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UV LED Light Efficiency in Aflatoxin M1 Reduction in Bovine Milk

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Abstract

Bovine milk is a food consumed by people of different age groups and especially by children, as it provides essential nutrients to the diet. In Brazil, milk is one of the most important products of Brazilian agriculture, being essential in the generation of employment and income. However, when there is contamination by toxic agents, such as aflatoxin (AFL) which is carcinogenic to humans and animals, there is a risk to the health of the consumer. Therefore, the objective of this work was to analyze the efficiency of the application of UV LED light as a tool to reduce aflatoxin M1 (AFM1) in bovine milk produced in the state of Amazonas, Brazil. Concerning time in 3-, 6- and 9-minutes, the reduction rates of 75.5, 97.3, and 94.1% were observed, respectively. The use of UV LED light was effective in reducing the concentration of AFL studied to be evaluated on a production scale.

Keywords: HPLC, mycotoxin, dairy food

1. Introduction

Bovine milk is an important source of protein in human nutrition, as it provides macro and micronutrients for growth and maintenance of health. The composition of bovine milk is influenced by genetic factors, stage of lactation, health and nutritional status of the animal and type of technological treatments (Ghafoori et al., 2022). It has economic importance as a source of income and its quality must meet health safety parameters, such as the presence of toxic agents.

Safety in its production has raised research for health protection, as both the domestic market and exports can be affected by mycotoxins, which are carcinogenic substances for humans and animals. It is estimated that more than 25% of world agricultural production is contaminated with mycotoxins and the occurrence of mycotoxins in milk has been studied over the world (Hasninia et al., 2022; Hassan et al., 2018).

The most important mycotoxins in food are aflatoxins (AFL), produced by species of the genus *Aspergillus*, for example. Aflatoxin B1 (AFB1) is considered the most potent with mutagenic, teratogenic, and carcinogenic action. When animals ingest contaminated food, AFL are metabolized, biotransformed and transferred to products, such as milk, in the form of aflatoxin M1 (AFM1), thus becoming a risk to human health. The quantification of this transfer provides information about the relationship of the levels of contamination in feed and the resulting contamination in milk (Zentai et al., 2023).

In this way, the studies of interest in AFM1 in milk identified that the pasteurization, sterilization, preparation, and storage of products derived from milk is stable. In Brazil, there is little research on AFM1 in bovine milk, with studies in the states of São Paulo and Paraná (Dos Santos et al., 2016; Shundo et al., 2016). In pure state, AFL are extremely stable at temperatures up to 200°C and are not affected by cold. Therefore, when subjected to various heat treatments, these were not efficient for the degradation of these mycotoxins. However, the literature describes the ability of an optical source such as light emitting diode (LED) to decrease the concentration of AFM1 in milk samples as a promising alternative for the reduction of AFL in contaminated food (Nguyen et al., 2022).

Therefore, the objective of this work was to evaluate the efficiency of UV led light in contaminated samples of bovine milk as a pilot evaluation for future application on an industrial scale.

2. Method

Sampling: bovine milk samples, free of AFM1, obtained directly from producers in the state of Amazonas-Brazil, with previous analysis by high performance liquid chromatography (HPLC) were analyzed. The samples (N=3) in triplicate were contaminated with AFM1 standard, divided for three different times of duration of application of the UV LED light, corresponding to 3, 6 and 9 minutes. After application of the UV LED light system, the samples were quantified using high performance Liquid Chromatography (HPLC). The temperature was monitored using ice, placed on the base of the container to a depth of approximately 5 mm underneath the milk samples. In 3 and 6 min the temperature stayed between 20 and 25°C, and in 9min it reached 35° C.

2.1 AFM1 Quantification:

The samples were analyzed in triplicate, according to AOAC (2016): for extraction 100mL of the filtered and centrifuged sample. Then, the fat layer was removed, and the skimmed milk submitted to the Aflastar immunoaffinity column (Romerlabs®). In the columns, the samples were washed twice with 10 mL of water and pressed air to remove residual water from the column. The eluent solution used was Acetonitrile 1 mL – Methanol 50%-50% and the solvent was evaporated under nitrogen flow at 50 °C in an evaporator and the residue reconstituted in 0.5 mL of mobile phase to be injected into the liquid chromatograph. The chromatogram of the AFM1 standard is shown in Figure 1.

