USE OF PULSED ELECTRIC FIELDS (PEF) TO MITIGATE AFLATOXIN B1 IN FRUIT JUICE-MILK BASED BEVERAGES

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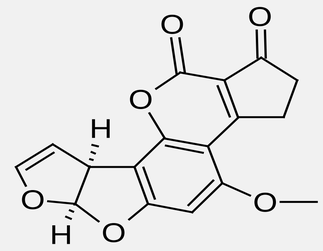
Introduction

Consumers' demand for fresh fruits and vegetables has increased over the last years seeking healthy beneficial effects attributed to their high content in micronutrients and bioactive compounds with antioxidant and free-radical scavenging properties. In order to obtain fresh-like products, several innovative food processing technologies have emerged such as pulsed electric fields (PEF) (Sánchez-Moreno et al., 2009). PEF technology involves the application of electrical treatments of different electric field strength (1–40 kV/cm) for short periods of time to a product placed between two electrodes. PEF treatment constitutes an effective tool for inactivating microorganisms at low temperatures with a minimum impact on food nutritional and functional characteristics (Knorr et al., 2011; Gabrí´c et al., 2018). Compared with thermal treatments, PEF-processed juices allowed for more retention of biologically active compounds such as vitamins, carotenoid, anthocyanins, lycopene, ascorbic acid and organoleptic characteristics. PEF has been applied in food industry to sterilize foods such as vegetables, fruit juices, milk, and liquid eggs (Knorr et al., 2011). More recently, these technologies have been explored by various authors as useful tool for removing foods contaminants, such as mycotoxins (Vijayalakshmi et al., 2017 and 2018; Gavahian et al., 2020). Mycotoxins are toxic natural contaminants of food and feeds produced by various fungi and are linked with a variety of adverse health effects in humans and animals. *Aspergillus* genera is responsible of aflatoxins (AFs) production, being AFB1 among the most potent mutagenic and carcinogenic substances known (Marín et al., 2013).



AFB1

Objective

The aim of the present study is to explore the potential of PEF technology on AFB1 reduction in fruit juice milk-based beverages and to compare it with the effect of the traditional thermal processing.

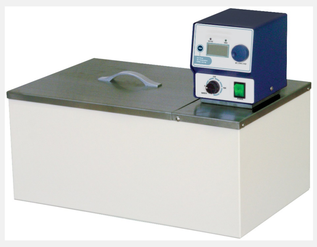
Materials and Methods

Samples preparation and treatment

Orange juice/ milk and strawberry juice/milk beverages were prepared and spiked with AFB1 at concentration of 100 µg/L, then samples were treated by PEF under conditions of field strength of 3 Kv /cm and specific energy of 500 KJ/kg. The effect of thermal treatment at 90 °C during 21 s has also been explored.

Table 1. Quantities for the ingredients of the different formulations for 100 mL.

Ingredients	Orange juice/milk beverage	Strawberry juice/milk beverage
Fruit juice	30 mL	30 mL
Skim milk	20 mL	20 mL
Bottled water	50 mL	50 mL
Pectine	0.3 g	0.3 g
Sugar	7.5 g	7.5 g
Citric acid	0.1 g	0.1 g

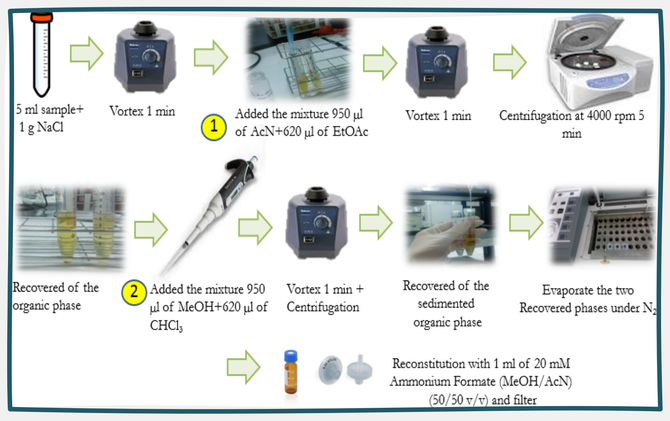


Thermal treatment

PEF treatment

DLLME extraction

Figure 1. Mycotoxins extraction procedure.



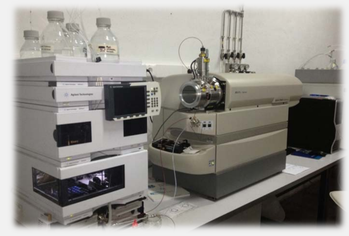
HPLC-MS/MS-IT determination

Table 2. Chromatographic conditions.

Column	Gemini NX C18 (150mm x 4,6mm 5µm)
Flow	0, 25 ml/ min
Injection volume	20 µl
Mobile phase	Mobile phase A: H_2O 5mM Ammonium Formate 0.1% Formic Acid Mobile phase B: MeOH 5mM Ammonium Formate 0.1% Formic Acid

Table 3. Quantification and confirmation transitions of AOH monitored fragments, retention time (Rt) and analytical parameters obtained.

