

VARIATION OF AFLATOXIN M1 IN MILK AT DIFFERENT DOSES OF ULTRAVIOLET RADIATION

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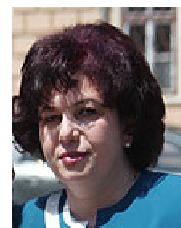
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REZUMAT. Lucrarea se bazează pe contaminarea artificială a laptelui de vacă, cu soluție de aflatoxină M1, cu o concentrație de 0,1(100 ng L⁻¹), de 2 ori mai mare decât nivelul admis de Directiva (CE) 1881/2006, având în vedere nivelul maxim de la contaminatorii normali din produsele alimentare (aflatoxina M1) și iradierea sa cu radiații UV pentru studierea și evaluarea detoxificării variației aflatoxinei M1 de la laptele contaminat artificial. Îndepărtarea aflatoxinei M1 din mostra laptelui contaminat cu radiații UV este încă satisfăcătoare și indică un tratament eficient.

Cuvinte cheie: aflatoxine, lapte, radiații UV, contaminare.

ABSTRACT: This research focuses on cow milk artificial contamination with aflatoxin solution M1, at a concentration of 0.1 ppb (100 ng L⁻¹), twice as much as the level admitted by the Regulation (CE) no. 1881/2006 regarding the maximum levels for certain contaminants from alimentary products, such as M1 aflatoxin and its irradiation with UV radiations for evaluation and study of detoxification variation of aflatoxin M1 from the artificial contaminated milk. Removal of aflatoxin M1 from contaminated milk sample by UV radiations is still satisfactory and indicates treatment efficiency

Keywords: aflatoxin, milk, UV radiations, contamination

1. INTRODUCTION

Mycotoxins are secondary metabolites produced by various fungi (Schmidt-Heydt & Geisen, 2007). Aflatoxins are fungal metabolites produced by three species of *Aspergillus*, namely *A. flavus*, *A. parasiticus* and *A. nomius*. *A. flavus* produces only B aflatoxins, while the other two species produce both B and G aflatoxins. One of the mycotoxins, aflatoxin M1 (AFM1) is the hydroxylated metabolite of aflatoxin B1 (AFB1) and can be found in milk and subsequently in other dairy products when lactating animals are fed with contaminated feedstuffs (Prandini, et al., 2009). About 1-2% of AFB1 in animal feed is transformed to AFM1 in milk; it may vary from animal to animal, from day to day and from milking to the next. 12-24 h after the first AFB1 ingestion, the toxin can be detected in the milk (Ebrahim 2010). The occurrence of aflatoxin M1 in milk is transitory in nature and reaches maximum within two days after the intake of the contaminated commodity (Hussain & Anwar, 2008).

Aflatoxins represent a serious risk for animal and human health, especially for children, who are the major milk consumers (Rosi, et al., 2007). The hepatotoxic, genotoxic, carcinogenic, teratogenic, immunosuppressive and antinutritional effects of aflatoxins are well documented (Wangikar, Dwivedi, Sinha, Sharma, & Telang, 2005; Williams, et al., 2004). Aflatoxins are considered to be human liver carcinogens, AFB1 being the most potent. AFM1 has a potency approximately one order of magnitude lower than that of AFB1 (Tajkarimi, et al., 2008)

The presence of AFM1 in milk and dairy products can be a potential threat to the health of consumers (Manetta, et al., 2009). Exposure to AFM1 through milk products is a serious problem for public health. Several countries have established regulatory limits for AFM1 in raw milk and milk products, which vary from country to country (Ruangwises & Ruangwises, 2010). At present, aflatoxin presence in feed, milk and dairy products can be systematically controlled in Europe and other developed countries (Yaroglu, Oruc, & Tayar,

2005). The European Community has set the maximum permitted level for AFM1 in raw milk and heat-treated milk at $0.05 \mu\text{g L}^{-1}$ (EC, 2006)

Various physical, chemical and biological agents have been used to detoxify aflatoxins from food and feed materials (Basappa & Shantha, 1996; Rustom, 1997). However, no universally applicable, effective and practical methods are currently available (Peltonen, El-Nezami, Salminen, & Ahokas, 2000). Ultraviolet (UV) energy can be used effectively to inactivate aflatoxin M1 in milk (Yousef & Marth, 1986). UV irradiation has been discovered for many years as an effective physical method to destroy aflatoxins for its photosensitive property (Liu, et al., 2010). Rate of degradation was a function of the film thickness and depth of penetration of the rays when operating conditions were held constant (Li & Bradley Jr, 1969).