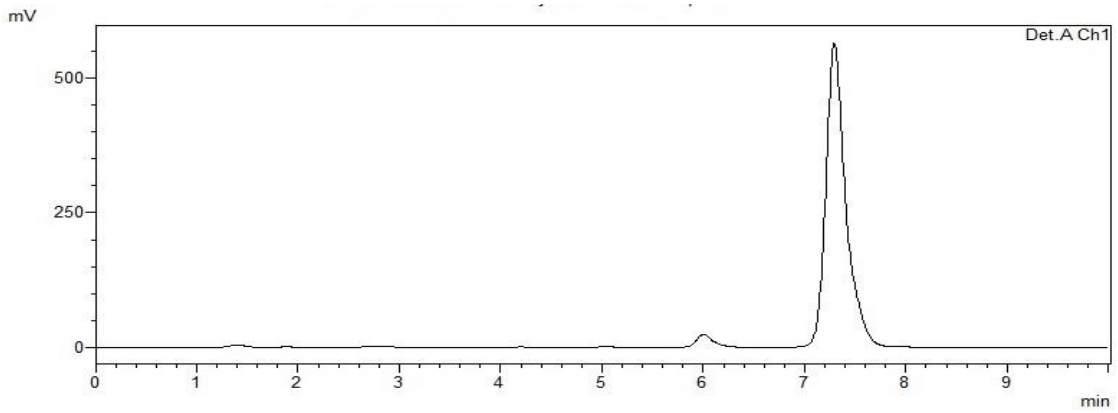

Figure 1. Chromatogram of AFM1 standard

2.2 Validation

A calibration curve was created to determine linearity and interval, with all injections performed in duplicate. Solutions were prepared at concentrations of 0.125, 0.5, 1.9, 2.5, 5.0 and 10.0 μg/mL. According to Brazilian legislation (Brasil, 2017), the correlation coefficient must be > 0.990, and in we obtained a value of 0.9966 being, which is therefore in accordance with the recommendations.

The working range at standard concentrations from 0.125 to 10 µg/mL enabled the detection of AFM1 within the limits of interest. The limit of detection (LD) was obtained with successive known concentrations of standard solution, injected in decreasing order to obtain the limit of detection, which was 0.0625 µg/ml. Figure 2 shows the LD in the chromatogram.

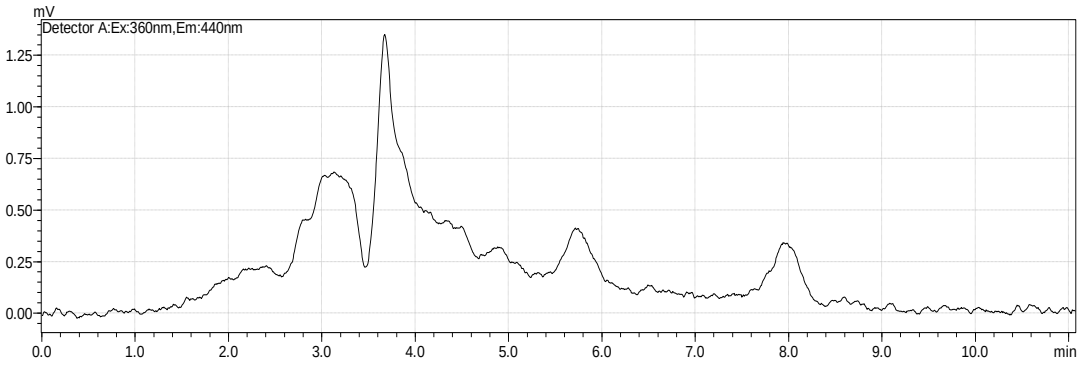

Figure 2. Chromatogram of the limit of detection for AFM (7.977 min. of retention time).

2.3 UV LED Light

The adapted method of Stanley (2020) was applied, with 50 ml added in 250 ml glass beakers to provide greater possibility of contact (depth=5 mm). For light radiation, a beam system was used as an UV LED light source producing a radiation of 365nm. Measured by a high sensitivity system to measure the irradiance of the UV LED light system on the surface of the test solution.

2.4 Acidity %

The milk acidity was quantified by titration with NaOH according to the AOAC 947.05 method (AOAC, 2016), to assess whether the variable would be altered in such a way as to impair the quality characteristics of bovine milk.

3.Results and Discussion

3.1 UV LED Light

There was a decrease in the AFM1 content, and the average result is reported in Table 1, indicating the duration of the zero-time light-emitting diode. The results with a reduction of 75.5, 97.3, and 94.1%, respectively for the time ranges of 3, 6 and 9 min.