Mycotoxin	Quantification Transition	Confirmation Transition	Rt	SSE (%)	LOD	LOQ	Recovery (%)		
							50	100	200
	m/z	m/z	min		µg/L	µg/L	µg/L	µg/L	µg/L
AFB1	313 > 285	313 > 241	7.41	48	0.3	1.0	111	64	115



Results

Figure 2. Chromatogram of the AFB1 in orange juice/milk beverage treated by PEF vs. non-treated.

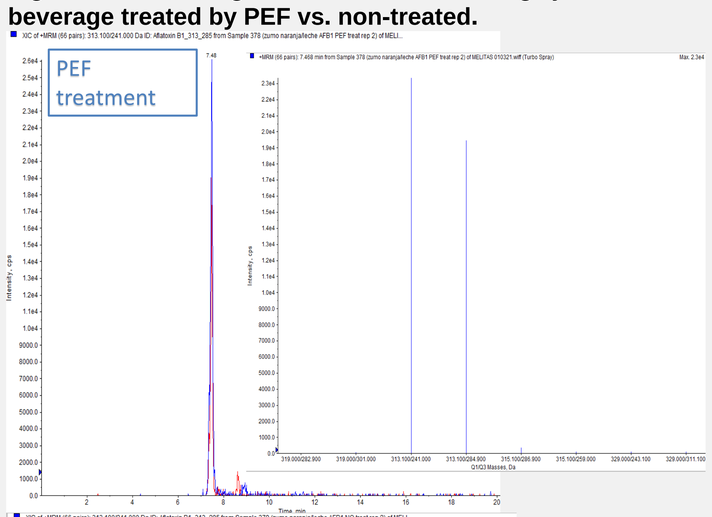


Figure 3. Percentages of AFB1 reduction (%) after PEF treatment.

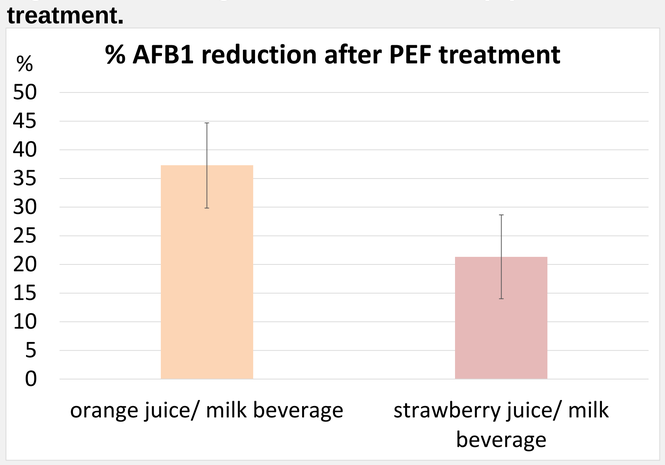


Table 4. Contents of AFB1 obtained after PEF and thermal treatments in different fruit juice milk-based beverages spiked at 100 µg/L.

Mycotoxin	Contents (µg/L) after PEF Treatment		Contents (µg/L) after thermal treatment	
	Orange juice/milk beverage	Strawberry juice/milk beverage	Orange juice/milk beverage	Strawberry juice/milk beverage
AFB1	63±7	79±7	100.00±4	100.00±4

Conclusions

- **The results revealed a significant AFB1 reduction after PEF treatment, with reduction percentages up to 37% in orange juice/milk beverage and 21% in strawberry juice/milk beverage.**
- **Thermal treatment did not reach any AFB1 reduction in both juice models, being PEF technology more effective in AFB1 mitigation.**
- **PEF treatment could be an effective tool in AFB1 mitigation.**

Acknowledgements

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References

Gabrí´c, D.; Barba, F.; Roohinejad, S.; Gharibzahedi, S.M.T.; Radoj˘cin, M.; Putnik, P.; Bursa´c Kova˘cevi´c, D. (2018). Journal of Food Process Engineering, 41, e12638. https://doi.org/10.1111/jfpe.12638.

Gavahian, M., Pallares, N., Al Khawli, F., Ferrer, E., Barba, F. J. (2020). Trends in Food Science & Technology, 106, 209-2018. https://doi.org/10.1016/j.tifs.2020.09.018.

Knorr, D., Froehling, A., Jaeger, H., Reineke, K., Schlueter, O., Schoessler, K. (2011). Annual review of food science and technology, 2, 203-235.

Marin, S., Ramos, A. J., Cano-Sancho, G., Sanchis, V. (2013). Food and Chemical Toxicology, 60, 218-237. https://doi.org/10.1016/j.fct.2013.07.047.

Sanchez-Moreno, C., De Ancos, B., Plaza, L., Elez-Martinez, P., Cano, M. P. (2009). Critical reviews in food science and nutrition, 49(6), 552-576. https://doi.org/10.1080/10408390802145526.

Vijayalakshmi, S., Nadanasabhapathi, S., Kumar, R., Kumar, S., Reddy, R. (2017). Journal of Food Processing and Preservation, 41, e13230, doi:10.1111/jfpp.13230.

Vijayalakshmi, S., Nadanasabhapathi, S., Kumar, R., Kumar, S.S. (2018). Journal of Food Science and Technology, 55, 868–878, doi:10.1007/s13197-017-2939-3.