Because aflatoxins are photosensitive (Liu, et al., 2010; Yousef & Marth, 1986), our aim was to study the detoxification variation of aflatoxin M1 in milk at different doses of ultraviolet (UV) energy at different volumes of milk.

2. MATERIALS AND METHODS

Sample preparation

Samples of milk come from various manufacturers of milk being purchased from the market and before starting the experiment milk was tested for presence of residual aflatoxin M1

Aflatoxin M1 was obtained from Sigma (St. Louis, MO), was prepared in a methanol/chloroform mixture, $10 \mu\text{g mL}^{-1}$ concentration and is kept frozen at -20°C . Because AFM1 is photosensitive, the solutions were stored in aluminum foil-wrapped vials, and were not exposed to daylight or placed close to fluorescent light sources. The solution of aflatoxin M1 was added (with stirring) to each sample to provide a calculated concentration of M1 in the milk of 0.1 ppb (100 ng L^{-1}) level at twice the maximum permitted by EC (2006). The samples were stored frozen in the dark until used.

Irradiation of samples

Ultraviolet Apparatus: An apparatus to irradiate milk was assembled as illustrated in **Figure 1**. The source of UV energy was a UV lamp (Suzhou Xicheng Lighting Co., Ltd, Jiangsu, China) with a

power of $0.05 \mu\text{W m}^{-2}$. The main wavelength of the lamp is 365 nm. The lamp was either rested on the edge of the glass tray or elevated to give a distance between lamp and milk layer of 25 mm. The sample has been inserted between two parallel plates (a plate of stainless steel and glass plate).

UV Treatment: Different volumes of contaminated milk sample (10 mL to 70 mL) was exposed to UV irradiation (365 nm) for 1 min and residual AFM1 content was measured at the end of each exposure. The sample was placed in experimental device (Figure 1) at varying distances from a 1 to 7 mm. Experimental data on inactivation of aflatoxin M1 in sheets were passed at different radiation doses (DOSE 1 = $0.0125 \mu\text{W m}^{-2}$ equivalent to 25% the lamp power), (DOSE 2 = $0.0250 \mu\text{W m}^{-2}$ equivalent to 50% of lamp power), (DOSE 3 = $0.0375 \mu\text{W m}^{-2}$ equivalent to 75% lamp power) respectively (DOSE 4 = $0.0500 \mu\text{W m}^{-2}$ equivalent to 100% of lamp power). Meanwhile, control experiments in the dark (blank experiments) under the same conditions were carried out in parallel for comparison without the application of light or AFM1.

Analysis of aflatoxin

Aflatoxin AFM1 concentrations of all samples was carried out with a commercial competitive ELISA kit described by (R-Biopharm, 2007). A suitable sample aliquot was centrifuged at $2.800 \times g$ at 4°C for 10 min, and 0.1 mL of the supernatant was used for the ELISA determination, which was carried out as recommended by the kit supplier. The absorption intensity was found to be inversely proportional to AFM1 concentration in the sample.

Statistical analysis

All analytical determinations were performed at least in triplicate. Values of different parameters were expressed as the mean \pm standard deviation. Statistical analysis was conducted using the IBM SPSS Statistics version 20 packet program for Windows (SPSS, Chicago, IL, USA). Data obtained were subjected to analysis of variance (ANOVA). Means were considered statistically different at 95% confidence levels. Where there were significant differences between the means, the new Duncan's multiple range test (Daniel & Cross, 2009) was used to separate them.

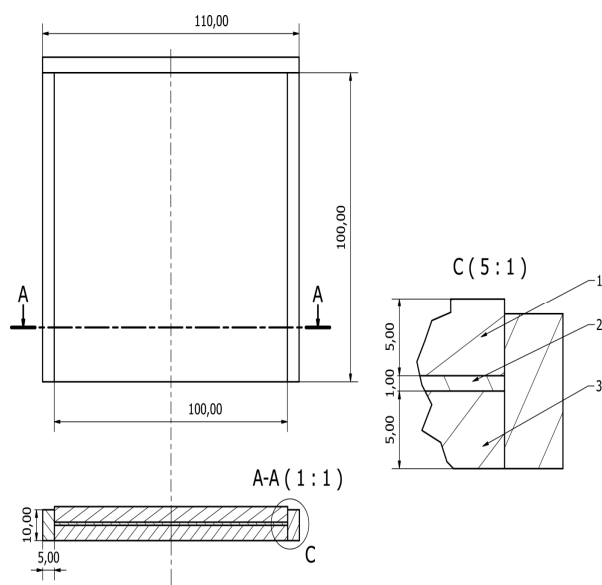


Fig. 1 Detailed figure of the experimental device: 1- glass plate (100 x 100 x 5 mm), 2 – layer of milk (1-7 mm), 3- stainless plate (100 x 100 x 5 mm)