Table 1. Performance of UV LED application in AFM1 decreasing in bovine milk.

Variable	**Time (min)**			
	0	3	6	9
AFM1 Concentration (µg/Kg)	1352.8	331.4	36.5	37.34

To illustrate the contamination of the study, figure 3 shows the chromatogram of AFM1 at times 0 and the time of 3 min. of application of the UV LED light.

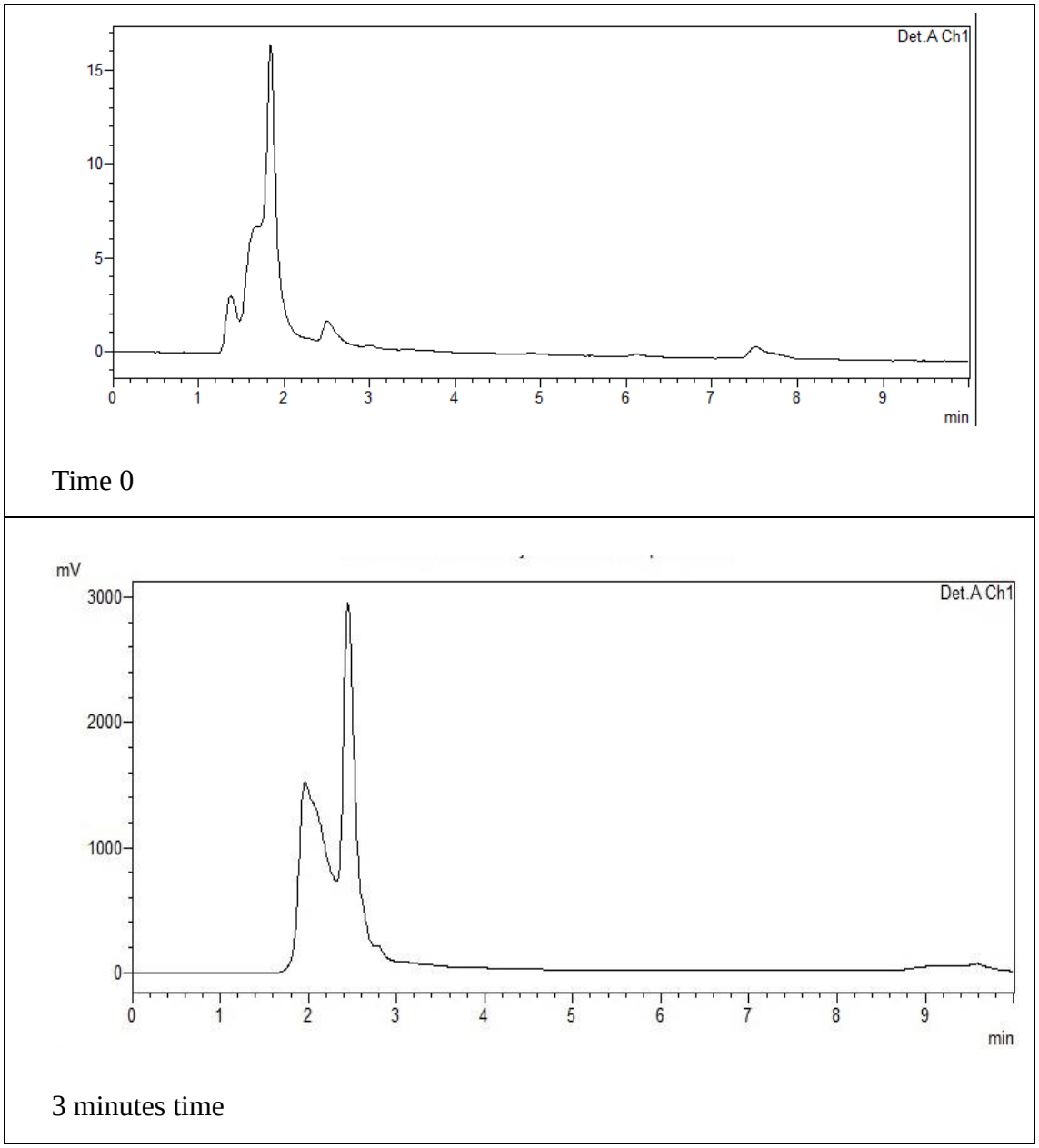

Figure 3. Chromatogram of AFM1 in different time of UV LED light application

The data obtained were more efficient than reported by Kurup et al. (2022) who applied the UV LED light technique (365 nm) in whole bovine milk and obtained a reduction of AFM1 of 65.7±1.65% (at 857 mJ/cm^2), in relation to control. In the work by Nguyen et al (2022) the authors used UV light at 254 nm and it was found that short-wave ultraviolet radiation (UVC) reduced up to 50% of AFM1 in milk after 20 min of treatment regardless of the initial AFM1 contamination level. Treatment time, depth of samples, and stirring were all found to significantly ($p < 0.05$) enhance the reduction of AFM1.

