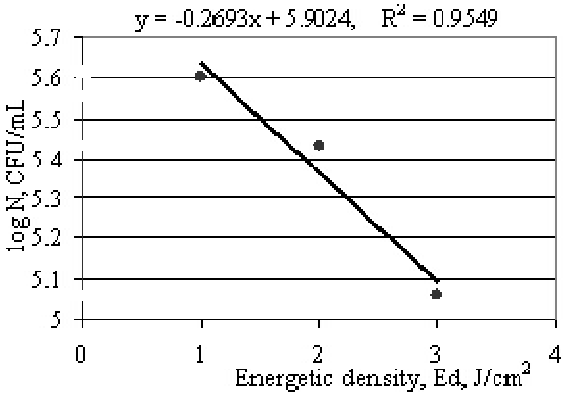

Figure 4. Viability variation of *A. flavus* spores against energetic densities for 2x10^{-3}s of ILP treatment

To compare the variation of viable spores number expressed as cfu/mL for all energetic densities applied during the intense light pulsed treatment Fig. 5 was plotted.

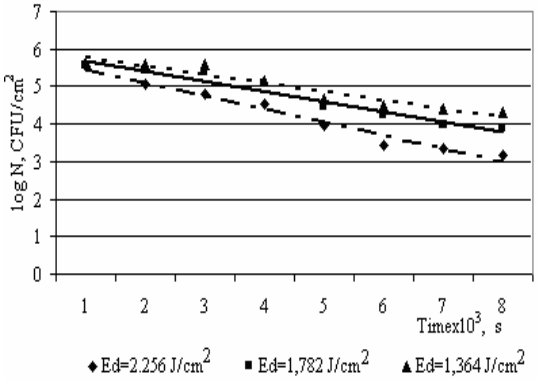

Figure 5. The variation of viability for *A. flavus* spores against all the energetic densities

From this graph it could be noticed that the lowest survival rate of mould spores after the treatment was registered for the 2.256 J/cm^2 energetic density and the highest survival rate was obtained for 1.364 J/cm^2. Thus, this means that a faster reduction of cells` number with the increase of the energetic densities could be obtained. For all the experimental results the R-square values (Table 1) were calculated.

From the Table 1 it can be observed the significant correlations coefficients obtained for the loglinear equations at 1400 V tension and very significant correlations at 1600 and 1800 V.

Table 1

The equations values for all energetic densities

Tension, U, V	*Energetic density, Ed, J/cm^2*	*R-square value*	*Linear equation*
1400	1.364	0.9272	y = -0.2235x + 5.987
1600	1.782	0.9556	y = -0.2745x + 5.9717
1800	2.256	0.9774	y = -0.3574x + 5.8363

The logistic equation was also applied to better describe the inactivation of *A. flavus* spores at different tensions. The nonlinear regression analysis using SAS System for Windows 9, software was applied. The convergence criterion was met for all experimental tension applied and the results are indicated in Table 2.

To check the significance of the parameters, Fisher factor (F) was calculated. For 1400 V a value of 601.84 was obtained, while for 1600 V an F value of 680.41 and for 1800 a smaller value was registered, namely 233.72 ($p< 0.05$).

Table 2

The equations values for all energetic densities

Tension, U, V	*Logistic equation*	*Parameter*	*STE (standard error)*	*Confidence limits*
1400	$\lg(N) = \lg(N_0) - \frac{1.2373}{1+\exp(4.1815 - 335.2t)}$	a	0.038	-1.3351…-1.1396
		b	0.5532	2.7596…5.6034
		c	48.8528	227.6…478.8
1600	$\lg(N) = \lg(N_0) - \frac{1.6595}{1+\exp(3.2525 - 252.6t)}$	a	0.0585	-1.8089…-1,5092
		b	0.3291	2.4067…4.0984
		c	28.7912	178.6…326.6
1800	$\lg(N) = \lg(N_0) - \frac{2.4605}{1+\exp(2.0495 - 182.1t)}$	a	0.1854	-2.9371…-1.9838
		b	0.3105	1.2514…2.8476
		c	37.7529	90.1744…274.0

In all the cases F was higher than F_{crit} and indicated a highly significance of the model. Table 2 presents the nonlinear regression equations and the parameters obtained for the survival of *Aspergillus flavus* spores.

When comparing 1400 V with 1600 V tension, almost the same rate of increase/decrease was observed in the a, b, and c parameters {1.3412; 0.777; 0.7151}, as for 1600 V compared with 1800 V, respectively {1.483; 0.6301; 0.7209}.

4. Conclusion

The research demonstrated that the intense light pulses treatment is inactivating the *Aspergillus flavus* spores at the surface.
The exposure time and the energetic density have a synergistic effect on spores` inactivation. Loglinear equations were used to model the inactivation at different energetic densities.
The logistic model was applied to predict the inactivation of the *Aspergillus* spores at different tensions from 1400V to 1600V and the model parameters were estimated.

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