3. RESULTS

Table 1 The variation of the efficiency of inactivation of *M₁* aflatoxin at different volumes of milk

Layer of milk between plates mm	Volume of milk mL	DOZE 1 UV $\bar{x} \pm S_r^*$ ng L ⁻¹	DOZE 2 UV $\bar{x} \pm S_r^*$ ng L ⁻¹	DOZE 3 UV $\bar{x} \pm S_r^*$ ng L ⁻¹	DOZE 4 UV $\bar{x} \pm S_r^*$ ng L ⁻¹
1	10	42.2	29.5	12.5	2.7
2	20	50.7	37.3	30.6	10.4
3	30	50.3	40	30	12.7
4	40	51.8	40	31	12.6
5	50	51.6	40.1	31.1	12.7
6	60	52.6	42.1	33.1	15.8
7	70	53.7	43.2	34.2	16.9

* Standard deviation, calculated from results generated under repeatability conditions.

4. DISCUSSION

Table 1 shows the results for AFM1 found.

These results are comparable o what was observed by xyz.

Table 2, it is observed that the total aflatoxin level was significantly decreased to 77% in 30 min ($P < 0.05$) and to 87.8% in 1 h ($P < 0.01$) upon UV exposure.

Aflatoxins are reported to be sensitive to UV radiation and may lead to the formation of less toxic photo-degradation products (Tripathi & Mishra, 2010) The efficiency of irradiation for:

DOSE 1 UV, which corresponds to a power of $0.0125 \mu\text{W m}^{-2}$, equivalent to 25% of the light bulb, the efficiency in the first case is of 57% when the layer of milk between the two plates is 1mm; in other cases, the efficiency is low, so that the inactivation of M1 aflatoxin is made in proportion from 40% to 50%.

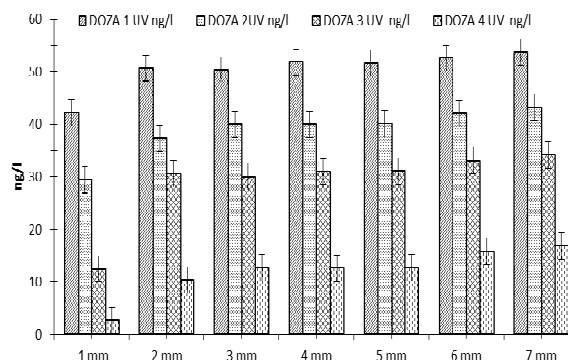


Fig. 2. The variation of inactivation at different degrees of irradiation

- DOSE 2 UV, which corresponds to a power of $0.0250 \mu\text{W m}^{-2}$, equivalent to 50% of the light bulb, the efficiency in the first case is of 70% when the layer of milk between the two plates is 1mm; in other cases, the efficiency is from 56% to 63%.

- DOSE 3 UV, which corresponds to a power of $0.0375 \mu\text{W m}^{-2}$, equivalent to 75% of the light bulb, the efficiency in the first case is of 88% when the layer of milk between the two plates is 1mm; in other cases, the efficiency is from 65% to 71%.

- DOSE 7 UV, which corresponds to a power of $0.0500 \mu\text{W m}^{-2}$, equivalent to 100% of the light bulb, the efficiency in the first case is of 96% when the layer of milk between the two plates is 1mm; in second case, the efficiency is 89%, while in the other cases the efficiency is from 82% to 85%.

5. CONCLUSIONS

The experimental data obtained in this research gives valuable information regarding some parameters of the process of treating milk with UV radiations in order to inactivate aflatoxin M₁.

Treating milk with UV can be a viable alternative for the elimination or the reduction of the levels of undesired microorganisms in the detriment of current thermal treatments. However, UV radiation can affect the characteristics of the product by generating free radicals and organic photochemical reactions in the case in which the doses of irradiations are not properly controlled.

The study of the inactivation method will lead to enhancing this method to an industrial scale through the use of UV reactors, so that the kinetic parameters of inactivation of aflatoxin M₁ in these reactors will be more reliable and more precise than the parameters obtained through traditional methods

Encouraging the widespread use of these techniques to reduce milk and feed aflatoxin will lead to lower consumption of contaminated milk and dairy product quality and human health.

6. ACKNOWLEDGEMENT

The authors are grateful to The Executive Unit for Financing Higher Education, Research, Development and Innovation (UEFISCDI), Romania, for access to information resources available through the ANELiS project.

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