Other methods have also been used to mitigate AFM1 contamination in milk such as the use

of lactic acid bacteria. Seyedjafarri (2021) used *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. Bulgaricus (1:1) and bacteria showed the higher binding ability between 90- 100% AFM1 in milk samples. Wochmer et al. (2019) used the probiotic *Lactobacillus acidophilus* to reduce AFM1 Comparing with the positive control, the AFB1 bioaccessibility ranged from 23.68 to 72.67% and for AFM1 was 0%. The probiotic, isolated or combined with prebiotics, was efficient in mycotoxin reduction. In addition, research by the authors cited above needed about of treatment/incubation to obtain the reduction of AFM1, while using the light emitting diode brings efficient rates using 3min.

The UV LED light technique as an AFM1 mitigation method can help contamination prevention actions based on data that there is AFM1 contamination in bovine milk in Brazil. Becker-Algeri et al. (2020) analyzed samples from southern Brazil and detected AFM1 in 13.2% of the samples. Goncalves et al. (2017) indicated that AFM1 is present in bovine milk in 40.4% of samples from small rural properties in southern Brazil. Considering that AFM1 contamination may be present in different Brazilian geographic regions, mitigation actions are necessary, such as good practices in animal feeding management (Jiang et al., 2021).

3.2 Acidity%

Considering that the use of LED UV light could change other characteristics of the milk, the acidity content was analyzed at each time of the test. According to Brazilian legislation, milk acidity is a quality and safety parameter that must be between 0.14 and 0.18 expressed in grams of lactic acid/100 mL (Brasil, 2018). According to the legislation, the range for acidity in is 0.14-0.18 g of lactic acid/100 mL and at the end of the test, the 9-min sample showed 0.16 g of lactic acid/100mL.

The fact that there is a change in the acidity values can be attributed to the heat treatment to which the milk was indirectly submitted during the application of the LED UV light. On the other hand, when milk has its temperature increased and is stored, the carbohydrate present is degraded and converted into acids, which explains why the change in the titratable acidity value occurred. Also, since LED UV light could make environments and products free of contaminants, when applied to milk samples, it is possible that there was a decrease or not of microorganisms present, interfering with acidity (Mubeen et al., 2020).

As recommendation, we suggest a systematic control program for safe livestock feed to be introduced by public health authorities (de Freitas et al., 2018). By monitoring the analysis of the samples, it will be possible to express the level of contamination of AFL present in milk and its derivatives from samples collected according to the region, milk producer, before and after treatment with UV LED light in bovine milk to the reduction of AFM1 without altering its properties such as acidity and protein content.

4. Conclusion

The efficiency of UV LED light in reducing the concentration of AFM1 in samples of bovine milk using 3, 6 and 9min was tested. The results showed that there was a percentage reduction of over 75% in all tests, showing the possibility of using it to reduce the risk of this mycotoxin. The results for acidity attended the limit according to the Brazilian regulation.

However, there are few works associated with ways to reduce AFM1 in milk and its derivatives, with a proposed method using exposure to UV LED light. Therefore, further studies are needed to assess the level of mycotoxin degradation in modified mycotoxins as future work.

References

AOAC- Association of Official Analytical Chemists (2016). Acidity of Milk, Titrimetric Method AOAC Official Method 947.05 Association of Official Analytical Chemists, Washington, DC.

Becker-Algeri, T. A., Souza, C., Bortoli, K., Castagnaro, D., Scaglioni, P. T., Drunkler, D. A., Dors, G., Valderrama, P. & Badiale-Furlong, E. (2020). Seasonal variation of milk quality: Physicochemical, microbiological, and toxicological, *J. of food safety*, *40*, 12662. https://doi.org/10.1111/jfs.12796.

Brasil (2019). *Ministério Agricultura, Pecuária e Abastecimento. Instrução Normativa N° 76, de 26/11/2018*. Aprova os Regulamentos Técnicos que fixam a identidade e as características de qualidade que devem apresentar o leite cru refrigerado, o leite pasteurizado e o leite pasteurizado tipo A. Edição 230 Seção 1, p. 9., Brasília (DF). 30/11/2019, 6p.

de Freitas, C. H., Gonçalves, C. L., & Nascente, P. S. (2018). Aflatoxins B1 and M1: risks related to milk produced in Brazil. *Annals of Microbiology*, *68*, 793-802. https://doi.org/10.1007/s13213-018-1395-2

Dos Santos J. S., Granella, V., Pigatto, G. M., Reiniger, L. R. S., & Costabeber, I. H. (2016). Aflatoxin M 1 in pasteurized and raw milk from organic and conventional systems. *J. für Verbraucherschutz und Lebensmittelsicherheit*, *11*, 299-304. https://doi.org/10.1007/s00003-016-1039-z.

Ghafoori, Z., Tehrani, T., Pont, L., & Benavente, F. (2022). Separation and characterization of bovine milk proteins by capillary electrophoresis-mass spectrometry. *Asian J. of Dairy and Food Research*., *40*, 30-34. https://doi.org/10.1002/jssc.202200423

Gonçalves, L., Dalla Rosa, A., Gonzales, S. L., Feltes, M. M. C., Badiale-Furlong, E., & Dors, G. C. (2017). Incidence of aflatoxin M1 in fresh milk from small farms. *Food Sci. Technol*., *37*, 11-15. https://doi.org/10.1590/1678-457X.06317

Hasninia, D., Salimi, G., Bahrami, G., Sharafi, K., Omer, A. K., Rezaie, M., & Kiani, A. (2022). Human health risk assessment of aflatoxin M1 in raw and pasteurized milk from the Kermanshah province, Iran. *J. of Food Composition and Analysis*, *110*, 104568.

Hassan, Z. U., Al-Thani, R., Atia, F. A., Almeer, S., Balmas, V., Migheli, Q., & Jaoua, S. (2018). Evidence of low levels of aflatoxin M1 in milk and dairy products marketed in Qatar. *Food control*, *92*, 25-29.

Jiang, Y., Ogunade, I.M., Vyas, D., & Adesogan, A. T. (2021). Aflatoxin in Dairy Cows: Toxicity, Occurrence in Feedstuffs and Milk and Dietary Mitigation Strategies. *Toxins*, *13*, 283. https://doi.org/10.3390/ toxins13040283

Mubeen, R., Hassan, A., Rashid, Z., Nisa, S. U., Saeed, F., Afzaal, M., & Tufail, T. (2020). Comparative Analysis of UV Treatment and Heat Treatment on Fatty Acids Profile of Milk. *Research Square*. https://doi.org/10.21203/rs.3.rs-42271/v1

Nguyen, T., Palmer, J., Loo, T., Petcu, M., Newson, H. L., & Flint, S. (2022). Investigation of UV light treatment (254 nm) on the reduction of aflatoxin M1 in skim milk and degradation products after treatment. *Food Chemistry, 390,* 133165. https://doi.org/10.1016/j.foodchem.2022.133165

Seyedjafarri, S. (2021). Detoxification of Aflatoxin M1 in Milk by Lactic Acid Bacteria. A*sian J. of Dairy and Food Research*, *40*, 30-34. https://10.18805/ajdfr

Shundo, L., Almeida, A. P., Alaburda, J., Lamardo, L. C. A., Navas, S. A., & Ruvieri, V. (2016). Occurrence of aflatoxin M1 in bovine milk samples consumed in different regions of Brazil. *Rev Inst Adolfo Lutz, 75*, 1704.

Stanley, J., Patras, A., Pendyala, B., Vergne, M. J., & Bansode, R. R. (2020). Performance of 607 a UV-A LED system for degradation of aflatoxins B 1 and M 1 in pure water: kinetics 608 and cytotoxicity study. *Sci. Rep., 10*, 1-12. https://doi.org/10.1038/s41598-020-70370-x

Wochmer, K. F., Moreira, M. C. C., Kalschne, D. L., Colla, E., & Drunkler, D. A. (2019). Detoxification of Aflatoxin B_1 and M_1 by Lactobacillus acidophilus and prebiotics in whole cow's milk. *J. of Food Safety, 39*. https://doi.org/10.1111/jfs.12670

Zentai, A., Józwiak, A., Süth, M., & Farkas, Z. (2023). Carry-Over of aflatoxin B1 from feed to cow milk- a review. *Toxins, 15*, 195. https://doi.org/10.3390/toxins15030195